FISH COMMUNITIES AND CONSERVATION OF AQUATIC LANDSCAPES IN NORTHEASTERN MESOAMERICA

by

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This research is dedicated to Belize:
a place and a people that have given me so very much.

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Table of Contents

Dedication………………………………………………………………………………ii
Acknowledgements…………………………………………………………………iii
List of Figures…………………………………………………………………………vi
List of Tables…………………………………………………………………………vii
Abstract………………………………………………………………………………viii

Chapter 1. Introduction………………………………………………………………1
    Literature cited…………………………………………………………………6

Chapter 2. Relative influences of catchment- and reach-scale abiotic factors on fish communities in rivers of northeastern Mesoamerica…………………………………8
    Abstract………………………………………………………………………………8
    Introduction…………………………………………………………………………9
    Methods……………………………………………………………………………12
    Results………………………………………………………………………………19
    Discussion…………………………………………………………………………24
    Literature cited…………………………………………………………………39

Chapter 3. Spatiotemporal dynamics of the spread of African tilapias (Pisces: *Oreochromis* spp.) into rivers of northeastern Mesoamerica…………………………43
    Abstract……………………………………………………………………………43
    Introduction………………………………………………………………………44
    Methods……………………………………………………………………………46
    Results……………………………………………………………………………53
    Discussion………………………………………………………………………56
    Literature cited…………………………………………………………………72

Chapter 4. Application of species distribution models and conservation planning software to the design of a reserve network for the riverine fishes of northeastern Mesoamerica……………………………………………………………………………77
    Abstract……………………………………………………………………………77
    Introduction………………………………………………………………………78
    Methods……………………………………………………………………………83
    Results……………………………………………………………………………91
    Discussion………………………………………………………………………94
    Literature cited………………………………………………………………107

Chapter 5. Conclusion……………………………………………………………116

List of Figures

Figure

2.1 Study area showing elevation, major drainage basins, important geographic features, and locations of all sampling sites………………………………………………………………………………… 34

2.2 The percentage of total variance explained by different scales of environmental data for fish presence-absence, relative abundance, and community metrics using partial CCA………………………………………….. 35

2.3 CCA bi-plot of species with catchment-scale environmental influences (a) and reach influences (b) based on presence or absence data………………… 36

2.4 CCA bi-plot of species with catchment-scale environmental influences (a) and reach influences (b) based on relative abundance data…………………… 37

2.5 CCA bi-plot of community metrics with catchment-scale environmental influences (a) and reach influences (b)………………………………………… 38

3.1 Study area showing elevation, major drainage basins, important geographic features and the locations of all sampling sites used for habitat suitability modeling with Maxent……………………………………………………… 68

3.2 River habitats predicted to be suitable for tilapias (dark lines), and habitats that were not predicted as suitable (gray)……………………………………... 69

3.3 The chronology of spread of tilapias in Belize based on fishermen's responses. Bottom graph shows detections of tilapia in new river reaches through time (right axis), and river discharge (left axis) at a station on the lower Belize River near Crooked Tree………………………………………… 70

3.4 Map of drainage basins (numbers), their invaded status (confirmed present = shaded; anecdotal report of presence = diagonal lines; no confirmed or anecdotal presence = no fill), and relative accumulative cost for inter-basin crossings (colored lines)………………………………………………………. 71

4.1 Study area showing elevation, hydrography, and the locations of all sampling sites used for presence-only modeling with Maxent…………………………….. 102

4.2 Environmental risk surface generated from agriculture and urban polygons, road lines, and village points available in GIS (left)……………………………... 103

4.3 Freshwater focal areas defined using 2 runs of Marxan. The first run defined focal areas for migratory species (dark gray areas), which demand a special class of critical management zone……………………………………………… 104

4.4 The focal reserve network overlaid by managed and intact protected areas… 105

4.5 The final portfolio including critical management zones (2 classes) and catchment management zones…………………………………………………. 106

List of Tables

Table

2.1 Mean, standard error (SE) and range of values for each catchment and reach variable………………………………………………………………… 28
2.2 Species codes, names, class, number of sites recorded as present (N), and mean relative abundance (proportion of all fish counted) of the 32 fish species used in the analysis…………………………………………………….. 31
2.3 Fish community metrics, their abbreviations, and summary statistics for each…………………………………………………………………………... 33
3.1 Environmental variables prepared for entry into Maxent models of tilapia habitat……………………………………………………………………….. 62
3.2 Initial introduction sources reported by fishermen, and possible dispersal mechanisms and/or routes for initial spread into each drainage basin in Belize……………………………………………………………………………. 65
3.3 Estimated dispersal rates calculated for the river systems for which multiple years of observation were recorded……………………………………………. 66
3.4 Summary table of crossings between drainage basins (Figure 3.4), and the associated elevational ranges, distances between river networks, and accumulative cost measure from cost surface analysis………………………… 67
4.1 Environmental variables prepared for entry into MaxEnt models of fish species distributions……………………………………………………………… 99
4.2 Intensity and influence distances assigned to different risk elements used to create an environmental risk surface…………………………………………... 101

ABSTRACT

FISH COMMUNITIES AND CONSERVATION OF AQUATIC LANDSCAPES IN NORTHEASTERN MESOAMERICA

by

Peter C. Esselman

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Tropical river conservation is a global priority because these rivers support high biodiversity and rural livelihoods, and contribute to maintenance of marine ecosystems. A challenge to river conservation in tropical developing countries is the paucity of scientific information to assist with conservation planning at appropriate spatial scales. This research attempted to alleviate some of the information scarcity impeding conservation of rivers draining to the coast of Belize in northeastern Mesoamerica. This work drew on field and museum collections of freshwater fishes to: (1) investigate the influences of reach- and catchment-scale environmental conditions on fish communities; (2) document spatial and temporal patterns of spread of an invasive fish, African tilapia (*Oreochromis* spp.), and make recommendations for its control; and (3) design a freshwater protected areas network in a riverine context. Environmental characteristics across scales described a large portion of total fish assemblage variation (64%), and catchment-scale factors explained a greater percentage of total variance (25%) than reach-scale environment (14%). The high correlation between assemblage patterns and catchment-scale factors suggests that fish conservation efforts are most appropriately conceptualized at this scale. A reconstructed spatial chronology of tilapia spread suggested that the invading population experienced an initial lag period before advancing from its initial home range, and that Nile tilapia (*O. niloticus*) is now widely distributed in the coastal plain rivers of at least 9 of 29 drainage basins. The study revealed unintentional releases from aquaculture and flooding as the two most likely dispersal mechanisms, leading to recommendations focused on (1) prevention of tilapia spread to un-invaded systems, and (2) control of aquaculture activities. Riverine conservation areas comprising 11% of the study area that had high fish biodiversity and low human influence were identified using conservation planning software and species distribution models for 63 fishes. Management zones were specified to mitigate threats to conservation areas, protect fish movement corridors, and target basin management. Despite chronic information limitations, this work demonstrates how limited field data, interviews with resource users, and modeling can be used to create biologically realistic hypotheses about ecological reality that can serve as a starting point for conservation planning in rivers.

Chapter 1

Introduction

Human influence on the world's terrestrial, freshwater, and marine biomes is extensive and growing (Vitousek et al. 1997, Halpern et al. 2008). Already, as much as 50% of the Earth's land surface has been transformed by human actions (Vitousek et al. 1997), 54% of the surface water supply is used by humans (Postel et al. 1996), and 41% of the ocean is affected by direct anthropogenic influence (Halpern et al. 2008). And human population continues to grow. Human domination of ecosystems at this scale has severe consequences for life as we know it, including permanent loss of species to extinction, long term climate and weather changes, and disruption of services provided to us by ecosystems. Few ecosystems are more affected, and also more important to life on the planet, than freshwater ecosystems.

Fresh water is one of the most crucial natural resources, and is in increasingly scarce supply due to growing appropriation for human uses (Postel et al. 1996). Humans derive essential goods and services from freshwater ecosystems in the form of water to drink, fish to eat, and more complex benefits such as flood moderation, drought protection, maintenance of food webs, waste processing, and nutrient delivery to the coast to support fisheries productivity (Postel and Richter 2003). The large array of goods and services provided to humans by fresh water go beyond water availability. Many of the benefits are directly reliant upon the existence of healthy, well-functioning ecosystems with a diversity of native species, intact food webs, and intact natural habitats (Postel and Richter 2003). For this reason, conservation of freshwater ecosystems, and the species they contain, is a societal imperative.

Relative to other biomes, freshwater ecosystems are disproportionately species rich, and also disproportionately threatened by human activities. Approximately 6% of the 1.75 million species known to science exist in the 0.8% of the planet occupied by freshwaters (Dudgeon 2000). Certain taxonomic groups are particularly rich, such as freshwater fishes, which comprise about 45% of the approximately 28,900 fish species known to science (Leveque et al. 2008). Tropical freshwater ecosystems, though poorly known, exhibit particularly high levels of endemism and species richness (Dudgeon 1999, 2000, Benstead et al. 2003, Strayer et al. 2004, Leveque et al. 2008). For instance, more than 70% of the above mentioned freshwater fish diversity occurs in the tropics (Leveque et al. 2008), a number that grows annually as new species are described (Stiassny 1999).

The wealth of freshwater biodiversity on the planet is increasingly threatened by humans. Freshwaters are most vulnerable to land use change, biotic exchange, and climate change (Sala et al. 2000, Revenga and Kura 2003). High global rates of land cover conversion to human uses, and non-indigenous species spread has already led to rates of decline in freshwater biodiversity that exceed declines in even the most seriously affected terrestrial ecosystems (Ricciardi and Rasmussen 1999). Species loss from freshwater ecosystems in tropical latitudes has not been estimated accurately, but may be similarly high to the rates of 4% per decade estimated for freshwater animals in North America (Ricciardi and Rasmussen 1999, Dudgeon et al. 2006).

The practical challenges to conserving freshwater ecosystems are numerous. One challenge is the high species turnover among drainage basins and water bodies that are separated by hard barriers to dispersal such as drainage divides or salt water (Sheldon 1988, Pusey and Kennard 1996). To account for high turnover, conservation efforts must be spatially distributed to protect the majority of species and maintain community diversity. River conservation is further challenged by the open, highly directional nature of flowing water systems, and their tendency to integrate conditions from their entire upstream catchment (Pringle 1997). This can lead to a spatial disjunction between stress origins and the locations of important species, communities, or habitats. The high degree of connectivity further complicates conservation efforts when migratory species are present that move longitudinally in the channel to fulfill their life cycles (Welcomme 1979). Given the spatially dispersed nature of stress origins, the wide-ranging habitat needs of many species, and community distinctiveness across drainage divides, conservation of freshwater ecosystems necessitates adoption of a landscape perspective (Moss 2000). Conservation of freshwaters at the landscape level implies that actions are planned and implemented at the basin- or multi-basin level, and that science to support this action is also focused at this level (Angermeier and Winston 1999, Fausch et al. 2002).

An additional challenge to river conservation is the often severe limitation on the amount of scientific information available to support sound conservation decision making. Information limitation is particularly acute in the tropics where species diversity is the highest. As observed by Dudgeon et al. (2006), “The manifest knowledge impediment in Asia and elsewhere in the tropics limits both attempts to quantify the freshwater biodiversity crisis and the ability to alleviate it”. The ability to undertake conservation in tropical developing countries is specifically impeded by a limited understanding of how tropical aquatic ecosystems function, the paucity of baseline research, insufficient human technical capacity and technology, and limited investment in research and monitoring (Pringle et al. 2000, Wishart et al. 2000). Such conditions necessitate that conservation assessments proceed based on incomplete or inadequate information about species and ecosystems. Enhanced conservation science in tropical aquatic ecosystems is a high global research priority if the asymmetry between high conservation need and low information availability is to be alleviated (Dudgeon et al. 2006).

The research presented in this dissertation was designed to meet the challenges mentioned above by providing landscape-scale information about ecosystems, threats, and conservation strategies to alleviate the information scarcity present in the tropical developing country of Belize. Belize is a small independent country on the Caribbean coast of Central America, with a population of 312,000 people. Freshwater ecosystems are very important to Belize, in part because Belize receives high levels of rainfall each year (up to 4 m annually), and also because rivers have a strong influence on Belize’s coastal zone, which contains the longest barrier reef in the Western Hemisphere. The 220 km-long Mesoamerican Barrier Reef System stretches the entire coast of Belize and is recognized by the United Nations as a World Heritage Site. Increased awareness of the connectivity between upland human activities and coastal/marine ecosystem integrity—all mediated by the extensive river network that integrates the landscape—has made river conservation a high national and regional priority among nations of the Mesoamerican Barrier Reef area (Nunny et al. 2001, Esselman et al. 2006, Bailey et al. 2007).

Fish communities have been frequently used as indicators of overall river health, because they occupy almost all trophic levels and habitats in aquatic systems, are sensitive to environmental or chemical changes to their habitat, and are long-lived and thus integrate river conditions over timescales that are meaningful to management (Karr 1981, 1987). Because they indicate river conditions, fishes can be seen as a vehicle through which landscape conservation can be accomplished in the ridge-to-reef context of the Mesoamerican Barrier Reef. To protect fish communities it is necessary to guard rivers from upland activities and direct negative influences. In this way, fishes are a conservation target that is aligned with the larger goals of river conservation and coastal zone protection from river-borne threats. This dissertation attempts to alleviate some of the information scarcity to support strategic conservation of rivers by focusing on protection of freshwater fishes.

The overarching goals of this work were: (1) to describe fish communities relative to dominant environmental gradients at the national scale; and (2) to address specific problems in fish conservation that have direct relevance to ongoing conservation efforts in Belize. Accordingly, three empirical studies were undertaken that are presented as chapters of this dissertation:

- Chapter 2 places fish communities into their landscape context by describing fish communities and investigating the ability of abiotic factors at several scales to explain variation in assemblage composition across the study area.
- Chapter 3 investigates the dynamics of spread of a potentially serious biological invader—African tilapia (*Oreochromis* spp.)—which also presents interesting fisheries management challenges. This chapter reconstructs the chronology and rates of invasion, predicts eventual range limits, and makes recommendations for tilapia control.
- Chapter 4 draws on species distribution models for 63 native fish species to recommend Mesoamerica's first freshwater protected areas network. This chapter considers the unique connectivity inherent to river networks to create a network of conservation areas that strives to meet the challenge of place-based conservation in dynamic aquatic landscapes.

I conclude with a synthesis of my research findings, and suggestions for productive future research directions. This work applies scientific research techniques to answer questions with direct application to river conservation planning and action. At a broader scale, it is a study in how limited data availability can be overcome to advance watershed science in the type of information-poor setting that is all too common in many countries facing conservation crises around the world.

Literature cited

Angermeier, P. L. and M. R. Winston. 1999. Characterizing fish community diversity across Virginia landscapes: Prerequisite for conservation. *Ecological Applications* **9**:335-349.

Bailey, M., P. H. Kirshen, E. Spang, S. Morrison, C. Pizarro, G. Kayser, G. Cid, and A. Parish. 2007. *Rapid assessment of anthropogenic impacts on select transboundary watersheds of the Mesoamerican Barrier Reef System (MBRS) region*. National Oceanic and Atmospheric Administration and Tufts University.

Benstead, J. P., P. H. De Rham, J. L. Gattolliat, F. M. Gibon, P. V. Loiselle, M. Sartori, J. S. Sparks, and M. L. J. Stiassny. 2003. Conserving Madagascar's freshwater biodiversity. *Bioscience* **53**:1101-1111.

Dudgeon, D. 1999. *Tropical Asian Streams : Zoobenthos, Ecology and Conservation*. Hong Kong University Press, Hong Kong.

Dudgeon, D. 2000. The ecology of tropical Asian rivers and streams in relation to biodiversity conservation. *Annual Review of Ecology & Systematics* **31**:239-263.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**:163-182.

Esselman, P. C., M. C. Freeman, and C. M. Pringle. 2006. Fish-assemblage variation between geologically defined regions and across a longitudinal gradient in the Monkey River Basin, Belize. *Journal of the North American Benthological Society* **25**:142-156.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience* **52**:483-498.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. P. Madin, M. T. Perry, E. R. Selig, M. Spalding, R. Steneck, and R. Watson. 2008. A global map of human impact on marine ecosystems. *Science* **319**:948-952.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. *Fisheries* **6**:21-27.

Karr, J. R. 1987. Biological monitoring and environmental assessment: a conceptual framework. *Environmental Management* **11**:249-256.

Leveque, C., T. Oberdorff, D. Paugy, M. L. J. Stiassny, and P. A. Tedesco. 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia* **595**:545-567.

Moss, B. 2000. Biodiversity in fresh waters - an issue of species preservation or system functioning? *Environmental Conservation* **27**:1-4.

Nunny, R., M. Santana, P. Stone, D. Tillet, and D. Walling. 2001. *An investigation of the impact on reef environments of changing land-use in the Stann Creek district of Belize*. The Watershed Reef Interconnectivity Scientific Study (WRIScS) EU Project No. B7-6200/97-02VII/ENV.

Postel, S. and B. D. Richter. 2003. *Rivers for Life: Managing Water for People and Nature*. Island Press, Washington, D.C.

Postel, S. L., G. C. Daily, and P. R. Ehrlich. 1996a. Human appropriation of renewable fresh water. *Science* **271**:785-788.

Pringle, C. M. 1997. Exploring how disturbance is transmitted upstream: Going against the flow. *Journal of the North American Benthological Society* **16**:425-438.

Pringle, C. M., F. N. Scatena, P. Paaby-Hansen, and M. Nunez-Ferrera. 2000. River conservation in Latin America and the Caribbean. Pages 41 - 77 *in* P. J. Boon, B. R. Davies, and G. E. Petts, editors. *Global Perspectives on River Conservation: Science, Policy, and Practice*. John Wiley and Sons Ltd., New York.

Pusey, B. J. and M. J. Kennard. 1996. Species richness and geographical variation in assemblage structure of the freshwater fish fauna of the Wet Tropics region of northern Queensland. *Marine and Freshwater Research* **47**:563-573.

Revenga, C. and Y. Kura. 2003. *Status and trends of biodiversity of inland water ecosystems*. Secretary of the Convention on Biological Diversity, Montreal, Canada.

Ricciardi, A. and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. *Conservation Biology* **13**:1220-1222.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - global biodiversity scenarios for the year 2100. *Science* **287**:1770-1774.

Sheldon, F. 1988. Conservation of stream fishes: patterns of diversity, rarity, and risk. *Conservation Biology* **2**:149-156.

Stiassny, M. L. J. 1999. The medium is the message: freshwater biodiversity in peril. Pages 53-71 *in* J. Cracraft and F. T. Grifo, editors. *The Living Planet in Crisis: Biodiversity Science and Policy*. Columbia University Press, New York, NY.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *Bioscience* **54**:429-439.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. *Science* **277**:494-499.

Welcomme, R. L. 1979. *Fisheries Ecology of Tropical Floodplain Rivers*. Longman, London, UK.

Wishart, M. J., B. R. Davies, P. J. Boon, and C. M. Pringle. 2000. Global disparties in river conservation: 'First world' values and 'third world' realities. Pages 353 - 369 *in* P. J. Boon, B. R. Davies, and G. E. Petts, editors. *Global Perspectives on River Conservation: Science, Policy, and Practice*. John Wiley and Sons Ltd., New York.

Chapter 2

Relative influences of catchment- and reach-scale abiotic factors on fish communities in rivers of northeastern Mesoamerica

Abstract

Fish assemblages at the reach scale within a river network are structured relative to local abiotic factors that are, in turn, constrained at the larger scales of the valley segment or catchment. While abiotic factors important to fish assemblages at the reach scale are well understood, studies of larger scale constraints have yielded variable conclusions, spurring a need for further studies in new settings and biogeographic contexts. This study investigated the relative importance of catchment- and reach-scale abiotic factors to variation in fish assemblage composition in rivers of northeastern Mesoamerica. I assembled catchment and reach environment, and fish datasets for 72 sampling sites on main stem rivers of 12 of the 16 major watersheds draining to the coast of Belize. Partial canonical correspondence analysis was used to determine the unique effects of catchment- and reach-level descriptors on fish presence and absence, relative abundance, and community summary metrics. Results showed that, combined, catchment and reach variables explained a large portion of the total variation in the fish assemblage data (57 to 73%), and that catchment environment explained a greater portion of variation (24 to 26%) than reach environment (9 to 20%). Variables representing landscape position (local elevation, watershed area) and its reach-level correlates (channel width, depth variation, and substrate composition) related most strongly to the fish assemblage data. The results suggest that landscape-scale factors have a stronger relative influence on assemblages than environmental conditions at the reach scale. These results contrast with past findings, and suggest that biodiversity conservation efforts must aim to protect ecological integrity across a positional gradient from the mountains to the sea, and that the catchment scale or larger is a biologically meaningful scale for fish conservation planning and coordination to begin in northeastern Mesoamerica.

Introduction

Ecologists have long sought to identify factors that regulate patterns in local community composition and structure through space and time (e.g., Ricklefs & Schluter 1993). This task has become more urgent in recent decades as it has become increasingly apparent that threats to global biodiversity have reached crisis levels (Sala et al. 2000, Dudgeon et al. 2006). An emergent perspective from this pursuit is that local diversity is often the result of a nested hierarchy of abiotic constraints that spans multiple spatio-temporal scales (Allen & Starr 1982). In stream and river ecosystems, numerous authors have postulated that assemblages at fine scales within a river reach are structured relative to local abiotic factors that are, in turn, constrained at the larger scales of the valley segment or catchment (Frissell et al. 1986, Townsend 1996, Poff 1997). A number of studies have substantiated this concept by demonstrating the multi-scalar influences that abiotic factors have on varied taxa across biogeographic contexts (e.g., Johnson et al. 2007). Much work also points to the overriding influence that landscape modification by humans can have on habitat and organisms at every scale (reviewed in Allan 2004). Still, the majority of studies of organism-environment relationships have focused at small spatio-temporal extents that limit our ability to discern large-scale controls (Dunham & Rieman 1999, Fausch et al. 2002, Durance et al. 2006), and create a mismatch between the scale of ecological knowledge and the scales at which management interventions must occur to protect and restore ecosystem integrity (Wiens 2002, Lake et al. 2007). Thus, studies that characterize community variation across relevant spatial scales can be an important pre-requisite to aquatic conservation efforts (Angermeier & Winston 1999), and should help to advance a more generalized understanding of aquatic community regulation.

Past studies of fishes in streams and rivers have led to a generalized understanding of abiotic correlates to variation in fish assemblages. At the reach scale, local channel and hydraulic conditions have been linked to fish community attributes in temperate and tropical streams (Gorman & Karr 1978, Schlosser 1982, Statzner et al. 1988, Angermeier & Schlosser 1989, Angermeier & Winston 1998). Studies in disparate geographies show consistent patterns of assemblage variation along longitudinal gradients of increasing stream order (Schlosser 1982, Lamouroux et al. 2002). These studies indicate the importance of local habitat and landscape position, but do not provide deeper insights into possible direct or indirect constraints placed on local assemblages by factors at larger scales.

Research into the relative influence of factors across scales has yielded interesting, and highly variable, conclusions that can help with the formation of hypotheses in unstudied landscapes. Studies have shown that local assemblage variation often corresponds strongly to variation in landscape characteristics (Angermeier & Winston 1999), particularly in landscapes with high degrees of land use conversion to anthropogenic uses (Roth et al. 1996, Allan et al. 1997, Wang et al. 2003). Other works suggest that segment- or reach-scale factors relate strongly to biotic patterns, in isolation from and in interaction with factors at the larger scale of the segment, catchment, or biogeographic region (Wang et al. 2003, Johnson et al. 2007). Consistent with nested hierarchy theory, a number of works indicate that site-specific environmental conditions and biota are dependent upon regional- or watershed-scale characteristics (e.g., Brazner et al. 2005) and that abiotic factors combined across nested spatial scales often explain a high portion of variation in fish assemblage patterns (~40 to 60%; Wang et al. 2003, Johnson et al. 2007, Stewart-Koster et al. 2007). Finally, several works suggest that landscape factors often have less explanatory power than reach-scale variables (Lyons 1996, Lammert & Allan 1999, Wang et al. 2003, Johnson et al. 2007), except in situations where landscapes are heavily dominated by anthropogenic land cover types. In human-dominated landscapes, conditions at the catchment scale have been shown to override the influence of local and riparian environmental conditions on stream fishes (Roth et al. 1996, Allan et al. 1997).

Multi-scale studies of fish assemblages are virtually non-existent in watersheds of tropical Mesoamerica, but provide opportunities for testing the generality of nested hierarchy concepts. As in temperate regions, stream fish assemblages of Mesoamerica are structured in part by local habitat conditions (Bussing & Lopez 1977, Gorman & Karr 1978, Winemiller 1983, Angermeier & Schlosser 1989), and assemblages also vary predictably across longitudinal gradients (Angermeier & Karr 1983, Lyons & Schneider 1990, Winemiller & Leslie 1992, Rodiles-Hernandez et al. 1999, Esselman et al. 2006).

Yet, virtually nothing has been published that simultaneously examines the influences of abiotic factors at multiple spatial scales on Mesoamerican fish assemblages.

The present study investigates the relative importance of catchment- and reach-scale abiotic factors to variation in fish assemblage composition in streams and rivers of northeastern Mesoamerica. For the purposes of this study, catchment-scale factors are defined as those that measure integrated conditions in the landscape upstream or downstream of a given sampling location (e.g., proportion of different geologies in the watershed upstream of a location), or the position of a location within the landscape (e.g., geographic latitudinal, or distance from the river mouth). In contrast, reach-scale factors describe local habitat conditions (e.g., channel morphology, substrates, fish cover, etc.) in 300 m to 1300 m sections of the river channel that encompass multiple riffle/run/pool sequences in streams and small rivers, or several river bends in larger river habitats that are free of runs and riffles.

To date, only two studies in northeastern Mesoamerica have examined the relationships between assemblages and environmental conditions. These studies indicate the potential importance of distance from the sea, temperature, and salinity (Schmitter-Soto & Gamboa-Perez 1996), and the constraints that surface geology may put on site-level physicochemistry and fish assemblage structure (Esselman et al. 2006). Based on these results and the findings from elsewhere reviewed above, I hypothesized that: (1) reach-scale abiotic conditions will account for more assemblage variation than catchment conditions, because of relatively low levels of landscape conversion to human uses; (2) longitudinal position in the catchment will explain a substantial portion of compositional and community variation, both directly and indirectly through interactions with local habitat conditions; and (3) latitude, which corresponds to a strong change in precipitation in the study area (see description of study area below), will correlate to compositional patterns driven by north-to-south species turnover across biogeographic boundaries. To test these hypotheses, I examined the proportion of variance in fish community data explained by catchment- and reach-scale environmental factors, and identified the individual variables with the highest influence on community patterns at both scales.

Methods

Study area and sample sites

The assessment was carried out in the domestic and international waters that drain to the coast of Belize, including portions of southern Mexico and northeastern Guatemala. Sixteen major catchments and numerous small coastal drainages are contained within this 45,750 km^2 area (Figure 2.1). These catchments vary in size, traverse a variety of geologic types and topographies, and drain a diversity of land cover types (Lee et al. 1995, Esselman & Boles 2001). Twelve of the 16 major rivers are high-gradient, low pH streams that originate in the metamorphic and volcanic rocks of the Maya Mountains (Figure 2.1), then flow through landscapes dominated by limestone and sedimentary rock before entering the coastal plain and discharging into the shelf lagoon that separates the coast from the Mesoamerican Barrier Reef system. The northernmost rivers originate in karst hills, drain the low relief limestone platform of the Yucatan Peninsula, and discharge into Chetumal Bay, which in turn connects to the shelf lagoon. The three southernmost rivers begin in Guatemala and flow eastward to the Gulf of Honduras. In addition to flowing waters, the study area has an abundance of fresh and brackish water lagoons and wetlands that include swamp forests, herbaceous marshes, and open water areas, often in association with riverine habitats (Esselman & Boles 2001).

Daily mean temperatures are warm throughout the year, ranging from a low of 16ºC in the winter to a high of 33ºC in summer months (Hartshorn et al. 1984). A strong north-to-south precipitation gradient exists, with the northern portion of the study area receiving approximately 1000 mm of rain annually and the southern portion receiving up to 4000 mm (Wilson 1980). The timing of precipitation is seasonal, with a dry season lasting from January to May and a wet season from June to November. The area is highly prone to hurricanes in the late summer and early fall (Wilson 1980).

Miller (1966) called northern Mesoamerica a “strong center of evolution” for fishes, because of its many endemic genera and species. The area is particularly rich in poeciliids (>34 species) and cichlids (>44 species) (Miller 1966, Bussing 1976), and contains at least 130 species of freshwater fishes in 34 genera and 10 families (Bussing 1976), a number that rises to over 200 species when predominately marine fishes that inhabit freshwaters are included (Miller 1966). One hundred and twenty-six fish species have been reported from the catchments of the study area (Schmitter-Soto & Gamboa-Perez 1996, Greenfield & Thomerson 1997, Schmitter-Soto 1998, Esselman et al. 2006).

Seventy-two sampling sites were selected on the main stems of 3rd through 6th order rivers in 12 of the major catchments in the study area (Figure 2.1). Fifty-three of these sites were identified by locating a random start point within the first 5,000 m of habitat upstream from the river mouth, then placing a site each 20 river km along the main stem channels and major tributaries. These sites were sampled for fishes and habitat during base flow conditions in 2006 and 2007. An additional 19 sites were used from a dataset assembled in 2000 (Esselman et al. 2006). These sites were selected randomly within physiographic strata representing distinct combinations of stream size, gradient, and underlying geology within one basin in the southern part of the study area (Monkey River; Figure 2.1).

Fish sampling

Fishes were collected using backpack and boat electrofishing during base-flow conditions in the dry season months between January and May. The goal at any given site was to capture as many species as possible in all habitats present. In wadeable rivers with riffle-run-pool morphology, reach lengths were 39 times the mean stream width, and a single electrofishing pass was performed through all available habitats using a Smith Root® 12 battery powered backpack electrofisher. In riffles, fishes were shocked downstream into a 2 m x 5 m seine (5-mm mesh) blocking the channel, while in runs and shallow pools, samples were collected by shocking and dip-netting fishes near cover, from areas free of cover, and from within the water column. All captured fishes were placed in 5 gallon buckets with aerators, and processed frequently to avoid stress. Slight distinctions existed in the fishing approaches used between the electrofishing conducted in 2006-2007 and that done in 2000 in the Monkey River. In the Monkey River, backpack electrofishing was used to sample both coastal plain and mountain sites, whereas all coastal plain sites were sampled by boat electrofishing in 2006-2007. Additionally, the coastal plain samples in the Monkey River study were collected during the moonless portion of the night to capture more taxa, and deep pools in the coastal plain were also fished by angling and trotlines to add species for the presence/absence analysis.

Boat electrofishing was conducted at 38 of the 72 sampling sites (Figure 2.1) using a Smith-Root GPP 5.0 generator-powered electrofisher deployed from booms on an aluminum boat in reaches that were uniformly 1300 m in length. Within this reach, a single pass was made along one bank, and fishes were placed in an aerated live-well. A single boat electrofishing pass generally involved no less than 3,000 seconds of shocking time.

Most fishes captured were identified to species in the field using the dichotomous key of Greenfield and Thomerson (1997), and released after positive identification. Individuals with uncertain identifications or for which meristic counts or microscope diagnostics were necessary were euthanized and preserved in 10% formalin for later examination. Voucher collections for the 2000 study were deposited at the Georgia Museum of Natural History (Athens, Georgia, USA), and for 2006-2007 at University of Michigan Museum of Zoology (Ann Arbor, Michigan, USA). Given that I only sampled during dry season conditions with a single electrofishing pass at each site, my sample represents a snapshot of dry season fish assemblages, and is not considered an exhaustive census of all species present at a given site.

Reach and riparian environmental variables

At each sampling site where I fished, information was also collected about channel morphology, substrates, fish cover, bank conditions, riparian width, and riparian land cover. Methods differed slightly between wadeable and non-wadeable sites. In wadeable sites, thirteen transects separated by a distance equal to three times the mean stream width were established at each site. At each transect, wetted width, water depth, fish cover type and extent, and habitat type (riffle, run, pool) were determined. Depth and dominant substrate type were recorded at five equidistant points across each transect. Fish cover types were assigned a categorical value from 0 to 4 according to the percentage of a 10- m wide zone centered on each transect line occupied by each cover type (0 = cover type absent, 1 = 0–10% covered, 2 = 10–40%, 3 = 40–75%, 4 = 75%; after Lazorchak et al. (1998). The area of bottom covered by large woody debris (> 0.30 m diameter) in the river channel was estimated and normalized based on reach length to calculate $m^2 \cdot 100\ m^{-1}$ of channel. Electric conductivity, pH, and water temperature were measured once at each site using hand-held water quality meters manufactured by Hanna Instruments®. At each transect, bank stability was categorically estimated according to the percentage of bank composed of unconsolidated bare soil (0 – 25%; 26 – 50%; 51 – 75%, or 76 – 100%). Riparian forest width and cover types were also estimated at each transect within 15 m from the top of the bank away from the river channel.

At non-wadeable sites, 10 transects were established at intervals of 130 m along the river channel. Channel dimensions, substrates, habitat types, large woody debris, water quality, bank stability, and riparian width and cover were assessed in the same manner as wadeable sites. Fish cover was assessed using the same categories as above within a 4 m by 10 m zone along each bank centered on the transect line.

Catchment environmental variables

To capture landscape-level variation in the catchments up- and downstream of each sampling site, I assembled a geospatial database that consisted of 25 variables representing watershed location and landscape position, climate, surficial geology, land use, upstream road density in catchment, and distance to nearest human settlement (Table 2.1). These variables represented conditions local to each site as well as integrated over the entire upstream watershed. Details on the preparation of these data can be found in Chapter 3.

Surficial geology of the study area consists of five different classes. The Maya Mountains (Figure 2.1) are composed of variably metamorphosed argillacous and arenaceous sediments dated from the Pennsylvanian period, with local intrusions of granite, and some extrusive volcanic rocks. Surrounding the Maya Mountains, and composing much of the northern part of the country are Cretaceous and early Tertiary limestones, dolomites, and breccias (called Cretaceous limestone in Table 2.1). To the south of the Maya Mountains, underlying the southernmost six drainage basins, is a distinctive series of shales, turbidites, sandstones, conglomerates, and mudstones of late Cretaceous and early Tertiary age, known as the Toledo Formation. In the late Tertiary, layers of marls, limestone, and gypsum were deposited in several areas north of the Maya Mountains on the Yucatan Platform (called Tertiary sedimentary in Table 2.1). Finally, much of the coastal plain of the area is covered in Quaternary alluvium from river terraces, sand bars, and calcareous sand and mud (called Quaternary alluvium in Table 2.1).

Data analysis

Prior to analysis, I summarized my datasets (Tables 2.1 – 2.3). For each site, I averaged measures taken across all transects to calculate the following metrics: means of width, depth, fish cover rating, riparian width, and bank stability; coefficient of variation for depths; percent composition of habitat types, substrates, fish cover types, and riparian land uses; Shannon diversity of fish cover and substrate types; and sum of the area of stream bottom covered by logs divided by 0.01 times the reach length for each site. For my fish data, I summarized the numeric abundance of each species at each site, and calculated the following site-level community metrics: richness, Shannon diversity index, percent top carnivore individuals and species, percent migratory individuals and species, percent narrowly endemic individuals and species, percent poeciliid individuals and species, and percent cichlid individuals and species. Narrow endemics were defined as those whose entire range occupies three or less of the freshwater ecoregions defined for Mesoamerica by Abell et al. (2008).

After summary, I organized the environmental datasets into separate matrices for catchment variables and reach/riparian variables (heretofore referred to as reach variables). Riparian variables were combined with reach variables because there were very few (n = 5), and they were measured in the field at the reach scale [versus in a broader longitudinal and lateral sense using remote sensing data, e.g., Lammert & Allan (1999)]. I also organized three fish data matrices: species presence/absence, relative abundance by count, and the community summary metrics (Table 2.3).

For my analysis, I used a variance decomposition technique known as partial constrained ordination (Borcard et al. 1992), which uses canonical correspondence analysis (CCA) or redundancy analysis (RDA) to determine the unique effect of catchment- and reach-level descriptors on community composition. Constrained ordination is a multivariate approach that constrains species data by linear combinations of environmental variables (ter Braak & Prentice 1988), and allows for easy interpretation of relationships between environmental factors and whole assemblage composition or structure. RDA is the constrained ordination approach used to describe species that respond linearly to environmental gradients. CCA is the equivalent procedure for species data that exhibit unimodal responses to gradients. Partial CCA and RDA are *a posteriori* techniques that partition variation in species datasets into individual components that describe pure influences and interactions of sets of predictor data (Borcard et al. 1992, Anderson & Gribble 1998). In the present implementation, I partitioned the variation in fish presence-absence, relative abundance, and community data sets that were explained by pure catchment and reach-scale influences and their interactions.

The analysis had two steps using CCA and/or RDA: (1) data reduction and (2) variance partitioning. I ran two processes to reduce the master catchment and reach datasets to a smaller number of variables with a low degree of collinearity. In the first process, I calculated pair-wise Pearson correlation coefficients for each combination of predictors in the catchment and reach datasets independently. I identified variable pairs with Pearson correlation coefficients greater than 0.65, and removed one of the two variables in each pair that I felt were the poorer predictors of fish assemblages. Next I ran CCA analysis (using Canoco for Windows© v.4.02) for each environmental dataset paired with each of the three fish datasets using the automatic stepwise forward selection procedure available in Canoco v. 4.02. I retained only those variables that were significantly correlated with one of the first 3 CCA axes.

Before running the second step of the analysis, I ran detrended correspondence analysis (DCA) to determine the appropriate constrained ordination technique (CCA or RDA) to use in the partial constrained ordination. The gradient length of species composition along the first DCA axis allows for an estimation of whether the species (or community) responses to the environmental data are likely to be linear or unimodal. Data with greater than 2 standard deviations (SD) of turnover along the first DCA axis are likely to respond unimodally and should be used with CCA, while data with less than 2 SD of turnover on that axis are likely to respond linearly to environmental gradients and should be used with RDA (ter Braak 1995). Each of the three fish datasets had greater than 2 SD of turnover on the first DCA axis, so CCA was determined as the appropriate technique in each case.

CCA was used to estimate the variation in assemblage characteristics explained by catchment and reach variables. To do this I combined the reduced catchment and reach matrices into a single dataset and paired it with each of the three fish data sets. I then used the approach of Borcard et al. (1992) to partition total variance of each fish data set into components that were explained by catchment and reach-level predictors. For each of the three fish datasets, two bi-plots were created to display correlations between the locations of species or community metrics and environmental variables from the catchment and reach datasets. Correlations between environmental factors and species or community metrics were inferred from visual interpretation of plots, with specific attention to the direction and length of environmental vectors (shown as lines with arrows in the plots; e.g., Figure 2.3) in relation to species or community metrics (shown as triangles in the plots, e.g., Figure 2.3). Longer environmental vectors in the plots were interpreted as having a stronger correlation with the species or community points found on the same axis as that vector.

Results

Catchment, reach, and fish characteristics

Sampling sites occurred across a range of abiotic conditions (Table 2.1), and sites ranged widely in terms of their positions on main stem rivers within a mostly forested landscape. At the catchment scale of measurement, sites were located from 1 to 367 river km from the river mouth across an elevational range of 2 to 468 masl. Consistent with the strong north-to-south precipitation gradient of the study area, historical mean annual upstream precipitation values ranged from 1,260 mm to 3,747 mm. Cretaceous limestone was the most common geologic type, averaging 36% of the surficial geology cover in watersheds of the study sites, and forest was by far the most common land cover type (mean percent forest in catchment = 76%) followed by undifferentiated agriculture (18%).

Consistent with the catchment data, most sites were located on small rivers that spanned an array of geomorphic and chemical conditions. Wetted channel widths varied from 2 m to 67 m wide with an average width of 28 m across sites. On average, river substrates were dominated by sand, followed by cobble, gravel and clay. Water chemistry indicated a wide range of ion contents with conductivity ranging from 22 to 2,531 $\mu S \cdot cm^{-2}$, and pH values from 6 to 9. These numbers are likely to be reflective of the variation between acidic rocks of the Maya Mountains and calcareous rocks that dominate much of the rest of the area. Consistent with the observation that the landscape was highly forested, riparian zones had a high percent of forest cover, with mean forested widths of 12 m (out of a maximum of 15 m). On average, only about 9% of riparian buffers were occupied by current human activities.

The fish assemblage data were reflective of the biogeographic context and the fact that the rivers of the study area are connected directly to the sea. A total of 74 fish species and 24,590 individuals were collected at 72 sites. Thirty-five of the species were from marine families that can tolerate full seawater but also use freshwater (Miller 1966). An additional 11 rare species occurred at less than five sites and were included in the calculation of community summary metrics only. After removal of rare species and non-migratory species from marine families, 32 species remained (Table 2.2), which were used in the analysis of environmental influences on fish presence-absence and relative abundances. Within this group, 11 families were represented, with Cichlidae (13 spp.), Poecillidae (6 spp.) and Characidae (3 spp.) being the most diverse. Two species exhibited clear numerical dominance: the central tetra (*Astyanax aeneus*; mean relative abundance = 0.26), and the shortfin molly (*Poecilia mexicana*; mean relative abundance = 0.16). Community metrics (Table 2.3) indicated a strong numeric representation by cichlids and poeciliids in their relative richness and relative abundance. Top carnivores, migratory fishes, and endemics generally comprised low proportions of species and individuals captured at a site. Species richness (with marine families excluded) ranged from two at high elevation sites to 22 in large coastal plain rivers, with an average of 15 species per site. The large influence by marine species in the fish data is indicative of the direct linkages between all rivers and the Caribbean Sea. The high relative richness of cichlids and livebearers is consistent with the biogeographic context of northeastern Mesoamerica (Miller 1966).

Variable selection

Variables were selected after reduction of the dataset using (1) removal of inter-correlated variables, and (2) forward stepwise CCA. Correlation reduction eliminated five variables from the catchment dataset (distance to ridge, local precipitation, slope in catchment, local temperature, and % volcanic rock in catchment), and four variables from the reach dataset (average depth, % silt substrates, % run habitat, % riffle habitat). After correlation reduction, but prior to variable reduction with CCA, there were 20 variables in the catchment dataset, and 23 in the reach dataset. Stepwise CCA runs identified the catchment and reach variables that related significantly to the first 3 CCA axes of fish data set ordination (Table 2.1). Based on significant correlations with the three fish datasets, 12 of the 20 catchment variables were retained for further examination of the fish presence-absence data, 10 for relative abundance, and eight for community characteristics. In the reach variable reduction process, 10 variables were retained for the fish presence or absence dataset, 11 for the abundance dataset, and 11 for the community characteristics dataset.

Ability of environmental variables to explain fish assemblage variation

The CCA results describing variation explained by catchment and reach variables showed that environmental factors at these scales combined explained a relatively large portion of the total variation in the fish assemblage data—62%, 57%, and 73% for the presence-absence, abundance, and community data sets respectively (Figure 2.2). When the variation was partitioned into the portions explained by only catchment variables, only reach variables, and the interaction of the two, catchment variables explained 24%, 24%, and 26% of variance for the presence-absence, abundance, and community data sets respectively, and reach variables explained 9%, 12%, and 20% (Figure 2.2). The interactions of catchment and reach scale variables explained 29%, 21%, and 27% percent of variation for the presence-absence, abundance, and community datasets, similar to the amount explained by catchment variables alone.

Relations between environmental variables and fish assemblages

An examination of bi-plots from the CCA runs with the reduced environmental datasets and the fish datasets revealed a number of interrelationships between co-occurring species and community metrics and environmental factors at the catchment and reach scales (Figures 2.3 – 2.5). For the presence-absence dataset, at least three species-environment associations were evident. The first association was between the southern endemic chisel-tooth cichlid (*Cichlasoma boucourti*) and golden firemouth cichlid (*Thorichthys aureus*), increasing percentage of Toledo Formation rock, and decreasing distance from sea (Figure 2.3a, upper left). The Toledo Formation is the geologic type that underlies the southernmost six catchments in the study area, and thus corresponds to the species distributions of those fishes limited to the south. At the reach level (Figure 2.3b) these species were weakly correlated to increasing percent clay substrate.

The second association was a strong correlation between increasing latitude and decreasing catchment rainfall, which defines species that occurred in the northern and southern parts of the study area respectively. Species on the low latitude/high rainfall end of this gradient included the machaca (*Brycon guatemalensis*) and the blackbelt cichlid (*Vieja maculicauda*) (Figure 2.3a, left side). The high latitude/low rainfall end of this gradient correlated to the presence of the redhead cichlid (*Vieja synspila*), Jack Dempsey (*Rocio octofasciata*), and the Guatemalan chulin (*Rhamdia guatemalensis*). In the same direction as the high latitude/low rainfall group, but in stronger association with watershed area, was a group of fishes captured frequently in the large lowland rivers of northern Belize, such as the Mayan cichlid (*Cichlasoma urophthalmus*), Peten molly (*Poecilia petenensis*), blue catfish (*Ictalurus furcatus*), and Nile tilapia (*Oreochromis niloticus*) (Figure 2.3a upper right side). At the reach scale, this high latitude/large watershed group correlated to channel characteristics typical of rivers with larger watersheds—greater channel widths and decreased percent boulder substrates (Figure 2.3b upper right).

A third association was apparent between mountain river species, including the mountain mullet (*Agonostomus monticola*), twospot livebearer (*Heterandria bimaculata*), filespine chulin (*Rhamdia laticauda*), and green swordtail (*Xiphophorus helleri*), and increasing local elevation (Figure 2.3a, bottom left). At the reach scale the mountain group correlated strongly to increasing variation in depths and decreasing channel widths (Figure 2.3b). In sum, patterns in species presence-absence were most strongly tied to a latitude/precipitation gradient, watershed area, and elevation, with reach associations to channel size, depth diversity, and substrates.

The relative abundance data served to reinforce the importance of catchment variables representing landscape position (latitude, distance from sea, local elevation), watershed size, and reach variables representing river size and substrate as drivers or proxies for the drivers of community composition. As in the presence-absence data, the percent of Toledo Formation geology correlated to the southern endemic cichlids, and also to the blackbelt cichlid (*V. maculicauda*) and machaca (*B. guatemalensis*), which are found in greatest abundances in the southern part of the study area (Figure 2.4a, top left). Both the blackbelt cichlid and the machaca are widely distributed in Central America south to Panama, but may fall out of the northern Belize fauna as a result of habitat changes created by the disappearance of mountainous topography, or in the case of the blackbelt cichlid, because of competition with congeneric species like the redhead cichlid (*V. synspilum*) that are highly successful in the Yucatan Platform rivers. Again, relationships between this group and reach variables were weak or absent. A second association was evident between several species and decreasing local elevation and distance from sea (Figure 2.4, center left). These include the false firemouth cichlid (*Amphilophus robertsoni*) and the obscure swamp eel (*Ophisternon aenigmaticum*). At the reach level, these species were correlated positively with river width and pH, and negatively with the percent forest in the riparian zone (Figure 2.4b). The association between high latitude, large river habitats and northern lowland river fishes was evident in the abundance data (Figure 2.4a, bottom), as was also apparent in the presence-absence bi-plot. No reach-level variables appeared to be directly associated with northern lowland river fishes. Finally, the only two species that are commonly found in the constrained and hydraulically stressful conditions in high elevation streams—the green swordtail (*X. helleri*) and twospot livebearer (*H. bimaculata*)—were correlated strongly to increasing local elevation, with only a weak correlation to increasing riparian forest and decreasing wetted width.

Community metrics were correlated to a more balanced mix of catchment and reach factors that included geology, local elevation, and latitude at the catchment scale, and, at the reach level, channel morphology and substrates. In the bi-plot of community metrics with environment (Figure 2.5), the southern endemic species were again linked to Toledo Formation geology with a weaker relationship to clay substrates in the reach. Poeciliid relative richness and abundance were strongly correlated to local elevation, decreasing percent pool habitat, and decreasing channel width. Migratory species were correlated to decreasing percentages of limestone, increasing depth variability, and decreasing percentages of clay substrates. This may reflect the preference of the migratory species for the high gradient rivers of the Maya Mountains, away from the Cretaceous limestone deposits of the north and the foothills. Carnivore relative abundances and richness were linked most strongly to increasing latitude and decreased percentages of boulder habitats, indicating that abundance of top predators (e.g. pike killifish, bay snook, yellowjacket cichlid) were greatest in larger river habitats in the northern parts of the study area. Cichlid species were linked to increasing percent Cretaceous limestone, decreasing local elevation, and, at the local level, increasing wetted width and percent pool habitats. This reflects a preference by cichlids for larger coastal plain habitats, where they are primarily found below 100 masl (Bussing 1998).

Finally, species richness and diversity were correlated with decreasing local elevation and increasing wetted width.

Discussion

My results affirmed the existence of strong and weak multi-scale relationships between environmental conditions and fish assemblages in the small coastal neo-tropical rivers of northeastern Mesoamerica. The first hypothesis, that reach-scale abiotic conditions should account for more assemblage variation than catchment conditions, because of relatively low levels of human landscape domination, was not well supported by the results. Contrary to expectations, catchment scale environmental factors accounted for substantially more variation than reach variables (Figure 2.2). At least two explanations may account for this contradiction of findings from other settings. First, the direct downstream connection of all rivers to the Caribbean Sea led to the presence of a large number of marine fishes in the assemblage (35 species), which were dropped from this analysis. However, these fishes may exert a strong influence on the remaining freshwater fishes via competition and/or predation. Because most of these fishes occur in the near shore river reaches above the estuary (Esselman et al. 2006), there is a strong positional dimension to their presence, and also presumably on the influence that they may exert. Thus, the positional influence of estuarine species on the freshwater fishes examined here may explain the finding that catchment-scale factors, such as position in catchment, described more variation than reach-scale factors. A second possible explanation for the greater amount of variance explained by catchment scale factors may relate to the spatial extent of the study area that spans strongly varying geologic, climatic, and physiographic types defined by the transition from the drier Yucatan Platform to the wetter Maya Mountains and Toledo Formation. This physiographic heterogeneity was likely to be more strongly reflected in the catchment-level dataset, which in turn may have inflated the importance of catchment-scale environmental factors in the analysis of assemblage variation. Further analysis within more homogeneous boundaries (e.g., only Maya Mountain rivers, only Yucatan Platform rivers), may lead to conclusions more typical of past studies with greater relative amounts of variance described by reach factors.

The second hypothesis, that longitudinal position in the catchment should explain a substantial portion of compositional and community variation, both directly and indirectly through interactions with local habitat conditions, also was supported. Variables that represented longitudinal position in the catchment (versus geographic longitude) were distance from sea, local elevation, and watershed area. Collectively, these were among the strongest correlates to fish assemblages of all the environmental conditions. Local elevation exhibited particularly strong correlations with all of the fish datasets (Figures 2.3 – 2.5), and was consistent with the reach-scale variables that correlated most strongly to species locations in the bi-plots (channel width, substrate sizes, depth variation). Several previous studies in small Mesoamerican coastal drainages suggested the importance of longitudinal position in the catchment to fish assemblage composition. For instance, Winemiller and Leslie (1992) showed predictable longitudinal variation across a freshwater-marine ecotone that correlated to reach conditions like habitat size and salinity, while other authors have shown an inverse relationship between richness and distance from sea (Lyons & Schneider 1990, Rodiles-Hernandez et al. 1999, Esselman et al. 2006). The present study reinforces the importance of landscape position, which itself may serve as a proxy for the finer scale correlates that exert more direct influences on Mesoamerican fish assemblages.

The third hypothesis, that latitude should correlate to compositional patterns driven by north-to-south species turnover across biogeographic boundaries, was strongly supported by the results. Latitude varied inversely with precipitation and corresponded to clear northern and southern species groups (Figures 2.3, 2.4). The correlation between the Toledo Formation rocks underlying the six southernmost rivers and the endemic cichlid fauna (e.g., golden firemouth cichlid, chisel-tooth cichlid) also has a latitudinal dimension to it (Figure 2.4). My results confirmed the distinctiveness of northern and southern faunal elements. The fauna of the deep south may have a particularly distinctive biogeographic history that could stem from allopatric speciation within the Rio Dulce/Rio Polichic basin immediately to the south of the study area, with later dispersal into the southernmost drainages studied here via headwater stream capture, or movement along the coast. Latitudinal species turnover and the presence of narrowly distributed endemics in the southern part of the study area suggest that conservation efforts should focus species protection efforts across this gradient, with special focus on the narrowly endemic fishes of the southern part of the area.

The findings provide an interesting contrast with the few previous studies that evaluated multi-scale environmental influences on river fish assemblages using similar methods. In the present study, environmental conditions across scales explained 57% to 73% of the total variation in fish assemblage composition. Similar studies have reported 40% to 50% (Wang et al. 2003), 56% (Johnson et al. 2007), and 44% to 52% (Stewart-Koster et al. 2007). This indicates that abiotic controls, particularly those at the catchment scale, may be particularly influential on the fish assemblages of the study area. In contrast to the present study, other studies have found that landscape factors often have less explanatory power than reach scale variables (Lyons 1996, Lammert & Allan 1999, Wang et al. 2003, Johnson et al. 2007), though it has also been observed that land-use in human-dominated landscapes can override the influence of local and riparian habitat on stream fishes (Roth et al. 1996, Allan et al. 1997). The majority of the afore mentioned studies were carried out in streams and rivers of the glacially influenced Midwestern United States, so may not be valid comparisons for the estuarine influenced short gradient tropical rivers and neotropical fish assemblages studied here. The finding of stronger catchment influence comes with an important caveat. Wiley et al. (1997) cautioned that inadequate sampling in time or space can inflate estimates of spatial variance attributed to large scale spatial factors. Because I only sampled each site on one occasion, and do not know the influence on sample density on the sensitivities of species responses to reach factors, these may contribute to my observation that catchment factors explain greater variation than reach factors in the study area.

This study is notable for its lack of a signal of human influence on fish assemblages at catchment or riparian scales. Very few indicators of human activities on the landscape (e.g., percent urban land cover, Table 2.1) made it into the reduced datasets. Instead, the strong influence of catchment factors occurred in a setting with very low rates of forest conversion to agriculture, urban, or other human uses (Table 2.1). This finding contrasts with the suggestions of workers in other geographies that reach-scale environment has greater explanatory power until human activities greatly modify land cover patterns, at which point landscape-scale variables become more important (Wiens 2002, Wang et al. 2003). Here I have shown that in watersheds with relatively little land cover conversion, landscape-scale factors may have a stronger relative influence on assemblages than environmental conditions at the reach scale. This does not imply that reach-scale controls are weak. When reach scale factors are considered in combination with the interaction terms (Figure 2.2), they explain between 33% and 47% of the total variance in the presence-absence, relative abundance, and community data. Further research that includes more spatial and temporal replication would be useful to further explore the strength of environmental controls at different scales in the nested spatiotemporal hierarchy.

I have presented strong correlative evidence of nested environmental controls on fish assemblages of northeastern Mesoamerica. These results suggest the importance of landscape position as a large scale spatial control, followed by habitat size and substrate at the reach scale, with little obvious influence of riparian habitats. This work has several implications for aquatic conservation in the study area. The first is that aquatic systems are almost wholly structured relative to natural (versus anthropogenic) gradients, suggesting that the fish communities of the area may still be in relatively natural condition. Second, results suggest the existence of a unique narrow endemic species assemblage in southern Belize which suggests that the area has a biogeographic history that is distinct from the rest of the study area. Taxonomic inventories in this poorly sampled area could lead to new species discoveries, and aquatic protection efforts are warranted to ensure persistence of large populations of these endemics. Finally, because landscape position may be an important large scale control, biodiversity protection efforts must consider how to maintain ecological integrity across a positional gradient from the mountains to the sea and account for north-to-south species turnover. Finally, this works supports the idea that the catchment scale or larger is an appropriate scale for fish conservation planning to begin in northeastern Mesoamerica.

Table 2.1. Mean, standard error (SE) and range of values for each catchment and reach variable. Variables that were retained in theanalysis (marked with an X) were significantly correlated ($p < 0.05$) with fish variables in CCAs between the two environment datasets and three fish datasets, which contained information on presence/absence (PA), relative abundance (ABUN), and community metrics (COMM).

Variable	Description	Mean + SE	Range	PA	ABUN	COMM
Watershed location and landscape						
lat	Latitude	844011±7615	735683 - 990141	X	X	X
long	Longitude	-2087964±2843	-2143562 - -2040908	X	X	
distsea	Distance downstream to sea (km)	89±10	1 - 367	X	X	X
area_shed	Upstream watershed area (km^2)	1647±319	0 - 9813	X	X	
elev_shed	Average catchment elevation (masl)	306±19	6 - 720	X		
elev_loc	Local elevation (masl)	59±11	2 - 468	X	X	X
slope_loc	Local slope (degrees)	4.1±0.6	0 - 26			
temp_shed	Average annual air temp in catchment (º C)	23±0	21 - 26		X	
prec_shed	Average annual rainfall in catchment (mm)	2050±63	1260 - 3747	X	X	X
Watershed surficial geology and soils						
alluv	% Quaternary alluvium	12±2	0 - 100			
limestn	% Cretaceous limestone	35±3	0 - 100		X	X
sedim	% Tertiary sedimentary	3±1	0 - 72			
toledo	% Toledo Formation	3±1	0 - 53	X	X	X
Watershed land use and human influence						
agric	% agriculture land use	18±2	0 - 100			
urban	% urban land use	0.49±0.28	0 - 16			X
forest	% forest land use	74±2	0 - 100	X	X	
savanna	% savanna	1±0.28	0 - 13	X		

wetland	% wetland	0.41±0.16	0 - 7	X		
snear_dist	Linear distance to nearest human settlement (km)	4312±477	468 - 26196	X		X
roadprop	Percent of pixels in catchment with roads present	0.84±0.1	0 - 4			
Reach channel morphology						
wetwidt	Mean wetted width (m)	28±2	2 - 67	X	X	X
depcovar	Coefficient of variation of water depth (m)	77±3	32 - 149	X		X
wdratio	Ratio of width to depth (ratio)	34±2	7 - 103			
pool	% stream reach that is pool	63±4	0 - 100		X	X
Reach substrate						
bedrck	% bedrock substrate in reach	7±2	0 - 56			
boulder	% boulder substrate in reach	5±1	0 - 33	X	X	X
cobble	% cobble substrate in reach	14±2	0 - 68		X	X
gravel	%gravel substrate in reach	13±2	0 - 55			
sand	% sand in reach	23±3	0 - 91	X	X	
clay	% clay in reach	6±2	0 - 80	X	X	X
H'sub	Shannon diversity of substrates	0.79±0.05	0 - 1.67		X	
Fish cover						
logs	Logs > 0.30 m diameter and > 1 m long (number 100 m-1)	8±1	0 - 29			X
sumfcov	Mean fish cover (index)	35±2	7 - 105			

H'cover	Shannon diversity of fish cover types	1±0	0 - 2			
Stream bank condition						
bnkstab	Mean bank stability rating (index)	1.30±0.04	1.00 – 2.23			
Reach water quality and temperature						
cond	Conductivity (µSm · cm^{-2})	437±66	22 - 2531	X		X
pH	pH	7±0	6 - 9	X	X	
temp	Water temperature (º C)	27±0	21 - 33	X	X	X
Riparian land uses						
ripwidth	Width of forested buffer (up to 16 m from stream)	12±0	1 - 16	X	X	X
ripforest	% forested within 16 m of bank	66±4	8 - 100		X	
ripdisturb	% disturbed vegetation types (human or natural) within 16 m of bank	23±3	0 - 92	X		
riphuman	% human cover types (agriculture and other within 16 m of bank	9±2	0 - 85			
ripagric	% agriculture within 16 m of bank	6±1	0 - 54			

Table 2.2. Species codes, names, class, number of sites recorded as present (N), and mean relative abundance (proportion of all fish counted) of the 32 fish species used in the analysis. Species with less than five observations were considered rare and were only used to calculate community metrics, but not included in the relative abundance matrix. Class refers to whether or not a species is a carnivore with a trophic level greater than 4 (C), a sub-regional endemic (E), or migratory (M). Class designations were used to calculate community metrics (Table 3).

Code	**Common name**	**Species name**	**Class**	**N**	**Mean Rel. Abun.**
Aaen	Central tetra	*Astyanax aeneus*		70	0.262
Bgua	Machaca	*Brycon guatemalensis*		23	0.041
Hcom	Mayan tetra	*Hyphessobrycon compressus*		41	0.057
Ifur	Blue catfish	*Ictalurus furcatus*		5	0.004
Rgua	Guatemalan chulin	*Rhamdia guatemalensis*		42	0.028
Rlat	Filespine chulin	*Rhamdia laticauda*		42	0.035
Rten	Dogtooth rivulid	*Rivulus tenuis*		1	
Bbel	Pike killifish	*Belonesox belizanus*	C	53	0.011
Glum	Sleek mosquitofish	*Gambusia luma*		45	0.035
Gsex	Teardrop mosquitofish	*Gambusia sexradiata*		2	
Gyuc	Yucatan mosquitofish	*Gambusia yucatana australis*		1	
Hbim	Twospot livebearer	*Heterandria bimaculata*		38	0.072
Pmex	Shortfin molly	*Poecilia mexicana*		66	0.164
Ppet	Sailfin molly	*Poecilia petenensis*		6	0.024
Pter	Mountain molly	*Poecilia teresae*	E	1	
Porr	Mangrove molly	*Poecilia orri*		1	
Pfai	Picotee livebearer	*Phallichthys fairweatheri*		1	
Xhel	Green swordtail ()	*Xiphophorus helleri*		38	0.066
Xmac	Southern platyfish	*Xiphophorus maculatus*		2	
Asp1	Belize silversides	*Atherinella sp. 1*		40	0.03
Oaen	Obscure swamp eel	*Ophisternon aenigmaticum*		55	0.011

Pcro	Burro grunt	*Pomadasys crocro*	C, M	15	0.007
Arob	False firemouth cichlid	*Amphilophus robertsoni*		55	0.026
Cboc	Chisel-tooth cichlid	*Cichlasoma bocourti*	E	5	0.017
Csal	Yellowbelly cichlid	*Cichlasoma salvini*		67	0.059
Curo	Mayan cichlid	*Cichlasoma urophthalmus*	C	18	0.013
Cspi	Blue-eye cichlid	*Cryptoheros spilurus*		64	0.078
Onil	Nile tilapia	*Oreochromis niloticus*		10	0.011
Pfri	Yellowjacket cichlid	*Parachromis friedrichsthalii*	C	12	0.006
Pspl	Bay snook	*Petenia splendida*	C	34	0.014
Roct	Jack Dempsey	*Rocio octofasciata*		19	0.012
Taur	Golden firemouth cichlid	*Thorichthys aureus*	E	7	0.1
Tmee	Firemouth cichlid	*Thorichthys meeki*		42	0.128
Vint	Northern checkmark cichlid	*Vieja intermedia*		9	0.037
Vgod	Southern checkmark cichlid	*Vieja godmanni*	E	4	
Vmac	Blackbelt cichlid	*Vieja maculicauda*		27	0.061
Vsyn	Redhead cichlid	*Vieja synspila*		34	0.076
Amon	Mountain mullet	*Agonostomus monticola*	M	22	0.043
Jpic	Hog mullet	*Joturus pichardi*	M	4	
Gdor	Bigmouth sleeper	*Gobiomorus dormitor*		44	0.023
Aban	Green river goby	*Awaous banana*	M	23	0.012

Table 2.3. Fish community metrics, their abbreviations, and summary statistics for each.

Variable	**Abbrev**	**Mean**	**SD**	**Min**	**Max**
Number of fish species	rich	15.13	3.83	2	22
Shannon diversity index	divers	1.83	0.38	0.58	2.49
Top carnivore individuals, %	pccarin	2.23	2.43	0	13.76
Top carnivore species, %	pccarsp	11.62	7.20	0	25.00
Migratory individuals, %	pcmigin	1.99	4.35	0	21.83
Migratory species, %	pcmigsp	5.38	6.56	0	25.00
Narrowly endemic individuals, %	pcendin	1.45	4.73	0	24.64
Narrowly endemic species, %	pcendsp	1.39	4.55	0	20.00
Cichlid individuals, %	pccicin	31.45	16.64	0	66.36
Cichlid species %	pccicsp	36.03	11.63	0	62.50
Poeciliid individuals, %	pcpeoin	26.91	21.97	0	100.00
Poeciliid species, %	pcpeosp	24.56	11.41	0	100.00

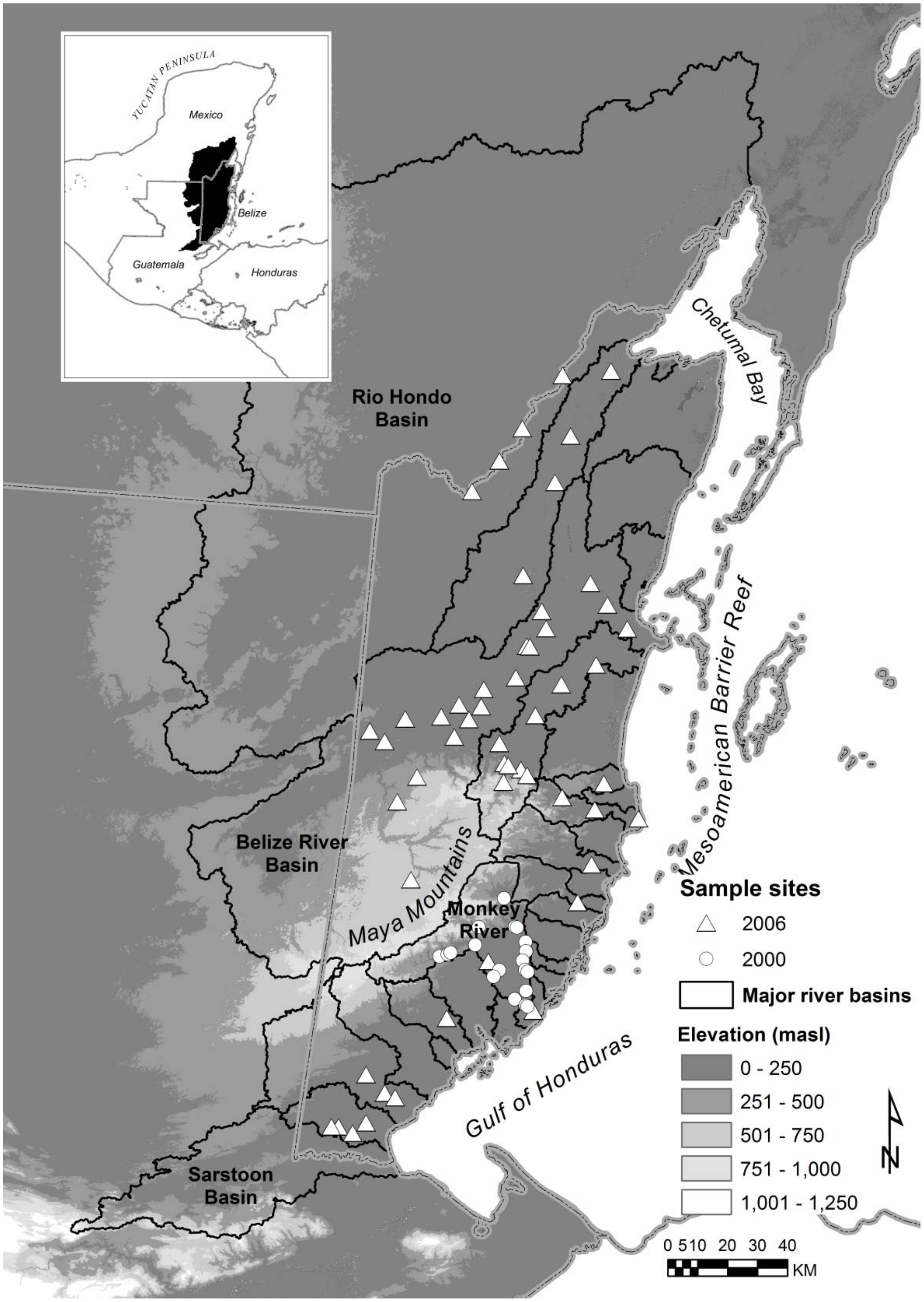

Figure 2.1. Study area showing elevation, major drainage basins, important geographic features, and locations of all sampling sites. Sampling locations in the Monkey River from 2000 are identified with a white circle, while those sampled in 2006-7 are marked by a white triangle.

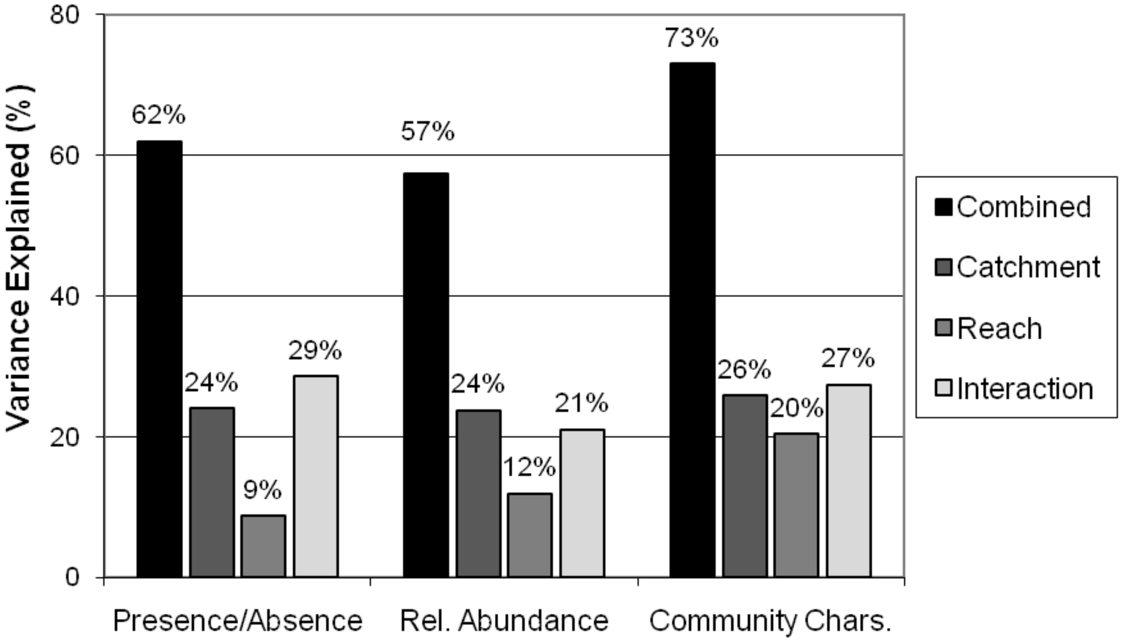

Figure 2.2. The percentage of total variance explained by different scales of environmental data for fish presence-absence, relative abundance, and community metrics using partial CCA. “Combined” represents the sum of pure catchment influence, pure reach, and interactions between catchment and reach.

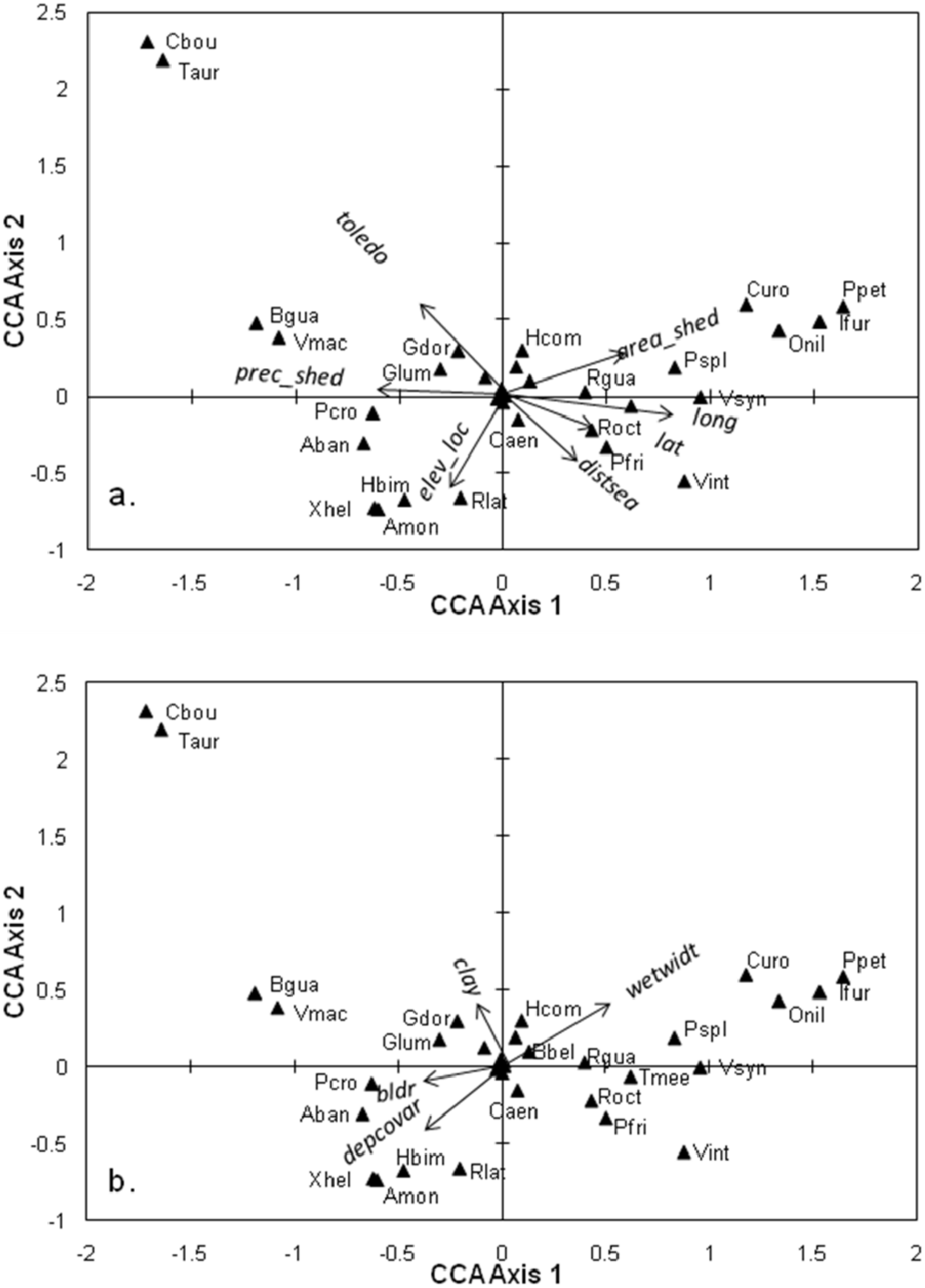

Figure 2.3. CCA bi-plot of species with catchment-scale environmental influences (a) and reach influences (b) based on presence or absence data. Triangles indicate fish species labeled with species codes from Table 2. Arrows indicate the direction of increasing values of the environmental variables, and the length of arrows indicates the degree of correlation of the variable with community data.

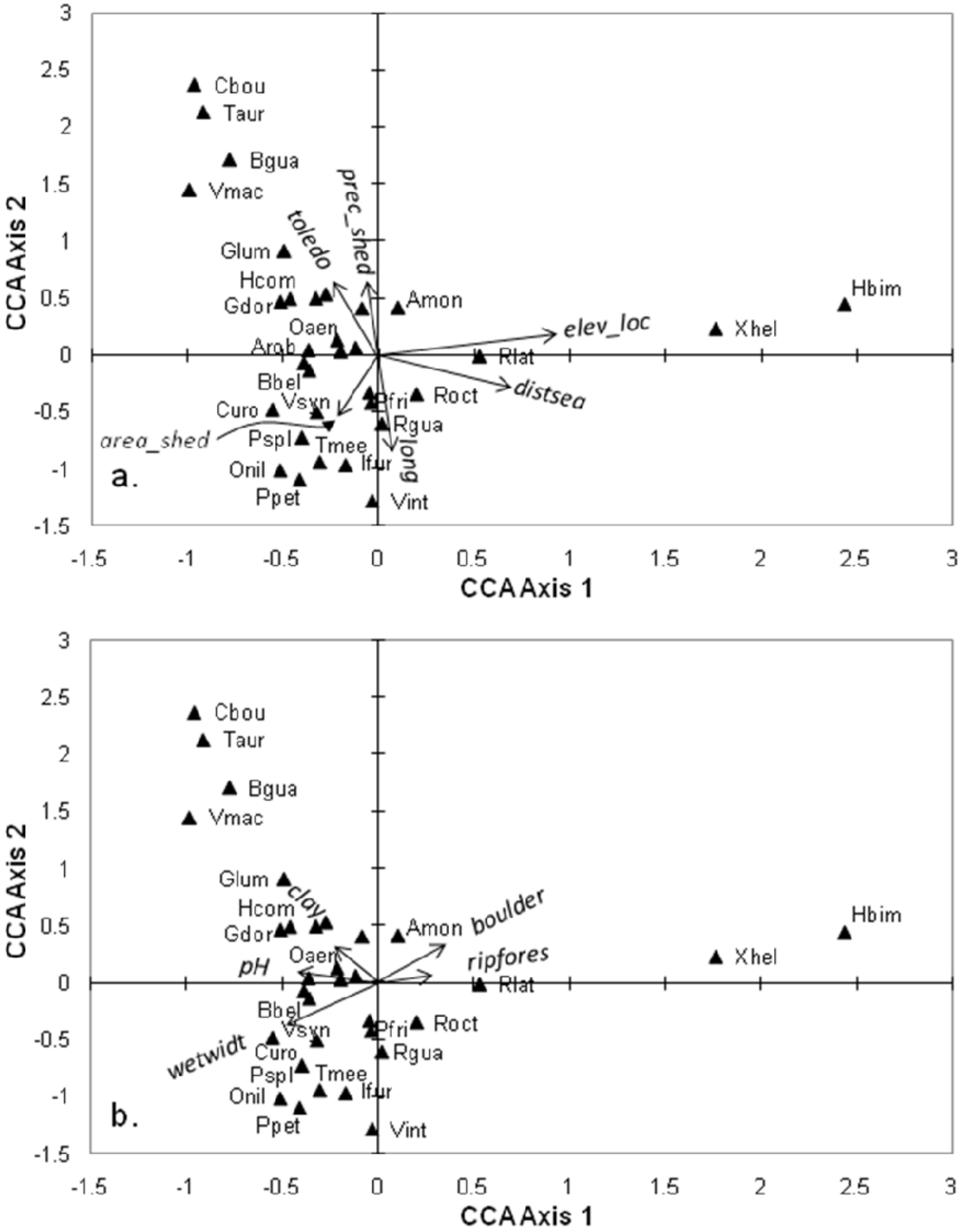

Figure 2.4. CCA bi-plot of species with catchment-scale environmental influences (a) and reach influences (b) based on relative abundance data. Triangles indicate fish species labeled with species codes from Table 2. Arrows indicate the direction of increasing values of the environmental variables, and the length of arrows indicates the degree of correlation of the variable with community data.

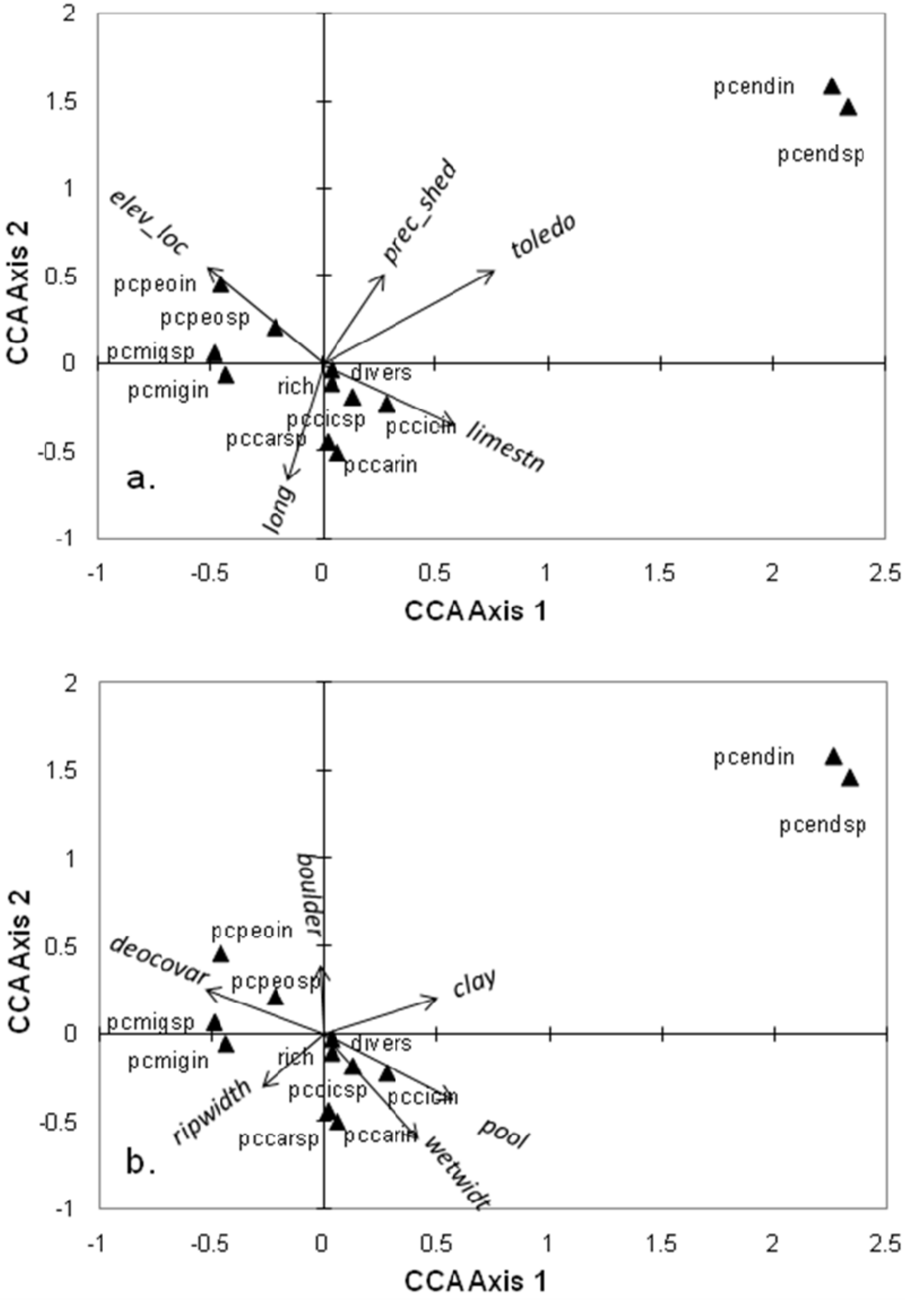

Figure 2.5. CCA bi-plot of community metrics with catchment-scale environmental influences (a) and reach influences (b). Triangles indicate fish species labeled with species codes from Table 2. Arrows indicate the direction of increasing values of the environmental variables, and the length of arrows indicates the degree of correlation of the variable with community data.

Literature cited

Abell, R., M. Thieme, C. Revenga, M.T. Bryer, M. Kottelat, N. Bogutskaya, B. Coad, N. Mandrak, S. Contreras Balderas, W.A. Bussing, M.L.J. Stiassny, P.H. Skelton, G.R. Allen, P. Unmak, A. Naseka, R. Ng, N. Sindorf, J. Robertson, E. Armijo, J.V. Higgins, T.J. Heibel, E. Wikramanayake, D. Olson, H.L. Lopez, R.E. Reis, J.G. Lundberg, M.H. Sabaj Perez, & P. Petry. 2008. Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *Bioscience* **58:**403 - 414.

Allan, J. D. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology Evolution and Systematics* **35**:257-284.

Allan, J. D., D. L. Erickson, and J. Fay. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* **37**:149-161.

Allen, T. F. H. and T. B. Starr. 1982. *Hierarchy: Perspectives for Ecological Complexity*. University of Chicago Press, Chicago, IL.

Anderson, M. J. and N. A. Gribble. 1998. Partitioning the variation among spatial, temporal and environmental components in a multivariate data set. *Australian Journal of Ecology* **23**:158-167.

Angermeier, P. L. and J. R. Karr. 1983. Fish communities along environmental gradients in a system of tropical streams. *Environmental Biology of Fishes* **9**:117-135.

Angermeier, P. L. and I. J. Schlosser. 1989. Species-area relationships for stream fishes. *Ecology* **70**:1450-1462.

Angermeier, P. L. and M. R. Winston. 1998. Local vs. regional influences on local diversity in stream fish communities of Virginia. *Ecology* **79**:911-927.

Angermeier, P. L. and M. R. Winston. 1999. Characterizing fish community diversity across Virginia landscapes: Prerequisite for conservation. *Ecological Applications* **9**:335-349.

Borcard, D., P. Legendre, and P. Drapeau. 1992. Partialling out the spatial component of ecological variation. *Ecology* **73**:1045-1055.

Brazner, J. C., D. K. Tanner, N. E. Detenbeck, S. L. Batterman, S. L. Stark, L. A. Jagger, and V. M. Snarski. 2005. Regional, watershed, and site-specific environmental influences on fish assemblage structure and function in western Lake Superior tributaries. *Canadian Journal of Fisheries and Aquatic Sciences* **62**:1254-1270.

Bussing, W. A. 1976. Geographic distribution of the San Juan ichthyofauna of Central America with remarks on its origin and ecology. Pages 157-175 *in* T. B. Thorson, editor. *Investigations of the Ichthyofauna of Nicaraguan Lakes*. University of Nebraska, Lincoln, Nebraska.

Bussing, W. A. 1998. Peces de las aguas continentales de Costa Rica. *Revista De Biologia Tropical* **46**:1-459.

Bussing, W. A. and M. I. Lopez. 1977. Distribucion y aspectos ecologicos de los peces de las cuencas hidrograficas de Arenal Bebederoy Tempisque, Costa Rica. *Revista de Biologia Tropical* **25**:13-37.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**:163-182.

Dunham, J. B. and B. E. Rieman. 1999. Metapopulation structure of bull trout: Influences of physical, biotic, and geometrical landscape characteristics. *Ecological Applications* **9**:642-655.

Durance, I., C. Lepichon, and S. J. Ormerod. 2006. Recognizing the importance of scale in the ecology and management of riverine fish. *River Research and Applications* **22**:1143-1152.

Esselman, P. C. and J. D. Allan. In review. Application of species distribution models and conservation planning software to the design of a reserve network for the riverine fishes of northeastern Mesoamerica. *Freshwater Biology*.

Esselman, P. C. and E. Boles. 2001. Status and future needs of limnological research in Belize. Pages 35 - 68 *in* R. G. Wetzel and B. Gopal, editors. *Limnology in Developing Countries Volume 3*. International Association for Limnology.

Esselman, P. C., M. C. Freeman, and C. M. Pringle. 2006. Fish-assemblage variation between geologically defined regions and across a longitudinal gradient in the Monkey River Basin, Belize. *Journal of the North American Benthological Society* **25**:142-156.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *Bioscience* **52**:483-498.

Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmental Management* **10**:199-214.

Gorman, O. T. and J. R. Karr. 1978. Habitat structure and stream fish communities. *Ecology* **59**:507-515.

Greenfield, D. W. and J. E. Thomerson. 1997. *Fishes of the Continental Waters of Belize*. University of Florida Press, Gainseville, FL.

Hartshorn, G., L. Nicolait, L. Hartshorn, G. Bevier, R. Brightman, J. Cal, A. Cawich, D. Weyer, W. H., and A. Wright. 1984. *Belize Country Environmental Profile: A Field Study*. USAID, Belize City, Belize.

Johnson, R. K., M. T. Furse, D. Hering, and L. Sandin. 2007. Ecological relationships between stream communities and spatial scale: implications for designing catchment-level monitoring programmes. *Freshwater Biology* **52**:939-958.

Lake, P. S., N. Bond, and P. Reich. 2007. Linking ecological theory with stream restoration. *Freshwater Biology* **52**:597-615.

Lammert, M. and J. D. Allan. 1999. Assessing biotic integrity of streams: effects of scale in measuring the influence of land use/cover and habitat structure on fish and macroinvertebrates. *Environmental Management* **23**:257-270.

Lamouroux, N., N. L. Poff, and P. L. Angermeier. 2002. Intercontinental convergence of stream fish community traits along geomorphic and hydraulic gradients. *Ecology* **83**:1792-1807.

Lazorchak, J., D. J. Klemm, and D. V. Peck. 1998. Environmental Monitoring and Assessment Program for Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams.*in* EPA, editor. Office of Research and Development, US Environmental Protection Agency, Cincinnati, OH.

Lee, M. D., J. D. Stednick, and D. M. Gilbert. 1995. *Belize Environmental Water Quality Monitoring: Final Report*. USAID-NARMAP Project to Government of Belize, Belmopan, Belize.

Lyons, J. 1996. Patterns in the species composition of fish assemblages among Wisconsin streams. *Environmental Biology of Fishes* **45**:329-341.

Lyons, J. and D. W. Schneider. 1990. Factors influencing fish distribution and community structure in a small coastal river in southwestern Costa Rica. *Hydrobiologia* **203**:1-14.

Miller, R. R. 1966. Geographical distribution of Central American freshwater fishes. *Copeia*:773-802.

Poff, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society* **16**:391-409.

Ricklefs, R. E. and D. Schluter. 1993. *Species Diversity in Ecological Communities.* Univeristy of Chicago Press, Chicago, IL.

Rodiles-Hernandez, R., E. Diaz-Pardo, and J. Lyons. 1999. Patterns in the species diversity and composition of the fish community of the Lacanja River, Chiapas, Mexico. *Journal of Freshwater Ecology* **14**:455-468.

Roth, N. E., J. D. Allan, and D. L. Erickson. 1996. Landscape influences on stream biotic integrity assessed at multiple spatial scales. *Landscape Ecology* **11**:141-156.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - global biodiversity scenarios for the year 2100. *Science* **287**:1770-1774.

Schlosser, I. J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. *Ecological Monographs* **52**:395-414.

Schmitter-Soto, J. J. 1998. Catalogo de los peces continentales de Quintana Roo. El Colegio de la Frontera Sur, San Cristobal de las Casas, Mexico.

Schmitter-Soto, J. J. and H. C. Gamboa-Perez. 1996. Composicion y distribucion de peces continentales en el sur de Quintana Roo, Peninsula de Yucatan, Mexico. *Revista De Biologia Tropical* **44**:199-212.

Statzner, B., J. A. Gore, and V. H. Resh. 1988. Hydraulic stream ecology: observed patterns and potential applications. *Journal of the North American Benthological Society* **7**:307-360.

Stewart-Koster, B., M. J. Kennard, B. D. Harch, F. Sheldon, A. H. Arthington, and B. J. Pusey. 2007. Partitioning the variation in stream fish assemblages within a spatio-temporal hierarchy. *Marine and Freshwater Research* **58**:675-686.

ter Braak, C. J. F. 1995. Ordination.*in* R. H. G. Jongman, C. J. F. Ter Braak, and O. F. R. van Tongeren, editors. *Data Analysis in Community and Landscape Ecology*. Cambridge University Press, New York, NY.

ter Braak, C. J. F. and I. C. Prentice. 1988. A Theory of Gradient Analysis. Pages 271-317 *in* M. Begon, A. H. Fitter, E. D. Ford, and A. MacFadyen, editors. *Advances in Ecological Research*. Academic Press, London.

Townsend, C. R. 1996. Concepts in river ecology: pattern and process in the catchment hierarchy. *Archive fur Hydrobiologie* **(Suppl.)**:3-21.

Wang, L. Z., J. Lyons, P. Rasmussen, P. Seelbach, T. Simon, M. Wiley, P. Kanehl, E. Baker, S. Niemela, and P. M. Stewart. 2003. Watershed, reach, and riparian influences on stream fish assemblages in the Northern Lakes and Forest Ecoregion, USA. *Canadian Journal of Fisheries and Aquatic Sciences* **60**:491-505.

Wiens, J. A. 2002. Riverine landscapes: taking landscape ecology into the water. *Freshwater Biology* **47**:501-515.

Wiley, M., S. Kohler, and P. Seelbach. 1997. Reconciling landscape and local views of aquatic communities: lessons from Michigan trout streams. *Freshwater Biology* **37**:133-148.

Wilson, E. M. 1980. Physical geography of the Yucatan Peninsula. Pages 5-40 *in* E. H. Moseley and E. D. Terry, editors. *Yucatan: A World Apart*. University of Alabama Press, Alabama.

Winemiller, K. O. 1983. An introduction to the freshwater fish communities of Corcovado National Park, Costa Rica. *Brenesia* **21**:47-66.

Winemiller, K. O. and M. A. Leslie. 1992. Fish assemblages across a complex, tropical freshwater marine ecotone. *Environmental Biology of Fishes* **34**:29-50.

Chapter 3

Spatiotemporal dynamics of the spread of African tilapias (Pisces: *Oreochromis* spp.) into rivers of northeastern Mesoamerica

Abstract

Tropical freshwater ecosystems harbor high levels of biodiversity, and are increasingly threatened by biological invasions. African tilapiine fish in the genus *Oreochromis* now occur in the tropical and sub-tropical parts of more than 100 countries outside of their native ranges in Africa, and research on their invasions is largely lacking. I investigated the spatiotemporal patterns of tilapia spread into river ecosystems in northeastern Mesoamerica drawing on the limited information sources that were available. The study area consisted of 29 drainage basins spanning a diversity of topographic and geologic types from the low lying limestone platform of the Yucatan Peninsula to the deeply incised ancient sedimentary, volcanic, and granitic rocks of the Maya Mountains. Habitat suitability models for tilapias were created from geospatial data about abiotic determinants of species distributions and from species occurrence data, and fishermen were interviewed to reconstruct the chronology of tilapia spread into this suitable habitat. Tilapia (*Oreochromis* sp.) presence was confirmed at 78 sites in 9 of 29 drainage basins in the study area, with the majority of individuals being identified as Nile tilapia (*O. niloticus*). Habitat suitability modeling predicted that 7,510 linear km of river habitat in the study area were vulnerable to colonization by tilapias, predominately in mid- to low elevation main stem rivers from sea level up into mountain valleys to 277 meters above sea level. The reconstructed spatial chronology of spread showed that the invasion that started in 1990 and progressed slowly (2 km yr^{-1}) through an establishment phase, before rapid expansion (~30 km yr^{-1}) between 1996 and 2002, after which the number of new detections slowed. Human movement of fish for aquaculture was identified as a primary cause of dispersal, with flooding as an important secondary cause, and the shortest paths across low elevation drainage divides between major basins were identified as potential corridors for intermittent tilapia spread during flooding. Based on this research, I make recommendations for tilapia management in areas of aquaculture, fisheries, and law and policy development.

Introduction

Tropical freshwater ecosystems, though poorly known, exhibit high levels of endemism and species richness (Dudgeon 1999, 2000, Benstead et al. 2003, Strayer et al. 2004, Leveque et al. 2008). More than 70% of the 13,000 fish species that occur in freshwaters live in the tropics (Leveque et al. 2008), a number that grows annually as new species are described (Stiassny 1999). While species loss from freshwater ecosystems in tropical latitudes has not been estimated accurately, extinction rates for freshwater animals in N. America have been estimated at an alarming 4% per decade (Ricciardi and Rasmussen 1999), and rates in the tropics may be similarly high (Dudgeon et al. 2006). Biological invasions by aquatic non-indigenous species are thought to be particularly important drivers of biodiversity loss in freshwater ecosystems (Sala et al. 2000). Given high levels of freshwater biodiversity and endemism and the importance of biological invasions as a threat, research on biological invasions into tropical freshwater ecosystems is a clear conservation research priority.

Yet, research on non-indigenous species invasions into tropical freshwaters is scarce, with a few notable exceptions (e.g., Nile perch in Lake Victoria; Balirwa et al. 2003). For instance, African tilapias in the genus *Oreochromis* have a nearly pan-tropical distribution (Welcomme 1988, Froese and Pauly 2008) in more than 100 countries outside of their native ranges in Africa, but research on their invasions is largely lacking. The work that has been completed shows that tilapias can have numerous detrimental effects on ecosystems into which they are introduced (reviewed by Canonico et al. 2005), including local extinctions of native species (Twongo 1995, Goudswaard et al. 2002), predation on eggs and young of other fishes (Arthington and Bluhdorn 1994), altered nutrient dynamics and eutrophication (Starling et al. 2002), de-vegetation of extensive areas of lake bottom, introduction of non-indigenous parasites to other fishes (McCrary et al. 2001), and food web alterations (Taylor et al. 1984). Tilapias can colonize a wide array of habitats in subtropical or tropical rivers, lakes and wetlands, brackish coastal lagoons, and estuaries (Phillippart and Ruwet 1982). Their ability to colonize a broad range of habitats can be attributed to broad physiological tolerances, and lead to high rates of successful establishment in ecosystems outside their native range (Courtenay 1997). High colonization success mixed with the potential to cause ecosystem damage makes control of tilapia spread a high management priority for aquatic ecosystem protection at every scale.

Few studies have documented the process of tilapia dispersal, rates of spread, and habitat suitability. Such information can be extremely useful to the development of management interventions to protect native ecosystem integrity (Sharov and Liebhold 1998, Everett 2000, Floerl and Inglis 2005). For non-air breathing fishes, the water's edge creates a hard barrier to species movement, thus confining natural dispersal to the permanent or intermittent interconnecting streams and rivers between water bodies (Lodge et al. 1998). Many studies have examined the influence of dispersal and colonization dynamics on the survival and distribution of fish species in lakes and rivers (e.g., Barbour and Brown 1974, Tonn et al. 1990, Schlosser 1991, Dunham and Rieman 1999). These studies show that fish spread to new habitats and successful colonization are dependent on the dispersal abilities of the species, the configuration and connectivity of suitable habitats, and the local habitat conditions present in new habitats (Moyle and Light 1996, Havel et al. 2002, With 2002). Past studies of tilapias in wet tropical environments suggest that human translocation is the primary cause of dispersal, and point to flooding as an important secondary dispersal mechanism that flushes tilapias from aquaculture ponds into natural water bodies and across swampy drainage divides (Arthington and Bluhdorn 1994, Schmitter-Soto and Caro 1997).

The tropical countries being invaded by tilapias are often faced with limited availability of baseline data, insufficient human technical capacity and technology, and low investment in research and monitoring (Pringle et al. 2000). The end result is that critical information for management is often unavailable. This is the case with the Mesoamerican country of Belize, where African tilapias have been expanding their range since the early 1990's (Esselman and Boles 2001). With limited economic development options, Belize is evaluating the expansion of tilapia aquaculture as a way of generating foreign income. Given that tilapias have yet to colonize the whole country, landscape-scale information about occupied habitats, unoccupied suitable habitats, spread rates, and dispersal routes and drivers would be particularly useful to Belizean decision makers at this point in time.

In this study, landscape scale habitat and tilapia occurrence data were used to create habitat suitability models, and investigate the spatiotemporal patterns of spread into these habitats. The specific goals were to:

1. Train and validate a species distribution model to predict habitat vulnerability to tilapias in rivers.
2. Reconstruct the chronology of tilapia invasion into aquatic habitats, and make initial observations about drivers and rates of spread.
3. Identify possible natural dispersal corridors.
4. Synthesize the above information into a set of management recommendations to inform tilapia control strategies in Belize.

I investigated these questions in the absence of historical fish census data, which were last collected in Belize in the 1970s. Instead, I relied on the information sources that were available to me: geospatial and remote sensing data about possible abiotic determinants of species distributions, species occurrence data collected in recent field sampling, and interview and catch data from artisanal fishermen.

Methods

Study Area

The assessment was carried out in the domestic and international waters that drain to the coast of Belize, including portions of southern Mexico and northeastern Guatemala. This 45,750 km^2 area comprises 29 drainage basins including 16 major basins and 13 small coastal tidal creeks (Figure 3.1) (Lee et al. 1995). These basins vary in size, and drain a variety of geologic, soil, and terrestrial land cover types (Lee et al. 1995, Esselman and Boles 2001). Twelve of the 16 major rivers originate in the Maya Mountains (Figure 3.1) as high-gradient streams within granite and metamorphic geologies, after which they traverse limestone-dominated landscapes, build up alluvial plains and wetlands, and discharge into the shelf lagoon that separates the coast from the Mesoamerican Barrier Reef. The two northernmost rivers in the area (Rio Hondo and New River; Figure 3.1) originate in karst hills, drain the low relief limestone platform of the Yucatan Peninsula, and discharge into Chetumal Bay, which then connects to the shelf lagoon. The headwaters of the rivers in the three southernmost basins (S to N; Sarstoon, Temash, and Moho Rivers) begin in Guatemala and flow eastward to the Gulf of Honduras. The Sarstoon River (demarcating the southern border of Belize) originates in Guatemala, within the mountain range of the Sierra de Santa Cruz. In addition to flowing waters, the study area has an abundance of fresh and brackish water lagoons and wetlands that include swamp forests, herbaceous marshes, and open water areas, often in association with riverine habitats (Esselman and Boles 2001).

Daily mean temperatures are warm throughout the year, ranging from a low of 16ºC in the winter months to a high of 33ºC in summer (Hartshorn et al. 1984). A strong north-to-south precipitation gradient exists, with the northern portion of the study area receiving approximately 1000 mm of rain annually and the southern portion receiving up to 4000 mm annually (Wilson 1980). The timing of precipitation is strongly seasonal, with a dry season lasting from January to May, and a wet season from June to November, during which intense floods can occur. The area is highly prone to hurricanes in the late summer and early fall (Wilson 1980).

Northern Mesoamerica has been called a “strong center of evolution” for fishes, because of its many endemic genera and species (Miller 1966). The Caribbean slope drainages of Belize, Guatemala, and Honduras share a common biogeographic history and contain a high diversity of poeciliids (34 species, 15 genera) and cichlids (44 species, 5 genera) (Miller 1966, Bussing 1976). 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 predominately marine species that inhabit freshwaters are included (Miller 1966). One hundred and twenty-six of these species have been reported in the study area, including at least 15 native species in the family Cichlidae, shared with tilapias (Schmitter-Soto and Gamboa-Perez 1996, Greenfield and Thomerson 1997, Schmitter-Soto 1998, Esselman et al. 2006).

Overview of approach

My analysis consisted of three parts. First, I used recently collected species occurrence data together with geospatial data about landscape-scale habitat factors to create a species distribution model (SDM) to estimate potential habitat suitability for tilapias. SDMs generalize the empirical relationships between species collection localities and underlying habitat conditions to predict the probability of species occurrence within a landscape area. SDMs have the potential to use incomplete information, such as historical point occurrence data from only a part of a species’ range, to generate spatial predictions of the realized niche of a species relative to the environmental variables in the model (Guisan and Zimmermann 2000). I used SDMs trained from data collected inside the invaded range of tilapias to predict habitat suitability for tilapias in the entire study area. Second, I used interview data about the locations and years that fishermen first detected tilapia in their catches to infer the spread of tilapia from 1990 through 2007. This spatial chronology of spread was used to make a first estimate of the rates of tilapia expansion in individual drainage basins. Fisherman observations on possible dispersal mechanisms were considered in conjunction with my spread chronology to infer probable dispersal mechanisms and routes between basins. Finally, I used hydrographic and topographic data to infer natural colonization routes using a GIS-based approach known as cost surface analysis. All of the above were synthesized to make management recommendations for tilapia management.

Data sources

Three primary data sources were used in this study: (1) geospatial data representing large scale habitat factors that were used as independent variables in SDMs; (2) tilapia occurrence data collected through field sampling and surveys with fishermen, and (3) fishermen interview data.

The geospatial database consisted of 33 variables prepared as individual raster layers for possible inclusion in SDM development (Table 3.1). This database consisted of variables representing landscape position, geology, climate, hydrology, soils, watershed characteristics, and human influence on the landscape. The cumulative upstream influence of different variables was represented using the weighted flow accumulation tool in the spatial analyst extension of ArcGIS 9.2® (ESRI Corp., Redlands, CA, USA). Weighted flow accumulation performs a downstream summation of pixel numeric values within a grid of equally sized cells, drawing on a flow direction grid to infer downstream directionality. This is in contrast to raw flow accumulation—the count of all pixels upstream of a given pixel in a map grid. To calculate proportional variables (e.g., upstream proportion of a geology class), binary grids showing the presence or absence of each geological or soils class were created, used as the weight grid in the weighted flow accumulation process, and then divided by raw flow accumulation to yield a representation of the proportion of the catchment in a given feature class. Mean upstream values of continuous variables were calculated by the same process, except that I used continuous (e.g., mean annual temperature) instead of binary values in the weight grid. All layers consisted of a uniform extent and cell size (30 m x 30 m) and were masked after processing to constrain the datasets to only those pixels underlying a river segment.

To represent risk of environmental degradation at specific locations along the river network, I created an environmental risk surface (ERS) to define the upstream risk of environmental degradation following the method outlined by Schill & Raber (2008). An ERS is a modeled composite raster surface that is created in GIS to combine information about the extents and relative intensities of perceived environmental risks to aquatic ecosystems. The first step in the ERS development process was to identify and map potential risk elements. Based on available data I selected current agriculture and urban land cover types (polygons), roads (lines), and the locations of villages (points). Each risk element was assigned an intensity value and influence distance. The intensity value is a relative measure of intensity on a 0 to 100 scale with 100 being the strongest. Influence distance represents the “maximum distance over which the feature has a negative impact on biodiversity” (Schill & Raber, 2008). The assignment of intensity and distance values (Table 4.2) was a logical process informed by the literature, and by professional judgment. In particular, I drew on observations made by Allan (2004) about the relative influences of agriculture versus urban land uses on aquatic biological integrity, and I assumed that the influence distance of human communities was positively correlated with human population size or population density (Table 4.2). The Protected Areas Tools for ArcMap 9.2 (v. 2.0; http://www.gispatools.org) were used to attribute each risk element with their intensity and distance values. Each feature was buffered by the distance of influence, and converted to a raster grid. Within the buffer, a linear decay function was used to simulate the decline of intensity away from the actual location of each risk type. This process was used to create a raster layer for each risk factor, and then the values were summed across all grids to yield a cumulative estimate of environmental risk. To make this surface relevant to riverine ecosystems, the cumulative ERS was used as the weight grid in weighted flow accumulation to estimate cumulative downstream risk. Finally, the weighted flow accumulation grid was divided by raw flow accumulation to calculate the “relative upstream risk intensity” scaled by contributing area (Figure 4.2).

Georeferenced data on tilapia occurrences were collected using backpack electrofishing, boat electrofishing, and surveys with fishermen. Electrofishing sites were defined systematically each 20 km along the river channel from a random starting point in the first 5000 river meters. The reach length of a backpack electrofishing site was 39 times the mean stream width, within which a single electrofishing pass was performed with a Smith-Root® 12 battery powered electrofisher. Boat electrofishing reaches were all 1300 m long, within which a single pass was made along a single bank using a Smith-Root® GPP 5.0 generator powered electrofisher deployed with booms from the front of a 14 foot aluminum boat. Interviews and/or catch surveys were conducted with 40 fishermen in villages on the main stems of all major rivers (except Rio Sarstoon) about the presence of tilapias in their fishing grounds. All interviews used a standardized approach and were conducted by the same three individuals. Interviewers located fishermen in each village through referrals from local residents, administered a standard questionnaire, and, when possible, collected voucher specimens from catches to confirm tilapia presence and species identity. When voucher specimens were not available, fishermen were asked to identify tilapia from an unlabeled laminated card with pictures of all of Belize’s native cichlids plus adult *Oreochromis niloticus* and *Oreochromis mossambicus* to confirm that they were reporting on tilapias. All fishermen were asked to mark the river reaches where they captured tilapias on photocopied 1:50,000 color topographic maps. They were also asked to share: (1) the locations and years of tilapia first detection in their catches; (2) hypotheses about initial sources of introduced individuals; (3) observations about dispersal; and (4) general patterns of tilapia abundance through time. A GIS shapefile was created with the tilapia presence localities from all methods combined.

Species distribution model development and validation

Because I mixed data from different methods and could not reliably estimate species absence, a modeling approach was selected that required presence-only data on species occurrences. Maximum entropy, or Maxent, is a mathematical approach to predicting an unknown probability distribution that estimates the most uniform distribution (e.g., the one with maximum entropy) across a defined area subject to the constraints imposed by environmental conditions (Phillips et al. 2006). The software used (Maxent software for species habitat modeling, version 3.2.19; http://www.cs.princeton.edu/~schapire/maxent/) iteratively adjusts weights associated with each environmental variable to maximize the probabilities under the species presence locations. The output of a Maxent model is a continuous surface of probability values between 0 and 1, with higher values indicating a higher suitability for tilapias. Several recent studies have shown Maxent to consistently outperform other presence-only techniques (Phillips et al. 2004, Elith et al. 2006, Hernandez et al. 2006, Phillips et al. 2006).

Models were trained with the 78 localities where fish were collected or confirmed throughout the study area and a reduced set of predictor variables. Principal components analysis (PCA) was used to summarize the predictor dataset and select eight variables that loaded heavily (>0.30) on the axes with observed eigenvalues exceeding those expected under the broken stick distribution (McGarigal et al. 2000; Table 3.1). PCA was run for all environmental variables (using PC-ORD, version 4.10, MjM Software Design, Gleneden Beach, Oregon) at the tilapia presence localities. The eight selected variables were then used to train a model of tilapia habitat suitability, which was then projected to all river habitats. A decision threshold was applied to convert the continuous output to a binary prediction of habitats as “suitable” or “unknown suitability”. The minimum probability value under a known presence locality (e.g., the minimum known suitable condition) was used as a threshold.

Two approaches were used to evaluate the performance of the model. The first was the area under the curve (AUC) of a receiver operator characteristic (ROC) plot. An ROC plot displays the relationship between model sensitivity (the true positive fraction) and 1-specificity (the false positive fraction) across all presence-absence thresholds, and the area under the ROC function provides a single threshold-independent measure of overall accuracy (Fielding and Bell 1997). The second validation approach was a threshold-dependent operation known as k-fold cross-validation that uses the binary suitable/unknown suitability classification to measure model performance (Fielding and Bell 1997). In k-fold cross validation, multiple models are trained with a fixed portion of all presence data points that are partitioned from the master dataset, and the remaining points are used for testing. In this case 75% of the points were used to train the model and the remaining 25% were used to test model performance. The proportion of known presence localities incorrectly classified as “unknown suitability”, also known as the extrinsic test omission rate, is used as the performance measure. To get a robust estimate of test omission, 100 runs with different training partitions were made and the mean omission rate and standard error reported.

Chronology of spread

The chronology of spread was mapped in increments based on the dates and locations of first detection in fishermen's catches. Cumulative new detections were plotted against time for a graphical representation of progress. Initial estimates of rates of spread were made within each drainage basin by calculating the distance between the reach where tilapia was first detected and the reach where detections were made in subsequent years. This distance was divided by the number of years separating the detection events to yield an estimate of $km \cdot yr^{-1}$. An implicit assumption in this rate calculation is that fishermen detected tilapias near the furthest extent of their spread in any given year, and that they attributed the year accurately. Because of these potential sources of error, I used this number as a rough first approximation to establish a range of possible rates, rather than a precise estimate.

Identification of possible natural dispersal routes

Fishes have the ability to move between connected water bodies, and the intermittent heavy flooding common to the study area has the potential to disperse fish within drainage basins and across low lying drainage divides. To identify possible inter-basin crossing points for natural dispersal, I used ArcMap 9.2 to run cost surface analysis (CSA) to identify least cost paths between riverine habitats in adjacent drainages. CSA is a spatially-explicit method for estimating the permeability of complex landscapes for organism movement (Gonzales and Gergel 2007). Inputs to the analysis include a cost surface that represents the impedance to movement of a species across a landscape, and the output is an accumulative cost surface where grid values represent the sum of costs to move between cells, and a line representing the least cost path through the accumulative cost surface.

ArcGIS 9.2 implements a Cost Distance function that iteratively determines the shortest weighted distance from each cell in a grid to adjacent cells that lie closer to a predefined end point in the grid. In each iteration, all cells that are adjacent to the source cell are assigned weighted distance values, calculated as the sum of cost values of the source cell and the adjacent cell, divided by the distance between the centers of each cell. This process proceeds until all cells in a grid connecting a start point and an end point have been assigned weighted cost values. The path between the start and end points with the least accumulative weighted cost is then defined as the least cost path. Because temporary inter-basin connections during flooding are only likely to occur across areas with very low elevational relief, I used a digital elevation model as the cost surface, leading to definition of paths between basins that overcome the shortest distance and the least cumulative elevational change. I used publicly available 90-m resolution shuttle radar topography mission (SRTM) elevation data to define least cost paths. SRTM data measure the elevation of the highest surface that was sensed first by the radar sensor on the space shuttle (e.g., forest canopy, buildings). Thus, in areas with high forest canopies, cost values may accumulate more rapidly than they would otherwise, potentially affecting the resulting least cost paths. I conditioned my elevation cost surface to impose zero cost to organisms traveling through mapped water bodies (rivers, canals, lakes, ponds, or lagoons). With a starting point on the lower reach of each river main stem, costs only accrued in cells where no river line or water body was located. After least cost path lines were determined, the range of accumulative cost underlying each line was summarized to aid with interpretation of possible natural dispersal routes.

Results

At 61 of the 78 localities where tilapias were recorded as present, whole fish vouchers or photographic vouchers were collected and the identities of the fish species were assessed using keys available on www.fishbase.org. Of the 61 vouchers, all but three of the fish were identified as Nile tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] based on external morphological characteristics. Two others were pink hybrid tilapias with likely direct origins as aquaculture stock, or within several generations of reproduction in the wild (Majumdar et al. 1997). The third individual, collected from a rice irrigation ditch on the Rio Hondo floodplain, was identified as Blue tilapia [*Oreochromis aureus* (Steindachner, 1864)] based on external morphological characteristics. However, given the long history of mixing and manipulation of aquaculture stocks and tilapias' propensity to hybridize (Costa-Pierce 2003), fish identified as Nile tilapia may still be hybrids.

Habitat vulnerability to tilapia colonization

The model of tilapia habitat suitability predicted that 7,510 linear km of river habitat in the study area were vulnerable to colonization by tilapia (Figure 3.2). This represents approximately 24% of all river length (31,257 river km) in the study area. Suitable habitats were present in mid- to low elevation (0 - 277 meters above sea level) main stem rivers (mean upstream watershed area = 566 km^2), extending from the river mouth up inside the margins of mountain valleys (0 - 446 km from sea). These results are consistent with expectations that tilapias would be widely distributed within the study area.

The model performed well according to both of the metrics used to judge its validity. The AUC was 0.97, suggesting that the model could correctly order randomly selected presence and background locations 97% of the time. The extrinsic omission rate from k-fold cross-validation also showed very good performance. The extrinsic omission rate was 0.04 (±0.01 SE), meaning that, on average, the model correctly predicted presence localities 96% of the time—strong results given the validation measures chosen.

Chronology of spread and potential drivers

According to fishermen, the tilapia invasion in Belize began in 1990 in Crooked Tree Lagoon in the Belize River system (Figure 3.3). Rio Hondo was the next river system where tilapias were detected in 1995 in three locations in the middle reaches, followed by Belize River habitats nearest to Crooked Tree Lagoon in 1996. By 1998, tilapias had spread to several more reaches within the Belize River basin, and were first detected in the Sibun River basin to the south of the Belize River, and in the Monkey River in southern Belize. In the following year, tilapias were detected in Moho River, and in two lagoon systems on the northeastern coastal plain (Progresso Lagoon and Shipstern Lagoon). By 2000, tilapias reached a small coastal drainage east of Crooked Tree Lagoon, and were well-dispersed within the Belize River system. In 2002, tilapias were first detected in the New River basin, and in 2004, in the North Stann Creek basin (Figure 3.3). Anecdotal reports suggested that three other basins (Manatee River, Mullins River, and Sarstoon River) may also have tilapias in them, though I was unable to confirm these reports (Figure 3.4).

Interview results confirmed the importance of flooding as a dispersal mechanism. The initial spread of tilapia into Belize in 1990 corresponds with a very large flood event in that year (Figure 3.3). Furthermore, the largest single-year jumps in new tilapia detections in Belize occurred in 1998 and 2000 when Hurricanes Mitch and Keith caused high flood pulses in the Belize River valley and throughout the country (Figure 3.3). Acknowledging the potential for a delay in fisherman detection after the initial arrival of a propagule, it is also possible that the tilapias first reported in Moho River, Progresso Lagoon, and Shipstern Lagoon in 1999 may have dispersed with Hurricane Mitch floods in 1998, and the spread of tilapias to New River may be associated with Hurricane Keith.

The primary cause of tilapia presence in the rivers of the study area was reported as pond aquaculture that was conducted in flood prone areas (Table 3.2, Figure 3.4). Based on interview data, I can hypothesize that initial propagules originated from flooded aquaculture ponds in six of the nine major basins where tilapias were confirmed to be present (Table 3.2). There is no evidence of direct stocking of natural waterways in the Belize portion of the study area, however on the Mexican side of Rio Hondo it is not uncommon for stocking to occur in sinkholes and wetlands (Schmitter-Soto and Caro 1997). It can be hypothesized that the initial propagules arrived in the other three systems through natural dispersal, particularly during large flood events. Pathways identified include (a) intermittently inundated low gradient drainage divides; (b) interconnected wetland complexes that join during floods; (c) a man-made canal that connects the Belize River system to the Sibun River and the Northern and Southern Lagoons; and (d) dispersal at sea. It has yet to be proven that the Nile tilapia disperses through sea water, but as a euryhaline species it has been shown in laboratory conditions to survive in salinities up to 30 ppt (Phillippart and Ruwet 1982), and the closely related Mossambique tilapia (*O. mossambicus*) has been captured in full sea water (Lobel 1980). After Hurricane Keith, two fishermen reported that they captured tilapias in their fish traps in the brackish waters of the Bay of Chetumal (Figure 3.1). They believed that the fish were pushed out of the mouth of Rio Hondo by flooding and dispersed into river and creek mouths along the margin of the Bay.

My coarse assessment of dispersal rates included five estimates in different years in the Belize River, and one estimate each from four other rivers. Dispersal rates ranged from 2.48 km yr^{-1} to 115.66 km yr^{-1}, with a mean of 31.65 km yr^{-1} (Table 3.3).

Least cost paths between drainage basins

Paths of least elevational resistance were identified between all drainage basins (Figure 3.4, Table 3.4). These data show that at least 11 potential low-elevational crossing points exist between basins, where flood waters may disperse tilapias, in addition to the Burdon Canal. Importantly, several low cost linkages exist between currently invaded basins and adjacent uninvaded systems. These are potential routes for future spread. Additionally, several of the crossings represent man-made linkages between basins via canals and agricultural ditches that are very likely to serve as movement corridors (Figure 3.4).

Discussion

> *"Tilapias have repeatedly demonstrated their ability to establish sustaining, feral populations almost everywhere they have been translocated into suitable climates." (Courtenay 1997)*
>
> *"Today, the tilapiine fishes are likely the world's most widely distributed exotic fish species, having invaded every tropical and subtropical environment to which they have gained access". (Costa-Pierce 2003)*

Tilapias have extraordinary potential to establish populations in non-native settings (Courtenay 1997, Costa-Pierce 2003). Given this, then it seems that only two factors may limit further tilapia range expansion: (1) unsuitable environmental conditions within newly accessed ecosystems, and (2) limitations on dispersal (human and natural). While the first factor is difficult to control and manage, the second option is at least partially within human control, and can thus be managed to impede tilapia spread.

Habitat vulnerability to colonization

Prediction of non-indigenous species distributions and spread through time is important for development of management interventions (Sharov and Liebhold 1998, Tobin et al. 2007). SDMs have been used often to infer potential future distributions of invasive species as they spread through a landscape (e.g., Drake and Bossenbroek 2004, Vander Zanden et al. 2004, Loo et al. 2007). Models implemented for this purpose use information about hypothesized niche parameters to predict the spatial limits of the realized niche (Peterson 2003). Such models do not predict spread explicitly, but attempt to define the spatial limits of habitat where a species' spread will stop.

My prediction of potential distributions of tilapias in Belize drew on coarse-scale indicators of landscape position, climate, geologic substrates, and human influence to predict tilapia habitat suitability. As observed by Rondinini et al. (2006), the predictions yielded by habitat suitability models are likely to fall somewhere between the true limits of a species' potential range (i.e., its extent of occupancy; EOO), and the locations within a species' range where it may actually occur (its area of occupancy; AOO). Given the coarse nature of the input data, it is likely that my model more closely approximates potential EOO, and that tilapias may be patchily distributed within the habitats predicted as suitable to them. While my models performed very well given the internal validation procedures I selected, they are a reflection of the training data that were available—namely, localities collected on main stem rivers and tributaries, with a bias toward collection sites in Belize. The Mexican portion of the study area was underrepresented, which may have led to an underestimation of tilapia EOO in the Rio Hondo basin. A more extensive presence dataset would likely expand the predicted EOO, rather than diminish it. Thus, the predictions represent a conservative estimate of habitat vulnerability to tilapia colonization. Future efforts should move towards better predictions of EOO and AOO through expansion of the variety of habitats represented in the presence dataset.

Spread of tilapia

The invasion process has been characterized as being composed of three sequential phases: (i) an initial establishment phase with low spread rate; (ii) an expansion phase where rates of spread increase; and (iii) a saturation phase where spread rates plateau (Shigesada and Kawasaki 1997). A lag period is often observed in the establishment phase, which has been variously attributed to slow initial population growth, rapid evolution among rare or newly created genotypes, an Allee effect, or the pressures that environmental or demographic stochasticity can place on a small colonizing population (Mack et al. 2000, Taylor and Hastings 2005). Spread rates may slow or halt as a population nears the geographic limits of available suitable habitats in the saturation phase of an invasion (Shigesada and Kawasaki 1997).

My data may exhibit several of these invasion stages. The plot of first detections through time (Figure 3.3) exhibits a pattern consistent with those predicted for the three invasion stages, with a slow initial rise in detections (establishment) followed by rapid increase (expansion), and leveling (saturation). An initial lag phase is clearly suggested by the data, which seems to transition between the establishment and expansion phases around 1996. The transition from the lag phase to the expansion phase may occur when a small initial population increases in size until emigration occurs as a result of high population density or stochastic natural environmental forces (Shigesada and Kawasaki 1997). Interviews with fishermen in Crooked Tree Lagoon support the idea that tilapia populations increased dramatically in this wetland system prior to their presumed emigration and first detection outside of the lagoon in 1996. Crooked Tree fishermen reported catching only a few tilapias in 1990, half of a 50 lb rice sack in 1991, and “many sacks” in 1994. An establishment-to-expansion transition is also suggested by the increasingly rapid spread rates for the Belize River system, with estimates of 2 and 13 km yr^{-1} for the two river reaches where tilapias were detected in 1996, and much higher rates of 20 to 33 km yr^{-1} in 1998 to 2002 (Table 3.3). The expansion phase of tilapias in Belize was clearly assisted by human- and flood-aided dispersal, and the subsequent establishment and spread from peripheral populations may have created multiple foci of invasion (Figure 3.3).

Based on the evidence, it is difficult to conclude if the leveling of the detections through time curve represents a true saturation of available habitats. The evidence does seem to suggest that tilapias have colonized much of the habitat available in the New, Belize, Sibun, and Moho River systems (Figure 3.3, top right), lending support to the idea that these systems are nearing saturation. However, many basins—particularly the small river systems draining east off of the Maya Mountains—have no reported incidence of tilapias despite the presence of suitable habitats. Thus, if tilapias are close to a saturation phase it is because they have yet to reach un-colonized basins, where the expansion would continue.

Expansion into un-colonized basins could occur through human assisted or natural dispersal. Cost surface analysis suggests the existence of numerous low-elevation, short-distance connections between colonized and un-colonized basins (Figure 3.4). It is currently not possible to distinguish which of these, if any, represent valid movement corridors, indicating another important area of future research.

Local ecological knowledge gathered in interviews with fishermen was a key source of information on tilapia spread in the study area. Knowledge from local fishermen has been shown in past studies to complement quantitative science by providing concordant and additional information about fish population and community patterns (Johannes

1978, 1981, Neis et al. 1999, Aswani and Hamilton 2004, Fraser et al. 2006, Garcia-Quijano 2007), and an abundance of research has reinforced the scientific accuracy of local and traditional fact-based claims. Previous works comparing local knowledge to mensurative scientific studies have shown that fishermen can recognize taxa, have accurate knowledge on fish behavioral traits and spatiotemporal changes in fish assemblage composition (Poizat and Baran 1997), and can accurately attribute causation to complex limnological occurrences (Calheiros et al. 2000). Yet, a substantial body of research also identifies the limitations of humans to accurately translate their experiences into explicit information, because of human tendencies toward judgmental biases, difficulties at understanding complex probabilities, and poor abilities to learn about complex systems (Fazey et al. 2006). In this case, several sources of bias may have affected my results. First, fishermen may have had difficulty attributing accurate dates to the year of first occurrence. Second, the accuracy of claims about the origins of propagules may be based on anecdotes rather than direct observation. Finally, it is possible that fishermen may have misrepresented the locations where they capture tilapias, because of poor map reading skills, exaggeration, or other factors. To the extent possible, the vouchers, catch data, and electrofishing data collected in this study were able to verify (1) fishermen's' abilities to identify tilapia correctly, and (2) some of the presence localities claimed by fishermen. Despite these weaknesses, local ecological knowledge was useful for assembling a general picture of invasion progress, and hypotheses about tilapia spread in this information poor context.

Tilapia management

Tilapia invasion is well underway in the study area, and given tilapias' potential to disrupt ecosystems and important services that ecosystems provide to humans (Canonico et al. 2005), they are a species that must be managed aggressively and quickly. This research supports several conclusions that should be incorporated into management guidelines. First, a large percentage of available habitat in the study area was predicted to be suitable for tilapias, and when tilapias have been given access to these habitats in the past, they have colonized successfully. Second, aquaculture activities were identified as a primary cause of dispersal, and flooding as an important secondary cause. Third, tilapia species diversity was low, suggesting that genetic diversity may also be limited. Fourth, there were a number of watersheds thought to have no tilapia present in them as of 2007.

Several authors have observed that tilapia eradication is an ambitious and highly unlikely management goal (Riedel 1965, Arthington and Bluhdorn 1994, McCrary 2007), particularly after an invasion is well-advanced within a drainage basin. Eradication may be particularly untenable in a place like Belize where poverty rates are high and government agencies are chronically underfunded to undertake resource-intensive efforts. Thus, it may be more reasonable to consider low-cost interventions (e.g., modifications to fisheries regulations) to reduce populations where they exist, and prevention of tilapia spread into uncolonized ecosystems as management goals.

Aquaculture, as the likely main culprit for tilapias' existence and spread throughout Belize, must be regulated far more carefully in the future. In particular I echo recommendations of McCrary (2007) in calling for: (1) the use of totally closed aquaculture systems and a ban on cage aquaculture; (2) siting restrictions for pond culture away from flood prone areas; (3) a strict ban on tilapia cultivation and transportation in watersheds where they have not yet colonized; and (4) limitation of the genetic stocks used in aquaculture to those varieties already introduced into the natural waters of the country. Colonization of a river network by tilapia does not mean that aquaculture promoters should be released from responsibility for controlling further unintended releases of tilapias into water bodies. Subsequent releases into already colonized areas may still exacerbate the severity of the invasion, by augmenting populations and/or increasing genetic diversity.

Fisheries activities can and should be used to manage tilapias. I recommend the following fisheries interventions: (1) reduction of tilapia source populations through intensive harvest of habitats known to be infested with tilapias; (2) protection and/or active augmentation of large predators on tilapias (e.g., tarpon and snooks); (3) evaluation and modification of gear restrictions to target tilapia size classes that exceed maximum sizes of native cichlid fishes. Tilapias outgrow many other fishes, so mesh sizes could be adjusted to target tilapias and avoid native by-catch (Pet et al. 1995).

The watersheds draining to the coast of Belize are fortunate in the relative scarcity of invasive species present relative to many other locations in the world (e.g., De Silva 1987, Courtenay 2007). One lesson from the unchecked spread of tilapias is that lax rules for importation of non-indigenous species can lead to widespread invasions, and to ecological and economic damage (Pimentel et al. 2005). Currently there is little legislation in Belize to guard against importation of additional varieties of tilapias or other invasive organisms such as aquarium fishes (McCalla 1995), or to regulate aquaculture activities. The final recommendation is that a thorough review is conducted leading to the strengthening of national laws to prevent importation and spread of non-indigenous species (tilapias or otherwise). Only by careful management of non-indigenous species transport into the waters of Belize can the country avoid some of the costly and catastrophic invasions that have occurred elsewhere in tropical freshwater systems.

Table 3.1. Environmental variables prepared for entry into Maxent models of tilapia habitat. Variables in bold represent those that were selected for entry into the model after using PCA to summarize data set. The first five axes explained a total of 62% of the variance. See Chapter 2 for descriptions of geology classes.

Variable (units)	Min	Max	Mean	PC 1	PC 2	PC 3	PC 4	PC 5
Average annual air temp in catchment (degrees C)	**21.86**	**26.00**	**23.87**	**-0.18**	**0.08**	**0.14**	**0.14**	**0.40**
Average annual rainfall in catchment (mm)	1,259.56	3,317.92	1,969.14	-0.04	-0.41	-0.11	-0.04	0.11
Average catchment elevation (masl)	1.50	591.44	222.84	0.36	-0.03	0.06	0.10	-0.03
Average catchment slope (degrees)	**0.00**	**18.00**	**7.09**	**0.33**	**-0.12**	**0.05**	**0.16**	**-0.06**
Average local annual air temperature (º C)	21.98	26.00	24.79	-0.13	0.12	0.15	0.22	0.36
Average local annual rainfall (mm)	1,258.00	3,867.00	2,029.64	-0.04	-0.25	0.14	0.32	0.09
Local elevation (masl)	**0.00**	**157.00**	**33.33**	**0.32**	**0.02**	**-0.02**	**-0.21**	**0.17**
Local slope (degrees)	0.00	32.00	3.13	0.19	-0.01	0.07	-0.09	0.14
Flow accumulation (10^3 pixels)	**0.01**	**10,892.7**	**2,042.57**	**0.06**	**0.32**	**0.08**	**0.17**	**-0.24**
Horizontal land distance to next perennial lake (km)	0.07	93.14	21.91	0.31	0.10	-0.08	-0.16	0.17
Surface area of nearest lake (km2)	0.52	40.12	5.23	-0.21	-0.01	0.03	-0.14	0.04
Distance downstream to sea (km)	**1.49**	**348.60**	**102.24**	**0.24**	**0.18**	**0.07**	**-0.23**	**0.24**

Upstream distance to furthest basin divide (km)	0.17	497.69	130.84	0.10	0.30	0.09	0.20	-0.22
Catchment geology proportions								
Quaternary alluvium	**0.00**	**1.00**	**0.23**	**-0.23**	**-0.03**	**0.05**	**-0.36**	**-0.12**
Cretaceous limestone	0.00	1.00	0.47	-0.02	0.25	-0.14	0.19	0.17
Lavas-pyroclastics-volcanic sediments	0.00	1.00	0.22	0.27	-0.19	0.21	0.08	-0.07
Tertiary sedimentary	0.00	0.89	0.05	-0.06	0.03	0.04	0.04	0.00
Toledo Formation	0.00	1.00	0.03	-0.01	-0.10	-0.36	0.15	0.07
Catchment soil proportions								
Cambisol - Leptosol - Vertisol	**0.00**	**0.93**	**0.08**	**0.02**	**-0.12**	**-0.42**	**0.21**	**0.07**
Fluvisol - Cambisol - Vertisol	0.00	0.26	0.01	0.05	-0.12	0.09	0.09	-0.23
Gleysols	0.00	0.35	0.02	-0.05	0.12	0.07	0.09	-0.05
Gleysols – Fluvisols	0.00	0.44	0.07	-0.14	0.02	0.05	-0.07	0.24
Gleysols – Vertisols	0.00	0.99	0.02	-0.02	0.08	-0.11	-0.17	-0.21
Gleysols - Vertisols - Fluvisol	0.00	1.00	0.13	-0.21	-0.17	0.08	-0.26	-0.11
Leptosol	0.00	0.22	0.01	0.05	-0.08	-0.30	0.11	0.09
Leptosol – Cambisol	0.00	1.00	0.22	0.24	-0.25	0.18	0.13	-0.08

Leptosols – vertisols	0.00	0.92	0.42	0.01	0.39	0.07	-0.02	0.05
Litosol – Cambisol	0.00	0.15	0.00	0.04	-0.10	0.08	0.08	-0.23
Catchment landuse proportions of:								
Agriculture	0.00	1.00	0.21	0.08	0.09	-0.37	-0.08	-0.22
Urban	0.00	0.05	0.00	-0.01	0.06	-0.16	-0.13	-0.21
Linear distance to nearest human settlement (km)	0.27	8.80	2.82	-0.10	-0.21	0.10	0.05	0.04
Proportion of pixels in catchment with roads present	0.00	0.06	0.01	-0.05	0.01	-0.40	0.14	0.05
Mean upstream risk intensity	**0.00**	**135.00**	**19.88**	**0.00**	**0.06**	**-0.33**	**0.13**	**-0.25**

Table 3.2. Initial introduction sources reported by fishermen, and possible dispersal mechanisms and/or routes for initial spread into each drainage basin in Belize. Map ID numbers identify basins in Figure 4. Status codes are: P = tilapia present; AN = Anecdotal reports of tilapia; ? = unknown.

Map ID	Drainage Basin	Status	Hypothesized introduction source(s)	Possible natural dispersal route(s)
1	Rio Hondo	P	• Tilapia cage culture in river • Aquaculture ponds on floodplain or tributaries	
2	New River	P	• Dispersal from adjacent basin	• Belize River • Progresso Lagoon • Chetumal Bay
3	Progresso and Shipstern Lagoons	P	• Aquaculture near Progresso Lagoon • Dispersal from adjacent basin	• New River • Chetumal Bay
4	Northern River	P	• Dispersal from adjacent basin	• Belize River • Freshwater Creek
5	Belize River	P	• Aquaculture ponds on floodplain or tributaries	
6	Sibun River	P	• Dispersal from Belize River • Aquaculture ponds on floodplain	• Burdon Canal
7	Manatee River	AN	• Dispersal from adjacent basin	• Burdon Canal
8	Mullins River	AN	• Dispersal from adjacent basin	• Southern Lagoon
9	North Stann Creek	P	• Aquaculture ponds on floodplain or tributaries	
12	Big Creek	?		
13	Sennis River	?	• Aquaculture ponds on floodplain (1997)	
14	Pine Ridge Creek	?		
15	Monkey River	P	• Dispersal from adjacent basins • Tilapia stocking in lagoon near tributary	• Sennis River
19	Moho River	P	• Aquaculture ponds on tributary in Guatemala	
21	Sarstoon River	AN		

Table 3.3. Estimated dispersal rates calculated for the river systems for which multiple years of observation were recorded. Distances tilapia moved along the river channel were calculated between the first reported establishment site and reaches that were reported to be established later in time. This value was divided by the number of years separating the two observations.

Dispersal route	**Distance (km)**	**Years**	**Rate (km yr^{-1})**
Crooked Tree to Mussel Creek	14.90	6	2.48
Crooked Tree to Belize River main stem (lower)	79.95	6	13.33
Crooked Tree to Belize River main stem (upper)	178.83	8	22.35
Crooked Tree to Mopan Branch	333.78	10	33.38
Crooked Tree to Macal Branch	250.62	12	20.89
New River (upper to lower)	115.66	1	115.66
Sibun River (lower to upper)	74.74	2	37.37
Monkey River (lower to upper)	35.15	8	4.39
Moho River (upper to lower)	69.93	2	34.96
		Mean	**31.65**

Table 3.4. Summary table of crossings between drainage basins (Figure 3.4), and the associated elevational ranges, distances between river networks, and accumulative cost measure from cost surface analysis.

Crossing	**Elev. Range (m)**	**Length (km)**	**Cost**
1 to 2	6	24.08	32,225
2 to 3	15	1.49	8,760
2 to 5	26	18.60	21,573
4 to 5	8	34.52	19,274
7 to 8	3	1.30	1,596
8 to 9	9	17.95	14,265
9 to 10	11	6.76	16,936
10 to 11	7	0.65	7,058
14 to 15	11	0.24	3,960
15 to 16	7	0.52	13,469
16 to 17	7	15.13	18,589
17 to 18	3	0.29	9,594
18 to 19	20	1.14	30,475
19 to 20	4	0.40	12,575
20 to 21	13	1.09	2,153

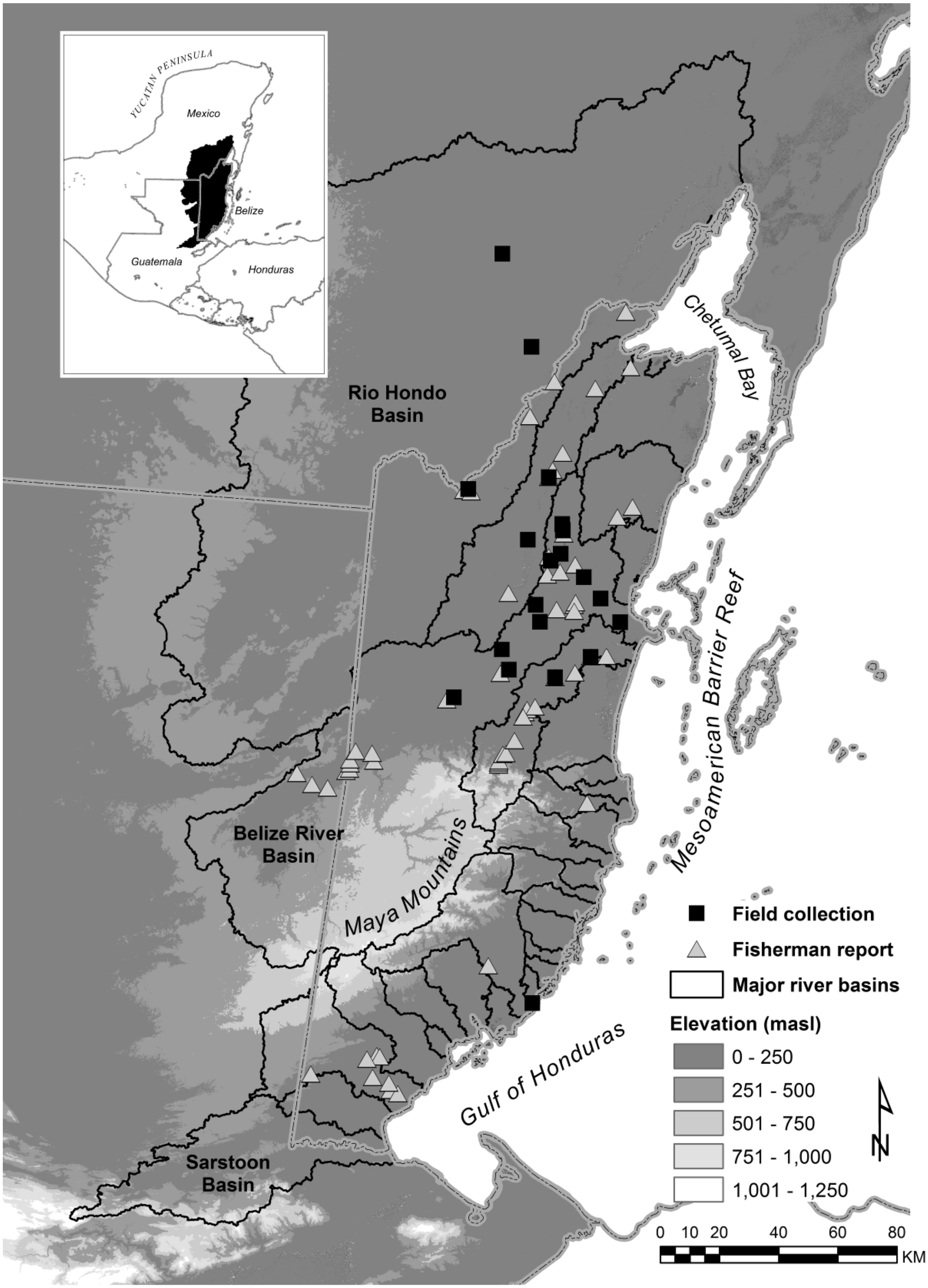

Figure 3.1. Study area showing elevation, major drainage basins, important geographic features and the locations of all sampling sites used for habitat suitability modeling with Maxent.

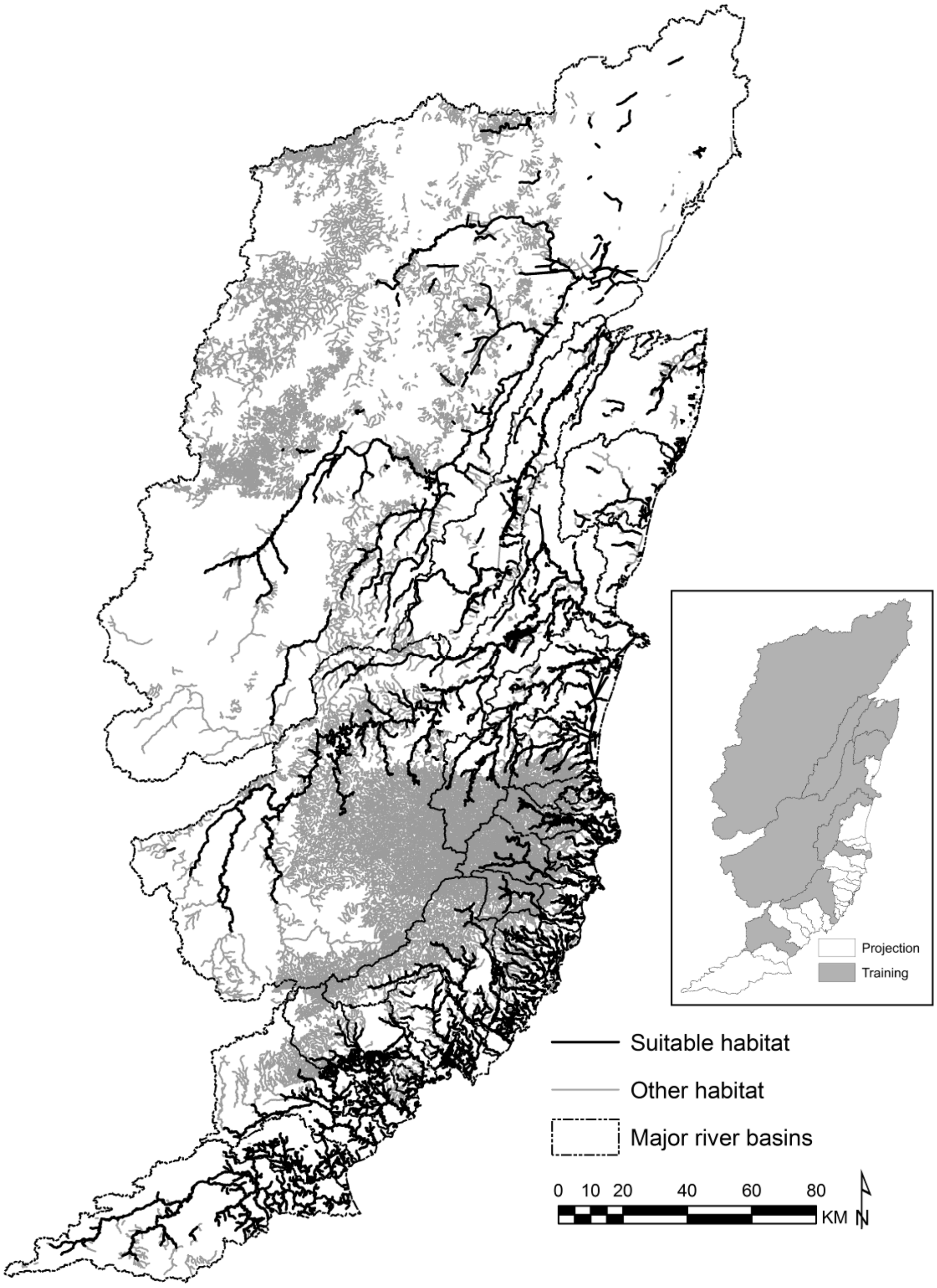

Figure 3.2. River habitats predicted to be suitable for tilapias (dark lines), and habitats that were not predicted as suitable (gray). Inset map shows basins with confirmed tilapia presence (shaded), occurrence points from where were used to train models and the project suitability into unshaded basins.

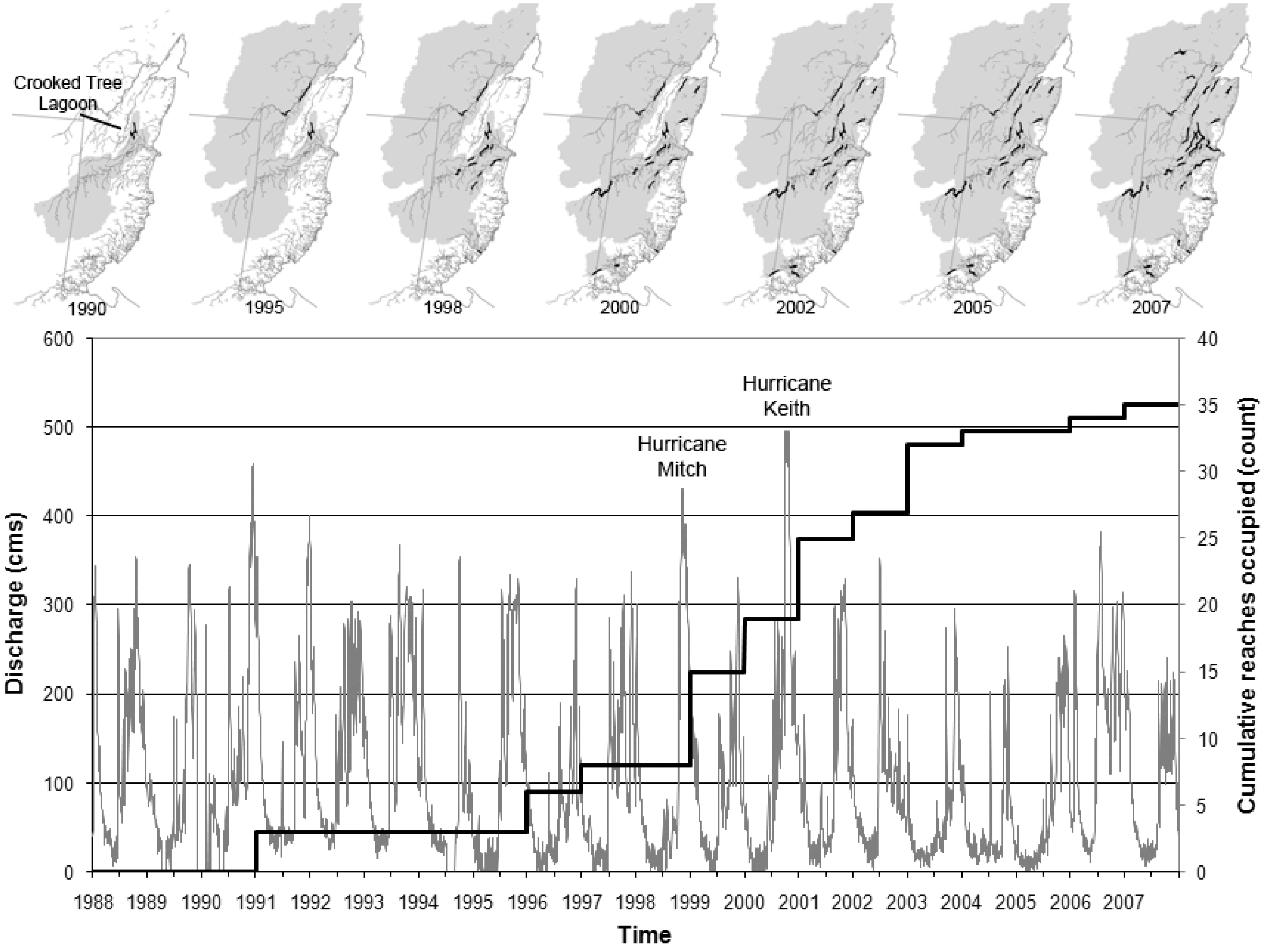

Figure 3.3. The chronology of spread of tilapias in Belize based on fishermen's responses. Bottom graph shows detections of tilapia in new river reaches through time (right axis), and river discharge (left axis) at a station on the lower Belize River near Crooked Tree. Discharge values are in cubic meters per second (cms), and the years of Hurricanes Mitch and Keith are labeled. The initial introduction of tilapia and the two largest jumps in detections all followed large flood events (>400 cms). The small maps across the top of the figure show a time series of colonized reaches (dark black lines) starting with the colonization of Crooked Tree Lagoon. Shaded gray areas show presence of tilapia within drainage basins through time. Note the increasing area in gray as tilapia spread throughout the area.

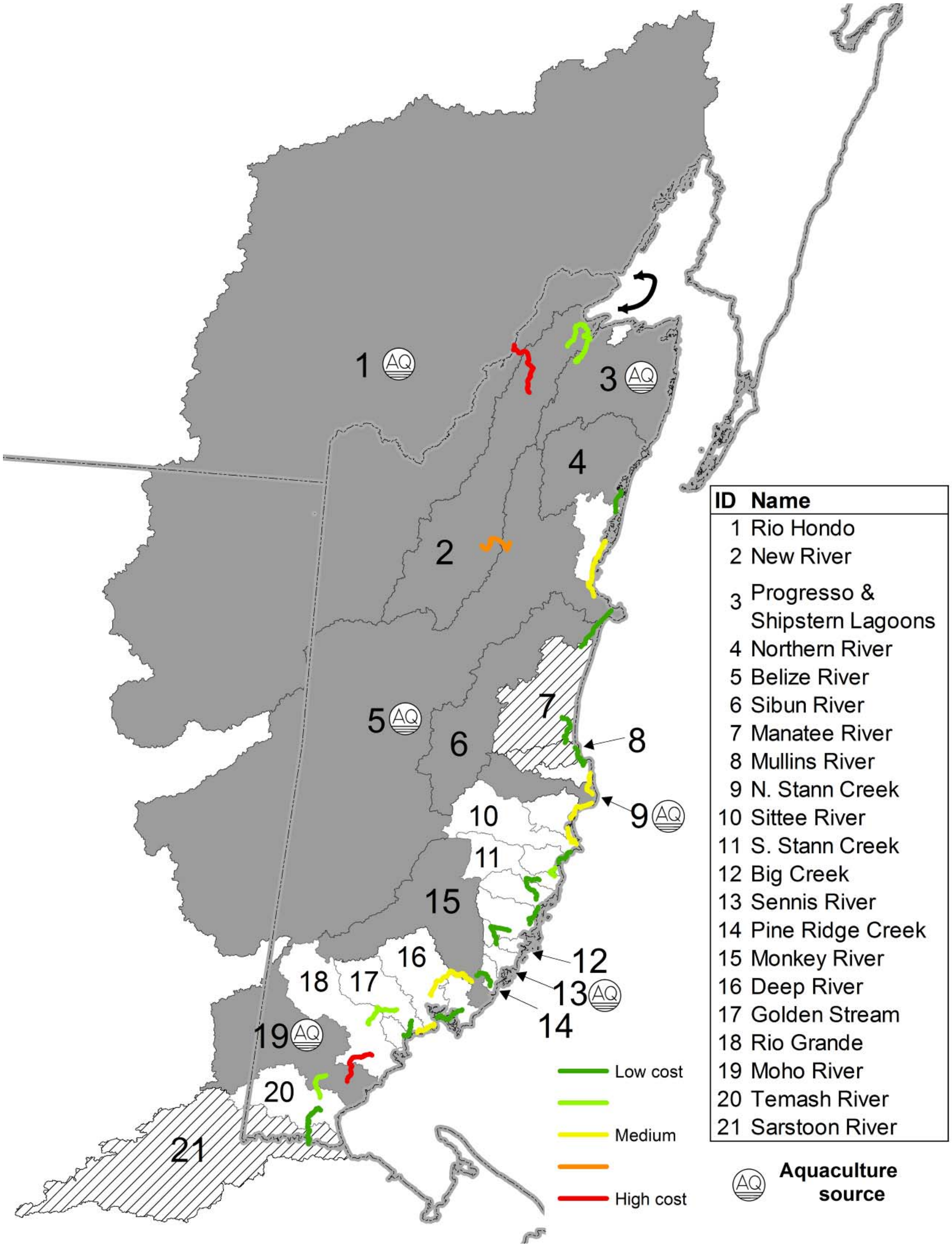

Figure 3.4. Map of drainage basins (numbers), their invaded status (confirmed present = shaded; anecdotal report of presence = diagonal lines; no confirmed or anecdotal presence = no fill), and relative accumulative cost for inter-basin crossings (colored lines). Table 3.4 contains summary statistics for each crossing. Those basins where aquaculture ponds within them were thought to be the initial source of propagules are also marked.

Literature Cited

Arthington, A. H. and D. R. Bluhdorn. 1994. Distribution, genetics, ecology and status of the introduced cichlid, *Oreochromis mossambicus*, in Australia. *Mitt. Internat. Verein. Limnol.* **24**:53-62.

Aswani, S. and R. J. Hamilton. 2004. Integrating indigenous ecological knowledge and customary sea tenure with marine and social science for conservation of bumphead parrotfish (Bolbometopon muricatum) in the Roviana Lagoon, Solomon Islands. *Environmental Conservation* **31**:69-83.

Balirwa, J. S., C. A. Chapman, L. J. Chapman, I. G. Cowx, K. Geheb, L. Kaufman, R. H. Lowe-McConnell, O. Seehausen, J. H. Wanink, R. L. Welcomme, and F. Witte. 2003. Biodiversity and fishery sustainability in the Lake Victoria Basin: An unexpected marriage? *Bioscience* **53**:703-715.

Barbour, C. D. and J. H. Brown. 1974. Fish species diversity in lakes. *American Naturalist* **108**:473-489.

Benstead, J. P., P. H. De Rham, J. L. Gattolliat, F. M. Gibon, P. V. Loiselle, M. Sartori, J. S. Sparks, and M. L. J. Stiassny. 2003. Conserving Madagascar's freshwater biodiversity. *Bioscience* **53**:1101-1111.

Bussing, W. A. 1976. Geographic distribution of the San Juan ichthyofauna of Central America with remarks on its origin and ecology. Pages 157-175 *in* T. B. Thorson, editor. *Investigations of the Ichthyofauna of Nicaraguan Lakes*. University of Nebraska, Lincoln, Nebraska.

Calheiros, D. F., A. F. Seidl, and C. J. A. Ferreira. 2000. Participatory research methods in environmental science: local and scientific knowledge of a limnological phenomenon in the Pantanal wetland of Brazil. *Journal of Applied Ecology* **37**:684-696.

Canonico, G. C., A. Arthington, J. K. McCrary, and M. L. Thieme. 2005. The effects of introduced tilapias on native biodiversity. *Aquatic Conservation-Marine and Freshwater Ecosystems* **15**:463-483.

Costa-Pierce, B. A. 2003. Rapid evolution of an established feral tilapia (*Oreochromis* spp.): the need to incorporate invasion science into regulatory structures. *Biological Invasions* **5**:71-84.

Courtenay, W. R. 1997. Tilapias as non-indigenous species in the Americas: enviuronmental, regulatory and legal issues. Pages 18-33 *in* B. A. Costa-Pierce and J. E. Rakocy, editors. *Tilapia Aquaculture in the Americas, Vol. 1*. World Aquaculture Society, Baton Rouge, Louisianna.

Courtenay, W. R. 2007. Nonindigenous fishes. Pages 109-122 *in* D. Simberloff, D. C. Schmitz, and T. C. Brown, editors. *Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida*. Island Press, Washington, D.C.

De Silva, S. 1987. Impact of exotics on the inland fishery resources of Sri Lanka. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **28**:273-293.

Drake, J. M. and J. M. Bossenbroek. 2004. The potential distribution of zebra mussels in the United States. *Bioscience* **54**:931-941.

Dudgeon, D. 1999. *Tropical Asian Streams : Zoobenthos, Ecology and Conservation*. Hong Kong University Press, Hong Kong.

Dudgeon, D. 2000. The ecology of tropical Asian rivers and streams in relation to biodiversity conservation. *Annual Review of Ecology & Systematics* **31**:239-263.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C.

A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**:163-182.

Dunham, J. B. and B. E. Rieman. 1999. Metapopulation structure of bull trout: Influences of physical, biotic, and geometrical landscape characteristics. *Ecological Applications* **9**:642-655.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. M. Overton, A. T. Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz, and N. E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**:129-151.

Esselman, P. C. and J. D. Allan. In review. Application of species distribution models and conservation planning software to the design of a reserve network for the riverine fishes of northeastern Mesoamerica. *Freshwater Biology*.

Esselman, P. C. and E. Boles. 2001. Status and future needs of limnological research in Belize. Pages 35 - 68 *in* R. G. Wetzel and B. Gopan, editors. *Limnology in Developing Countries Volume 3*. International Association for Limnology.

Esselman, P. C., M. C. Freeman, and C. M. Pringle. 2006. Fish-assemblage variation between geologically defined regions and across a longitudinal gradient in the Monkey River Basin, Belize. *Journal of the North American Benthological Society* **25**:142-156.

Everett, R. A. 2000. Patterns and pathways of biological invasions. *Trends in Ecology and Evolution* **15**.

Fazey, I., K. Proust, B. Newell, B. Johnson, and J. A. Fazey. 2006. Eliciting the implicit knowledge and perceptions of on-ground conservation managers of the Macquarie Marshes. *Ecology and Society* **11**.

Fielding, A. H. and J. F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation* **24**:38-49.

Floerl, O. and G. J. Inglis. 2005. Starting the invasion pathway: the interaction between source populations and human transport vectors. *Biological Invasions* **7**:589-606.

Fraser, D. J., T. Coon, M. R. Prince, R. Dion, and L. Bernatchez. 2006. Integrating traditional and evolutionary knowledge in biodiversity conservation: a population level case study. *Ecology and Society* **11**.

Froese, R. and D. Pauly. 2008. FishBase.

Garcia-Quijano, C. G. 2007. Fishers' knowledge of marine species assemblages: Bridging between scientific and local ecological knowledge in southeastern Puerto Rico. *American Anthropologist* **109**:529-536.

Gonzales, E. K. and S. E. Gergel. 2007. Testing assumptions of cost surface analysis-a tool for invasive species management. *Landscape Ecology* **22**:1155-1168.

Goudswaard, P. C., F. Witte, and E. F. B. Katunzi. 2002. The tilapiine fish stock of Lake Victoria before and after the Nile perch upsurge. *Journal of Fish Biology* **60**:838-856.

Greenfield, D. W. and J. E. Thomerson. 1997. *Fishes of the Continental Waters of Belize*. University of Florida Press, Gainseville, FL.

Guisan, A. and N. E. Zimmermann. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* **135**:147-186.

Hartshorn, G., L. Nicolait, L. Hartshorn, G. Bevier, R. Brightman, J. Cal, A. Cawich, D. Weyer, W. H., and A. Wright. 1984. *Belize Country Environmental Profile: A Field Study*. USAID, Belize City, Belize.

Havel, J. E., J. B. Shurin, and J. R. Jones. 2002. Estimating dispersal from patterns of spread: spatial and local control of lake invasions. *Ecology* **83**:3306-3318.

Hernandez, P. A., C. H. Graham, L. L. Master, and D. L. Albert. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* **29**:773-785.

Johannes, R. E. 1978. Reproductive strategies of coastal marine fishes in the tropics. *Environmental Biology of Fishes* **3**:65-84.

Johannes, R. E. 1981. Working with fishermen to improve coastal fisheries and resource-management. *Bulletin of Marine Science* **31**:673-680.

Lee, M. D., J. D. Stednick, and D. M. Gilbert. 1995. *Belize Environmental Water Quality Monitoring: Final Report*. USAID-NARMAP Project to Government of Belize, Belmopan, Belize.

Leveque, C., T. Oberdorff, D. Paugy, M. L. J. Stiassny, and P. A. Tedesco. 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia* **595**:545-567.

Lobel, P.S. 1980. Invasion by the Mozambique tilapia (*Sarotherodon mossambicus*) of a Pacific atoll marine ecosystem. *Micronesica* **16**:349-355.

Lodge, D. M., R. A. Stein, K. M. Brown, A. P. Covich, C. Bronmark, J. E. Garvey, and S. P. Klosiewski. 1998. Predicting impact of freshwater exotic species on native biodiversity: Challenges in spatial scaling. *Australian Journal of Ecology* **23**:53-67.

Loo, S. E., R. P. Keller, and B. Leung. 2007. Freshwater invasions: using historical data to analyse spread. *Diversity and Distributions* **13**:23-32.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**:689-710.

Majumdar, K. C., K. Nasaruddin, and K. Ravinder. 1997. Pink body colour in Tilapia shows single gene inheritance. *Aquaculture Research* **28**:581-589.

McCalla, W. 1995. *Compendium on Environmental Protection and Natural Resource Legislation in Belize.* Department of Environment, Government of Belize, Belmopan, Belize.

McCrary, J. K. 2007. Tilapia (Teleostei: Cichlidae) status in Nicaraguan natural waters. *Environmental Biology of Fishes* **78**:107-114.

McCrary, J. K., E. P. Vandenberghe, K. R. McKaye, and L. J. Lopez Perez. 2001. Tilapia cultivation: a threat to native fish species in Nicaragua. *Encuentro* **58**:9-19.

McGarigal, K., S. Cushman, and S. Stafford. 2000. *Multivariate Statistics for Wildlife and Ecology Research*. Springer-Verlag, New York, NY.

Miller, R. R. 1966. Geographical distribution of Central American freshwater fishes. *Copeia*:773-802.

Moyle, P. B. and T. Light. 1996. Fish invasions in California: Do abiotic factors determine success? *Ecology* **77**:1666-1670.

Neis, B., D. C. Schneider, L. Felt, R. L. Haedrich, J. Fischer, and J. A. Hutchings. 1999. Fisheries assessment: what can be learned from interviewing resource users? *Canadian Journal of Fisheries and Aquatic Sciences* **56**:1949-1963.

Pet, J. S., C. Petsoede, and W. L. T. Vandensen. 1995. Comparison of Methods for the Estimation of Gillnet Selectivity to Tilapia, Cyprinids and Other Fish Species in a Sri-Lankan Reservoir. *Fisheries Research* **24**:141-164.

Phillippart, J.-C. and J.-C. Ruwet. 1982. Ecology and distribution of tilapias. Pages 15-59 *in* R. Pullin, Lowe-McConnell, R.H., editor. *The Biology and Culture of Tilapias*. ICLARM, Manila, Phillipines.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190**:231-259.

Phillips, S. J., M. Dudik, and R. E. Schapire. 2004. A maximum entropy approach to species distribution modeling. Pages 655 - 662 Proceedings of the 21st International Conference on Machine Learning. ACM Press.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**:273-288.

Poizat, G. and E. Baran. 1997. Fishermen's knowledge as background information in tropical fish ecology: a quantitative comparison with fish sampling results. *Environmental Biology of Fishes* **50**:435-449.

Pringle, C. M., F. N. Scatena, P. Paaby-Hansen, and M. Nunez-Ferrera. 2000. River conservation in Latin America and the Caribbean. Pages 41 - 77 *in* P. J. Boon, B. R. Davies, and G. E. Petts, editors. *Global Perspectives on River Conservation: Science, Policy, and Practice*. John Wiley and Sons Ltd., New York.

Ricciardi, A. and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. *Conservation Biology* **13**:1220-1222.

Riedel, D. 1965. Some remarks on the fecundity of Tilapia (T. mossambica Peters) and its introduction into Middle Central America (Nicaragua) together with a first contribution towards the limnology of Nicaragua. *Hydrobiologia* **25**:357-388.

Rondinini, C., K. A. Wilson, L. Boitani, H. Grantham, and H. P. Possingham. 2006. Tradeoffs of different types of species occurrence data for use in systematic conservation planning. *Ecology Letters* **9**:1136-1145.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - global biodiversity scenarios for the year 2100. *Science* **287**:1770-1774.

Schlosser, I. J. 1991. Stream fish ecology: a landscape perspective. *Bioscience* **41**:704-712.

Schmitter-Soto, J. J. 1998. Catalogo de los peces continentales de Quintana Roo. El Colegio de la Frontera Sur, San Cristobal de las Casas, Mexico.

Schmitter-Soto, J. J. and C. I. Caro. 1997. Distribution of tilapia, *Oreochromis mossambicus* (Perciformes : Cichlidae), and water body characteristics in Quintana Roo, Mexico. *Revista de Biologia Tropical* **45**:1257-1261.

Schmitter-Soto, J. J. and H. C. Gamboa-Perez. 1996. Composicion y distribucion de peces continentales en el sur de Quintana Roo, Peninsula de Yucatan, Mexico. *Revista De Biologia Tropical* **44**:199-212.

Sharov, A. A. and A. M. Liebhold. 1998. Bioeconomics of managing the spread of exotic pest species with barrier zones. *Ecological Applications* **8**:833-845.

Shigesada, N. and K. Kawasaki. 1997. *Biological Invasions: Theory and Practice*. Oxford University Press, Oxford, UK.

Starling, F., X. Lazzaro, C. Cavalcanti, and R. Moreira. 2002. Contribution of omnivorous tilapia to eutrophication of a shallow tropical reservoir: evidence from a fish kill. *Freshwater Biology* **47**:2443-2452.

Stiassny, M. L. J. 1999. The medium is the message: freshwater biodiversity in peril. Pages 53-71 *in* J. Cracraft and F. T. Grifo, editors. *The Living Planet in Crisis: Biodiversity Science and Policy*. Columbia University Press, New York, NY.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *Bioscience* **54**:429-439.

Taylor, C. M. and A. Hastings. 2005. Allee effects in biological invasions. *Ecology Letters* **8**:895-908.

Taylor, J. N., W. Courtenay Jr, and J. A. McCann. 1984. Known impacts of exotic fishes in the continental United States. Pages 322-373 *in* W. R. Courtenay and J. R. Stauffer, editors. *Distribution, Biology, and Management of Exotic Fishes*. Johns Hopkins University, Baltimore.

Tobin, P. C., A. M. Liebhold, and E. A. Roberts. 2007. Comparison of methods for estimating the spread of a non-indigenous species. *Journal of Biogeography* **34**:305-312.

Tonn, W. M., J. J. Magnuson, M. Rask, and J. Toivonen. 1990. Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *American Naturalist* **136**:345-375.

Twongo, T. 1995. Impact of fish species introductions no the tilapias of Lakes Victoria and Kyoga.*in* T. J. Hart, editor. *The Impact of Species Changes in African Great Lakes*. Chapman & Hall, London.

Vander Zanden, M. J., J. D. Olden, J. H. Thorne, and N. E. Mandrak. 2004. Predicting occurrences and impacts of smallmouth bass introductions in north temperate lakes. *Ecological Applications* **14**:132-148.

Welcomme, R. L. 1988. *International Introductions of Inland Aquatic Species*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Wilson, E. M. 1980. Physical geography of the Yucatan Peninsula. Pages 5-40 *in* E. H. Moseley and E. D. Terry, editors. *Yucatan: A World Apart*. University of Alabama Press, Alabama.

With, K. A. 2002. The landscape ecology of invasive spread. *Conservation Biology* **16**:1192-1203.

Chapter 4

Application of species distribution models and conservation planning software to the design of a reserve network for the riverine fishes of northeastern Mesoamerica

Abstract

A major challenge in the design of freshwater protected area networks is how to incorporate the high degree of connectivity inherent in freshwater ecosystems where strong terrestrial-aquatic linkages cause local biota and habitats to be influenced by conditions integrated over the entire upstream drainage network. A suite of tools are available for predicting spatial patterns in freshwater biodiversity, and for optimizing the selection of conservation areas that protect freshwater biodiversity. The aims of this study were to use existing tools and methods to design a freshwater reserve network that specifies locations with high fish biodiversity and low human influence that deserve direct protection, and to recommend an extended network of management zones that considers issues of connectivity, exogenous threats, and basin management. Fish species occurrence data from historical and recent sampling were used together with landscape-scale habitat data to generate species distribution models for 63 freshwater fish species. Predicted species distributions were used as conservation features with conservation planning software to select an efficient reserve network of conservation focal areas that protected 15% of the predicted range of each species with special emphasis on migratory species, apex predators, and endemics. Risk from environmental degradation in the upstream drainage network was accounted for by creating a cumulative risk surface that estimated the intensity of human use of the landscape, which was propagated downstream with flow direction information derived from a digital elevation model. The final reserve network consisted of conservation focal areas for migratory and non-migratory species that occupied 11% of the study area, or approximately 4,935 km^2, and was 51% contained within existing protected areas. The final reserve network contained more than 20 distinct focal areas with high species richness and low human influence, 4,927 linear km of rivers thought to be critical to the maintenance of these focal areas as riparian buffers and migration corridors, and the

watersheds that drain to each of these reserve components. This study provides an example of how characteristics of flowing water ecosystems can be incorporated into systematic conservation planning. Use of species distribution models for fishes allowed for a biogeographically realistic representation of biodiversity patterns in the reserve selection process. Creative application of reserve design software allowed for representation of riverine connectivity in a way that respected topographic barriers to species movement, and my representation of mean upstream environmental risk offers benefits over other published examples in that it provides a direct representation of risk magnitudes that incorporates longitudinal connectivity. Finally, definition of critical management zones (riparian buffers and migration corridors) and watersheds created a final reserve that protects high numbers of species in the focal areas where they occur, and also encompasses off-site habitats that contribute to species persistence and abatement of spatially distant threats. I call for critical evaluation of the logistical implications of focal areas linked with extended management zones for reserve management, and call for a continued expansion of freshwater reserve network design in general.

Introduction

The flow path of water along topographic gradients and the capacity of flowing water to transport dissolved and particulate matter result in strong terrestrial-aquatic linkages where downstream habitats in rivers are influenced by conditions integrated over the entire upstream drainage network (Hynes, 1975; Stoms et al., 2005; Meyer et al., 2007). Riverine biotic assemblages, in turn, are strongly associated with local abiotic factors that are constrained by landscape variables at the larger scales of the valley segment or watershed (Frissell et al., 1986; Townsend, 1996; Poff, 1997), and are closely linked to upstream-downstream and river-floodplain connectivity (Vannote et al., 1980; Pringle, 1997; Ward & Wiens, 2001). Owing to this high degree of connectivity, conservation of an important freshwater feature may involve place-based interventions to mitigate stressors in geographically disparate sites up- or downstream of the feature of interest (Lake, 1980; Skelton et al., 1995; Pringle, 1997; Moyle & Randall, 1998). This contrasts with place-based interventions in terrestrial environments where sufficiently sized and managed protected areas can simultaneously protect biodiversity targets and exclude local threats to these targets (Possingham et al., 2006). While fundamental differences exist between place-based conservation strategies in terrestrial and aquatic environments, it is certain that aquatic conservation efforts can benefit from the long history of debate and the development of tools for systematic conservation planning (Margules & Pressey, 2000) on land and at sea.

Protected areas networks are a central strategy for biodiversity conservation. The contemporary design of protected area portfolios is often aided by software tools that help identify reserve networks to efficiently protect representative biodiversity (Sarkar et al., 2006). These software tools operate in such a way as to generate reserve network solutions that satisfy the many criteria that should be considered in protected area planning (see review by Ardron, Possingham & Klein, 2008). While various authors have explored the concept of freshwater protected areas (Saunders, Meeuwig & Vincent, 2002; Linke et al., 2007; Nel et al., 2007; Moilanen, Leathwick & Elith, 2008; Roux et al., 2008), the highly dynamic and interconnected nature of freshwater ecosystems creates unique challenges to their protection using secured parcels of land. In a recent exploration of the potential for protected areas for freshwater ecosystem protection, Abell, Allan & Lehner (2007) argued that traditional concepts of protected areas do not translate well to freshwater ecosystems. Instead of forcing freshwaters "into the terrestrial mold", they propose a three-part vocabulary to describe freshwater management units that better capture the complexity of using place-based strategies to conserve freshwaters. The central unit of this new vocabulary is a freshwater focal area; the specific location of a freshwater feature in need of protection (e.g., a richness hotspot, a critical habitat for spawning, sub-population of a threatened or endemic species). Focal areas are complemented with critical management zones—places that are fundamentally important to maintain the functionality of the focal areas (e.g., a migration corridor for fishes). Finally, a catchment management zone is the entire upstream catchment of a focal area or critical management zone where integrated catchment management principles (e.g., land use planning to reduce non-point source pollution loads) should be applied. In this paper, I adopt the conceptual framework of Abell et al. (2007) and some of the tools of systematic conservation planning to define a network of conservation sites in tropical riverine ecosystems of northeastern Mesoamerica.

The ability to undertake systematic conservation planning in developing countries is impeded by several distinct challenges, including a limited understanding of how aquatic ecosystems in these places function, the paucity of baseline research, limited human technical capacity and technology, and limited investment in research and monitoring (Pringle *et al.*, 2000; Wishart *et al.*, 2000). These conditions pose challenges to assembling the basic information necessary for use in conservation planning, particularly data about where biodiversity is located across a landscape. Of the information sources that can be used to represent spatial patterns in biodiversity, two sources are available in many developing countries: (1) geospatial (GIS) data about major habitat determinants; and (2) georeferenced species occurrence records of the places where species have been collected in historical biological surveys. These data sources can be combined to predict spatial variation in the locations of representative biodiversity.

Current theories on aquatic community assembly suggest that abiotic conditions occurring at multiple spatial scales limit the species from the regional species pool that will occupy local habitats within a lake or river (Tonn *et al.*, 1990; Poff, 1997). By identifying and mapping spatial variation in the abiotic factors hypothesized to filter species out of the regional pool, it is possible to create a spatial surrogate for biodiversity under the assumption that different habitat conditions will correspond with differences in ecosystem function and/or community composition (Higgins *et al.*, 2005). Such abiotic habitat classifications are commonly used in conservation assessments (e.g., Thieme *et al.*, 2007; Rivers-Moore, Goodman & Nel, in review). While the abiotic factors used in habitat classifications may represent important physical gradients to which species in the regional species pool respond, it is difficult for habitat classifications to represent landscape-scale variation in the regional species pool, which is often driven by a complex array of historical factors that influence speciation, colonization, and extinction, such as plate tectonics and genetic bottlenecks (Brown & Lomolino, 1998). Thus, abiotic surrogates for biodiversity fail to represent important ecosystem patterns that may be better represented with maps of current species distributions. Georeferenced species occurrence records, if numerous enough, can allow for realistic representations of species distributions with species distribution models (SDMs), therefore allowing for representation of the intra- and inter-basin patterns in the distributions of taxa in the regional species pool.

SDMs generalize the empirical relationships between species occurrence localities or abundance data and underlying habitat conditions to predict the probability of species occurrence or abundance within a given landscape area (Guisan & Zimmermann, 2000).

An important strength of SDMs is that they have the potential to use incomplete information, such as historical point occurrence data from only a part of a species' range, to generate spatially comprehensive predictions of the realized niche of a species relative to the environmental variables in the model (Guisan & Zimmermann, 2000). A strength of using SDMs in conservation planning is that the models act as "filters on habitat from a species-specific viewpoint" (Rondinini *et al.*, 2006), offering potential improvements in the biological realism of conservation plans over reliance only on abiotic factors assumed to be important to species. Similarly, SDMs, with accurate range limits may act as "filters on historical drivers of biogeography from a species-specific viewpoint", thus addressing one of the main weaknesses of the use of abiotic surrogates alone. A potential drawback of using SDMs is the difficulty of defining how well modeled species serve as proxies for unmodeled components of biodiversity. For instance, SDMs for the fish species in a given region may not adequately represent taxonomic diversity of aquatic macroinvertebrates or diatoms. Ultimately, if the goal is to develop reserve networks for all biodiversity, an approach that integrates biotic and abiotic proxies may yield the best results (Roux *et al.* 2008). However, in this paper I focus attention on how to integrate SDMs into the planning process in the absence of a habitat classification scheme.

Many techniques are available to model species distributions, with different requirements for input data, and differences in how they specify empirical relationships between predictor variables and species locations (Elith *et al.*, 2006). In a data-poor context such as exists in many tropical developing countries, it is important for the method selected to: (1) require presence-only data on species occurrences rather than presence and absence data, because in many cases it may be difficult to infer absences in a consistent manner from datasets collected by different methods or sampling intensities (Graham *et al.*, 2004); and (2) perform well with few sampling localities. As with the application of conservation planning software in aquatic contexts, it is important for SDMs to be implemented with consideration of the directional and highly connected nature of aquatic ecosystems.

A variety of reserve design programs use algorithms to determine the minimal set of sites in which conservation features of interest can be adequately encompassed within a reserve network. As used here, a conservation feature is "a measureable, spatially definable component of biodiversity that is to be conserved within a reserve network" (Ardron *et al.*, 2008). Conservation features are represented in reserve networks according to representation targets (e.g., 400 km of occupied river reach, 20% of its range) that are ideally set according to ecological criteria (e.g., viability assessments). The end goal of the planning process is to define a reserve network solution that adequately accounts for all relevant conservation features within a network portfolio (Sarkar *et al.*, 2006). Marxan (Ball & Possingham, 2000) is a commonly used software package for designing efficient conservation area networks. Marxan's simulated annealing algorithm identifies a portfolio of planning units (e.g., catchments) that most efficiently meets predefined targets for protection of one or numerous conservation features, while also meeting the general criteria of reserve design, such as comprehensiveness, efficiency, complementarity, etc. (Ball & Possingham, 2000; Possingham, Ball & Andelman, 2000). Relative to the new vocabulary of Abell et al. (2007), Marxan is a suitable method for selecting freshwater focal areas—the specific locations with conservation features in need of protection—and because of its popularity among conservation planners, is a useful platform to help demonstrate expanded applications in freshwater.

I applied Marxan in conjunction with SDMs for inland fish species in the watersheds flowing to the coast of Belize to explore efficient networks of freshwater focal areas that protect the majority of fish species present. Using these focal areas and applicable concepts from aquatic conservation biology, I recommended critical management zones and catchment management zones that support persistence of specific feature groups within the fish community (migratory species, apex predators, and sub-regional endemics). In doing so, I explored (1) how SDMs can be applied in a data-limited aquatic context to enhance conservation planning for aquatic biodiversity; (2) how Marxan can be used to accommodate the unique constraints of reserve network design in flowing water ecosystems; and (3) how an effective reserve network may be structured in a way that is responsive to the realities of place-based conservation in an aquatic context.

Methods

Study Area

The assessment was carried out in the domestic and international waters that drain to the coast of Belize, including portions of southern Mexico and northeastern Guatemala. This 45,750 km^2 area comprises 16 major basins and many small coastal tidal creek basins (Figure 4.1). These watersheds vary in size, traverse a variety of geologies and soil types, and drain a diversity of terrestrial land cover types (Esselman & Boles, 2001). Twelve of the 16 major rivers originate in the Maya Mountains (Figure 4.1) as high-gradient, low pH streams draining granite and metamorphic geologic formations, after which they traverse limestone-dominated landscapes, build up alluvial plains, and ultimately discharge into the shelf lagoon that separates the coast from the Mesoamerican Barrier Reef. The two northernmost rivers in the area (N to S; Rio Hondo and New River) originate in karst hills, drain the low relief limestone platform of the Yucatan Peninsula, and discharge into Chetumal Bay, which in turn connects to the shelf lagoon. The headwaters of the rivers in the three southernmost basins (S to N; Sarstoon, Temash, and Moho Rivers) begin in Guatemala and flow eastward to the Gulf of Honduras. The Sarstoon River (demarcating the southern border of Belize) originates in Guatemala, within the mountain range of the Sierra de Santa Cruz. In addition to flowing waters, the study area has an abundance of fresh and brackish water lagoons and wetlands that include swamp forests, herbaceous marshes, and open water areas, often in association with riverine habitats (Esselman & Boles, 2001).

Daily mean temperatures are warm throughout the year, ranging from a low of 16ºC in the winter months to a high of 33ºC in summer (Hartshorn *et al.*, 1984). A strong north-to-south precipitation gradient exists, with the northern portion of the study area receiving approximately 1000 mm of rain annually and the southern portion receiving up to 4000 mm per year (Wilson, 1980). The timing of precipitation is seasonal, with a dry season lasting from January to May and a wet season from June to November. The area is highly prone to hurricanes in the late summer and early fall (Wilson, 1980).

Northern Mesoamerica has been called a “strong center of evolution” for fishes, because of its many endemic genera and species (Miller, 1966). The area encompassing the entire Yucatan Peninsula, Tabasco and part of Chiapas state in Mexico, and the Caribbean slope drainages of Belize, Guatemala and Honduras shares a common biogeographic history and contains a high diversity of poeciliids (34 species, 15 genera) and cichlids (44 species, five genera) (Miller, 1966; Bussing, 1976). 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 predominately marine species that inhabit freshwaters are included (Miller, 1966). One hundred and twenty-six of these species have been reported in the study area (Schmitter-Soto & Gamboa-Perez, 1996; Greenfield & Thomerson, 1997; Schmitter-Soto, 1998; Esselman, Freeman & Pringle, 2006).

Species distribution models

The goal of the species modeling process was to use species locality data to model broad scale patterns in species distributions from landscape predictor variables. My primary interest was to model potential species distributions relative to major abiotic factors that predict fish species distributions at large spatial scales. I selected a modeling approach that relies solely on species presence data (vs. presence and absence), because I wanted to combine datasets collected with different methods, and thus could not be relied on to consistently indicate the absence of a species from a sample (MacKenzie, 2005). A secondary interest was to find a modeling approach that performed well even with low sample sizes. I selected an approach known as maximum entropy or Maxent.

Maxent estimates the most uniform distribution (e.g., the one with maximum entropy) across a defined area subject to the constraints imposed by information available about environmental conditions underlying species locality points and randomly selected background points (Phillips, Anderson & Schapire, 2006). Several recent studies showed that Maxent consistently outperforms other modeling techniques (Elith et al., 2006; Hernandez et al., 2006; Phillips et al., 2006), and displays good predictive power even with low numbers of species occurrence localities (as low as five) (Hernandez et al., 2006; Pearson et al., 2007).

Inputs to Maxent are raster data layers of environmental attributes hypothesized as important to freshwater fish distributions, as well as georeferenced fish presence locality data, and the output is a probability occurrence value ranging from zero to one. A master independent variable database was generated (Table 4.1), summarized with principal components analysis (PCA), and reduced to seven model inputs. This database consisted of variables representing landscape position, geology, climate, hydrology, soils, and watershed characteristics. The cumulative upstream influence of different variables was represented using the weighted flow accumulation tool in the spatial analyst extension of ArcGIS 9.2® (ESRI Corp., Redlands, CA, USA). Weighted flow accumulation sums all pixel values in a weight grid upstream of a given pixel drawing on a flow direction grid to infer upstream directionality. This is in contrast to raw flow accumulation, which counts all pixels upstream of a given pixel in the landscape, rather than summing values from a weight grid. To calculate proportional variables (e.g., upstream proportion of a geology class), binary grids of each geological or soils class were created, used as the weight grid in a weighted flow accumulation, and then divided by raw flow accumulation to yield a representation of the proportion of the catchment in a given pixel type. Mean upstream values of continuous variables were calculated by the same process, except that I used continuous instead of binary values in the weight grid. All layers consisted of a uniform extent and cell size (30 m x 30 m) and were masked after processing to constrain the datasets to only those pixels underlying a river segment.

PCA was run for all environmental variables (using PC-ORD, version 4.10, MjM Software Design, Gleneden Beach, Oregon) at the 303 localities where fish were collected throughout the study area (Figure 4.1). I retained the first three principal component axes whose observed eigenvalues exceeded the eigenvalues expected under the broken stick distribution (calculated by PC Ord) (McGarigal, Cushman & Stafford, 2000). Of the variables with principal component loadings greater than 0.30, I selected the strongest loadings that I felt best represented factors known to influence river habitat and fish communities (Table 4.1).

The species occurrence data derived from four ichthyological studies conducted at different points in time. The first of these was the collections from 1970 to 1980 at 111 sites that led to the publication of the book *Fishes of the Continental Waters of Belize* (Greenfield & Thomerson, 1997). An additional 63 sites collected in the mid- to late-1990s in Mexico were added from collections housed at the University of Michigan and El Colegio de la Frontera Sur in Chetumal, Mexico (Schmitter-Soto & Gamboa-Perez, 1996; Schmitter-Soto, 1998). A third study added 21 sites collected in 2000 (Esselman *et al.*, 2006), and a final 108 sites were sampled as part of this study in 2006-2008. The final fish database contained 126 species collected from at least one of 303 sampling sites. Based on the results of Maxent performance studies with low sample sizes (Hernandez *et al.*, 2006), only those species for which five or more collection locations were available were used to develop SDMs (n = 63; Appendix 1).

Maxent species modeling software (v.3.2.1; www.cs.princeton.edu/~schapire/maxent/) was downloaded from the internet and run with the default parameters (Phillips *et al.*, 2006). From the point occurrence locality data and field studies, it was evident that 11 common species had range limits that occupied only a portion of the study area. For these species, the analysis was constrained to only those catchments where they were known to occur.

The area under the curve (AUC) of the receiver operating characteristic (ROC) plot was used to assess the accuracy of model predictions (Fielding & Bell, 1997). An ROC plot is created by graphing the fraction of true positive predictions (sensitivity) against the fraction of false-positive predictions across all presence-absence decision thresholds. An ROC curve that maximizes sensitivity at low values of the false-positive fraction is considered a good model and has a higher AUC (Fielding & Bell, 1997). AUC values usually range 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 1.0 indicates that the model can discriminate perfectly between true and false positive occurrences. For presence-only analyses, pseudo-absence data drawn randomly from the study area can be substituted for absence data, changing the interpretation of the ROC plot to the model's ability to distinguish presence from random rather than presence from absence (Phillips *et al.*, 2006).

Data used in the development of SDMs are often biased with respect to the range of environmental conditions where data were collected (Kadmon, Farber & Danin, 2003; Filipe *et al.*, 2004; Kadmon, Farber & Danin, 2004), which can reduce the predictive accuracy of the models (Kadmon *et al.*, 2003). I used a null model approach (following Raes & ter Steege, 2007) to test whether the sampling frame (n = 303 sites across all species) was environmentally biased by comparing the AUC value of a single SDM created from all of the sites to a confidence interval of AUC values constructed from 100 model runs with 303 randomly drawn data points from the entire study area. Because the AUC from the known collection localities fell outside the 95% confidence interval set by the null models, I concluded that my sampling frame was biased. Therefore, I tested the AUCs of SDMs for all 63 species against null models constructed from points drawn randomly from the 303 collection localities rather than from the entire range of environmental conditions, thus drawing the null model from an equally biased distribution (Raes & ter Steege, 2007). A SDM with an AUC that was significantly higher from the null model was concluded to perform significantly better than random, and was considered valid for the purposes of this planning exercise.

The continuous probability outputs of valid SDMs were classified into binary grids representing species presence and unknown presence localities using a numeric threshold. The minimum training presence value was used as the threshold, which is the lowest probability value under a known presence locality, or the least suitable habitat where the species is known to be present (Phillips *et al.*, 2006).

MARXAN Analysis

The Marxan simulated annealing algorithm was used to select a portfolio of freshwater focal areas. In each of many iterations (e.g., 200), the algorithm identifies a portfolio of catchments that efficiently meets predefined goals for protection of one or numerous conservation targets (Game & Grantham, 2008). The most efficient portfolio is the one that minimizes a measure of cost while still meeting conservation goals. Total cost is defined as:

$$\textit{Total Cost} = \sum \textit{Unit Cost} + \sum \textit{Species Penalties} + \sum \textit{Boundary Length}$$

where total cost is the objective function to be minimized; unit cost is the cost assigned to each planning unit based on some measure of the intensity of human activity in that unit (see below), species penalties are costs imposed for failing to meet representation goals, and boundary length is a cost determined by the outer boundary length of the portfolio. Simulated annealing attempts to minimize total portfolio cost by selecting the minimum set of planning units with the lowest total cost needed to meet all biodiversity goals, and by selecting units that are clustered together to minimize boundary length.

The planning units for this study were the 36,429 reach catchments that make up the river basins of the study area. A reach catchment, as used here, is the smallest drainage unit that encompasses an individual river segment between two tributaries. Reach catchments (heretofore called catchments) were derived from a 30 m digital elevation model using Arc Hydro tools (version 9) for ArcGIS 9.2. Marxan requires the input of a boundary file that contains information about connectivity between planning units to assist with aggregation of units in the final solution. I created a boundary file that respected basin divides by generating separate boundary files for each basin using the input generator for the Protected Areas Tools for ArcMap 9.2 (v. 2.0; /www.gispatools.org), then combining them into one file before input into Marxan. The net effect of this is that adjacent catchments separated by a drainage divide were not counted as connected in the selection of focal areas consisting of aggregations of catchments.

To define conservation features, I drew on knowledge of the species in the study area and on recent freshwater conservation assessments conducted by stakeholder groups in the study area to weigh migratory species, apex predators, and regional endemics more heavily in the Marxan solution according to their presumed conservation value. There are at least six migratory species present in the waters of the study area—four amphidromous and two catadromous (Appendix 1). Spawning and down-migration of fishes in the study area are thought to correspond with wet season flood events (Cruz, 1987; Cruz, 1989; Benstead *et al.*, 1999), and up-migration has been documented to occur in mixed-species post-larval movements that occur in the transition between the wet and dry season (Gilbert & Kelso, 1971; Winemiller & Leslie, 1992). These species utilize both fresh and salt water during their life cycles, and thus may serve as an indicator of the integrity of ridge-to-estuary connections in a ridge-to-reef conservation context (Esselman et al. 2006). As a result, The Nature Conservancy in Belize has identified migratory species as important biodiversity feature in recent planning exercises (P.C. Esselman, personal observation). Apex predators (defined here as species with trophic levels greater than 4.0 according to www.fishbase.org; Froese & Pauly, 2008) have been shown to play important roles in the maintenance of aquatic food web structure (Halpern *et al.*, 2005), and thus were given more importance in the selection of focal areas. Finally, the basins of the study area compose a large portion of the ranges of a number of narrowly distributed sub-regional endemics, whose viability depends upon their ability to persist there. These species were weighted according to the number of aquatic ecoregions (Abell *et al.*, 2008) that they occupy, with those species occupying only one of the 16 ecoregions found in Mesoamerica receiving the highest weight, and those occupying three or less ecoregions receiving a moderate weight (Appendix 1).

The representation target for each conservation feature was defined as a fixed proportion of the total predicted distribution. A goal of 15% of the range of each species was selected—an arbitrary number selected for purposes of management feasibility and because sufficient ecological information to specify the amount of habitat needed ensure species viability was lacking.

The unit cost term in Marxan's total cost function is often used to represent some measure of the cost of including a specific planning unit in the reserve system. In this study, I employed the logic that, given the number of conservation features occurring there, planning units with a higher risk of environmental degradation from human activities were less suitable for conservation and thus should carry a higher cost in the reserve selection process. To represent risk of environmental degradation, I created an environmental risk surface (ERS) to define the unit cost for each catchment following the method outlined by Schill & Raber (2008).

An ERS is a modeled composite raster surface that is created in GIS to combine information about the extents and relative intensities of perceived environmental risks to aquatic ecosystems. The first step in the ERS development process was to identify and map potential risk elements. Based on available data, I selected current agriculture and urban land cover types (polygons), roads (lines), and the locations of villages (points). Each risk element was assigned an intensity value and influence distance. The intensity value is a relative measure of intensity on a 0 to 100 scale with 100 being the highest. Influence distance represents the "maximum distance over which the feature has a negative impact on biodiversity" (Schill & Raber, 2008). The assignment of intensity and distance values was a logical process informed by the literature, and by professional judgment (Table 4.2). In particular, I drew on observations made by Allan (2004) about the relative influences of agriculture versus urban land uses on aquatic biological integrity, and I assumed that the influence distance of human communities was positively correlated with human population size or population density (Table 4.2). The

Protected Areas Tools for ArcMap 9.2 (v. 2.0; http://www.gispatools.org) were used to attribute each risk element with their intensity and distance values. Each feature was buffered by the distance of influence, and converted to a raster grid. Within the buffer, a linear decay function was used to simulate the decline of intensity away from the actual location of each risk type. This process was used to create a raster layer for each risk factor, and then the values were summed across all grids to yield a cumulative estimate of environmental risk (Figure 4.2). To make this surface relevant to riverine ecosystems, the cumulative ERS was used as the weight grid in weighted flow accumulation to estimate cumulative downstream risk. Finally, the weighted flow accumulation grid was divided by raw flow accumulation to calculate the "relative upstream risk intensity" scaled by contributing area (Figure 4.2).

Marxan can be parameterized to fix or exclude planning units into or from the final solution through the use of status codes assigned to each planning unit. I excluded all catchments in the Belize River watershed that lie upstream of an area of extensive hydropower development, and I also excluded those catchments where no fishes were predicted to occur. Finally, to increase the probability that the final portfolio overlaps with existing protected areas, I parameterized Marxan to select those catchments that intersected protected areas in the initial seed reserve network at the beginning of the Marxan run.

The data layers described above (catchments, boundary file, SDMs, ERS) were used to determine a portfolio of focal areas in a two-step process. The first step was to run Marxan to define the focal areas for migratory species only. This allowed us to define downstream migratory corridors as a critical management zone unique to these focal areas. In the second step, I fixed the migratory species focal areas into the final solution and ran Marxan for a second time to generate the final portfolio of focal areas that included the remaining 57 species. Each run consisted of 200 iterations of the simulated annealing algorithm. I used the technique recommended by Game & Grantham (2008; p. 23) to select the appropriate boundary length modifier (0.0001) for each of the Marxan runs.

When the final portfolio of focal areas was generated, I defined critical management zones and catchment management zones for each focal area. Migratory species utilize nearly the entire river continuum in the juvenile and adult phases of their life cycles and live in marine habitats during their larval and early juvenile stages, and thus have special needs as compared to non-migratory species (McDowall & Taylor, 2000). The downstream migration corridor that connects upland habitats to the sea was defined as the first critical management zone unique to migratory species.

Riparian buffers have been shown to be effective at protecting habitat quality in downstream river reaches (Naiman, Decamps & McClain, 2005), and thus were defined as the second critical management zone above all focal areas. It is difficult to derive rules of thumb for suitable buffer extent given the virtual absence of research on this topic in small tropical rivers, but several studies support the idea that longer buffer lengths correlate positively to increased biotic integrity (Barton, Taylor & Biette, 1985; Parkyn *et al.*, 2003). In an extensive study of the influences of riparian vegetation cover on stream thermal regimes in New Zealand, Rutherford *et al.* (1997) found that 1 to 5 km buffers were suitable to maintain low temperatures in small (1st to 3rd order) streams with low thermal inertia, while 10-20 km of forested buffer was necessary to maintain the natural thermal regime in 5th order rivers. For the purposes of this study I adopted a middle of the road scenario and delineated all riparian habitats within a 5 km zone upstream of all focal areas as critical management zones (which translates to greater than 5 river km because of channel sinuosity).

Catchment management zones were delineated as the entire watershed upstream of the most downstream focal area or critical management zones in a drainage basin.

Results

Species distribution models

Of 63 species for which sufficient data existed, the SDMs for all of them were judged as valid when compared to the 95% confidence interval created by the distribution of 100 null models for each species. In fact, the AUCs for all of these deviated strongly from their null models ($p<0.0001$), indicating that predictions performed significantly better than random.

Predicted range sizes relative to the total stream distance in the study area (31,257 linear km calculated from 1:50,000 scale maps) ranged from 376 km to 28,642 km

[*Cichlasoma bocourti* (Vaillant & Pellegrin, 1902) and *Heterandria bimaculata* (Heckel, 1848) respectively], with a mean predicted occupancy of 9,801 km (31% of available habitat). The modeled fish species occupied about half of the available elevational range (~1200 masl) from zero to 600 masl, with the majority of species limited to elevations below 200 masl (Appendix 1). Migratory species, with the exception of the opossum pipefish [*Microphis brachyurus* (Bleeker, 1853)], were distributed well into mountain areas where many protected areas are located. In contrast, most top predators inhabited the lower and estuarine reaches of watersheds. The narrow endemics—which comprised 12 of the 14 cichlids and 8 of the 11 poeciliids in the dataset—were often limited to the northern or southern part of the area and had ranges extending outside of the study area. The spatial distributions of species from the different target groups in this analysis led to the selection of a final reserve network that spanned from north to south across biogeographic barriers, and also across important environmental gradients like elevation.

Reserve network

The Marxan solution for migratory species focal areas defined a reserve network that successfully met the goal of 15% representation for all of the migratory species. The migratory species reserve network was aggregated in catchments located on the fringe of the Maya Mountain, in addition to three large consolidated focal areas in the coastal plain in south, central, and northern Belize, and several smaller focal areas in the northern interior (Figure 4.3). The combined migratory species focal areas occupied 1,579 km^2, or about 3.4% of the study area.

The final Marxan solution with 63 species included was more than three times larger than the initial migratory species network. This solution included a larger band of catchments on the fringes of the Maya Mountains, larger consolidated focal areas in the coastal plain and northwest portion of the study area, and numerous small focal areas in the deep north (Figure 4.3). In particular, more focal areas were identified in the two most northern watersheds, where migratory species are generally absent. However, many of these northern focal areas occurred in first order catchments draining karstic hills, which are likely to be dry for a portion of each year. The final network successfully met the 15% representation goal for 58 of the 63 species included as conservation features in the analysis, and occupied 4,935 km^2 or about 10.7% of the study area. Of the five species that did not receive 15% representation within the reserve network, three [*Cichlasoma bocourti*, *Thorichthys aureus* (Günther, 1862), and *Vieja godmanni* (Günther, 1862)] were narrowly distributed endemics with ranges limited to the southernmost three basins, and two were catfishes [*Ictalurus furcatus* (Valenciennes, 1840), *Sciades assimilis* (Günther, 1864)] with habitats constrained largely to main stem rivers. Of these, the only fish with less than 12% representation (8%) was *Thorichthys aureus*. This suggests that southern and main river habitats were slightly under-represented in the final reserve solution.

The feasibility of successful implementation of the focal area portfolio is likely to be strongly influenced by how well it corresponds to existing protected areas. Managed or unmanaged but intact protected areas occupy 14,667 km^2 or approximately 32% of the study area. These terrestrial reserves are concentrated in the rugged terrain of the Maya Mountains in the northwestern part of the study area, with a few also scattered across the coastal plains. Approximately 51% (2,529 km^2) of the focal areas occurred within existing protected areas (Figure 4.4). The majority of focal areas lying outside of protected areas are in the northern half of the study area in the coastal plains where protected areas are less frequent (particularly in Mexico).

Critical management zones were defined for each focal area (Figure 4.5). For those focal areas protecting migratory species, downstream migration corridors connecting to the sea were identified and mapped. There were 1,256 linear km of migration corridors between migratory species focal areas and the coastal margin (4% of river km in area). A buffer width of 100 m on either side of the river within these corridors added 242 km^2 of land to the portfolio. Inclusion of riparian corridors 5 km upstream of each focal area added 3,671 linear km of riparian corridors and 722 km^2 to the final reserve network. Riparian corridors tended to connect nearby focal areas to one another creating several “meta-reserves” in the northern part of the study area (Figure 4.5).

Catchment management zones, where integrated watershed management efforts are to be targeted, were defined from the lower extent of any given focal area or critical management zone, whichever was more downstream (Figure 4.5). Catchment management zones were defined for 88% of the study area (40,139 km^2), in large part because in 13 of the 16 major drainage basins, migratory corridors stretched all the way to the river mouth, thus incorporating entire basins in this management category (Figure 4.5).

Discussion

Given the importance of protected areas as general strategy for conservation (Groom, Meffe & Carroll, 2006), protected area networks can and should be an important strategy to protect representative freshwater biodiversity (Crivelli, 2002; Saunders *et al.*, 2002; Abell *et al*., 2007). Freshwater protected areas must not only incorporate important biodiversity features where they occur, but also consider physical directionality, connectivity, and the transfer of stressors through a river network (Lake, 1980; Saunders *et al.*, 2002). Various authors have proposed and demonstrated ways that these characteristics of freshwater ecosystems can be incorporated into protected area network design (Moyle & Yoshiyama, 1994; Filipe *et al.*, 2004; Linke *et al.*, 2007; Thieme *et al.*, 2007; Moilanen *et al.*, 2008), and there has been recent call for yet more focus on this topic (Abell *et al.*, 2007; Turak & Linke, in review). This paper presents a first implementation of the proposed system for freshwater reserve design suggested by Abell et al. (2007).

Enhanced planning in data-limited contexts

In most planning situations, data are more limited than a planning team may desire to represent spatial variation in biodiversity and habitats. This is especially true in tropical developing countries (Abell *et al.*, 2008), which have particularly extensive aquatic ecosystems and globally high levels of fish diversity and endemism (Amarasinghe & Welcomme, 2002; Abell *et al.*, 2008; Leveque *et al.*, 2008), but may also have less capacity to undertake systematic conservation planning (Pringle *et al.*, 2000; Wishart *et al.*, 2000). Thus, approaches are needed that maximize the utility of data types that are commonly available, like museum collection records and geospatial data. Internet-based data clearinghouses (e.g., the Global Biodiversity Information Facility; www.gbif.org) serve millions of georeferenced collection localities for fishes and other organisms to the public for easy manipulation in a GIS. In combination with geospatial data, these data sources can be used to create SDMs that add biogeographic realism to the planning process.

An imperfect but useful representation of reality

SDMs yield generalized estimates of the relationships between incomplete data about a species' niche, and the often-biased locality data where the species has been recorded (Guisan & Zimmermann, 2000). Bias and data quality is a particular challenge in many developing countries, because data are often assembled from *ad hoc* surveys, gray literature, and museum records, and may consist of only a few sampling points for some species. Furthermore, if collections were made prior to the use of global positioning systems (GPS) they may be imprecisely georeferenced (Hernandez *et al.*, 2008). It is necessary to identify and mitigate these issues to the greatest extent possible, and to interpret models conservatively when these problems cannot be avoided. It is also possible to factor out some forms of bias (e.g., Phillips & Dudik, 2008), as with the null model method employed here (Raes & ter Steege, 2007). Despite possible problems, SDMs frequently perform well when validated internally and against independent data (Elith *et al.*, 2006). For this reason, they have increasingly been used in reserve design (Cocks & Baird, 1989; Araujo & Williams, 2000; Williams & Araujo, 2000; Polasky & Solow, 2001; Filipe *et al.*, 2004), but their application to site selection in freshwater contexts has been limited (but see Filipe *et al.*, 2004; Linke *et al.*, 2007; Moilanen *et al.*, 2008; Hermoso, in review).

Systematic conservation planning in an aquatic context

Reserve design is often aided by software that helps identify reserve networks that efficiently protect representative biodiversity (Sarkar *et al.*, 2006). I chose a widely applied and available software package, Marxan, for my study, which was originally conceptualized for use in marine and terrestrial settings (Ball & Possingham, 2000). Translation of Marxan and other software tools (e.g., ZONATION; Moilanen *et al.* 2008) to flowing water settings poses some challenges. Among these are the representation of longitudinal and lateral connectivity in a watershed context, and the downstream influence of human activities in a watershed. I addressed these problems in conjunction with Marxan in three ways. First, connectivity between catchment planning units was represented in a way that respected major drainage divides, thus forcing Marxan to only define focal areas within and not across these major barriers to species movement. Second, relative magnitudes of environmental risk in the landscape were represented in a way that considered the potential downstream transfer of threats from human activities, and the influence that basin size might have on downstream accumulation of risk. Third, the focal area network defined by Marxan was post-processed to define complementary management zones designed to maintain high quality habitats, facilitate migration, and abate spatially disparate threats to species in freshwater focal areas.

Several other recent studies have addressed connectivity in freshwater systematic conservation planning. Linke et al. (2007) and Moilanen et al. (2008) incorporated more explicit representations of connectivity than used here by drawing on spatial databases with network topology to help define selection rules for planning units with the highest conservation values. Databases with network topology link each local catchment with all catchments in their up- and downstream watersheds, allowing for an accurate representation of connectivity across sequential catchments at the sub-basin level (Linke et al., 2007; Moilanen et al., 2008). Linke et al. (2007) enforced a decision rule that non-headwater catchments could not be protected without also protecting sub-catchments further upstream. Moilanen et al. (2008) used species-specific loss functions to adjust the conservation value of individual planning units based on the proportion of up- and downstream planning units left in the solution (e.g., as units were dropped from a solution, the estimated value of planning units in the same stream network were adjusted). In both cases, this led to the tendency to give priority to entire basins or sub-basins. A weakness of Marxan is that connectivity between planning units is enforced by a boundary file that only captures adjacency between two planning units rather than across multiple subsequent units. This prevents the direct enforcement of selection rules that extend beyond immediately adjacent planning units, and leads to a more limited representation of connectivity than the solutions of Linke et al. (2007) and Moilanen et al. (2008).

This deficiency may have been partly improved by the environmental risk surface that was used to assign a unit cost to each planning unit, which did incorporate an accurate representation of connectivity patterns across catchments. The result may have been that Marxan was guided to solutions somewhat similar to those that would have resulted from having decision rules enforced with a network topology. The relative upstream risk index guided network solutions away from catchments with greater risk from agriculture, roads, and human settlements in their upstream watersheds. Because higher risk areas tended to be found in the same sub-basins (Figure 4.2), use of the ERS was likely to have guided Marxan away from higher risk sub-basins, and toward sub-basins with lower cost across interlinked planning units. The ideal representation of connectivity would include accurate representation of network topology (as in Linke et al., 2007; Moilanen et al., 2008), and a realistic representation of how risk is propagated downstream through dendritic networks. Many countries, including, Belize and its neighbors, have yet to develop geodatabases with network typologies defined in them. As national databases improve, so can the representation of accurate riverine connectivity within planning exercises such as the one carried out here.

The representation of downstream transfer of combined anthropogenic risk offers benefits over examples found in the published literature on aquatic reserve planning. Contrasting studies have used: presence or absence of human activity in sub-basins as a measure of ecosystems intactness (Thieme *et al.*, 2007), categorical estimates of river integrity established through expert workshops (Nel *et al.*, 2007), consideration of single threats (e.g., water availability; Roux *et al.*, 2008), or no consideration of the locations of anthropogenic activities (Filipe *et al.*, 2004). In an approach more similar to that used here, Linke *et al.* (2007) also considered threats integrated over the upstream catchment. That study combined direct measures of catchment condition (e.g. nutrient and sediment budgets) and indirect measures (e.g., proportions of different land uses and road density) into orthogonal stressor gradients that were incorporated into a heuristic reserve design algorithm. While this scheme allowed for relative ranking of planning units in an ordinated multi-dimensional “risk space”, it did not provide a strong basis for estimating numeric risk magnitudes. In contrast, the approach of Schill and Raber (2008) used here offers a relatively direct representation of risk magnitudes in a directionally appropriate way that has good potential to be used in freshwater conservation planning.

This study presents the first practical assessment of the design framework of Abell *et al.* (2007), and the first published assessment of freshwater protected areas in Mesoamerica. Application of the framework of Abell *et al.* (2007) contributed relatively little added work to the reserve design process, and, when implemented on the ground, is likely to provide critical protections to species and habitats, without which persistence of species within the focal areas will be far more tenuous. When compared to reserve management in a terrestrial setting, however, implementation of Abell *et al.*’s three-part network is likely to create added layers of reserve management complexity, because a management presence will still be required within focal areas just like in terrestrial reserves, but additional management effort will also be needed to (1) successfully protect riparian corridors and longitudinal connectivity within them, and (2) coordinate large-scale landscape planning with public and private entities that have control over land use practices in catchment management zones. While an exploration of specific management issues is beyond the scope of this paper, a critical evaluation of the logistical, policy, and management dimensions of such an extended reserve network is warranted. Similarly, I echo the voices of others to call for further refinement and critical exploration of approaches to design efficient reserve networks for place-based conservation of freshwater biodiversity throughout the rivers of the world.

Table 4.1. Environmental variables prepared for entry into Maxent models of fish species distributions. Variables in bold represent those that were selected for entry into the SDMs after variable reduction with PCA. Eigenvector loadings for the first three axes are listed to the right. The first axis accounted for 22% of the variance, the second for 14%, and the third for 10%.

Variable (units)	**Min**	**Max**	**Mean**	**PC 1**	**PC 2**	**PC 3**
Average annual air temp in catchment (degrees C)	20	26	23	-0.12	-0.04	0.03
Average annual rainfall in catchment (mm)	117	4070	1886	0.17	**0.42**	0.13
Average catchment elevation (masl)	<1	1047	334	0.36	-0.14	0.04
Average catchment slope (percent)	0	36	9	0.34	-0.01	-0.01
Elevation of study reach (masl)	0	1051	240	**0.30**	-0.12	0.03
Flow accumulation (pixels)	0	18300373	174771	-0.12	**-0.32**	0.01
Horizontal land distance to next perennial lake (km)	0	120	33	0.31	-0.15	0.18
Surface area of nearest lake (km^2)	0.52	56.78	6.13	0.07	0.08	-0.07
Distance downstream to sea (km)	0	500	161	0.18	-0.30	0.07
Upstream distance to furthest basin divide (km)	0	504	13	-0.10	**-0.34**	0.02
Catchment geology proportions						
Alluvium	0	1	0.13	-0.20	0.19	**-0.32**
Limestone	0	1	0.47	-0.20	-0.24	0.28
Lavas-pyroclastics-volcanic sediments	0	1	0.34	**0.38**	-0.02	-0.18
Sedimentary	0	1	0.02	-0.09	-0.01	-0.07
Clastic sedimentary	0	1	0.03	-0.02	0.25	0.43
Catchment soil proportions						
Cambisol - Leptosol - Vertisol	0	1	0.06	-0.01	0.23	**0.49**
Fluvisol - Cambisol - Vertisol	0	1	0.04	-0.05	-0.21	-0.10

Gleysols	0	1	0.02	-0.05	-0.07	-0.02
Gleysols - Fluvisols	0	1	0.03	-0.13	-0.01	-0.07
Gleysols - Vertisols	0	1	0.03	-0.10	-0.04	-0.03
Gleysols - Vertisols - Fluvisol	0	1	0.03	-0.12	0.15	-0.25
Leptosol	0	1	0.02	-0.02	0.17	0.36
Leptosol - Cambisol	0	1	0.30	0.38	-0.02	-0.11
Leptosols - vertisols	0	1	0.38	-0.19	-0.35	0.13
Litosol - Cambisol	0	1	0.02	0.06	0.09	-0.15

Table 4.2. Intensity and influence distances assigned to different risk elements used to create an environmental risk surface.

Risk element	Class	Intensity	Influence distance
Agricultural land cover	Any agriculture	50	0
Urban land cover	High density (50-75 person km^{-1})	100	10000
	Medium density (16-50)	75	7000
	Low density (≤15)	65	5000
Roads	Track	10	5
	Dirt road	20	15
	2-lane highway	50	60
	4-lane highway	50	200
Villages	Small (≤2500 persons)	45	3000
	Large (>2500 persons)	55	3000

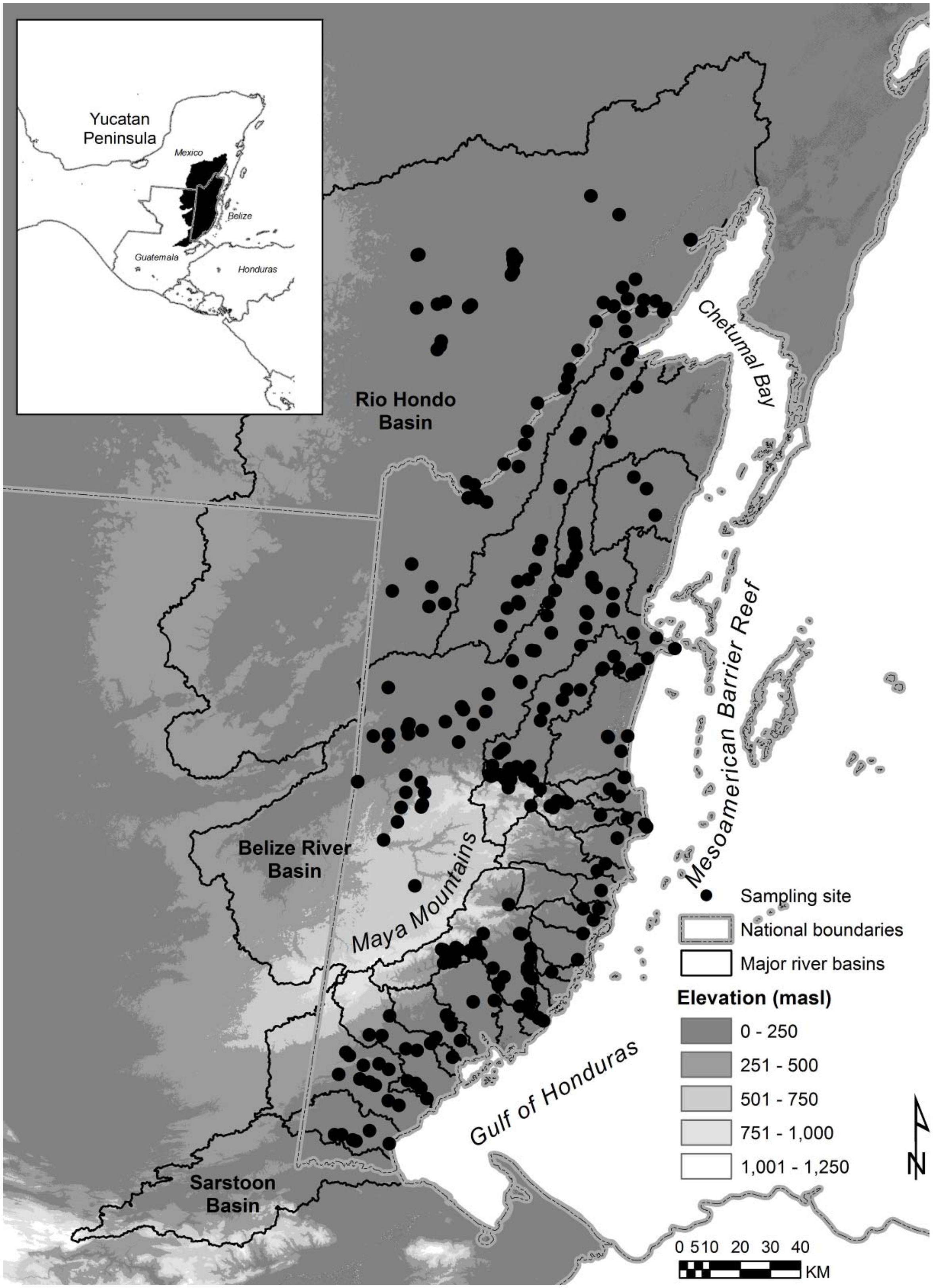

Figure 4.1. Study area showing elevation, hydrography, and the locations of all sampling sites used for presence-only modeling with Maxent.

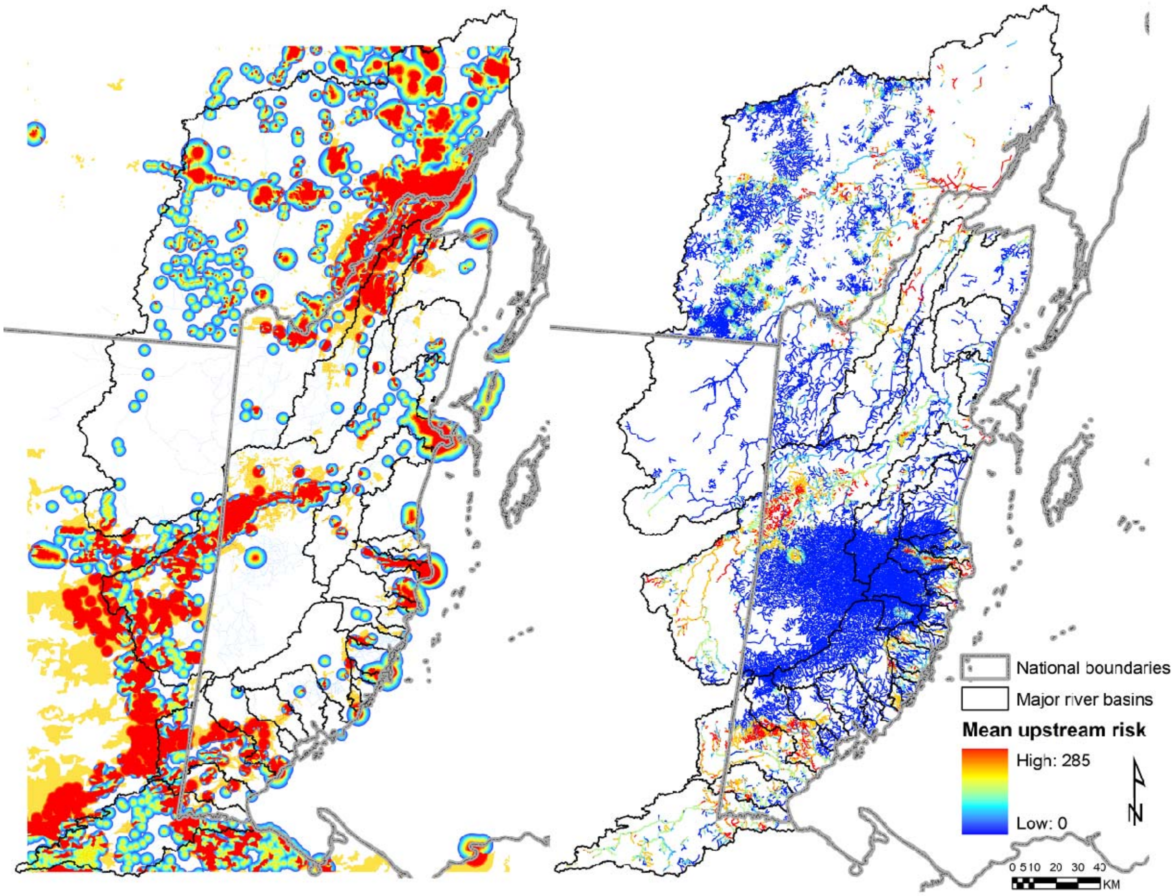

Figure 4.2. Environmental risk surface generated from agriculture and urban polygons, road lines, and village points available in GIS (left). This ERS was used as the weight grid in a weighted flow accumulation process to accumulate the intensity values in a downstream direction. The weighted flow accumulation was then divided by raw flow accumulation to give a measure of mean upstream risk intensity (right).

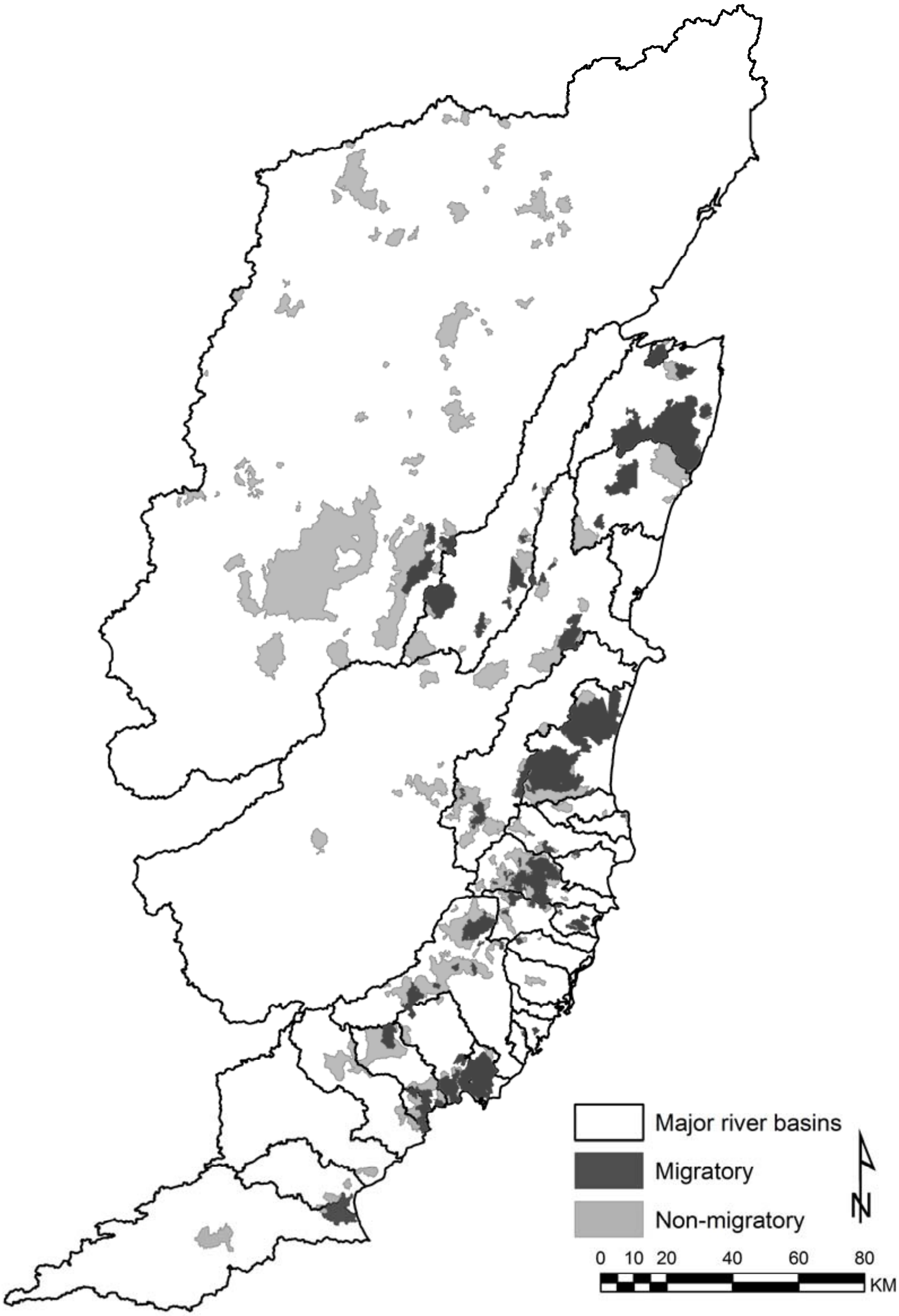

Figure 4.3. Freshwater focal areas defined using 2 runs of Marxan. The first run defined focal areas for migratory species (dark gray areas), which demand a special class of critical management zone. The second run (light gray areas) was constrained by the migratory species focal areas to select focal areas to protect the remainder of the 63 species evaluated.

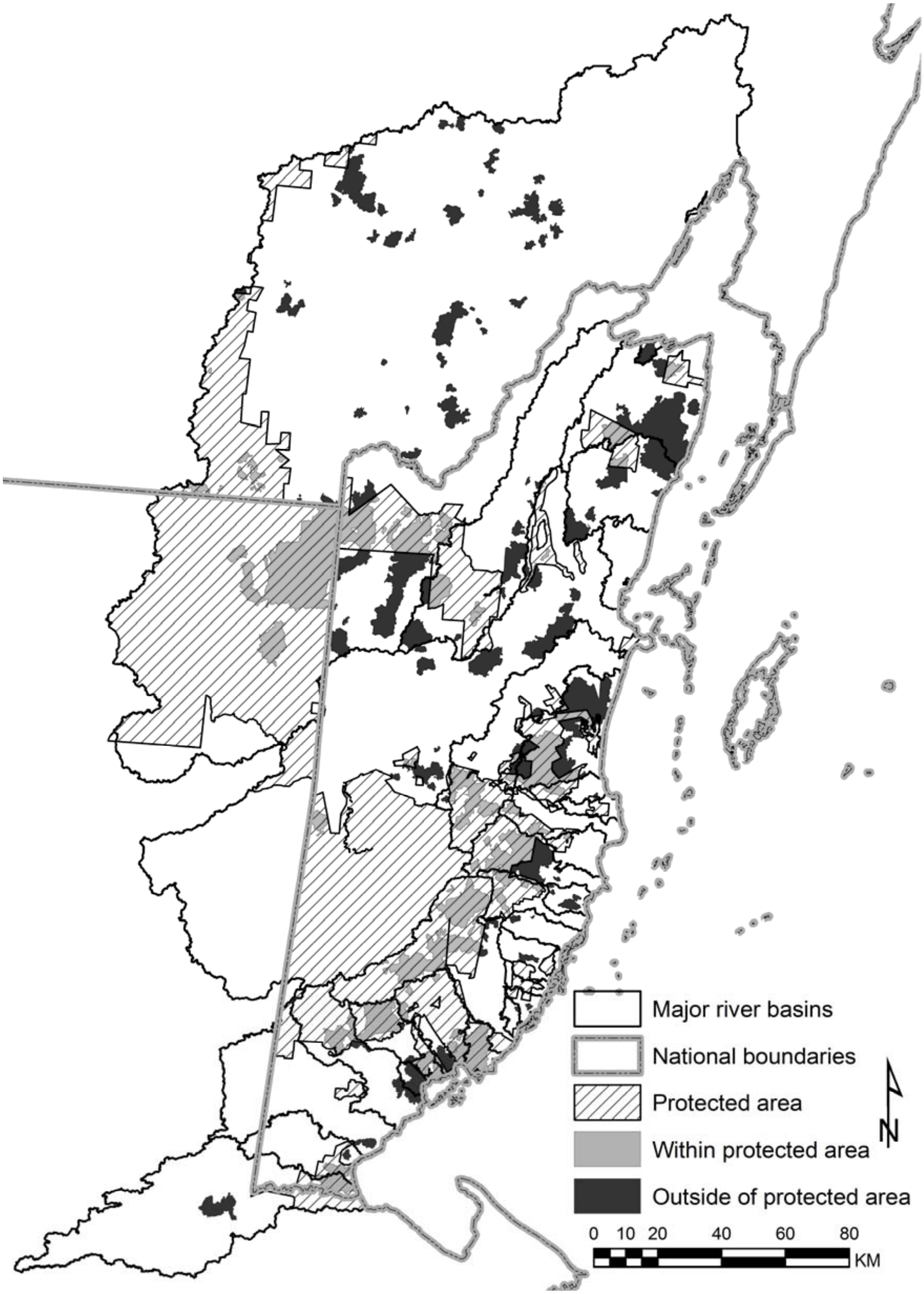

Figure 4.4. The focal reserve network overlaid by managed and intact protected areas. Approximately 51% of the selected focal areas were located within protected areas (dark gray areas vs. light gray areas which are outside of PAs).

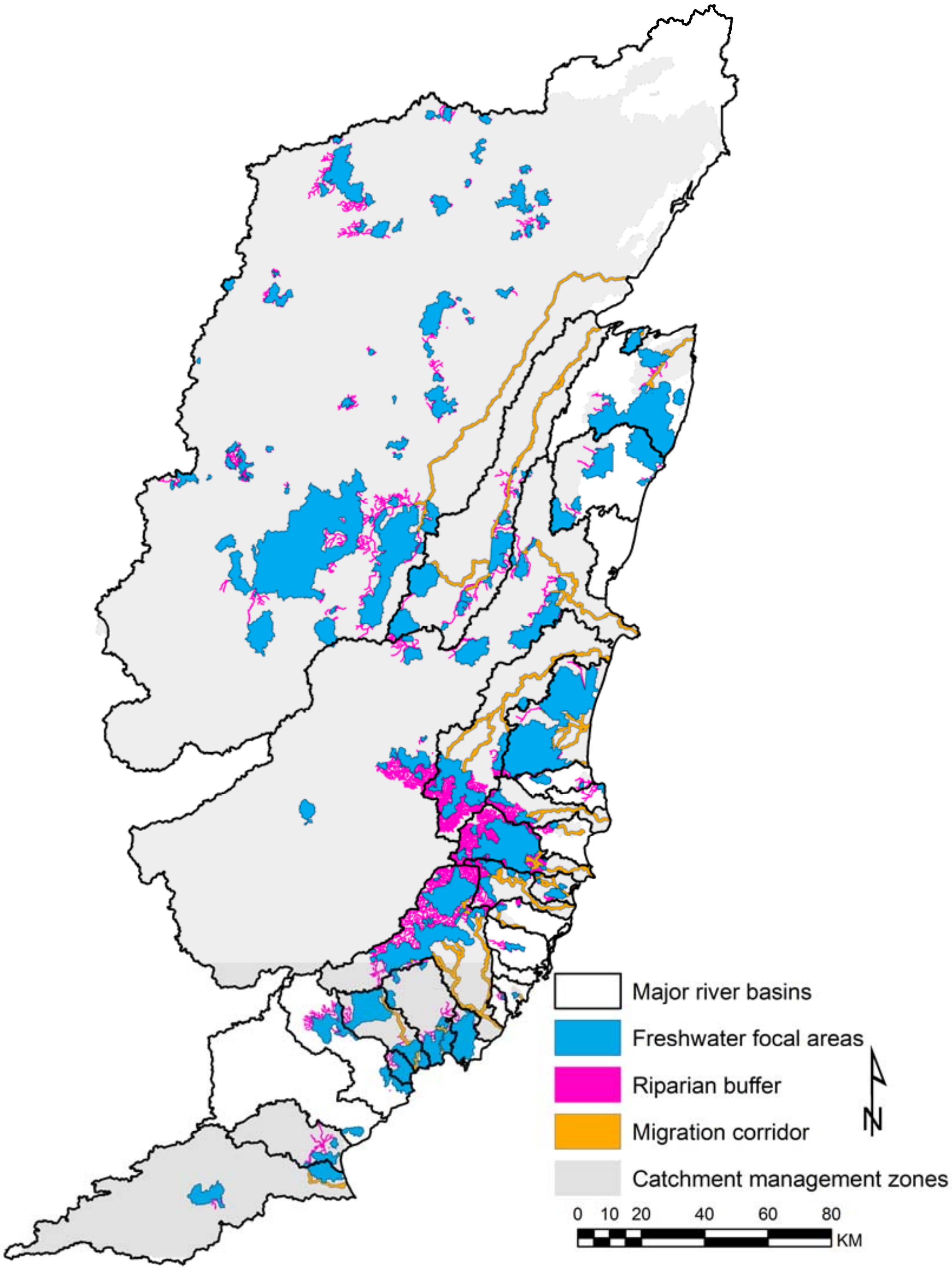

Figure 4.5. The final portfolio including critical management zones (2 classes) and catchment management zones.

Literature Cited

Abell, R., J. D. Allan, and B. Lehner. 2007. Unlocking the potential of protected areas for freshwaters. *Biological Conservation* **134**:48-63.

Abell, R., M. Thieme, C. Revenga, M. T. Bryer, M. Kottelat, N. Bogutskaya, B. Coad, N. Mandrak, S. Contreras Balderas, W. A. Bussing, M. L. J. Stiassny, P. H. Skelton, G. R. Allen, P. Unmak, A. Naseka, R. Ng, N. Sindorf, J. Robertson, E. Armijo, J. V. Higgins, T. J. Heibel, E. Wikramanayake, D. Olson, H. L. Lopez, R. E. Reis, J. G. Lundberg, M. H. Sabaj Perez, and P. Petry. 2008. Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *Bioscience* **58**:403 - 414.

Amarasinghe, U. S. and R. L. Welcomme. 2002. An analysis of fish species richness in natural lakes. *Environmental Biology of Fishes* **65**:327-339.

Araujo, M. B. and P. H. Williams. 2000. Selecting areas for species persistence using occurrence data. *Biological Conservation* **96**:331-345.

Ardron, J. A., H. P. Possingham, and C. J. Klein, editors. 2008. *Marxan Good Practices Handbook*. Pacific Marine Analysis and Research Association, Vancouver, BC.

Ball, I. and H. P. Possingham. 2000. *Marxan (mv.1.8.2): Marine Reserve Design Using Spatially Explicit Annealing*.

Barton, D. R., W. D. Taylor, and R. M. Biette. 1985. Dimensions of riparian buffer strips required to maintain trout habitat in southern Ontario streams. *North American Journal of Fisheries Management* **5**:364-378.

Benstead, J. P., J. G. March, C. M. Pringle, and F. N. Scatena. 1999. Effects of a low-head dam and water abstraction on migratory tropical stream biota. *Ecological Applications* **9**:656-668.

Brown, J. H. and M. V. Lomolino. 1998. *Biogeography*. Sinauer Associates, Sunderland, Mass.

Bussing, W. A. 1976. Geographic distribution of the San Juan ichthyofauna of Central America with remarks on its origin and ecology. Pages 157-175 *in* T. B. Thorson, editor. *Investigations of the Ichthyofauna of Nicaraguan Lakes*. University of Nebraska, Lincoln, Nebraska.

Cocks, K. D. and I. A. Baird. 1989. Using mathematical programming to address the multiple reserve selection problem: An example from the Eyre Peninsula, south Australia. *Biological Conservation* **49**:113 - 130.

Crivelli, A. J. 2002. The role of protected areas in freshwater fish conservation Pages 373 - 388 *in* M. J. Collares-Pereira, I. G. Cowx, and M. M. Coelho, editors. *Conservation of Freshwater Fishes: Options for the Future*. Fishing News Books, Oxford, UK.

Cruz, G. A. 1987. Reproductive biology and feeding habits of cuyamel, *Joturus pichardi* and tepemechin, *Agonostomus monticola* (Pisces, Mugilidae) from Rio Platano, Mosquitia, Honduras. *Bulletin of Marine Science* **40**:63-72.

Cruz, G. A. 1989. Sexually mature *Joturus picardi* (Pisces, Mugilidae) captured in Laguna de Brus and in the disemboguement of Rio Platano, Honduras *Revista De Biologia Tropical* **37**:107-108.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. M. Overton, A. T. Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz, and N. E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**:129-151.

Esselman, P. C. and E. Boles. 2001. Status and future needs of limnological research in Belize. Pages 35 - 68 *in* R. G. Wetzel and B. Gopal, editors. *Limnology in Developing Countries Volume 3*. International Association for Limnology.

Esselman, P. C., M. C. Freeman, and C. M. Pringle. 2006. Fish-assemblage variation between geologically defined regions and across a longitudinal gradient in the Monkey River Basin, Belize. *Journal of the North American Benthological Society* **25**:142-156.

Fielding, A. H. and J. F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation* **24**:38-49.

Filipe, A. F., T. A. Marques, S. Seabra, P. Tiago, F. Ribeiro, L. M. Da Costa, I. G. Cowx, and M. J. Collares-Pereira. 2004. Selection of priority areas for fish conservation in Guadiana River basin, Iberian Peninsula. *Conservation Biology* **18**:189-200.

Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmental Management* **10**:199-214.

Froese, R. and D. Pauly. 2008. www.fishbase.org.

Game, E. T. and H. S. Grantham. 2008. *Marxan User Manual for Marxan Version 1.8.10*. The University of Queensland Pacific Marine Analysis and Research Association, Vancouver British Columbia, Canada, St. Lucia, Queensland, Australia.

Gilbert, C. R. and D. P. Kelso. 1971. Fishes of the Tortuguero area, Caribbean, Costa Rica. *Bulletin of the Florida State Museum of Biological Sciences* **16**:1 - 54.

Graham, C. H., S. Ferrier, F. Huettman, C. Moritz, and A. T. Peterson. 2004. New developments in museum-based informatics and applications in biodiversity analysis. *Trends in Ecology & Evolution* **19**:497-503.

Greenfield, D. W. and J. E. Thomerson. 1997. *Fishes of the Continental Waters of Belize*. University of Florida Press, Gainseville, FL.

Groom, M. J., G. K. Meffe, and C. R. Carroll. 2006. *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Guisan, A. and N. E. Zimmermann. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* **135**:147-186.

Halpern, B. S., E. T. Borer, E. W. Seabloom, and J. B. Shurin. 2005. Predator effects on herbivore and plant stability. *Ecology Letters* **8**:189-194.

Hanski, I.A. & Gilpin, M.E. 1997. *Metapopulation Biology: Ecology, Genetics, and Evolution,* Academic Press, San Diego, CA.

Hartshorn, G., L. Nicolait, L. Hartshorn, G. Bevier, R. Brightman, J. Cal, A. Cawich, D. Weyer, W. H., and A. Wright. 1984. *Belize Country Environmental Profile: A Field Study*. USAID, Belize City, Belize.

Hermoso. in review. A new method of planning for fish habitat kilometers in an Iberian basin. *Freshwater Biology*.

Hernandez, P. A., I. Franke, S. K. Herzog, V. Pacheco, L. Paniagua, H. L. Quintana, A. Soto, J. J. Swenson, C. Tovar, T. H. Valqui, J. Vargas, and B. E. Young. 2008. Predicting species distributions in poorly studied landscapes. *Biodiversity Conservation* **17**:1353-1366.

Hernandez, P. A., C. H. Graham, L. L. Master, and D. L. Albert. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* **29**:773-785.

Higgins, J. V., M. T. Bryer, M. L. Khoury, and T. W. Fitzhugh. 2005. A freshwater classification approach for biodiversity conservation planning. *Conservation Biology* **19**:432-445.

Hynes, H. B. N. 1975. The stream and its valley. *Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie* **19**:1 - 15.

Jenkins, M. 2003. Prospects for biodiversity. *Science* **302**:1175-1177.

Kadmon, R., O. Farber, and A. Danin. 2003. A systematic analysis of factors affecting the performance of climatic envelope models. *Ecological Applications* **13**:853-867.

Kadmon, R., O. Farber, and A. Danin. 2004. Effect of roadside bias on the accuracy of predictive maps produced by bioclimatic models. *Ecological Applications* **14**:401-413.

Lake, P. S. 1980. Conservation. Pages 163 - 173 *in* W. D. Williams, editor. *An Ecological Basis for Water Resource Management*. Australian National University Press, Canberra, Australia.

Leveque, C., T. Oberdorff, D. Paugy, M. L. J. Stiassny, and P. A. Tedesco. 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia* **595**:545-567.

Linke, S., R. L. Pressey, R. C. Bailey, and R. H. Norris. 2007. Management options for river conservation planning: condition and conservation re-visited. *Freshwater Biology* **52**:918-938.

Loftus, W. F. and C. R. Gilbert. 1992. Mountain mullet, *Agonostomus monticola*. Pages 163-168 *in* C. R. Gilbert, editor. *Rare and endangered biota of Florida Volume 2: Fishes*. University of Florida Press, Gainesville, FL.

MacKenzie, D. I. 2005. What are the issues with presence-absence data for wildlife managers? *Journal of Wildlife Management* **69**:849-860.

Margules, C. R. and R. L. Pressey. 2000. Systematic conservation planning. *Nature* **405**:243-253.

McDowall, R. M. and M. J. Taylor. 2000. Environmental indicators of habitat quality in a migratory freshwater fish fauna. *Environmental Management* **25**:357-374.

McGarigal, K., S. Cushman, and S. Stafford. 2000. *Multivariate Statistics for Wildlife and Ecology Research*. Springer-Verlag, New York, NY.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman, and N. E. Leonard. 2007. The contribution of headwater streams to biodiversity in river networks. *Journal of the American Water Resources Association* **43**:86 - 103.

Miller, R. R. 1966. Geographical distribution of Central American freshwater fishes. *Copeia*:773-802.

Moilanen, A., J. Leathwick, and J. Elith. 2008. A method for spatial freshwater conservation prioritization. *Freshwater Biology* **53**:577-592.

Moyle, P. B. and P. J. Randall. 1998. Evaluating the biotic integrity of watersheds in the Sierra Nevada, California. *Conservation Biology* **12**:1318-1326.

Moyle, P. B. and R. M. Yoshiyama. 1994. Protection of aquatic biodiversity in California: A five-tiered approach. *Fisheries* **19**:6 -18.

Naiman, R. J., H. Decamps, and M. E. McClain. 2005. *Riparia: Ecology, Conservation, and Management of Streamside Communities*. Elsevier Academic Press, Burlington, MA.

Nel, J. L., D. J. Roux, G. Maree, C. J. Kleynhans, J. Moolman, B. Reyers, M. Rouget, and R. M. Cowling. 2007. Rivers in peril inside and outside protected areas: a systematic approach to conservation assessment of river ecosystems. *Diversity and Distributions* **13**:341-352.

Parkyn, S. M., R. J. Davies-Colley, N. J. Halliday, K. J. Costley, and G. F. Croker. 2003. Planted riparian buffer zones in New Zealand: do they live up to expectations? *Restoration Ecology* **11**:436-447.

Pearson, R. G., C. J. Raxworthy, M. Nakamura, and A. T. Peterson. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography* **34**:102-117.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190**:231-259.

Phillips, S. J. and M. Dudik. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* **31**:161-175.

Poff, N. L. 1997. Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society* **16**:391-409.

Polasky, S. and A. R. Solow. 2001. The value of information in reserve site selection. *Biodiversity and Conservation* **10**:1051-1058.

Possingham, H. P., I. R. Ball, and S. Andelman. 2000. Mathematical methods for identifying representative reserve networks. Pages 291-305 *in* S. Ferson and M. Burgman, editors. *Quantitative Methods for Conservation Biology*. Springer, New York.

Possingham, H. P., K. A. Wilson, S. J. Andelman, and C. H. Vynne. 2006. Protected areas: goals, limitations, and design. Pages 509 - 551 *in* M. J. Groom, G. K. Meffe, and C. R. Carroll, editors. *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA.

Pringle, C. M. 1997. Exploring how disturbance is transmitted upstream: Going against the flow. *Journal of the North American Benthological Society* **16**:425-438.

Pringle, C. M., F. N. Scatena, P. Paaby-Hansen, and M. Nunez-Ferrera. 2000. River conservation in Latin America and the Caribbean. Pages 41 - 77 *in* P. J. Boon, B. R. Davies, and G. E. Petts, editors. *Global Perspectives on River Conservation: Science, Policy, and Practice*. John Wiley and Sons Ltd., New York.

Raes, N. and H. ter Steege. 2007. A null-model for significance testing of presence-only species distribution models. *Ecography* **30**:727-736.

Revenga, C. and Y. Kura. 2003. *Status and trends of biodiversity of inland water ecosystems*. Secretary of the Convention on Biological Diversity, Montreal, Canada.

Rivers-Moore, N. A., P. S. Goodman, and J. L. Nel. in review. Scale-based freshwater conservation planning: Towards protecting freshwater biodiverstiy in KwaZulu-Natal, South Africa. *Freshwater Biology*.

Rondinini, C., K. A. Wilson, L. Boitani, H. Grantham, and H. P. Possingham. 2006. Tradeoffs of different types of species occurrence data for use in systematic conservation planning. *Ecology Letters* **9**:1136-1145.

Roux, D. J., J. L. Nel, P. J. Ashton, A. R. Deaconc, F. C. de Moor, D. Hardwick, L. Hill, C. J. Kleynhans, G. A. Maree, J. Moolman, and R. J. Scholes. 2008. Designing protected areas to conserve riverine biodiversity: Lessons from a hypothetical redesign of the Kruger National Park. *Biological Conservation* **141**:100-117.

Rutherford, J. C., R. J. Davies-Colley, J. M. Quinn, M. J. Stroud, and A. B. Cooper. 1997. *Stream shade: towards a restoration strategy*. Department of Conservation Wellington, New Zealand.

Sarkar, S., R. L. Pressey, D. P. Faith, C. R. Margules, T. Fuller, D. M. Stoms, A. Moffett, K. A. Wilson, K. J. Williams, P. H. Williams, and S. Andelman. 2006. Biodiversity conservation planning tools: Present status and challenges for the future. *Annual Review of Environment and Resources* **31**:123-159.

Saunders, D. L., J. J. Meeuwig, and A. C. J. Vincent. 2002. Freshwater protected areas: Strategies for conservation. *Conservation Biology* **16**:30-41.

Schill, S. and G. Raber. 2008. *Protected Area Tools (PAT) for ArcGIS 9.2 Version 2: User Manual and Tutorial*. The Nature Conservancy, Arlington, VA.

Schmitter-Soto, J. J. 1998. Catalogo de los peces continentales de Quintana Roo. El Colegio de la Frontera Sur, San Cristobal de las Casas, Mexico.

Schmitter-Soto, J. J. and H. C. Gamboa-Perez. 1996. Composicion y distribucion de peces continentales en el sur de Quintana Roo, Peninsula de Yucatan, Mexico. *Revista De Biologia Tropical* **44**:199-212.

Skelton, P. H., J. A. Cambray, A. Lombard, and G. A. Benn. 1995. Patterns of distribution and conservation status of freshwater fishes in South Africa. *South African Journal of Zoology* **30**:71-81.

Stoms, D. M., F. W. Davis, S. Andelman, M. H. Carr, S. D. Gaines, B. S. Halpern, R. Hoenicke, S. G. Leibowitz, A. Leydecker, E. M. P. Madin, H. Tallis, and R. R. Warner. 2005. Integrated coastal reserve planning: making the land-sea connection. *Frontiers in Ecology and the Environment* **3**:429-436.

Thieme, M., B. Lehner, R. Abell, S. K. Hamilton, J. Kellndorfer, G. Powell, and J. C. Riveros. 2007. Freshwater conservation planning in data-poor areas: An example from a remote Amazonian basin (Madre de Dios River, Peru and Bolivia). *Biological Conservation* **135**:484-501.

Tonn, W. M., J. J. Magnuson, M. Rask, and J. Toivonen. 1990. Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *American Naturalist* **136**:345-375.

Townsend, C. R. 1996. Concepts in river ecology: Pattern and process in the catchment hierarchy. *Archive fur Hydrobiologie* **(Suppl.)**:3-21.

Turak, E. and S. Linke. in review. Freshwater conservation planning: An introduction. *Freshwater Biology*.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. River continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**:130-137.

Ward, J. V. and J. A. Wiens. 2001. Ecotones of river ecosystems: role and typology, spatiotemporal dynamics, and river regulation. *Ecohydrology and Hydrobiology* **1**:25 - 36.

Williams, P. H. and M. B. Araujo. 2000. Using probability of persistence to identify important areas for biodiversity conservation. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**:1959-1966.

Wilson, E. M. 1980. Physical geography of the Yucatan Peninsula. Pages 5-40 *in* E. H. Moseley and E. D. Terry, editors. *Yucatan: A World Apart*. University of Alabama Press, Alabama.

Winemiller, K. O. and M. A. Leslie. 1992. Fish assemblages across a complex, tropical fresh-water marine ecotone. *Environmental Biology of Fishes* **34**:29-50.

Wishart, M. J., B. R. Davies, P. J. Boon, and C. M. Pringle. 2000. Global disparties in river conservation: 'First world' values and 'third world' realities. Pages 353 - 369 *in* P. J. Boon, B. R. Davies, and G. E. Petts, editors. *Global Perspectives on River Conservation: Science, Policy, and Practice*. John Wiley and Sons Ltd., New York.

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Appendix 1. Fish species used as conservation targets and environmental and biological data pertinent to this study. Occup. = Length of rivers predicted to be occupied in the study area from a classified SDM. Prop = the proportion of total river length in the study area predicted to be occupied by each species. Species penalty factor (SPF) took into account the migratory status (Mig.) and range (Range class) of a species and whether or not it is an apex predator (Trophic level > 4). Migratory species status codes are: CAT = catadromous life cycle; AMP = amphidromous life cycle. Range classes were as follows: 1 = Range limited to less than 1 freshwater ecoregion (Abell *et al.*, 2008); 2 = Range limited to less than 3 aquatic ecoregions; 3 = Range is regional (i.e., encompasses all or most of Central America or the Caribbean); 4 = range spans several regions (e.g., North to South America); 5 = range crosses the Atlantic Ocean. N is the number of localities used in model development. Trophic level entries marked by an asterisk (*) were inferred from congeneric species in the Fishbase database.

Family	**Species**	**Occup. (km)**	**Prop**	**Elev (masl)**	**Mig.**	**Troph. level**	**Range class**	**SPF**	**N**
Anguillidae	*Anguilla rostrata*	3,716	0.12	6 - 101	CAT[1]	3.67	5		5
Megalopidae	*Megalops atlanticus*	4,287	0.14	7 - 45		4.50	5	5	6
Clupeidae	*Dorosoma anale*	5,420	0.17	0 - 42		3.40	2	5	10
Clupeidae	*Dorosoma petenense*	10,970	0.35	0 - 135		3.09	4		57
Characidae	*Astyanax aeneus*	28,500	0.91	0 - 600		2.73	3		242
Characidae	*Brycon guatemalensis*	4,362	0.14	7 - 208		2.32	3		39
Characidae	*Hyphessobrycon compressus*	14,473	0.46	0 - 181		2.90*	2	5	110
Ictaluridae	*Ictalurus furcatus*	3,711	0.12	2 - 70		3.41	4		15
Ariidae	*Sciades assimilis*	4,385	0.14	0 - 29		3.62*	3		27
Pimelodidae	*Rhamdia guatemalensis*	24,306	0.78	0 - 453		3.20	4		116
Pimelodidae	*Rhamdia laticauda*	17,986	0.58	3 - 468		3.20*	3		81

Hemiramphidae	*Hyporhamphus roberti*	3,224	0.10	7 - 14		2.90*	4		5
Belonidae	*Strongylura timucu*	10,453	0.33	0 - 66		4.46	4	5	19
Rivulidae	*Rivulus tenuis*	8,876	0.28	0 - 194		3.19*	2	5	22
Cyprinodontidae	*Jordanella pulchra*	6,300	0.20	3 - 41		2.93*	1	10	7
Poeciliidae	*Belonesox belizanus*	16,266	0.52	0 - 356		4.00*	3	5	138
Poeciliidae	*Gambusia luma*	15,725	0.50	4 - 195		3.20*	2	5	96
Poeciliidae	*Gambusia nicaraguensis*	3,775	0.12	5 - 17		3.20	3		6
Poeciliidae	*Gambusia sexradiata*	17,516	0.56	0 - 70		3.50	3		40
Poeciliidae	*Gambusia yucatana*	15,712	0.50	0 - 162		3.20*	2	5	52
Poeciliidae	*Heterandria bimaculata*	28,642	0.92	0 - 600		2.00*	2	5	120
Poeciliidae	*Poecilia mexicana*	20,961	0.67	0 - 356		2.00	2	5	212
Poeciliidae	*Poecilia orri*	5,846	0.19	0 - 35		2.00*	2	5	13
Poeciliidae	*Poecilia petenensis*	7,309	0.23	3 - 135		2.00*	2	5	27
Poeciliidae	*Xiphophorus helleri*	25,663	0.82	0 - 533		3.19	2	5	62
Poeciliidae	*Xiphophorus maculatus*	21,233	0.68	0 - 350		3.20	2	5	22
Atherinidae	*Atherinella sp. 1*	13,687	0.44	0 - 145		2.68*	2	5	83
Syngnathidae	*Microphis brachyurus*	4,246	0.14	8 - 18	AMP[1]	3.32	5		5
Synbranchidae	*Ophisternon aenigmaticum*	13,468	0.43	2 - 194		3.50*	3		73

Centropomidae	*Centropomus ensiferus*	4,766	0.15	7 - 25		4.18	4	5	12
Centropomidae	*Centropomus parallelus*	7,203	0.23	7 - 14		4.21	4	5	6
Centropomidae	*Centropomus pectinatus*	4,615	0.15	7 - 21		4.03	4	5	8
Centropomidae	*Centropomus undecimalis*	5,091	0.16	3 - 23		4.08	4	5	12
Carangidae	*Oligoplites saurus*	3,551	0.11	7 - 14		4.48	4	5	6
Lutjanidae	*Lutjanus griseus*	4,674	0.15	7 - 23		4.25	4	5	16
Lutjanidae	*Lutjanus jocu*	5,032	0.16	7 - 16		4.30	4	5	9
Gerreidae	*Eucinostomus melanopterus*	4,268	0.14	3 - 26		3.39	4		25
Gerreidae	*Eugerres brasiliensis*	5,126	0.16	9 - 19		3.39	4		6
[illegible]lae	*Eugerres plumieri*	10,378	0.33	1 - 91		2.19	4		28
Haemulidae	*Pomadasys crocro*	5,967	0.19	9 - 168	AMP[1]	4.03	4		20
Sciaenidae	*Bairdiella ronchus*	4,638	0.15	3- 8		3.74	4		10
Cichlidae	*Amphilophus robertsoni*	14,597	0.47	0 - 168		3.23*	2	5	133
Cichlidae	*Cichlasoma bocourti*	376	0.01	7 - 29		≤3.99*	1	10	6
Cichlidae	*Cichlasoma salvini*	19,182	0.61	0 - 468		3.97	2	5	166
Cichlidae	*Cichlasoma urophthalmus*	11,428	0.37	0 - 124		4.11	3	5	76
Cichlidae	*Cryptoheros chetumalensis*	5,876	0.19	3 - 149		3.26*	1	10	18
Cichlidae	*Cryptoheros spilurus*	18,155	0.58	0 - 468		3.26*	2	5	142

Cichlidae	*Parachromis friedrichsthalii*	12,336	0.39	0 - 145		4.20	2	10	58
Cichlidae	*Petenia splendida*	15,931	0.51	0 - 162		4.50	2	10	104
Cichlidae	*Rocio octofasciata*	14,798	0.47	0 - 149		3.53	2	5	76
Cichlidae	*Thorichthys aureus*	873	0.03	7 - 233		2.00*	1	10	18
Cichlidae	*Thorichthys meeki*	13,416	0.43	0 - 135		2.00	2	5	150
Cichlidae	*Vieja godmanni*	782	0.03	7 - 233		2.00*	1	10	10
Cichlidae	*Vieja intermedia*	1,832	0.06	6 - 145		2.00*	2	5	19
Cichlidae	*Vieja maculicauda*	2,979	0.10	7 - 168		2.00	3		38
Cichlidae	*Vieja synspila*	12,175	0.39	0 - 181		2.00*	2	5	97
Mugilidae	*Agonostomus monticola*	4,671	0.15	16 - 195	AMP[2]	3.44	4		37
Mugilidae	*Joturus pichardi*	1,350	0.04	70 - 168	CAT[3]	2.37	4		6
Mugilidae	*Mugil curema*	4,111	0.13	7 - 14		2.00	4		5
Eleotridae	*Eleotris amblyopsis*	7,445	0.24	3 - 33		3.99	4		18
Eleotridae	*Eleotris pisonis*	7,444	0.24	8 - 33		3.66	4		7
Eleotridae	*Gobiomorus dormitor*	14,774	0.47	1 - 194		3.72	4		75
Gobiidae	*Awaous banana*	6,605	0.21	10 - 239	AMP[4]	2.00	4		41

[1] Greenfield & Thomerson, 1997
[2] Loftus & Gilbert, 1992
[3] Cruz, 1987

Chapter 5

Conclusion

The overarching goals of this dissertation were: (1) to describe fish communities relative to dominant environmental gradients at the national scale in Belize; and (2) to address specific problems in fish conservation that have direct relevance to ongoing conservation efforts in Belize. To meet these goals I employed a variety of analytical approaches and data sources to understand patterns of species distributions and to predict spatial limits of these distributions across aquatic landscapes.

The descriptive work on fish assemblages and the abiotic environment (Chapter 2) was carried out at two scales to test hypotheses generally oriented toward understanding the relative influences of catchment and reach characteristics on fish assemblage patterns. Results showed that catchment and reach variables, in combination, explained a large portion of the total variation in the fish assemblage data, suggesting that abiotic conditions across scales have a strong influence on fish assemblages. When variation in taxonomic membership among sites was partitioned into parts described by each scale of analysis, catchment environmental factors explained a greater portion of variation—particularly those variables representing landscape position, rather than geology or land use. Several reach-level correlates were also important, particularly those relating to channel morphology and substrates. From these results, it can be interpreted that landscape-scale factors have a stronger relative influence on assemblages than environmental conditions at the reach scale. One important question that remains is the degree to which the specific spatial configuration of sampling sites, or lack of temporal replication, may have biased results in favor of catchment explanatory capability. This question notwithstanding, the results of this work unambiguously pointed to the ability of landscape abiotic factors to explain variation in fish assemblages in different locations throughout the area.

The results of Chapter 2 revealed several important aspects about the fish community of the area. First, there is much species turnover across geographic latitude, a fact that may be linked to distinctions in the physiographic characteristics of northern (Yucatan Platform) and southern (Maya Mountains) parts of the study area. Second, there is a grouping of at least three fish species endemic to the southernmost 3 to 5 catchments that include the golden firemouth cichlid (*Thorichthy aureus*), the southern checkmark cichlid (*Vieja godmanni*), and the chisel-tooth cichlid (*Cichlasoma boucourti*). The presence of endemics here and not elsewhere may indicate a strong biogeographic disjunction that could lead to more species discoveries with increased sampling effort. Third, longitudinal position in catchments correlated strongly to taxonomic and functional composition changes within the community. Finally, given the high degree of correspondence between fish patterns and catchment scale influences, conservation efforts must focus first at this larger spatial scale before focusing on specific locations at the level of valley segment or reach.

The study of tilapia spread presented in Chapter 3 aimed to provide crucial context to the history of tilapias in Belize, and to identify spatial dimensions of spread and current landscape-level habitat occupancy. This chapter was innovative in its combination of low-tech and high-tech data sources from fishermen and GIS respectively, and provides a model of how best available information can be used to formulate hypotheses about ecological reality. The study confirmed that Nile tilapia (*Oreochromis niloticus*) is the common species present, and that they are widely distributed through nine drainage basins and potentially inhabit others. Given their impressive tolerances for environmental variability it is not surprising that species distribution models predicted tilapias to be present in virtually all coastal plain river channels. The reconstructed spatial chronology suggests that tilapias may have experienced an initial lag period before advancing from their initial area of establishment (Crooked Tree Lagoon) into other habitats. The study pointed to unintentional releases from aquaculture and flooding as the two most likely mechanisms for dispersal, leading to recommendations that focused largely on (1) keeping tilapia out of un-invaded systems, and (2) controlling aquaculture activities. Given the widespread nature of the global tilapia invasion, this research may provide a useful model for reconstructing spread and investigating habitat vulnerability to tilapias (or other aquatic non-indigenous species) in other locations.

Chapter 4 explored a method to define freshwater protected areas that integrated species distribution models and conservation planning software. The aim of this study was to demonstrate methods to (1) specify locations with high fish biodiversity and low human influence; and (2) recommend extended management zones to account for issues of connectivity, exogenous threats, and basin management. These aims were accomplished using the predicted ranges of 63 fish species as biodiversity features to be protected. This chapter is innovative in several ways. First, it is among the first efforts to conceptualize protected areas for Mesoamerican rivers. Second, the use of flow direction information in conjunction with other variables allowed for all aspects of the analysis to consider the directional connectivity present in rivers. As with the tilapia work, this chapter used software and computing technology to leverage available information to the greatest extent possible to create hypothetical representations of ecological reality that can serve as a starting point for conservation planning and further research. Such a contribution has potential applications in developing and developed countries alike.

Collectively the three chapters of this dissertation made contributions to our understanding of fish communities and their conservation in Belize, and more broadly to the empirical evaluation of nested hierarchy theory in aquatic ecosystems. Future research should build on this foundation, and the tools for leveraging available information demonstrated here should be evaluated in other information-poor settings. Recommended future research directions for each of the three components of this study are listed below.

Patterns and drivers of freshwater fish assemblage variation

1. Further investigations into the influences of multi-scalar environmental controls on species assemblages are warranted. In particular an examination of whether or not catchment-scale factors will still explain more assemblage variation than reach factors in more homogeneous landscape units may reverse my findings of greater catchment-scale influence, and reveal the reach level variables that have the most influence on fishes.
2. More intensive bio-inventory work is necessary in the four southernmost drainage basins (Rio Sarstoon, Temash River, Moho River, Rio Grande) to search for further endemic species.

3. The biogeographic history of the area deserves more detailed examination, and particularly the events that led to the presence of endemic species in only the southern three to five drainage basins.
4. Further investigation into the lack of a biological response to anthropogenic influences on the landscape. Is the community unaffected by the levels of stress present in the channel currently, or are they responding, but in a spatially localized way that was not picked up in the current analysis?
5. Surficial geology was shown in past studies to influence local physicochemical conditions in individual drainage basins, but was not shown to be important in the current research. Studies designed specifically to test for influences of geology on habitat and species are needed.

African tilapias in Belize

1. Research leading to a generalized understanding of the conditions that lead to tilapia domination in habitats is necessary. The current work investigated vulnerability to colonization, but not vulnerability to high tilapia relative abundance. Spatial models of tilapia abundance would be an important tool for conservation and fisheries managers.
2. Studies of tilapia as an economic fishery are needed to document production, biomass, catch, and optimal harvesting scenarios that target tilapias and not native fishes. Such a study could lead to the creation of targeted fisheries efforts in highly productive systems where tilapias dominate.
3. Investigations of the potential for tilapias to hybridize with native cichlid species are needed. This research concern grows from reports of fishermen of hybridization occurring in nature.
4. Documentation of tilapia genotypes present in northeastern Mesoamerica are necessary, and a genetic registry for new stocks coming into the area is needed.
5. Further studies of the environmental impacts of tilapias on (a) native competitor species; (b) predator populations including birds; and (c) nutrient cycling in ecosystems.

Freshwater protected areas

1. The protected area network recommended here should be further evaluated for its abilities to: (a) protect rare species; and (b) protect plants and invertebrates.

2. Further advancement of the connectivity concept incorporated into the protected areas assessment is necessary, particularly through creation of reach catchment layers with network topology defined that accurately describes catchment interconnectivity.
3. Integration of freshwater, marine, and terrestrial protected areas networks into one optimal scenario could lead to better decision making that can address connectivity and complementarity between ecosystem types (e.g., river to marine connections).
4. A recommendation of management best practices for each of the three management zones (focal areas, critical management zones, and catchment management zones) is needed.
5. A conservation prioritization of recommended focal areas would help with decision making process for moving from a conceptual protected area network to an network that can be implemented in steps.

In general, the amount of research focusing on the inland water ecosystems of northeastern Mesoamerica must increase dramatically. The contributions made by the current research are a start to understanding basic patterns in biodiversity, the threat of tilapia, and possible locations for more intensive conservation effort. However, many more threats exist, and the majority of taxa present in the aquatic systems have still not been documented in a systematic way at a scale appropriate to decision making. Only with a deepened understanding of patterns in biodiversity, threats, and the effectiveness of mitigation strategies will freshwater conservation in the basins of northeastern Mesoamerica be successful.