Riverine reserves: the conservation benefits of spatial protection for rivers in the context of environmental change

By

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Abstract

This dissertation explores the effects of community-initiated and enforced no-take conservation reserves in rivers of northwestern Thailand. There, like many other areas in Southeast Asia, humans have high nutritional reliance on inland fisheries, and in response to perceived declines in catches, communitites have responded by creating conservation reserves. These reserves, howerver, occur across a landscape that has also changed rapidly in past decades. It is because of the strong influence that watershed landuse has on rivers that spatial conservation for rivers has to date largely focused on terrestrial protected areas. This dissertation explores the effects of land-use strategies on water quality and ecosystem functioning (Chapter 1), the effects of riverine reserve creation on food webs (Chapter 2), develops a theory for riverine reserve design (Chapter 3), and finally tests this theory within a network of riverine reserves (Chapter 4).

I found that expansion of intensified agricultural practices in northern Thailand has increased in-stream nutrient concentrations sufficiently to shift background nutrient limitation states. Compared to rivers in low-intensity agricultural areas, which showed strict nitrogen limitation, rivers in areas with high fertilizer-intensive crop extent showed a shift towards nitrogen and phosphorus co-limitation. This likely results from broad use of nitrogen-rich fertilizers like urea throughout Thailand.

I also found community riverine reserves were highly effective at increasing fish species richness, density, and biomass relative to adjacent fished areas. The effects of harvest outside of reserves also had effects that cascaded down to benthic algae due to the alteration of fish community size structure and species composition. By surveying numerous reserves I found that attributes such as reserve size, duration of protection, and proximity to the nearest village had strong effects on the effects of reserves on species richness, density, and biomass. However, the specific reserve characteristics varied based on the outcome variable (e.g., species richness versus density). This empirical support for the success of even small riverine reserves is supported through my investigation of the underlying principles used to develop protected area design theory in other ecological realms (marine and terrestrial).

Introduction

Access to freshwater is a driving requirement for life on Earth, guiding processes on evolutionary time scales and affecting the ecological distributions and interactions of organisms we can observe at present. For thousands of years, human civilizations have flourished along rivers and collapsed as a result of their mismanagement. Despite our collective understanding of the importance of water for life, freshwaters remain among the most critically imperiled ecosystems on Earth (Strayer and Dudgeon 2010). The threats to inland waters are numerous, including the direct effects of diversion, abstraction, and flow modification and associated habitat loss, the direct and indirect effects of pollution, the loss of native species and the addition of non-native species, all within the context of a changing climate (Dudgeon et al. 2006, Vörösmarty et al. 2010). Given the great challenges presented by the state of the world's freshwaters, there is an urgent need for developing solutions to improve the quality of these ecosystems and ensure the continued provisioning of their critical resources.

Inland fisheries are one such resource, totaling an estimated 11.9 million tons in 2014 (FAO 2016), and providing critical nutrition to millions of people, particularly in low-income countries around the world (McIntyre et al. 2016). Management of inland fisheries, however, is a great challenge as a result of their diffuse nature: large numbers of individuals participate across numerous waterbodies. In high-income countries, top-down regulation and intensive stocking efforts have been widely employed to manage what have become largely recreational fisheries (Arlinghaus et al. 2002, Cowx et al. 2010). Where inland fisheries remain subsistence-based, such intensive management efforts face significant challenges (Welcomme et al. 2010).

However, local management of inland fisheries is often highly effective, and can take on a variety of forms (Baird 2006, Welcomme et al. 2010).

Although spatial protection has become fundamental to terrestrial and marine conservation efforts (Watson et al. 2014), the potential application of reserve thinking to freshwaters has yet to be fully realized (Saunders et al. 2002, Abell et al. 2007b, Loury et al. 2017). It is true that terrestrial conservation reserves can provide a measure of protection to the biodiversity of inland waters, yet this benefit is often limited as a result of the low concordance between areas of high biodiversity for terrestrial and freshwater systems (Abell et al. 2011). Additionally, the ability of spatial protection to confer real benefits to freshwater ecosystems is challenged by the fact that inland waters are affected by upstream sources of pollution, land-use change, flow modification, and species invasions (Nel et al. 2007). Thus, the systematic implementation of reserves in freshwater systems has been infrequent (Herbert et al. 2010), and the quantitative evaluation of any realized benefits resulting from spatial protection have been rarer still.

Throughout Southeast Asia, which has globally-high human dependence on inland fisheries (Hortle 2009), various communities have engaged in small-scale spatial protection for decades (Baird 2006). The duration of protection, and the opinion of local communities suggest that these areas provide important refuge for fish, especially during annual periods of low water (Baird and Flaherty 2005, Baird 2006). Research on these community-based conservation projects to date has been largely focused on reserves associated with deep-water habitats in the Mekong River (Baird and Flaherty 2005, Baird 2006). However, potentially thousands of small reserves exist in rivers throughout the region (Hogan 1997), often organized by individual communities who stand to receive any potential benefits from these largely uncoordinated conservation efforts. The abundance of reserves across the region provide a compelling justification for investigating both the long-term effects of intensive harvest in these systems and the capacity for grassroots reserves to maintain local biodiversity, which undergirds the region's highly productive fisheries.

While the focus of dissertation chapters 2, 3, and 4 is on small riverine reserves, my first chapter investigates the broad effect of land use on nutrient dynamics in rivers throughout northern Thailand (Koning et al. 2017). Specifically, I investigate the potential impacts of implementing land sparing versus land sparing land use strategies on aquatic systems within this rapidly changing region of the world. There has been much debate regarding how to balance the need for adequate food production to feed an ever-growing human population with maintaining habitable landscapes for wildlife (Phalan et al. 2011, Tilman et al. 2011). However, the potential impacts of either management strategy on freshwaters has largely been overlooked. By measuring nutrient concentrations within rivers covering a gradient of land use types, I found that areas of intensified agriculture, in line with land sparing strategies, has elevated nitrogen (NO_3-N) levels compared to areas of rotational or swidden cultivation, which more closely align with land sharing strategies. Notably, intensification had relatively no effect on phosphorus. Nutrient bioassays showed that increased nitrogen loading was sufficient in intensified agricultural systems to shift in-stream nutrient limitation from strict nitrogen limitation to co-limitation with phosphorus. Finally, analysis of rare earth elements from water and sediment samples provided strong evidence that elevated levels of nitrogen are associated with inorganic fertilization rather than natural sources. In the context of the subsequent chapters, the results of Chapter 1 serve as a reminder that spatial protection is no panacea for the numerous stressors to freshwater systems, especially the effects of increased fertilization associated with intensified forms of agriculture.

In my second chapter, I investigate the consequences of intensive fishing on the trophic dynamics of an upland Southeast Asian river, the Mae Ngao. I conducted surveys and experimental manipulations inside and outside of two community reserves in which harvest is prohibited. I found that community reserves had strong positive effects on the abundance and biomass of fish overall, and large-bodied (> 15 cm maximum length) fishes in particular. Small fish densities were higher outside of reserves, indicating a cascading effect of harvest on large-bodied fishes and consequent trophic release of small-bodied fishes. Strong harvest-induced effects on fish assemblage size structure did not, however, cascade to lower trophic levels. Experimental fish exclosures revealed that fish, both inside and outside of reserves, were equally effective at decreasing densities of macroinvertebrates relative to where fish were excluded. In turn, in the absence of fish, macroinvertebrates significantly decreased algal accrual on natural rock substrates and experimental tile substrates. Finally, the top-down fish–macroinvertebrate–algae cascade was strong enough to overwhelm the positive bottom-up effect on algae of release from nutrient limitation. The results of Chapter 2 provide strong evidence of the efficacy of small reserves to increase the density and biomass of fish relative to non-reserves. Interestingly, we found only indirect effects of the highly disparate reserve and non-reserve fish assemblages on lower trophic levels. This is likely due to the fact that harvest showed no effect on mean trophic position of the fish assemblage, despite strong effects on fish size. Thus, while small reserves have dramatic consequences for fish communities, high rates of omnivory and trophic redundancy likely dampen the trophic consequences of intensive fishing. Yet, the strong trophic interactions that we observed cascading from fish, regardless of size, to macroinvertebrates and algae are likely the result of intensified trophic interactions during the high-productivity dry season and preference for high-nutrient animal prey, even by large, omnivorous fishes.

The success of small reserves (< 1km in length) for increasing fish abundance and biomass observed while working on Chapter 2 drove the conceptual development of Chapters 3 and 4. In Chapter 3, I develop principles for the design of riverine reserve networks, building upon the protected area literature for both terrestrial and marine systems over the past 4 decades (Diamond 1975, Margules and Pressey 2000, Gaines et al. 2010). As cornerstones of modern conservation strategies (Margules and Pressey 2000), there is much to gained by the application and adaptation of spatial protection strategies to rivers (Abell et al. 2007b). However, given the hierarchical dendritic network structure of rivers, many of the same principles derived for two- or three-dimensional systems like forests or oceans have limited direct applicability (Erős et al. 2011b). Chapter three reviews the guiding principles for reserve design based on the species–area relationship, the species–energy relationship, meta-population theory, and the properties of dendritic networks to derive 10 principles to maximize the efficacy of riverine reserve networks for conserving freshwater biodiversity while also supporting inland fisheries.

My final chapter serves as an empirical test of reserve features shown to have positive benefits on the richness, abundance and biomass of fish in marine ecosystems. We conducted paired surveys of 23 reserves and non-reserves within the Ngao River Basin and related the reserve-specific fish response to attributes of each reserve. Specifically, we modeled the response ratios for reserve fish richness, density, and biomass to predictors relating to size, age, enforcement, isolation, and network position. We find that our different reserve response variables respond to particular reserve features differently based on key functional traits. For example, the reserve response of overall fish richness was best predicted by river discharge, but for small fish (<15 cm max length), richness decreased with increasing years of protection. The number of different features found to predict reserve success for each outcome variable and functional trait indicates the need for clearly defined targets prior to reserve implementation. Once defined, a network of reserves having different features can maximize the likelihood of achieving the desired conservation outcomes.

Taken together, the four chapters of my dissertation provide much-needed empirical evaluation of some of the threats facing freshwater ecosystems in Southeast Asia. My first chapter has implications for current land management strategies, particularly the shift towards intensified agriculture throughout the region. In the remaining chapters, I provide a preliminary empirical evaluation of the efficacy of small-scale riverine reserves to conserve species and maintain important ecosystem dynamics, develop a framework for riverine reserve design based on a broad body of ecological theory, and finally evaluate which features of riverine reserves predict their success at maintaining biodiversity and abundance of fish populations, with potential implications for supporting local food security. The threats to freshwaters are numerous, requiring the development of many solutions. It is my hope that the research presented here makes a significant contribution to the conservation of freshwater resources in this region of high resource demand, with potential applications to other systems facing similar challenges.

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Chapter 1

Aquatic ecosystem impacts of land sharing versus sparing: nutrient loading to Southeast Asian Rivers

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Abstract

As world food demand continues to increase, two broad strategies for agricultural production have been widely discussed: land sparing and land sharing. Reflecting tradeoffs between the extent and intensity of agricultural use, land sparing maximizes the ratio of conserved to agricultural land, while land sharing allows more extensive, nature-friendly transformation of the agroecological matrix. Freshwater ecosystems are rarely considered in weighing these strategies, despite being strongly affected by land use change. Here we analyze how shifting from extensive to intensive agricultural practices is altering dry season riverine nutrient availability and algal growth in upland Southeast Asia, which is experiencing rapid conversion from swidden—or shifting—cultivation to fertilized row-crops. In-situ algal growth assays and nutrient concentration data from eleven catchments representing a spectrum of land use and population densities show that intensive agriculture is associated with significantly elevated nitrate concentrations but no shift in phosphorus. As a result, nitrogen to phosphorus ratios increase dramatically, shifting algal growth toward co-limitation by nitrogen and phosphorus. Geochemical analysis of suspended solids suggested comparable geological inputs across basins, but dissolved rare earth elements reveal that inorganic fertilizers are changing water chemistry in parallel with land use. Taken together, our analyses suggest that the transition

from low-input land-sharing management (swidden) to high-input land-sparing practices (intensive row-crop agriculture) underway in Southeast Asia has profound consequences for river ecosystems. Such nutrient loading can affect river productivity and food webs, suggesting that land use conservation strategies for Southeast Asia need to account for impacts on freshwater ecosystems.

Introduction

By 2050 the global demand for food is expected to double from current levels due to increasing size and wealth of human populations (Tilman et al. 2011). To date, increases in human food demand have been met through two processes: intensification and extensification of agricultural systems. Intensification—boosting crop yields (i.e., harvest per hectare) via improved seed stocks, mechanization, irrigation, fertilizers, and pesticides (Matson et al. 1997)—has enhanced food security in much of the world but strongly displaces native biota (Phalan et al. 2011). Extensification involves expanding use of marginal or previously undeveloped lands, causing even greater loss of unaltered habitats (Tilman et al. 2011). Both patterns have been implicated as major drivers of Earth system change, threatening continued global environmental resilience by reconfiguring nutrient cycles, altering climate and land cover, and reducing genetic diversity (Poppy et al. 2014).

Conservationists have debated two main strategies for increasing global food security while maintaining biodiversity and critical ecosystem functions. Land sparing aims to increase production on existing cropland via intensification to provide adequate yields while minimizing the amount of land directly impacted by agriculture and thereby maximizing land set aside for strictly conservation purposes (Green et al. 2005). Critics of land sparing strategies often advocate a land sharing scheme, under which lower yields per area are accepted in exchange for expanding the extent of heterogeneous agroecological landscapes that maintain higher biodiversity (Perfecto & Vandermeer 2010). Although crop yields under low-impact management are typically lower than from intensive agriculture, the yield gap is sometimes small, and land sharing proponents argue that the benefits of improved ecosystem resilience (e.g., higher biodiversity, reduced soil loss, increased water retention; Ponisio et al. 2015) more than offset the impact of a larger agricultural footprint.

Theoretical considerations aside, current trends suggest that intensification in many regions has rarely led to land sparing and may in fact increase rates of deforestation (Ewers et al. 2009). Furthermore, on a global scale, opposing patterns of intensification and extensification exist for high- and low-income nations; in recent decades, high-income nations have decreased agricultural extent and fertilizer use even as most low- and mid-income nations have seen increases in both (FAO 2015). In Southeast Asia (SEA), forest loss has often been attributed to the decline of swidden cultivation practices and the transition to permanent, intensive agriculture (Schmidt-Vogt et al. 2009). Swidden—or shifting cultivation—refers to agricultural systems that use fire to clear land for short-term farming, then rely on fallowing and forest regeneration to restore soil fertility (Mertz et al. 2009). Increased access to markets has aided the observed transition in land-use practices, however governmental promotion of industrial agriculture and other policies promoting land sparing strategies are major drivers of reduced swidden agricultural extent in SEA and elsewhere (van Vliet et al. 2012). In some cases, regional governments have long prohibited swidden agriculture but have only recently begun to enforce these laws by actively removing people from working swidden landscapes, restricting access to fallowing swidden fields, and encouraging intensive cultivation practices on remaining lands

(Fox et al., 2009). Transitions from swidden practices to more intensive agriculture have resulted in reduced terrestrial biodiversity (Scales & Marsden 2008), increased rates of pesticide and fertilizer use (Lal 2000), and altered hydrology (Ziegler et al. 2009).

The detrimental consequences of high-input, intensive agriculture for freshwater ecosystems in developed nations are well known (Carpenter et al. 1998), but studies on the effects of land use on the biogeochemistry of tropical rivers are rare (Elser et al. 2007), despite high conversion rates of tropical forests (Gibbs et al. 2010). In tropical rivers, seasonal precipitation and runoff result in natural fluctuations in nitrogen (N) and phosphorus (P) yields that are dominated by biologically fixed N and weathered P, unlike most temperate rivers (Dumont et al. 2005; Harrison et al. 2005). Shifts toward intensive agriculture and urban land use change are expected to increase P availability relative to N, reducing N:P and increasing N limitation of primary production within tropical freshwater ecosystems (Downing et al. 1999). However, experimental enrichment studies have provided equivocal support for overall P limitation in the tropics (Elser et al. 2007).

This study aims to elucidate how ongoing land use changes in SEA are affecting riverine nutrient availability and the implications for algal growth that supports food webs. We analyzed land use and surveyed nutrient concentrations in 3 major SEA river basins, then experimentally tested the strength and nature of nutrient limitation of algal growth, and finally used geochemical analyses to distinguish natural and anthropogenic sources of nutrients. Specifically, we tested the following hypotheses: 1) intensive agriculture and human population densities are stronger predictors of nutrient concentrations than swidden agriculture, 2) nutrient loading from human populations and intensive agricultural practices alleviates nutrient limitation of algal growth, and

3) anthropogenic inputs rather than natural geochemistry or climate now govern the chemical differences among watersheds even in rural SEA.

Methods

We selected eleven sites in northern Thailand to include a representative range of human population densities and land use patterns within three major SEA river basins: the Salween, Chao Phraya, and Mekong (Fig. 1). Population density in the Chao Phraya (149 people km^{-2}) and Mekong basins (85 people km^{-2}) are higher than in the Salween basin (30 people km^{-2}; calculated from Varis et al. 2012), and the Salween has experienced substantially less intensive agricultural development. In total we selected 6 study sites in the Salween, 4 in the Chao Phraya, and a single site in the Mekong River Basin. Detailed descriptions of ecosystem and geochemical characteristics of the study region are found in the Supplemental Materials.

We performed standard physical and chemical field measurements to characterize each study site (Table 1; detailed methods in Supplemental Materials). Measurements were made once at each site, except for NO_3-N and SRP, which were measured twice, and are expected to be broadly representative of conditions during the long dry season (5–6 months per year) based on seasonal results from other tropical rivers (Li et al. 2014; Marwick et al. 2014; Roach et al. 2014). We investigated algal nutrient limitation using nutrient diffusing substrata (NDS; Tank et al. 2007) at all 11 sites within the Salween, Chao Phraya, and Mekong Basins between January and March 2013 (detailed methods in Supplemental Materials). We were able to analyze NDS from only 6 rivers (3 each from the Salween and Chao Phraya basins), as 5 experiments were removed by local people or smothered by drifting mats of filamentous algae. Of the 6 analyzed NDS experiments, two were replacements following the loss of original NDS units, resulting in incubation times ranging from 8-17 days. To accommodate disparities in experimental duration between sites, we compared treatment effects across sites using a time-standardized limitation index proposed by Osenberg and Mittelbach (1996) for nutrient enrichment experiments:

$$L = [\log_{10}(C_t') - \log_{10}(C_t)]/t$$

where L is the limitation index, C_t' is the chl *a* concentration of each nutrient amended treatment after *t* days, C_t is the chl *a* concentration of the corresponding within-block control treatment after *t* days, and *t* is the duration of the experiment in days. Prior to standardization, chl *a* concentrations were strongly related to duration of deployment ($F = 114.8$, $df = 1, 142$, $p < 0.001$), but time-standardization eliminated this dependence ($F = 0.20$, $df = 1, 142$, $p = 0.65$) to enable comparisons across all study sites.

We delineated upstream catchments for NDS deployment sites using the drainage direction data layer from the HydroSHEDS dataset (Lehner et al. 2008). Land cover data were acquired from the Land Development Department of Thailand (LDDT) for 2009, generated at 1:25,000 scale from Landsat TM and ETM+ imagery and aerial photos. LDDT data are classified into 16 land cover classes, 15 of which were represented within the focal drainages (Supplemental Materials). Average population densities were calculated from the LandScan database for the year 2012 (Bright et al. 2012). The Salween mainstem site was not included in these catchment analyses because the vast majority of the basin lies outside the coverage of LDDT data.

To relate nutrient data to land cover, we first reduced the full suite of land cover classes to reflect our focus on human-dominated land uses. Of the six agricultural land cover classes present in the data set, we pooled five classes (rice paddy, field crop, orchard, horticulture, and perennials) into a single category—intensive agriculture—based on their active use of fertilizers and sustained land conversion. Swidden, classified following the UN FAO land cover classification scheme, was kept as a separate class of agricultural land use. We eliminated both primary and secondary forest classes because they showed a strong inverse relationship with agricultural land use. We excluded the remaining seven land classes because each accounted for < 4% of area in every drainage. Thus, our final land cover metrics were simply proportions of area under intensive agriculture or swidden cultivation.

We used regression models to first test the hypothesis that land use and population density affect nutrient availability. To test the effect of land use and population density on nutrient limitation of algal growth, our second hypothesis, we began by evaluating site-specific limitation status inferred following Tank and Dodds (2003) based on two-way analysis of variance (ANOVA) of algal growth response to nutrient enrichment followed by pairwise comparisons. Block effects were never statistically significant and were pooled with error terms in the final models. We then tested for overall patterns of algal growth response to nutrient enrichment across sites using linear mixed effect (LME) models to predict a nutrient limitation index based on N, P, river basin, and their interactions as fixed factors along with sites as a random factor. To test whether the magnitude of algal growth response was affected by nutrient availability, we regressed limitation indices by type (e.g., N limitation index) against mean nutrient concentrations. Finally, we tested our second hypothesis using LME to regress limitation indices, again by type, against intensive agriculture, swidden, and human population density independently and in combination, with sites as a random factor.

To distinguish nutrients originating from natural processes (e.g., weathering, N-fixation) versus anthropogenic inputs, we used rare earth elements (REEs). REEs are widely used as tracers of geochemical sources based on comparisons between dissolved and particulate samples, where solids represent the weathered parent material and solutes reflect additional inputs from human activities (Leybourne & Johannesson 2008). We used standard geochemical approaches to analyze patterns in REE concentrations among river basins (Supplemental Materials). We further investigated anthropogenic signatures in REE concentrations among sites by using non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarity, and then testing correlations between dissolved nutrient concentrations (NO_3, NH_4, and SRP) and ordination scores using permutation tests. We also tested for specific differences among basins in REE dissimilarity scores using MANOVA.

To meet assumptions of normality and homogeneity of variance for linear models, nutrient concentration data were $\log_{10}$ transformed. Ammonium data were $\log_{10}(NH_4+1)$ transformed to account for values of zero. All statistical analyses were conducted using the R statistical language (R Core Team 2015) and statistical packages contained therein (see Supplemental Materials).

Results

Nutrient concentrations were variable but generally low across sites (0.17–14.69 μmol NO_3-N L^{-1}, 0–2.72 μmol NH_4-N, and 0.05–0.72 μmol SRP; Table 1). N:P ratios (i.e., DIN:SRP) also varied widely from 2 to 147. NO_3-N concentrations and N:P molar ratios for sites from which NDS were recovered differed strongly by basin, although the range of nutrient concentrations overlapped among basins when all sites were considered. Mean NO_3-N concentrations from Chao Phraya NDS sites were ≥ 7.4 μmol N L^{-1}, resulting in N:P ratios > 24. Salween NDS sites had mean NO_3-N concentrations ≤ 0.7 μmol N L^{-1} and molar N:P ratios < 18. Differences in NO_3-N were significant between NDS sites in the two basins (Welch's two-sample t-test, $t = 5.6$, $p = 0.002$). For sites at which NDS were not recovered, the Lao River (M1) had the highest NO_3-N (14.69 μmol N L^{-1}) but comparable NH_4-N and SRP values to Chao Phraya and Salween sites, resulting in a substantially higher N:P of 147. The Nan River site (C4) had the lowest recorded NO_3-N (0.17 μmol N L^{-1}) and N:P (1.94), and the Salween River mainstem (S4) had comparatively high NO_3-N (10.25) and N:P (130).

Land use varied widely among the three study basins, and regressions of nutrient data against land use variables showed distinct relationships. Intensive agriculture was strongly positively correlated with NO_3-N ($F_{(1,8)} = 5.80$, $p < 0.05$) and DIN:SRP ($F_{(1,8)} = 11.98$, $p < 0.01$), and swidden was strongly negatively related to DIN:SRP ($F_{(1,8)} = 12.25$, $p < 0.01$). Population density was not significantly associated with nutrients in univariate models. Regressions of nutrient data against intensive agriculture, swidden, and population density significantly predicted NO_3-N, DIN, and DIN:SRP, and the relative magnitudes of standardized regression coefficients (β_{std}) suggested intensive agriculture was the dominant predictive term in each model (Supplemental Materials).

For all rivers, nutrient amendment led to increased algal accrual, providing experimental evidence that growth of algae in rivers in northern Thailand is limited by nutrient availability. For each of the three Chao Phraya sites, NP treatments accrued significantly more algae than all others (Fig. 2). Results of ANOVA indicated NP co-limitation in the Ping (C1) and Yom (C2) Rivers, primary-N and secondary-P limitation in the Wang River (C3); all Salween sites exhibited strict N limitation (Table 2). In fact, P treatments generally accrued less algae than controls (Fig. 2).

Mean N limitation indices were highest for Salween sites, P limitation indices were negative at all sites, and NP limitation indices did not differ between basins (Supplemental

Materials). LME models revealed no significant main effects of N addition, P addition, or basin on nutrient limitation indices across sites, however, significant interactions showed NP treatments had highest limitation indices across sites (i.e., greatest algal growth response), and that Salween sites had higher N and lower P limitation indices than Chao Phraya sites (Supplemental Materials).

Only N limitation indices were significantly predicted by nutrient availability. Across sites, N limitation was negatively related to NO_3-N concentrations, DIN concentrations, and DIN:SRP (Fig. 3a-c), but showed no relationship to NH_4-N concentrations. P limitation was not significantly related to SRP, and NP limitation was not significantly related to N or P concentrations. Land use also significantly predicted N limitation in LME models ($F_{2,\,4.80} = 6.5$, $p = 0.043$, $R^2c = 0.37$); N limitation was negatively related to percent intensive agriculture, positively related to percent swidden, but showed no relationship to population density (Fig. 3d-f). The magnitude of the standardized coefficient for intensive agriculture ($\beta_{std} = -0.497$) was associated with reductions in N limitations three times larger than the increases arising from swidden ($\beta_{std} = 0.134$), though neither was statistically significant. P limitation exhibited a negative relationship to percent swidden ($\beta_{std} = -0.741$, $F_{1,\,4.95.} = 15.24$, $p = 0.012$, $R^2c = 0.54$), but this result was confounded by the apparent inhibiting effect of P addition on algal growth. NP limitation was not significantly related to any land use variable.

Suspended sediment REE concentrations (REE_{SS}) were consistent across all sites, indicating that bedrock types are similar across the Chao Phraya, Mekong, and Salween Basins (Fig. 4a & Supporting Information). However, Chao Phraya REE concentrations from water samples (REE_{WAT}) were significantly lower than Salween (Welch's 2-sample t-test, $p < 0.05$) or Mekong samples, although concentrations were relatively low across all sites (Fig. 4b). Ratios of light REE to medium and heavy REE were highest from Chao Phraya rivers (Supplemental Materials). ANOVA of Bray-Curtis distance matrices revealed only a marginal difference among basins in REE_{SS} ($F = 2.8$, $R^2 = 0.41$, $p = 0.077$) but strongly significant differences among basins for REE_{WAT} ($F = 34.8$, $R^2 = 0.90$, $p = 0.002$). NMDS plots for REE_{SS} indicate broad overlap between sites within the Salween and Chao Phraya basins (Fig. 5a), in contrast with separation among basins in REE_{WAT} along NMDS axis 1 (Fig. 5b). For REE_{WAT}, both NMDS axis 1 and 2 separated REE by atomic mass, with light REE accentuated in Chao Phraya basin sites while Salween sites contained heavier elements (Fig. 5b). Nutrient data (NO_3-N, NH_4-N, SRP) showed no significant relationship to NMDS ordinations, however nutrient vectors strongly aligned with light REE along NMDS axis 2 for REE_{WAT} (Fig. 5b).

Discussion

Nutrient concentrations, experimental assays, and REE profiling together indicate that agricultural intensification is transforming river chemistry in SEA. Intensive agriculture was associated with elevated N concentrations and higher N:P, thereby alleviating N limitation of algal growth. REE analysis supported the conclusion that differences in nutrient patterns resulted from anthropogenic loading rather than underlying geological differences. We expect that continued transition from complex swidden agroecosystems to high-input, intensive cropping systems will profoundly affect aquatic ecosystems, regardless of benefits to terrestrial biodiversity on spared lands.

As expected, we found that land use strongly affected nutrient availability and limitation state. Intensive agriculture was significantly correlated with NO_3-N, but not NH_4-N or SRP, suggesting that nitrogen availability increases as land is converted from swidden to intensive agricultural use. Compared to areas with high intensive agriculture, river basins with high swidden extent had low dissolved N and correspondingly low N:P ratios (Table 1).The lack of N associated with swidden land use could reflect the rarity of inorganic fertilization usage or volatilization of N from biomass burning. Because P does not have a gaseous state, burning removes substantially less P and can lower ecosystem N:P (Toberman et al. 2014). The 6 sites from which NDS were recovered each showed significant nutrient limitation, but sites having higher intensive agriculture and NO_3-N concentrations (Chao Phraya Basin) also showed reduced N limitation relative to high swidden sites (Salween Basin), which were all strictly N-limited (Table 2, Fig. 3a,d). Surprisingly, nutrient concentrations were unrelated to human population densities in univariate models (Supplemental Materials). However, the two NDS sites in closest proximity to urban centers, the Yuam (S3) and Wang (C3), had the highest NH_4-N values (Table 1), suggesting a localized human population effect. We attribute the very low levels of NO_3-N in the Nan River (C4) to rapid uptake by a luxurious filamentous algal mat covering the riverbed, however, the source of high NO_3-N in the Salween (S4) is not obvious.

Phosphorus concentrations did not increase with intensive agricultural extent, nor was P limitation related to P availability. The latter may be explained by the apparent inhibition of algal growth on P-amended treatments, which has been observed in other NDS experiments (Elser et al. 2007). However, the lack of a relationship between P availability and the extent of intensive agricultural may reflect fertilization practices adopted by farmers in the region. N-P-K fertilizers are widely available and recommended by Thai agricultural extension offices. However, regional experts suggest that the higher cost of balanced fertilizers containing P (e.g., 16-16-16) compared with urea fertilizers (46-0-0), as well as the widespread use of urea as a supplemental fertilizer during the growing season, may yield very high net fertilizer N:P ratios (P. Gypmantasiri, personal communication). Such lack of access to phosphorus fertilizers is a broad pattern among developing countries (Peñuelas et al. 2013). Indeed, developed nations use 40% of global phosphorus fertilizer despite holding only 10% of agricultural lands (Foley et al. 2011). Another possible reason for the lack of correlation between SRP and intensive agriculture is the potential for rapid sorption of applied P to the highly weathered oxisol and ultisol clays common in Thai agricultural lands (Ziegler et al. 2000). Therefore, even balanced fertilizers may still elevate N:P ratios above the ideal Redfield ratio (16:1), as we observed in basins having higher intensive agriculture (Tables 1 & 2).

The fact that all study sites from which we recovered NDS showed significant nutrient limitation of algal accrual is notable for two reasons. First, results of NDS experiments from around the world suggest that many streams exhibit marginal or no nutrient limitation (Francoeur, 2001; Johnson et al., 2009; Tank & Dodds, 2003). In the two most extensive NDS experiments to date, which total 82 site deployments across 8 North American ecoregions, only 22 (26%) exhibited nutrient limitation of algal growth (Johnson et al., 2009; Tank & Dodds, 2003). Second, the observed shift in limitation state from N to N-P co-limitation or primary-N, secondary-P limitation with modest ambient increases in N concentrations (~7 μmol N L^{-1}) is striking. Average NO_3-N concentrations across all of our sites (5.2 μmol N L^{-1}) are considerably lower than the average reported by Lewis et al. (1999) for tropical rivers (8.6 μmol N L^{-1}), and far lower than observed in agricultural (93.4 μmol N L^{-1}) and urban (32.7 μmol N L^{-1}) streams in North America (Johnson et al. 2009). Apparently, relatively low levels of N loading are sufficient to move beyond strict N limitation given the low background levels of available nutrients in our study rivers (Table 1).

It should be noted that work occurred solely during the height of dry season when rivers were near base flow conditions. While NDS methods allow for direct comparison of limitation by N and P *in situ*, their results must be interpreted according to the limited spatial and temporal context in which the experiments were conducted (Tank and Dodds 2003). At other times of year, different nutrient limitation patterns might be observed due to strong seasonality in precipitation, cropping cycles, and fertilizer application. Nutrient concentrations have been shown to peak during low-water periods in tropical rivers in Peru and Venezuela (Roach et al. 2014), Kenya (Marwick et al. 2014), and China (Li et al. 2014). Unfortunately, year-round nutrient data are not available from our study sites or other SEA rivers, hence we cannot be sure that the dry season patterns of nutrient concentrations and limitation reported herein are representative of all seasons.

REE data nicely augmented the correlative approach to linking agricultural land use with riverine nutrients. Given that suspended solids indicated comparable background geochemistry among our focal basins (Figs. 4a, 5a), we could interpret REE_{WAT} as reflecting human influences. Their short residence time compared to suspended solids makes dissolved REEs sensitive to human inputs because natural concentrations are typically low in rivers having pH >7.75–8, which was the case for our study basins (Table 1). Furthermore, REE_{WAT} from Chao Phraya sites showed enrichment of light REE relative to medium and heavy REE, consistent with patterns in commercial fertilizers (Otero et al. 2005). For instance, concentrations of light REEs (La, Ce, and Nd, which form complexes with NO_3) dissolved in river water have increased due to fertilizer use in China (Zhang & Shan 2001), a major exporter of fertilizers to Thailand. Though we do not know the source of the fertilizers used in our study region, our findings are concordant with fertilizer-based light REE enrichment: Chao Phraya rivers have elevated light

REEs (Supplemental Materials), and there were strong associations between light REE and both NO_3-N and intensive agriculture (Fig. 5b). Taken together, these results provide strong geochemical evidence that differences in nutrient availability have anthropogenic origins.

Importantly, rising N concentrations were not paralleled by increased P availability in catchments with more intensive agriculture, so dissolved N:P increased with intensive agriculture. Accepted models describing anthropogenic eutrophication in freshwaters suggest that intensified agriculture should decrease N:P ratios due to the application of balanced chemical fertilizers and low N:P manures (Downing & McCauley 1992). These patterns apply well to areas like the Midwestern United States and Western Europe, which are global hotspots of both fertilizer application and livestock production. A similar model has been proposed for the tropics (Downing et al. 1999). However, in northern Thailand and many other tropical developing countries, fertilizer use in support of agricultural intensification does not necessarily connote high P inputs. Farmers may elect to rely primarily on N-rich manure, or switch to chemical fertilizers but with a preference for high N:P due to the cost premium for P compared to cheap urea. Overall, the SEA rivers that we studied remain sensitive to modest nutrient additions, in contrast to many rivers worldwide where decades of intensive agriculture and atmospheric N deposition have dramatically boosted background N levels (Dumont et al. 2005; Elser et al. 2009).

The increased N:P observed in intensive agricultural rivers in our study parallel stoichiometric shifts resulting from agricultural eutrophication observed in freshwaters worldwide (Peñuelas et al. 2013). Increased N:P ratios have been reported from temperate lakes resulting from atmospheric deposition (Elser et al. 2009) and agricultural land-use practices (Arbuckle & Downing 2001). Shifts in freshwater N:P have been shown to have bottom-up effects on algal growth rates and community composition, which can affect higher trophic levels. In turn, altering producer N:P can translate into differences in nutrient regeneration by consumers (Sardans et al. 2012), a potentially important ecosystem process in nutrient-limited SEA rivers. Thus, the full consequences of shifting ambient N:P due to agricultural intensification is unknown for these highly biodiverse river ecosystems.

Our inference that intensive agriculture is forcing the Chao Phraya basin toward an anthropogenic state of P limitation raises profound questions about the applicability of the land sparing versus sharing debate to aquatic ecosystems. Central to the land sparing paradigm is the notion that sustainable intensification can maintain or improve agricultural yields while using less land (Tilman et al. 2011). In SEA, governments have responded to increasing political and economic pressures to halt forest encroachment, enforce wildlife protection, and maximize carbon storage by discouraging swidden cultivation practices, particularly in protected areas (Schmidt-Vogt et al. 2009; van Vliet et al. 2012). Facing reduced access to land, many swidden farmers have adopted intensive agricultural practices to maximize yields on remaining tracts (Mertz et al. 2009). In our study basins where such policies have been enforced, fallow length has decreased or been eliminated, and some farmers have begun chemical fertilizer amendments to replace lost fertility. Government-sponsored agricultural promotions and subsidies have further favored cash crops that feature high nutrient demand (e.g., maize, cabbage, chilies). Indeed, economic motivations have driven widespread conversion of fallow lands to row crops even in the absence of land protections. These shifts undermine the benefits of land sparing policies as secondary forest is lost, previously untilled soils are cultivated, and usage of fertilizers and pesticides increases. Achieving sustainable intensification necessitates matching nutrient application rates to crop demands, but swidden farmers have little prior experience or access to extension offices to guide their use of chemicals as they shift toward intensive agricultural practices.

Applying accepted models of land use change (Allan 2004) and biogeochemical cycling (Downing et al. 1999) to our results from major SEA basins indicates that land sharing and sparing strategies may lead to dramatically different outcomes for tropical freshwater ecosystems. Low-intensity swidden systems maintain both low stream nutrient concentrations and ecosystem N:P, as N export decreases following initial clearing (Downing et al. 1999) and regular fire further reduces N availability (Toberman et al. 2014). In fallow years, soil organic carbon accumulates and vegetation regenerates, slowing runoff and enhancing groundwater recharge (Ziegler et al. 2009). Transition from swidden mosaics to blocks of spared forest and high-input agriculture will likely increase overall nutrient availability and N:P in streams (Downing et al. 1999) due to fertilizer use to replace lost soil fertility and nourish cash crops. The introduction of tilling in converted swidden landscapes, typically in high-slope areas, and the permanent reduction in ground cover leads to increased nutrient runoff to rivers, while also decreasing the ground water recharge that is critical for maintaining flows during the prolonged dry season (Ziegler et al. 2009).

Our caution regarding the benefits of land sparing strategies for river ecosystems parallels concerns about spillover effects within the terrestrial zone. Sparing land through agricultural intensification confers benefits for terrestrial biodiversity compared to land sharing strategies only for species that remain within protected areas (Phalan et al. 2011; Egan & Mortensen 2012). For example, habitat specialists with small ranges receive greater benefit from land sparing than habitat generalists that move extensively (Phalan et al. 2011). Spillover effects are unavoidable in freshwater ecosystems, where upstream–downstream connectivity transmits negative impacts through watersheds irrespective of habitat or protected area boundaries. Despite reducing the areal extent of agriculture, land sparing may substantially increase nutrient loading leading to negative spillover effects on all downstream aquatic habitats even when intensive agriculture occupies only a modest portion of the watershed. In contrast, swidden cultivation requires more space in order to allow natural nutrient regeneration processes to maintain soil fertility, but avoiding intense local application of nutrients appears to reduce the net load to rivers. Thus, even if land sparing strategies successfully protect terrestrial diversity, these benefits are unlikely to extend to aquatic organisms due to alteration of ecosystem chemistry and subsequent effects on freshwater ecosystem functioning.

Supplemental Materials

Additional descriptions of study sites (Appendix S1), physicochemical parameter measurements (Appendix S2), nutrient diffusing substrata methods (Appendix S3), limitation index standardization (Appendix S4), land use data (Appendix S5), rare earth element sample collection and analysis (Appendix S6), details of statistical analyses (Appendix 7), regression results for nutrient concentrations and land use data (Appendix S8), limitation indices by nutrient treatment and site (Appendix S9), and LME results for limitation index by nutrient treatment and river basin (Appendix S10) are available online. The authors are solely responsible for the content and functionality of these materials.

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Tables

Table 1. Physical and chemical characteristics of study sites.

Basin	River ID	River Name	Catchment Area (km^2)	% Int. Ag.	% Swidden	Discharge (m^3 sec^{-1})	Depth (m)	Water Temp. (˚C)	pH	TSS (mg L^{-1})	NO_3-N (μmol N L^{-1})	NH_4-N (μmol N L^{-1})	DIN (μmol N L^{-1})	SRP (μmol P L^{-1})	DIN:SRP (Molar)
Salween	S1	Malit*	1342.7	5.61	27.59	6.53	0.53	21.5 (1.3)	8.5	6.8	0.63	0.12	0.75	0.36	2.07
	S2	Ngao*	931.6	1.10	27.00	12.54	0.41	22.6 (1.1)	8.1	7.2	0.42	0.23	0.64	0.22	2.97
	S3	Yuam*	2601.5	4.56	11.85	4.86	0.35	24.5 (2.1)	8.2	11.8	0.39	2.39	2.85	0.17	17.12
	S4	Salween	262,700.0	-	-	-	-	-	-	9.5	10.25	0.12	10.37	0.08	130.15
	S5	Moei	11,009.7	13.24	12.34	-	-	-	-	28.1	0.97	0.23	1.20	0.05	23.63
	S6	Louie	60.7	0.05	43.40	0.42	0.24	19.8 (1.2)	-	-	2.35	0.31	2.66	0.72	3.69
Chao Phraya	C1	Ping*	769.4	16.00	2.56	1.93	0.43	25.3 (2.1)	7.9	6.0	11.31	0.62	11.93	0.13	91.30
	C2	Yom*	3287.0	15.09	4.24	7.71	0.57	27.2 (0.7)	8.2	22.8	8.93	1.11	10.04	0.23	43.58
	C3	Wang*	3361.2	13.64	3.34	2.29	0.22	28.0 (2.0)	8.0	12.3	7.40	2.72	10.13	0.41	24.43
	C4	Nan	2196.2	10.17	14.13	7.24	0.56	27.0 (1.6)	-	6.6	0.17	0.00	0.17	0.09	1.94
Mekong	M1	Lao	1288.3	38.60	2.49	4.08	0.84	26.9 (1.8)	-	16.1	14.69	0.86	15.55	0.11	147.06

Int. Ag.: intensive agriculture; TSS: total suspended solids; NO_3-N: nitrate; NH_4-N: ammonium; SRP: soluble reactive phosphorus; DIN: dissolved inorganic nitrogen equals the sum of NH_4-N and mean of NO_3-N; DIN:SRP is DIN divided by mean SRP by site. Parameter measurement methods are located in the Supplemental Materials.

* Denotes sites from which nutrient diffusing substrata (NDS) were recovered.

Table 2. Two-factor ANOVA results of chlorophyll *a* accrual on NDS.

Basin	River ID	Days	Treatment			Limitation Status
			N	**P**	**NP**	
			F, p-value	*F*, p-value	*F*, p-value	
Chao Phraya	C1	17	16.23, 6.6x10^{-4}	0.16, 0.70	30.71, 2.0x10^{-5} *	NP
	C2	16	7.89, 0.01	7.36, 0.01	9.45, 6.0x10^{-3} *	NP
	C3	15	24.27, 8.1x10^{-5} *	2.32, 0.14	8.47, 8.7x10^{-3} *	1° N, 2° P
Salween	S1	12	54.16, 4.1x10^{-7} *	14.19, 0.0012	*3.15*, 0.09	N
	S2	8	29.39, 2.6x10^{-5} *	5.73, 0.027	*0.44*, 0.51	N
	S3	8	20.37, 2.1x10^{-4} *	7.20, 0.014	*0.012*, 0.92	N

* Denotes significant ANOVA results for treatments having higher mean chl *a* relative to control treatments. Limitation status corresponds to interpretation of ANOVA results given in Tank and Dodds (2003).

Figure Captions

Figure 1. Map of study sites with regional map inset. Study basins from which NDS were recovered are shaded in light gray and agricultural land cover classes are depicted with pie charts. Site labels are by major river basin: C1-Ping; C2-Yom; C3-Wang; C4-Nan; M1-Lao; S1-Malit; S2-Ngao; S3-Yuam; S4-Salween; S5-Moei; S6-Louie.

Figure 2. Algal response (chl *a*) to nutrient enrichment in six tributaries of the Chao Phraya (top) and Salween Rivers (bottom). Bars are treatment means (± SE; n = 6). Letters above bars indicate statistically significant groupings (p-value < 0.05) based on Tukey's HSD method following two-way ANOVA. Nutrient treatments: C (white bars) = unamended control, N (light grey bars) = nitrogen (0.5 M KNO_3), P (dark grey bars) = phosphorus (0.5 M KH_2PO_4), NP (black bars) = nitrogen + phosphorus (0.5 M KNO_3 + 0.5 M KH_2PO_4).

Figure 3. Univariate regression results of nitrogen limitation index (N Lim Index) to nutrient data (a, b, c) and % intensive agriculture (d), % swidden (e), and population density (f) with site as a random blocking term. Filled circles are means for Chao Phraya sites, open circles are means for Salween sites, and error bars represent ±SE. Gray points are observed values. R^2c values are conditional coefficients of determination for mixed models. Site identifiers follow Table 1.

Figure 4. Mean ratio (±SE) of REE concentrations from (a) suspended solids (REE_{SS}) and (b) water (REE_{WAT}) samples to Post-Archean Australian Shale (REE_{PAAS}) from the Chao Phraya, Mekong, and Salween Basins. Note that water values are multiplied by 10^6 as REE_{WAT}

concentrations are much lower than REE_{SS}. REE are ordered by increasing atomic number; LREE = light REE, MREE = medium REE, and HREE = heavy REE.

Figure 5. Non-metric multidimensional scaling (NMDS) plots for ordination axes 1 and 2 for Chao Phraya (closed circles) and Salween (open circles) sites derived from rare earth elements (REE) concentrations in suspended solid (a) and water samples (b). NMDS is an ordination technique we used to generate synthetic axes (NMDS axis 1 and 2) for assessing similarities among sites based on REE concentrations. Nutrient data are displayed as environmental predictors of NMDS ordination scores; vector length corresponds to correlation strength. Site numbers as in Fig. 1.

Figure 1.

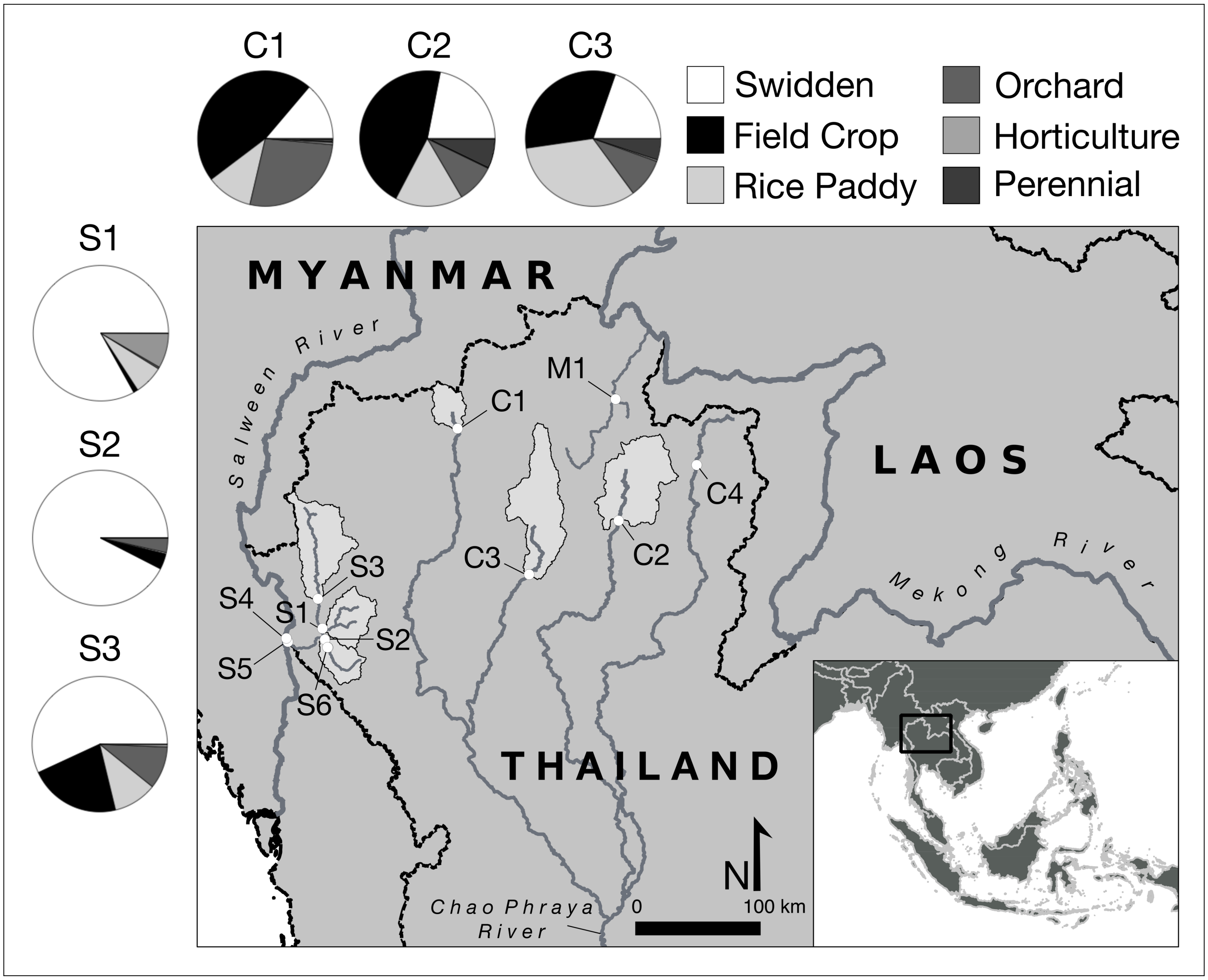

C1
C2
C3
Swidden
Field Crop
Rice Paddy
Orchard
Horticulture
Perennial
S1
S2
S3
MYANMAR
LAOS
THAILAND
Salween River
Mekong River
Chao Phraya River
M1
C1
C2
C3
C4
S1
S2
S3
S4
S5
S6
N
0
100 km

Figure 2.

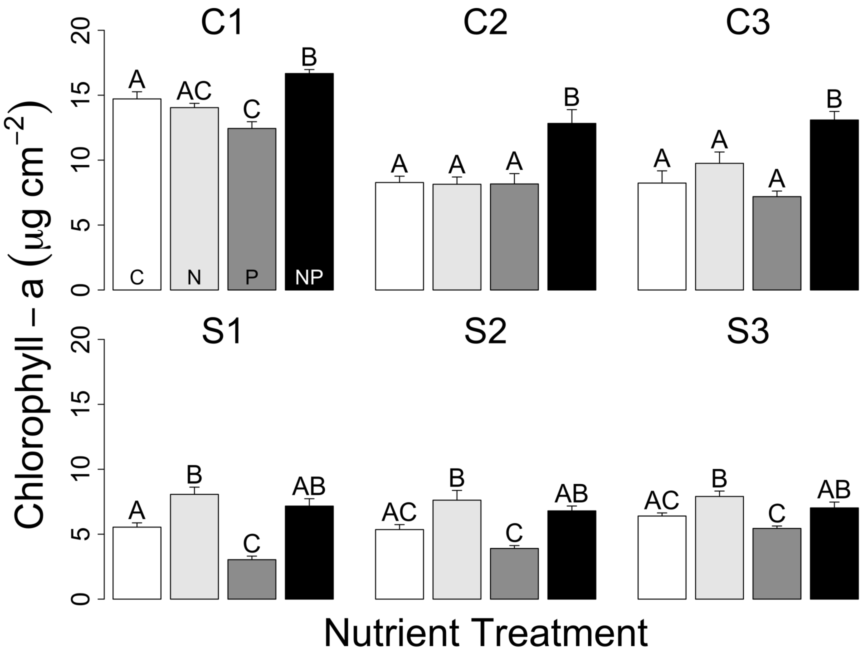

C1
C2
C3
S1
S2
S3
Chlorophyll – a (μg cm^{-2})
Nutrient Treatment
0
5
10
15
20
C
N
P
NP
A
AC
C
B
A
A
A
B
A
A
A
B
A
B
C
AB
AC
B
C
AB
AC
B
C
AB

Figure 3.

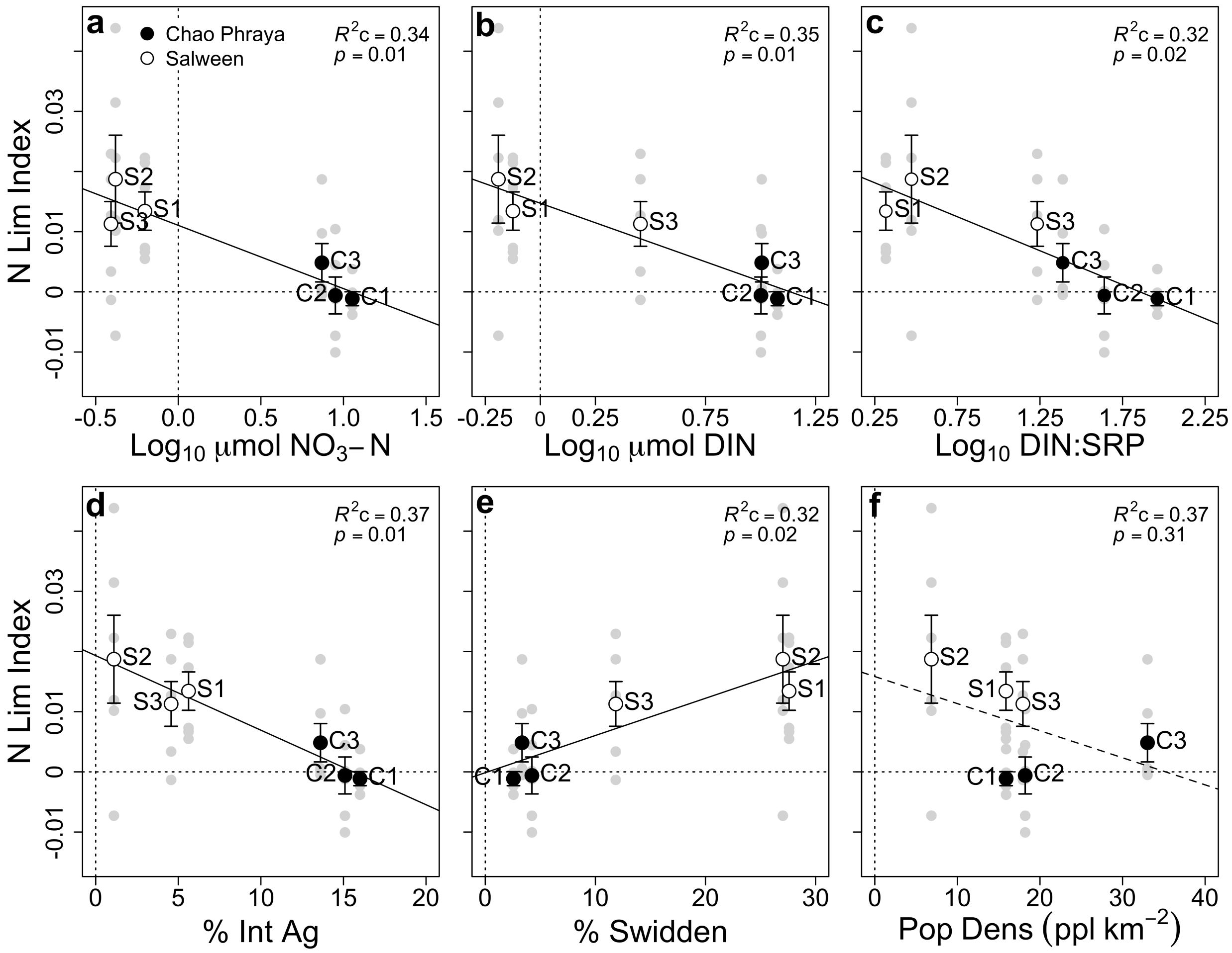

a
● Chao Phraya
○ Salween
R2c = 0.34
p = 0.01
b
R2c = 0.35
p = 0.01
c
R2c = 0.32
p = 0.02
d
R2c = 0.37
p = 0.01
e
R2c = 0.32
p = 0.02
f
R2c = 0.37
p = 0.31
N Lim Index
S1
S2
S3
C1
C2
C3
Log10 μmol NO3− N
Log10 μmol DIN
Log10 DIN:SRP
% Int Ag
% Swidden
Pop Dens (ppl km−2)
-0.01
0
0.01
0.03
-0.5
0.0
0.5
1.0
1.5
-0.25
0
0.25
0.75
1.25
0.75
1.25
1.75
2.25
0
5
10
15
20
30
40

Figure 4.

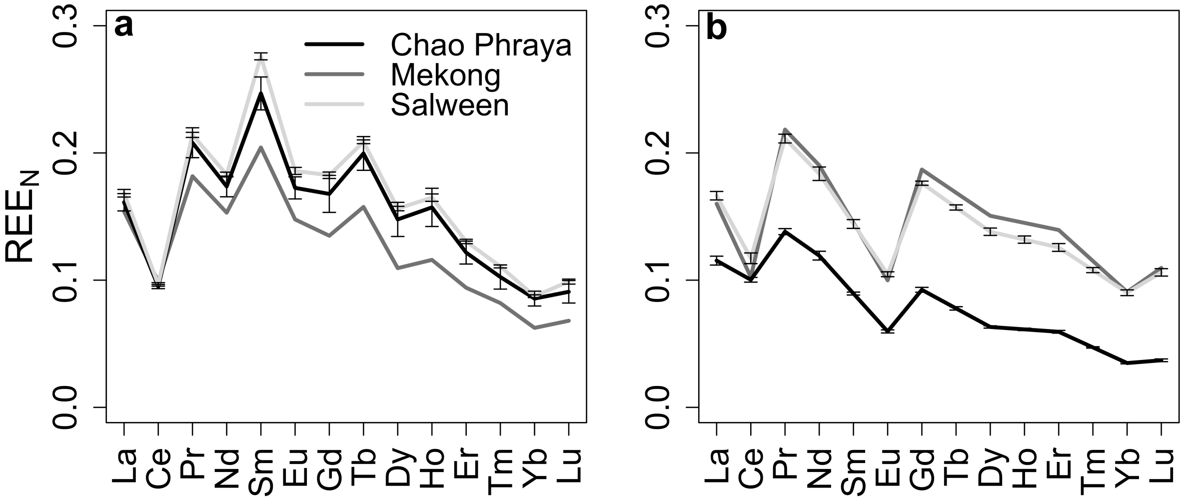
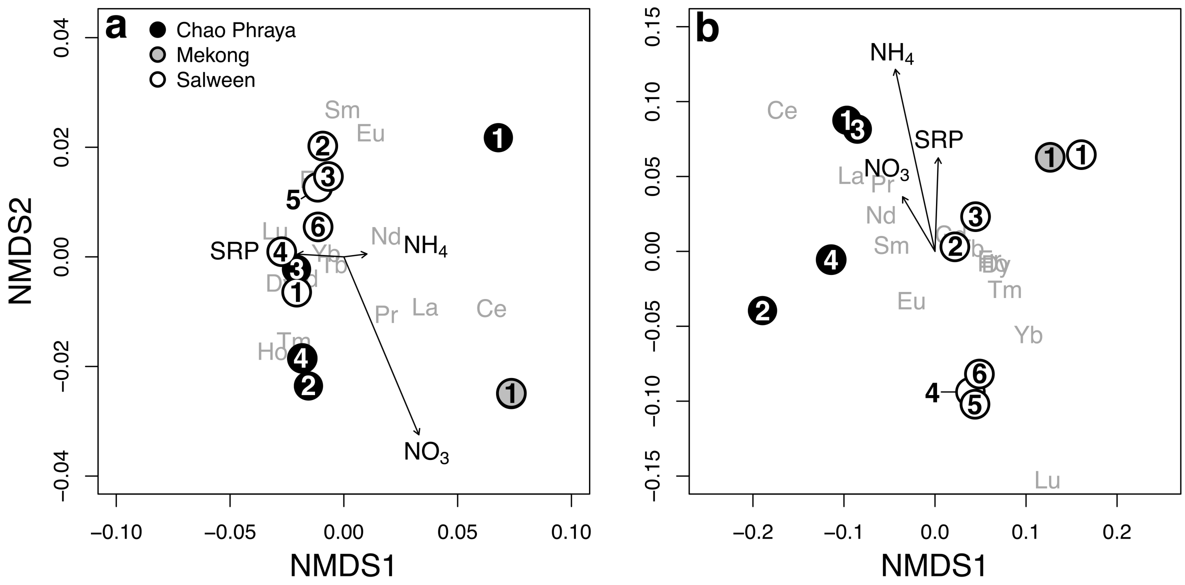

a
b
Chao Phraya
Mekong
Salween
REE_N
0.0
0.1
0.2
0.3
La
Ce
Pr
Nd
Sm
Eu
Gd
Tb
Dy
Ho
Er
Tm
Yb
Lu

Figure 5.


a
● Chao Phraya
● Mekong
○ Salween
Sm
Eu
Nd
NH4
SRP
Lu
Yb
Tb
Pr
La
Ce
Tm
Ho
NO3
NMDS1
NMDS2
0.04
0.02
0.00
−0.02
−0.04
−0.10
−0.05
0.00
0.05
0.10
b
NH4
Ce
SRP
NO3
La
Pr
Nd
Sm
Eu
Tm
Yb
Lu
0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
−0.2
−0.1
0.0
0.1
0.2
NMDS1

Supporting Information

Appendix S1. Site description details

Northern Thailand is mountainous with steep river valleys and many peaks exceeding 2000 m in elevation (Ridd et al., 2011a). The underlying geology of study river basins is the result of a complicated tectonic history, but is largely comprised of two tectonic blocks creating an overall north to south orientation of most river valleys (Ridd et al. 2011). Bedrock ages range from Devonian (~410 Mya) to a few million years old (Morley and Racey 2011, Ridd et al. 2011). Study basins contain silica-rich igneous (granite and acid volcanic) and metamorphic bedrock as well as sedimentary bedrock, including common and widespread occurrences of limestone and other carbonate-containing units (Ridd et al. 2011, Hartmann and Moosdorf 2012).

Northern Thailand experiences a monsoonal climate, having a wet season from May through October, and little to no rainfall from November through April. The dominant vegetation type in the region was historically deciduous dry forest, with swidden agriculture occurring in upland areas for centuries or millennia (Fox & Vogler, 2005) and extensive clearing of lowland forests for agriculture beginning around 1930 (Ramankutty & Foley, 1999). Many smaller streams stop flowing for part of the dry season, while larger rivers experience a single-peaked annual hydrograph with a gradual rise toward maximum flows in August–September and a more rapid descent to minimum flows in April–May. Our study was conducted near base flows during dry season (January-March 2013).

Appendix S2. Measurement of Physicochemical Parameters

Discharge and depth were measured once at each site during the study period following standard sampling protocols (Hauer & Lamberti, 2007). Total suspended solids (TSS) and pH were measured once using precombusted 47 mm ProWeigh glass fiber filters and an Oakton handheld meter, respectively. Water temperature was measured simultaneously at sites within each major basin at 5-minute intervals for 5-13 days using Hobo pendant temperature loggers. We collected water samples to measure ambient nitrate (NO_3-N) and soluble reactive phosphorus (SRP) during both the deployment and retrieval of nutrient diffusing substrata (NDS). Water samples were filtered (Whatman GD/X; 0.45 μm pore size), stored on ice in the field, and kept frozen until analysis by standard colorimetric methods. Ammonium (NH_4-N) concentrations were measured once at each site using the fluorometric method of Taylor et al. (2007) following sample refrigeration for < 48 hours.

Appendix S3. Nutrient Diffusing Substrate Methods

We followed the widely used nutrient diffusing substrata (NDS) protocol used by Tank et al. (2007). In this method, a mixture of agar and nutrient amendment is set into 30mL plastic cups that allow diffusion of nutrients through a fritted glass disc, which serves as the substrate for algal growth. At each site, we installed six randomized complete blocks of four treatments: nitrogen amendment (N; 0.5 M KNO_3), phosphorus amendment (P; 0.5 M KH_2PO_4), nitrogen plus phosphorus amendment (NP; 0.5M KNO_3 + 0.5M KH_2PO_4), and a control lacking nutrient addition (C). NDS were placed in shallow (< 50 cm), well-mixed, open-canopy reaches. Algal accrual on fritted glass discs was measured using area-specific chlorophyll *a* concentration (μg chl *a* cm^{-2}) determined in the field using a Turner Aquafluor following 24-hour extraction in cold 90% pH-buffered ethanol.

Appendix S4. Limitation index calculation

We standardized the effects of nutrient enrichment across treatments and sites using the limitation index proposed by Osenberg and Mittelbach (1996) for nutrient enrichment experiments across ecosystem types:

$$L = [\log_{10}(C_t{}') - \log_{10}(C_t)]/t$$

where L is the limitation index, $C_t{}'$ is the chl *a* concentration of each nutrient amended treatment after *t* days, C_t is the chl *a* concentration of the corresponding within-block control treatment after *t* days, and *t* is the duration of the experiment in days. Prior to standardization, chl *a* concentrations were strongly related to duration of deployment ($F = 114.8$, $df = 1, 142$, $p < 0.001$), but time-standardization eliminated this dependence ($F = 0.20$, $df = 1, 142$, $p = 0.65$) to enable comparisons across all study sites.

Appendix S5. Land use data for sites within the Chao Phraya, Salween, and Mekong River Basins.

	C1-Ping		C2-Yom		C3-Wang		S1-Malit		S2-Ngao		S3-Yuam	
Land Cover Type	km^2	%	km^2	%	km^2	%	km^2	%	km^2	%	km^2	%
Old Growth Forest	604.4	78.60	2484.0	75.57	2594.1	77.18	859.6	64.01	652.4	70.03	2134.6	82.05
Secondary Forest	3.6	0.47	98.3	2.99	25.7	0.76	22.3	1.66	11.7	1.25	1.6	0.06
Swidden Cultivation*	19.7	2.56	139.2	4.24	112.3	3.34	370.5	27.59	251.8	27.0	308.3	11.85
Intensive Agriculture*	122.8	16.00	496.1	15.09	457.5	13.64	75.5	5.61	10.3	1.10	118.9	4.56
Field Crop	66.4	8.63	288.3	8.77	186.3	5.54	4.9	0.36	0.8	0.09	56.5	2.17
Rice Paddy	15.8	2.05	102.7	3.12	187.2	5.57	31.3	2.33	9.4	1.01	54.9	2.11
Orchard	38.6	5.05	59.4	1.81	52.8	1.60	1.9	0.14	0.0	0.00	3.2	0.12
Horticulture	0.7	0.10	0.1	0.00	2.4	0.07	37.0	2.75	0.0	0.00	0.6	0.02
Perennial	1.3	0.17	45.6	1.39	28.8	0.86	0.4	0.03	0.0	0.00	3.7	0.14
Pasture/Farm House	0.1	0.01	0.5	0.02	0.5	0.01	0.1	0.01	0.0	0.00	0.1	0.00
Wetland	0.5	0.07	0.08	0.00	7.6	0.23	0.2	0.02	0.3	0.03	4.7	0.18
Water Body	1.4	0.18	10.4	0.32	33.0	0.98	2.6	0.19	1.7	0.18	6.1	0.23
Aquaculture	0.1	0.01	0.0	0.00	0.3	0.01	0.0	0.00	0.0	0.00	0.0	0.00
Aquatic Plant	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0	0
Urban	11.3	1.47	34.1	1.04	91.1	2.71	8.6	0.64	3.3	0.35	21.3	0.82
Misc.	4.8	0.63	24.4	0.74	39.2	1.17	3.4	0.26	0.3	0.03	6.0	0.23
TOTAL	769.0	100	3287.1	100	3361.3	100	1342.8	100	931.8	100	2601.6	100
Population Density * (people km^{-2})	15.90		18.21		33.02		15.88		6.85		17.93	

Intensive agriculture is the sum of indented land use categories.

* Denotes variables included in statistical models.

Appendix S5 Continued. Land use data for sites within the Chao Phraya (C), Salween (S), and Mekong (M) River Basins.

	M1-Lao		C4-Nan		S5-Moei		S6-Louie	
Land Cover Type	km^2	%	km^2	%	km^2	%	km^2	%
Old Growth Forest	657.1	51.00	1584.6	72.15	7765.1	70.53	30.0	49.42
Secondary Forest	27.3	2.12	17.5	0.80	174.8	1.59	4.3	7.04
Swidden Cultivation*	32.1	2.49	310.2	14.13	1359.0	12.34	26.3	43.40
Intensive Agriculture*	497.2	38.60	223.2	10.17	1456.8	13.24	0.03	0.05
Field Crop	164.6	12.78	88.6	4.04	986.3	8.96	0.0	0.00
Rice Paddy	188.2	14.61	83.2	3.79	332.0	3.02	0.03	0.05
Orchard	67.1	5.21	42.8	1.95	34.6	0.31	0.0	0.00
Horticulture	1.1	0.08	0.0	0.00	45.9	0.42	0.0	0.00
Perennial	76.2	5.92	8.6	0.39	58.0	0.53	0.0	0.00
Pasture/Farm House	0.5	0.04	0.4	0.02	0.3	0.00	0.0	0.00
Wetland	0.1	0.01	0.2	0.01	11.28	0.1	0.0	0.00
Water Body	7.4	0.57	7.62	0.35	41.0	0.37	0.0	0.00
Aquaculture	1.1	0.09	0.0	0.00	0.27	0.00	0.0	0.00
Aquatic Plant	0.01	0.00	0.0	0.00	0.0	0.00	0.0	0.00
Urban	48.9	3.79	35.9	1.63	171.1	1.55	0.05	0.09
Misc.	16.7	1.30	16.63	0.76	28.9	0.26	0.0	0.00
TOTAL	1288.3	100.0	2196.2	100.0	11,009.7	100.0	60.68	100.0
Population Density* (people km^{-2})	64.40		40.97		30.39		3.40	

Intensive agriculture is the sum of indented land use categories.
* Denotes variables included in statistical models.

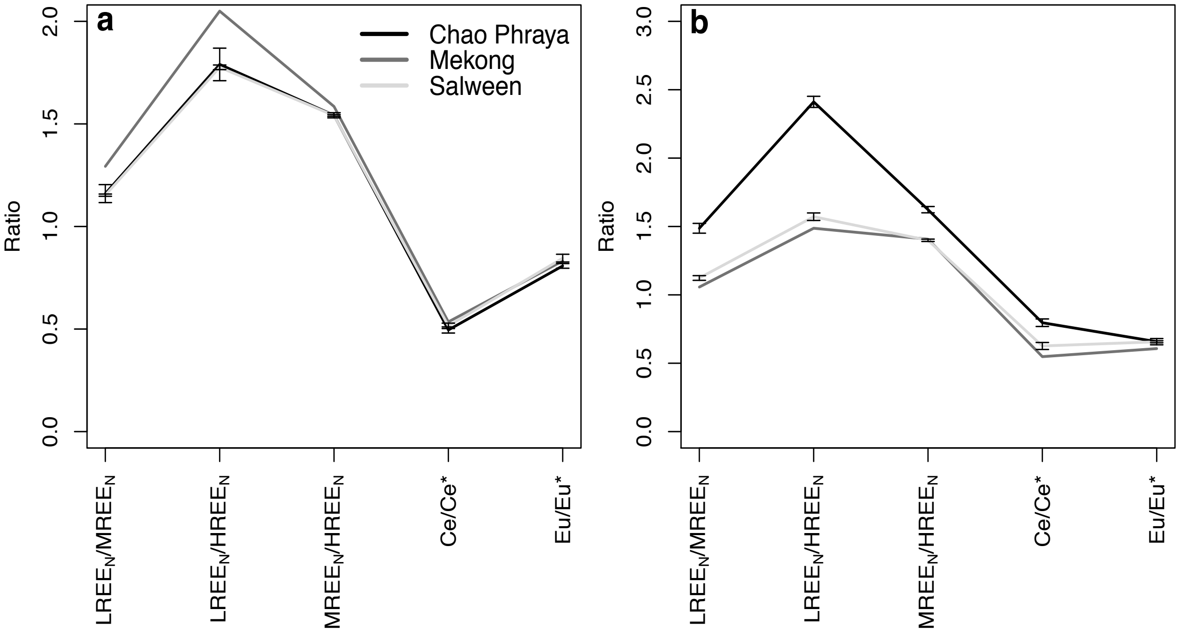
Appendix S6. Rare earth element (REE) sample collection and analysis

We collected water and suspended sediment samples once at each study site. Water samples for dissolved REE analysis were filtered (Whatman GD/X; 0.45 μm pore size), stored frozen, and acidified to pH 2 using ultrapure HNO_3. Suspended solid samples were collected on pre-combusted ProWeigh 47mm glass fiber filters, stored frozen, dried for 48 hours at 60˚C prior, and digested with ultrapure HNO_3 prior to analysis. Water and suspended solids samples were analyzed at the University of Massachusetts–Boston on a Perkin Elmer Sciex DRCII dynamic-reaction cell inductively-coupled plasma mass spectrometer. The instrument settings for all sample analyses were as follows: RF power was 1100 volts (V); nebulizer gas flow was 0.93 L/min; plasma gas flow was 15L/min; sample and skimmer cones were nickel; pulse stage was 950 V; analog stage was -1850 V; lens voltage was 6.75; 3 replicates were analyzed with 10 sweeps per reading; 1 MCA channel; dwell time per amu was 50 milliseconds (ms); integration time was 4000 ms; and we used peak hopping mode. Calibration standards and United States Geological Survey MAG-1 were analyzed every sixth sample.

We normalized data to Post-Archean Australian Shale (McLennan, 1989) prior to making comparisons among the following REE groups: light REEs (LREEs, average of La, Pr, Nd, and Sm), medium REEs (MREEs, average of Gd, Tb, and Dy), and heavy REES (HREEs, average of Ho, Er, Tm, Yb, and Lu) following Noack et al. (2014). Cerium (Ce) and europium (Eu) anomalies were calculated following Leybourne and Johannesson (2008): $Ce/Ce^* = Ce_N/(La_N \times Pr_N)^{0.5}$ and $Eu/Eu^* = Eu_N/(Sm_N \times Gd_N)^{0.5}$ with values of <1 constituting negative anomalies and values >1 constituting positive anomalies.

REE were depleted in all three major river basins relative to PAAS (Fig. 4). LREEs showed similar enrichment patterns relative to MREEs and HREEs in suspended sediment samples from

Salween and Chao Phraya rivers (see below panel a), however, LREE enrichment in water samples was significantly higher in Chao Phraya tributaries (panel b). All samples showed large negative anomalies for both Ce (Ce/Ce* = ~0.5) and Eu (0.72 – 0.75). The consistency of these patterns indicates that bedrock types contributing suspended particles are similar across basins, but suggests anthropogenic alteration of water chemistry.



Fractionation profile of PAAS-normalized REE concentrations for suspended solids (**a**) and water samples (**b**). LREE to MREE, LREE to HREE, MREE to HREE ratios were calculated following Noack et al. (2014), and cerium (Ce) and europium (Eu) anomalies calculated following Leybourne & Johannesson (2008).

Appendix S7: Details of Statistical Analysis

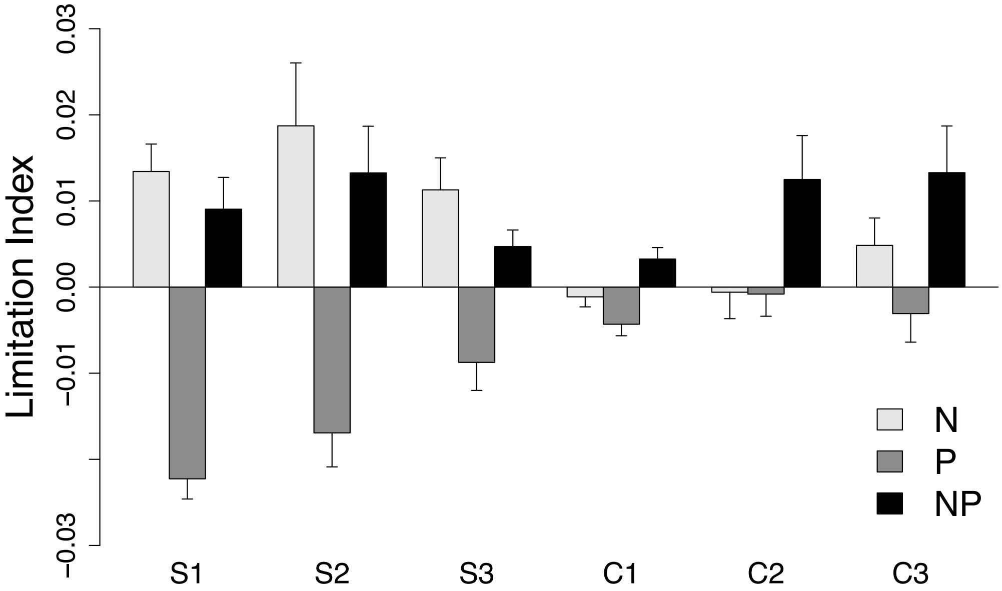
All statistical analyses were conducted in the R statistical language (R Core Team 2015). LME regressions were conducted using the *lme4* statistical package (Bates et al., 2014). Significance values were calculated using bootstrapped likelihood ratio *F*-tests with Satterthwaite approximated degrees of freedom comparing the fit of each model against the null model containing only the random term, site (Halekoh & Højsgaard, 2014). Model fits were evaluated using the conditional coefficient of determination (R^2c; Johnson 2014) for random slopes models implemented in the MuMIn package (Bartón, 2014). All REE statistical analyses were performed using the *vegan* package (Oksanen et al., 2013).

Appendix S8: Model results for multiple linear regression of river water nutrient concentrations by intensive agriculture, swidden, and population density

Nutrient	Intensive Agriculture			Swidden			Population Density			Full Model		
	β_{std}	t	p	β_{std}	t	p	β_{std}	t	p	F	p	$Adj\ R^2$
NO_3-N	1.603	3.74	<0.01	-0.051	-0.18	0.86	-1.154	-3.03	<0.05	6.76	<0.05	0.66
NH_4-N	-0.209	-0.29	0.78	-0.767	-1.63	0.15	-0.166	-0.26	0.81	1.02	0.45	0.01
DIN	1.387	2.88	0.03	-0.249	-0.80	0.45	-1.153	-2.69	<0.05	4.91	<0.05	0.57
SRP	0.340	0.49	0.64	0.440	0.98	0.37	-0.567	-0.91	0.40	1.28	0.37	0.08
DIN:SRP	1.151	3.70	<0.01	-0.456	-2.28	0.06	-0.814	-2.95	<0.05	14.64	<0.005	0.82

All nutrient values were $\log_{10}$ transformed, NH_4 was $\log_{10}(NH_4+1)$ to account for values of zero. β_{std} denotes standardized regression coefficients.

Appendix S9. Limitation index of nutrient treatments by sites



Limitation index of nutrient treatments relative to control and standardized to deployment duration (days). Bars are site means ±1 SE (n = 6).

Appendix S10. LME results for limitation index for the model containing fixed N, P, and Basin effects and a random blocking term, site

Random Effect	Variance	Std. Dev.			
Site	1.899×10^{-6}	0.001378			
Residual	5.695×10^{-5}	0.007546			
Fixed Effects	*Estimate*	*Std. Error*	*df*	*t*	*p*
N	0.00104	2.515×10^{-3}	130.3	0.41	0.68
P	-0.00274	2.515×10^{-3}	130.3	-1.09	0.28
Basin	-2.7×10^{-17}	2.756×10^{-3}	26.2	0.00	1.00
N × P	0.01138	3.557×10^{-3}	130.3	3.19	0.0002*
N × Basin	0.01344	3.557×10^{-3}	130.3	3.78	0.0002*
P × Basin	-0.01324	3.557×10^{-3}	130.3	-3.72	0.0003*
N × P × Basin	-8.7×10^{-4}	5.031×10^{-3}	130.3	-0.17	0.86

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Chapter 2

Grassroots reserves protect tropical river food webs

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Abstract: Intensive fishing is altering the functioning of aquatic ecosystems worldwide, threatening both biodiversity and food security. Conservation reserves are a cornerstone of ecosystem-based fisheries management in the world's oceans and have provided unique insights into food web responses to the removal large species. To evaluate their potential value in freshwaters, we used experimental manipulations within small, community-initiated reserves to test the cascading effects of fishing on trophic dynamics in a tropical river. Protection from harvest profoundly reconfigured fish communities—increasing richness, biomass, and body size—yet did not lead to higher mean trophic position or cascading effects on primary producers. Remarkably, omnivory and species richness appear to buffer trophic dynamics against even extreme fishing pressure. The effectiveness of these individual reserves is surprising given their small size, recent creation, and community enforcement. Potential synergistic benefits of a network of such community reserves could offer a new conservation model for protecting food webs and augmenting fishery yields in biodiverse tropical rivers.

Significance Statement: The success of the marine protected area paradigm is widely heralded, but its transferability to imperiled freshwater fisheries is uncertain. In Southeast Asia, local communities have established riverine reserves of their own accord. These reserves have been created in isolation rather than through systematic planning, yet we find strong ecological responses that have spillover benefits for fisheries. Our experimental manipulations and fish community profiles revealed two distinct cascading feeding interaction in this species-rich tropical river. Densities of large fish were up to 9 times higher and biomass of all fish was 13 times higher on average in reserves. These strong fish community responses suggest that riverine reserves may present a new paradigm for conservation of important freshwater biodiversity.

Keywords: inland fisheries, tropical river, trophic cascade, spatial protection, riverine reserve

Main Text: Overharvest of fisheries threatens biodiversity and food security in aquatic ecosystems worldwide (FAO 2016). Widespread fishery declines have led to the development of ecosystem-based management strategies, which aim to enhance harvest sustainability by ensuring ecosystem resilience (Pikitch et al. 2004, Halpern et al. 2010). Marine protected areas are central to ecosystem-based management of coastal oceans; they have led to increased fish density, biomass, and species richness within their boundaries (Lester et al. 2009) that can spill over to augment fisheries in adjacent unprotected areas (Gell and Roberts 2003). No-take reserves also offset selective depletion of large, predatory fishes (Micheli et al. 2004), thereby re-establishing important trophic interactions that shape community assembly and ecosystem processes (Mumby et al. 2006, Lester et al. 2009).

Despite their success in marine ecosystems, fishery reserves remain rare in freshwaters everywhere except Southeast Asia (SEA; Abell et al. 2007). Across SEA, hundreds of no-take areas along rivers have been designated and enforced by local communities in response to declining subsistence catches (Baird and Flaherty 2005). These inland fisheries provide critical food security to poor and under-nourished populations (McIntyre et al. 2016); annual harvest from the Lower Mekong Basin alone is estimated to be 2.2 million tons, providing the major source of animal protein for over 70 million people (Hortle 2009). The sustainability of such heavy exploitation is questionable (Allan et al. 2005), and the proliferation of riverine reserves throughout SEA indicates that communities are prepared to take substantive action to protect this critical resource. Yet principles of reserve design in oceanic and terrestrial ecosystems (Gaines et al. 2010) call into question the effectiveness of such small reserves for protecting fish and fisheries.

Conservation reserves have also provided critical insights into the cascading influence of predatory fishes in marine ecosystems, revealing that fishery benefits must be understood in a food web context (Mumby et al. 2006). The consequences of overharvest for trophic dynamics are less well known for freshwaters, particularly biodiverse tropical rivers and lakes (Allan et al. 2005). Theory suggests that high species richness and rates of omnivory among tropical fishes may buffer key ecosystem functions from intense harvest, while also reducing the strength of top-down trophic interactions (Polis et al. 2000, Bascompte et al. 2005). Empirical tests of these predictions have proven challenging for inland fisheries but are an essential part of understanding whether community-initiated reserves offer a viable model for protecting fish and food security in SEA and other productive tropical freshwater fisheries.

Here, we analyze existing riverine reserves in Thailand as a manipulation of the top predator (humans), then use multi-scale experiments to measure the cascading effects of intensive fishing on the food web that supports fishery productivity. We compared fish diversity, abundance, and biomass within two small reserves (< 1 km in length) to adjacent upstream and downstream reaches in a tributary of the Salween River, the largest free-flowing river remaining in Asia. Overlaid upon stark differences in fishing pressure created by no-take reserves, we isolated consumptive and non-consumptive effects of fish on benthic macroinvertebrates and algae using exclosures (Fig 1A). Within each exclosure, we tested the strength of top-down versus bottom-up effects on primary producers by experimentally manipulating nutrient availability to benthic algae. These hierarchical manipulations provide a strong test of the effects of intensive harvest on fish assemblage and trophic structure, the cascading effects on macroinvertebrates and algae, and the potential for riverine reserves to benefit both ecosystems and fisheries.

Results & Discussion

Reserve protection resulted in comprehensive shifts in fish assemblage structure and biomass, and also revealed the presence of a size-based trophic cascade resulting from harvest (Fig 1B). Protection was highly effective (Fig. 1C), but disproportionately benefitted large-bodied species (max. length > 15 cm), which achieved densities 5–9 times higher than in harvested areas (Fig 1D). Conversely, small (max. length ≤ 15 cm), lower-value fishes had 50–70% higher densities in heavily-fished areas around reserves

(Fig 1E). Although protection led to only moderate increases in aggregate fish density, reserves held over 10-fold higher fish biomass than harvested areas (Fig. 2A). Reserve assemblages were dominated by large-bodied *Hypsibarbus*, *Tor*, and *Neolissochilus* species that are prized by local communities, in contrast to the dominance of nemacheiline loaches, glass perch (*Parambassis vollmeri*), and spiny eels (*Mastacembelus armatus*) in unprotected reaches (Fig. 3B). The opposing shifts in densities of large- versus small-bodied fish densities across reserve boundaries—despite active harvest of all sizes and species of fish outside reserves—indicates that removing large fish triggers cascading effects on small fish that outweigh the direct removal of small fish through fishing (Fig 1B).

Experimental exclosures revealed a second cascade extending from fish to macroinvertebrates and algal biomass, and this pattern was unaffected by reserve protection. In fishless exclosures, macroinvertebrate densities were higher (two-way ANOVA, $F = 5.06$, $p = 0.036$; Fig. 1F), and algal accrual was lower on rocks (Fig. 1G) and nutrient-diffusing tile substrates (Fig. 1H). Both macroinvertebrate densities and algal accrual on tiles were comparable within and outside reserve zones, but algal accrual on natural rock substrates showed an interaction of protection and exclosure treatment (two-way ANOVA, $F = 3.99$, $p = 0.033$). While the qualitative pattern of fish–macroinvertebrate–algae cascade dynamics was unchanged (Fig. 1B), rocks exposed to fish in upstream and downstream zones had higher algal biomass than those in reserves, which were comparable to exclosure treatments in all zones (Fig. 1G). This interaction likely reflects the presence of additional non-consumptive effects outside reserves (McIntosh et al. 2002, Schmitz et al. 2004; *Supplementary Information*). Finally, on nutrient-diffusing tiles, algal biomass consistently increased with nutrients (three-way ANOVA, $F = 151.45$, $p < 0.001$) and decreased when fish were excluded ($F = 43.00$, $p < 0.001$), regardless of fishing (Fig 1H). However, the effect of providing algae with the growth-limiting nutrient (Hedge's $d_N = 0.75$; Gurevitch et al. 2000) was less than that of exposing macroinvertebrate grazers to fish ($d_G = 1.25$; *Supplementary Information*), indicating the primacy of top-down control of energy flow exerted by fish in this system.

The decoupling between the fishery-induced cascade, which reconfigured fish community size structure, and the background cascading effects of fish upon primary producers in this tropical river is remarkable (Fig. 1). Within the lower cascade, we observed comparably strong top-down effects in reserves and the depauperate reaches upstream and downstream (Fig. 1B), reflecting the omnivorous trophic strategy of many fishes in this food web, including large-bodied species (Fig. 3A). The ubiquity of the fish-invertebrate-algae cascade contrasts with the lack of detectable trophic cascades in previous tropical freshwater studies (Flecker 1996, Pringle and Hamazaki 1998), which has been interpreted as evidence that abundant omnivory and species richness preclude strong cascading interactions in the tropics (Shurin et al. 2010). Thus, our results present an apparent paradox: a fish assemblage characterized by widespread omnivory, which is associated with prevalence of weak species interactions (Gellner and McCann 2012), clearly exhibits cascade-type dynamics that are expected to arise from fewer, stronger species interactions (Polis et al. 2000). Whereas omnivores in previous studies show morphological adaptations for feeding effectively as both primary and secondary consumers (Flecker 1996, Pringle and Hamazaki 1998), the large-bodied omnivores that dominated reserve zones in our study river lack morphological specialization yet eat algae, invertebrates, and fish (Froese and Pauly 2017). Omnivorous fishes in reserves apparently fed preferentially on nutritious animal prey rather than algae, thereby reducing the abundance of macroinvertebrates (Fig 1F) and small invertivorous fishes (Fig 1E). In this way, strong trophic interactions emerged in reserves despite rampant omnivory and high species richness, giving rise to the strong, fish-driven cascade dynamics observed both inside and outside of reserves (Fig 1B).

The similar magnitude of top-down control of algal accrual in both depauperate fished zones and species-rich reserves challenges the common assumption that no-take reserves invariably enhance ecosystem functioning (Pikitch et al. 2004, Halpern et al. 2010). Outside reserves, high abundances of three specialist taxa (loaches, glass perch, spiny eels; 73% of observed fish) maintained cascade effects comparable to those of the more trophically diverse reserve fish assemblage that contained higher abundance and biomass of grazing fishes and piscivores (Fig 3). Despite the persistence of basic trophic dynamics, the reduced species richness and resulting increased interaction strengths within harvested fish assemblages may ultimately undermine long-term stability (McCann et al. 1998). Furthermore, the trophic plasticity of large-bodied omnivores, in contrast to more specialized invertivores, allows them to shift from being functionally secondary and tertiary consumers during the dry season to consuming terrestrial plants when increased turbidity and disturbance during the wet season reduce in-stream productivity (Correa and Winemiller 2014). Thus, while protection did not alter the lower cascade pattern, reserves may still enhance broader ecosystem resilience by conserving the functional diversity of fishes within their boundaries.

The disparity between fish assemblages separated by only an invisible boundary of fishing pressure supports the notion that humans act as super-predators (Darimont et al. 2015), having disproportionate impact on fish species with one key functional trait: large size. During field surveys, we regularly observed fish approaching 1 meter in length within reserves, but only 4 of 2,410 fish observed in harvested areas exceeded 40 cm. Unlike true "fishing-down" of the food web, the relative effect of harvest on fish density did not increase with trophic position, a second important functional trait often assumed to correlate with size. In fact, herbivore and omnivore densities responded most strongly to reserve protection (Fig. 3B). This surprising result is due to the lack of correlation between body size and trophic position observed in this food web (Pearson's $r = 0.05$; Fig. 3A).

In contrast to the paradigm of sustained harvest pressure decreasing mean trophic position (MTP) of fish communities that has emerged from two decades of research on predator collapses (fishing down the food web; Pauly et al. 1998) and increased harvest of species occupying lower trophic positions (fishing through the food web; (Essington et al. 2006)), our results more strongly align with recent models of MTP for systems in which harvest is based on availability—where harvest progresses from abundant, large-bodied species to rarer, hard-to-catch species—rather than trophic position (Branch et al. 2010). Such indiscriminate fisheries are common throughout Asia, and disproportionately impact slow-maturing, large-bodied species. Comparing harvested and protected assemblages in our study area indicates that fishing leads to the most dramatic declines in large fishes, which leaves MTP unaffected yet strongly reduces the trophic position range as both piscivores and herbivores are eliminated (Fig 3B). The consequent trophic release of small invertivores at intermediate trophic positions also leaves MTP unaltered. However, unlike some indiscriminate fisheries where harvest of small-bodied fishes can offset or exceed losses due to collapse of larger species (Szuwalski et al. 2017), widespread reports from fishers of reduced catch biomass following the decline of large species suggest that sustained harvest pressure exceeds even the capacity of fast-growing, small-bodied fishes to sustain fishery productivity in our study system.

Fishers regularly catch medium- and large-sized fish along reserve boundaries, indicating that these fish leave reserves at night, likely to forage. Such “spillover effects” are an essential aspect of the benefits of marine reserves, shown to boost both the biomass and sustainability of fisheries along coastlines and across reserve networks (Gell and Roberts 2003). Presumably, similar spillover benefits from riverine reserves serve to increase local harvests, but it is not yet clear whether the size and number of reserves is sufficient to maintain long-term food security. It also remains to be seen whether the growing network of small, community-initiated reserves in this region can maintain viable populations of large-bodied fishes and highly mobile predators. Given the low abundance of large predatory species within any single reserve, we suspect that extinction debts will become an issue as these reserves age.

Enthusiasm for the increased size and biomass of fishes visible from the bank in reserve zones (Fig. S1) has resulted in the designation of over 50 community-initiated reserves in our study catchment alone. Networks of reserves can have collective benefits that exceed expectations for individual protected areas, which has led to the development of principles to maximize both biodiversity and fishery benefits in marine ecosystems (Gaines et al. 2010). Our results demonstrate the potential for small reserves to serve as effective refugia from intense harvest, so adapting principles of marine protected area network design to river networks and their fish faunas is promising. In addition to its benefit to both fish and people in SEA, the grassroots conservation approach exemplified by the Salween reserves offers a potentially transferable model for conserving biodiversity while enhancing the sustainability of tropical river fisheries worldwide.

Materials and Methods

Both reserves surveyed in this study are on the Mae Ngao, a fourth-order river in the Salween River Basin along the border of Thailand and Myanmar (Burma). The mouth of the Mae Ngao River is located at 150 m ASL (17.868 N, 97.968 E) and drains approximately 1,000 km^2 of upland (max. elevation ~1,500 m ASL) mixed deciduous forest and swidden agriculture. Northern Thailand experiences a highly seasonal climate, with a dry season lasting from late November–April and a rainy season with maximum precipitation in August and September. We conducted the study during the dry season (January–April 2015), when the Mae Ngao's waters are relatively transparent. Dry season nutrient concentrations are low, and algal growth is limited by availability of nitrogen (Koning et al. 2017). Given the width of the river and floodplain, light is not thought to be a limiting factor in most reaches.

Our two study reserves were located adjacent to villages, Mae Louie (ML) and Sop Mae Peh (SMP), separated by 3.5 km of channel length. Within each reserve, we selected 4 sites for our paired manipulations: two sites approximately 50 m upstream and downstream of the boundaries, and the other 2 sites spaced evenly within the reserve. Outside of each reserve, we selected 4 sites upstream and 4 downstream at increasing distances (approximately 50, 100, 300, 700 m) from the reserve boundary. All surveys and experiments were performed at each of these 12 sites per reserve area (i.e., total of 24 sites). The specific placement of experiments was standardized by depth (~40 cm), substrate type (cobble and large gravel), and flow velocity (11-52 cm sec^{-1}) in every case.

To test the effects of fish on lower trophic levels, we used paired $1m^2$ fish exclosures and two-sided exclosure controls. Exclosure controls allowed fish to fully access experimental substrates from downstream, while retaining potential cage artifacts arising from flow obstruction (Fig. 1). At each site, we place one 4-sided exclosure and one 2-side exclosure side-by-side, randomly assigning exclosure position relative to the nearest bank. Exclosures and exclosure controls were constructed of plastic mesh (7 mm opening), balancing our aims of effectively excluding fish while allowing water flow. Mesh was attached to a rebar frame that extended from above the water surface to 20 cm beneath the substrate, thereby excluding both jumping and burrowing fishes. We removed debris (leaves and algae) from mesh twice per day for the duration of the experiment.

We created standardized experimental substrates to measure the potential cascading effects of fish on benthic macroinvertebrates and attached algae (periphyton). We standardized benthic substrates in each cage (20 cm × 30 cm × 10 cm) using 10 size-representative rocks having comparable algal biofilms. Two replicate cages of rocks were placed on the substrate of each 2-sided and 4-sided exclosure (n = 48). We harvested cages on day 18-19; macroinvertebrates were collected, preserved, and later enumerated to functional feeding group classifications. We sampled periphyton from 2 rocks in each cage and quantified algal biomass as areal ash-free dry mass.

We tested the relative strength of bottom-up (nutrient limitation) and top-down (grazing) controls on algal accrual using nutrient diffusing substrates, which contained replicate nutrient-enriched (nitrogern and phosphorus) and unenriched controls. We collected substrates after 20 and 25 days, analyzed algal accrual as ash-free dry mass, and compared algal accrual by nutrient treatment and exclosure to infer the effects of release from nutrient limitation and grazing respectively.

We were prohibited from using traditional methods of assessing fish communities (e.g., seining, electrofishing) within reserves, so instead we estimated fish densities and composition using two visual survey methods. We conducted 3, 50-m longitudinal snorkel surveys in deeper water (>60 cm) and 6 bank-to-bank lateral transects in shallower water (<60 cm). Together these methods captured the major habitats in our study system. We measured human fishing effort at sites inside and outside riverine reserves by counting fishing gears present along the river.

We tested for statistical differences in harvest effort (i.e., fishing gears), fish densities and biomass (total, size-based, and trophic position) using generalized additive models (GAMs) having negative binomial (fish density) and Tweedie (biomass) distributions. We tested differences among means using a Bonferonni correction for multiple comparisons by zones and evaluating the 95% confidence intervals (Fig 2B). Our experimental manipulations were analyzed as replicated split-plot, randomized complete-blocks using 2-way (zone and exclosure) and 3-way (zone, exclosure, and nutrient treatment) ANOVA. When interactions were not significant, we refit models with only main effects. We tested for differences among groups for all variables using Tukey-Kramer tests of least squares means (reported as grouping letters in Fig. 2).

Finally, we tested the relative strength of top-down versus bottom-up effects on algal biomass using Hedge's *d* calculation of effect size (Gurevitch et al. 2000) as detailed in (Hillebrand 2002).

Trophic positions for nearly all recorded fish species were determined using nitrogen stable isotopes from individuals sampled outside of reserve zones. For those species we were unable to collect (2 of 24 species counted in surveys), we used trophic position estimates from FishBase (Froese and Pauly 2017), which generally matched our estimated trophic positions for other species in this system. Species-specific maximum body lengths were taken from literature values summarized in FishBase (Froese and Pauly 2017). To estimate fish biomass, we estimated the average length of observed individuals for each fish species, then estimated the weight of average length fish using standard length-weight regression methods. We calculated total biomass by multiplying density estimates for each species at each site by the weight of the average-sized individual estimated as described.

We performed transformation-based redundancy analysis ordination (tbRDA; (Legendre and Legendre 2012)) on Hellinger-transformed density data using the 'vegan' package in R (Oksanen et al. 2017). For each site, we constrained the community matrix data by a matrix of density-weighted trophic position estimates and average maximum body lengths for each site. The overall test was significant ($F_{(4,19)} = 2.16$, $p = 0.001$, $R^2 = 0.31$). Density-weighted average maximum length and density-weighted trophic position were both highly significant in the model ($p = 0.001$) and strongly correlated with canonical axes 1 and 2 ($R^2 = 0.85$ and 0.83), explaining 17% and 8% of the total variance, respectively.

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Figure Captions

Figure 1. **(A)** Sampling framework and experimental design across spatial scales for variables of interest. Fish were surveyed along transects (dashed arrows) within reserve (RR), upstream (UP), and downstream (DN) reaches at 4-50m sites. Macroinvertebrate and algal sampling was conducted within paired 2-sided fish exclosure controls (left) and full fish exclosures (right) from natural rock and nutrient-diffusing tile substrates; nitrogen + phosphorus (NP) and unamended control (C). (**B)** Cascade effects of harvest on fish and fish on algal biomass. Solid arrows indicate direct effects (positive or negative), and dashed arrow indicates indirect effects. (**C–H)** Mean **(**±SD**)** effect of zone (colored bars; upstream [UP], riverine reserve [RR], and downstream [DN]) and exclosure treatment (gray bars) by trophic level. (**C**) Human harvest effort as number of fishing gears observed. (**D**) Large fish density. (**E**) Small fish density. **(F)** Mean macroinvertebrate density composed of grazing (GR), suspension feeding (SF), and gathering collector (GC) functional feeding groups. (**G**) Ash-free dry mass (AFDM) of algal biomass collected from rocks. Lighter paired bars are control treatments, darker bars are exclosures by zone. (**H**) AFDM collected from nutrient-diffusing tiles by nutrient treatment (C, control; NP, nitrogen + phosphorus). Letters denote significant group differences ($\alpha = 0.05$); NS = non-significant differences.

Figure 2. Reserve effects on fish density and biomass by functional traits: Size **(A)**, large fish > 15 cm, small fish ≤ 15 cm; Trophic position **(B)**, piscivores ($TP \geq 3.5$), omnivores ($2.5 \geq TP < 3.5$), herbivores ($TP < 2.5$). Asterisks indicate significant differences ($p < 0.05$); NS denotes non-significant difference. **(C)** Cumulative proportion of fish density

(solid lines) and biomass (dashed lines) for reserves and non-reserves by maximum length and trophic position. Vertical dashed lines are the categorical thresholds used in (A) and (B).

Figure 3. **(A)** Stable isotope-based trophic position estimates and $\log_{10}$ maximum length of study fishes. Species are represented by gray letters (full names in Supplement). Vertical and horizontal lines depict means of density-weighted $\log_{10}$ maximum lengths and trophic position by zone, respectively. **(B)** Redundancy analysis of fish densities by site. Points represent sites colored by zone; ellipses correspond to 1 SD around group centroids. Fish species as in (A) with arrow lengths corresponding to loading scores. Red arrows are constraining variables; density-weighted maximum length (Max Length) and trophic position (TP).

Figure 1.

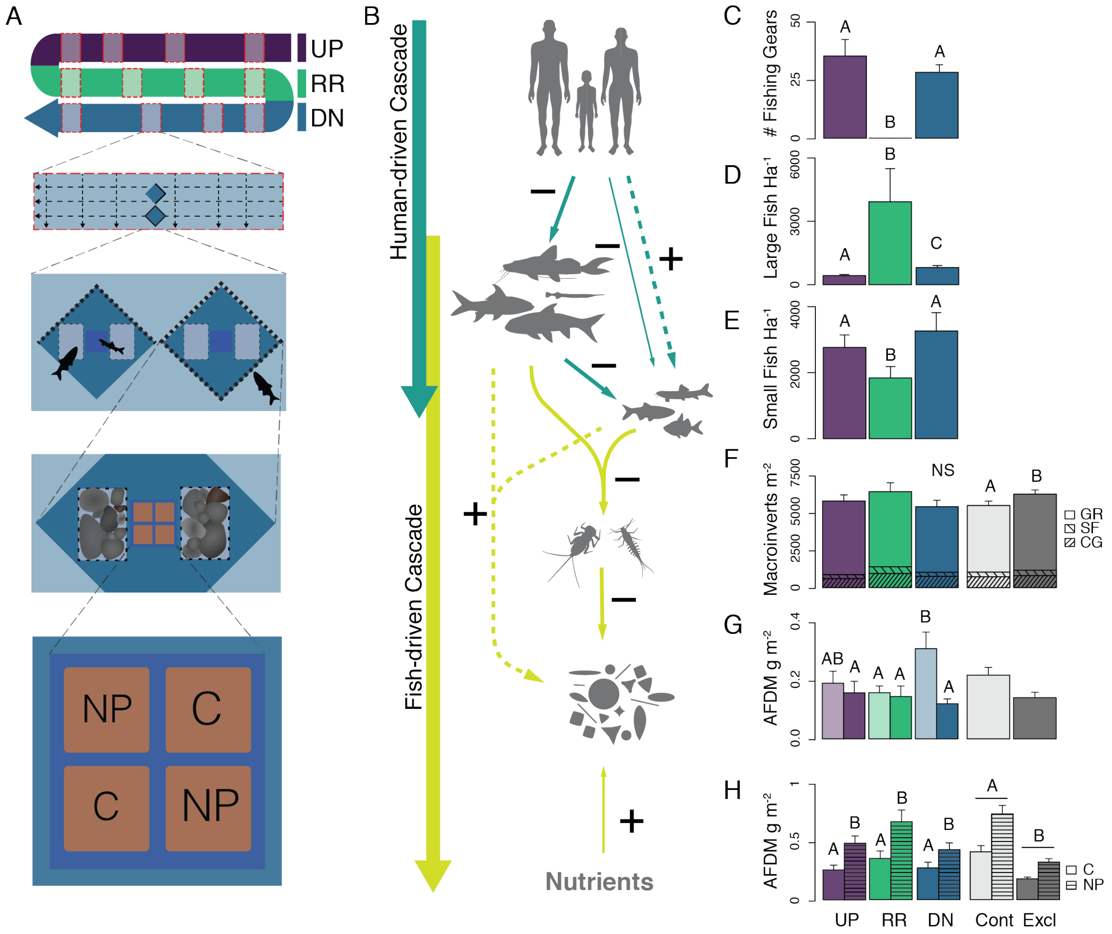

A
UP
RR
DN
NP
C
C
NP
B
Human-driven Cascade
Fish-driven Cascade
–
–
+
–
–
+
–
+
Nutrients
C
Fishing Gears
0
25
50
A
B
A
D
Large Fish Ha^{-1}
0
3000
6000
A
B
C
E
Small Fish Ha^{-1}
0
2000
4000
A
B
A
F
Macroinverts m^{-2}
0
2500
5000
7500
NS
A
B
☐ GR
▧ SF
▨ CG
G
AFDM g m^{-2}
0.0
0.2
0.4
AB
A
A
A
B
A
H
AFDM g m^{-2}
0
0.5
1
A
B
A
B
A
B
A
B
☐ C
⊟ NP
UP
RR
DN
Cont
Excl

Figure 2.

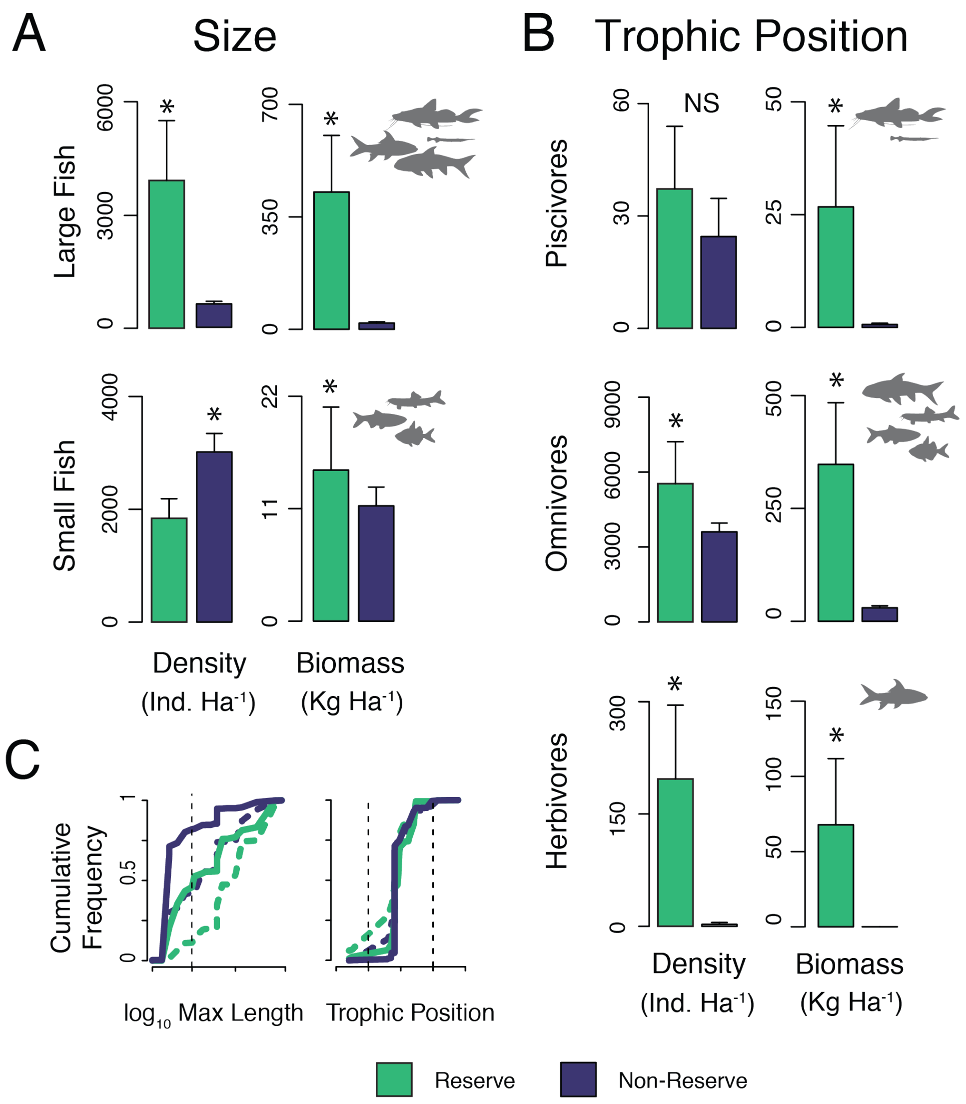
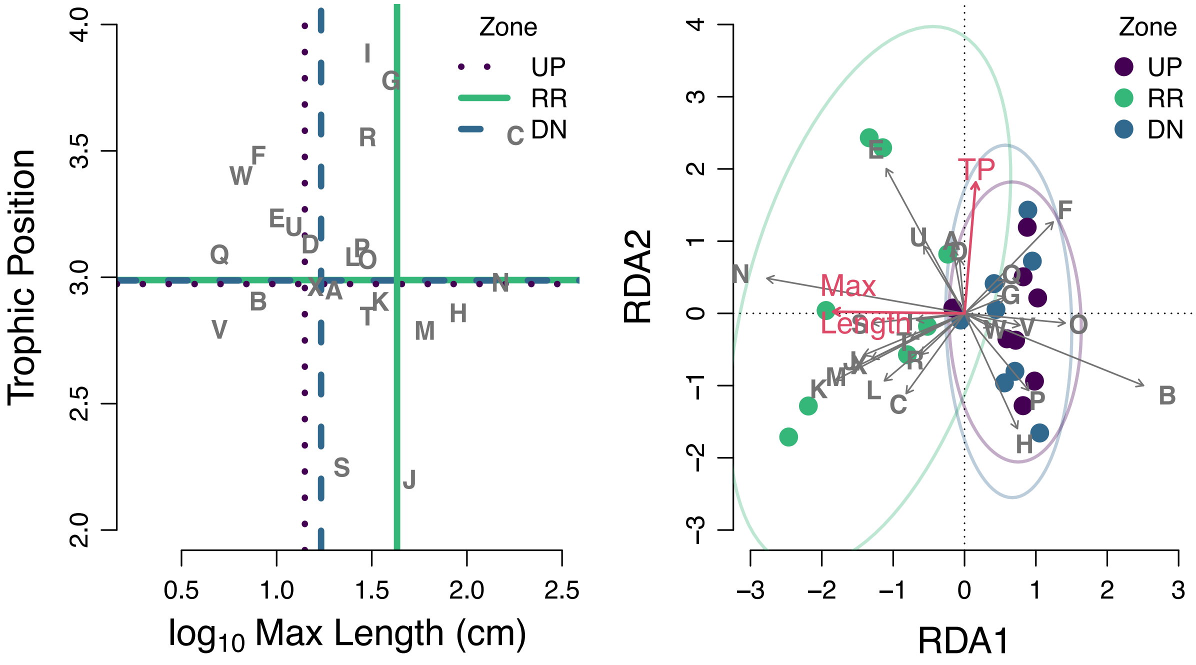

A
Size
B
Trophic Position
Large Fish
6000
3000
0
*
700
350
0
*
Small Fish
4000
2000
0
*
22
11
0
*
Density
(Ind. Ha-1)
Biomass
(Kg Ha-1)
Piscivores
60
30
0
NS
50
25
0
*
Omnivores
9000
6000
3000
0
*
500
250
0
*
Herbivores
300
150
0
*
150
100
50
0
*
Density
(Ind. Ha-1)
Biomass
(Kg Ha-1)
C
Cumulative Frequency
1
0.5
0
log10 Max Length
Trophic Position
🟩 Reserve
🟦 Non-Reserve

Figure 3.


Zone
UP
RR
DN
Trophic Position
log$_{10}$ Max Length (cm)
4.0
3.5
3.0
2.5
2.0
0.5
1.0
1.5
2.0
2.5
RDA2
RDA1
TP
Max Length
−3
−2
−1
0
1
2
3
4

Supplemental Information

Materials and Methods

Study System

Both reserves surveyed in this study are on the Mae Ngao, a fourth-order river in the Salween River Basin along the border of Thailand and Myanmar (Burma). The mouth of the Mae Ngao River is located at 150 m ASL (17.868 N, 97.968 E) and drains approximately 1,000 km^2 of upland (max. elevation ~1,500 m ASL) mixed deciduous forest and swidden agriculture. Northern Thailand experiences a highly seasonal climate, with a dry season lasting from late November–April and a rainy season with maximum precipitation in August and September. During the dry season, the Mae Ngao's waters are relatively transparent, but become highly turbid with the onset of rainy season due to suspended sediment. We observed a 4-meter rise and fall in river depth between January 2015 and April 2016, during which time daily mean water and air temperatures ranged from 18–31°C in water and 19–32°C in air. Stage height and air and water temperatures were measured continuously using two Onset Hobo U20 Water Level Loggers, one deployed in the river, and the second deployed in the shade to measure air temperature and barometric pressure for river stage height correction. Dry season nutrient concentrations are low in the Ngao River, and algal growth is limited by availability of nitrogen (Koning et al. 2017). Given the width of the river and floodplain, light is not thought to be a limiting factor in most reaches.

Experimental sites

We conducted our experiments in January-March 2015, in two segments of the Mae Ngao adjacent to two villages, Mae Louie (ML) and Sop Mae Peh (SMP), separated by 3.5 km of channel length. Each village managed their own riverine reserve. Inside each reserve, we selected 4 sites for our paired manipulations: two sites approximately 50 m inside the reserve boundaries (both upstream and downstream), and the other 2 sites spaced evenly along the length of the reserve. Outside of each reserve, we selected 4 sites upstream and downstream at increasing distances (approximately 50, 100, 300, 700 m) from the boundary to quantify attenuation of reserve effects with distance. We performed surveys and experiments at 12 sites at 2 villages for a total of 24 sites. The exact placement of experiments was based on standardizing the depth (~40 cm), substrate type (cobble and large gravel), and flow velocity (11-52 cm sec^{-1}) across all experiments.

Fish exclosures

To test the effects of fish on lower trophic levels, we used paired 1-m^2 fish exclosures and exclosure controls. Two-sided exclosures allowed fish to fully access experimental substrates from downstream, while controlling for potential caging artifacts related to upstream flow obstruction (Fig. 1). At each site, we place one 4-sided exclosure and one 2-side exclosure side-by-side, randomly assigning exclosure position relative to the nearest bank. Exclosures and exclosure controls were constructed of plastic mesh having a maximum aperture of 7 mm. This mesh size was chosen to be as large as possible to maximize flow-through yet exclude all species of fish. The plastic mesh was attached to a frame of welded rebar that was pounded into the substrate and extended from above the water surface to 20 cm beneath the substrate, thereby ensuring the exclusion of even jumping or burrowing fishes. To minimize flow obstruction by exclosures, we removed debris (leaves and algae filaments) twice per day for the duration of the experiment.

Caging Artifacts

To ensure that any observed differences in macroinvertebrate communities or algal accrual were attributable to experimental effects rather than experimental artifacts, we measured water velocity, depth, and light intensity in every experimental exclosure. Flow is a primary control on many structural attributes in river ecosystems, affecting substrate stability, habitat availability, detritus accumulation, and channel form. At the spatial and temporal scales most pertinent to our experiment, variance in flow velocity resulting from in-stream fish exclosures and stream depth would most strongly affect important factors like sedimentation rate and benthic shear stress, which could affect our response variables (biofilm biomass and macroinvertebrate abundance) directly or via alteration of fish behavior and abundance. We measured flow velocity (cm sec^{-1}) and substrate depth (cm) 15 cm inside each of the four corner posts of both 2- and 4-sided exclosures following standard protocols for wadeable streams (Hauer and Lamberti 2007). We also measured flow velocity 1 m upstream of each exclosure as a reference point. To statistically test for differential effects on flow velocity or substrate depth from 2- versus 4-sided exclosures, we constructed a linear mixed-effect model (LME) to independently regress velocity and depth on exclosure treatment (fixed) and individual sites (random). Flow velocity did vary by exclosure treatment (95% CI [0.03, 0.12], $R^2c = 0.72$); however, average velocity in 2-sided exclosures was only 5.6 cm sec^{-1} faster than in 4-sided exclosures. We expect that differences in water velocity experienced by biofilm and invertebrates near the bottom would be negligible, and certainly not strong enough to substantially affect the results of our experiment. Depth did not vary between treatments (95% CI [-0.01, 0.05], R^2c= 0.36).

Due to the relatively small aperture of the exclosure material (7 mm) we tested for potential differences in light availability between exclosure treatments. We interchanged 5 pairs of Hobo Pendant Temperature/Light loggers through all exclosure sites logging solar irradiance as broad-spectrum lux (lumens m^{-2}) every 10 minutes. Loggers were attached to rebar at substrate level and oriented upwards in the middle of each cage. For each day having an uninterrupted 24-hour record, we calculated a light dose (lumens m^{-2} day^{-1}) by integrating the area under the daily lux curve, yielding 118 daily light estimates from 48 exclosures. To test for differences between 2- and 4-sided exclosures in daily light dose, we constructed LME models to compare exclosure treatment (fixed) and sites (random), individual exclosure sub-site (random), and date (random). Daily light dose did not differ between exclosure treatments (95% CI [-0.069, 0.099], R^2c = 0.78). All LME models were constructed using the 'lme4' package (Bates et al. 2015) in R (R Development Core Team 2018), and lux data were log-transformed to meet model assumptions.

Benthic macroinvertebrate sampling units

We created standardized experimental substrates to measure the potential cascading effects of fish on benthic macroinvertebrates and attached algae (periphyton). We standardized benthic substrates in each cage by selecting 10 rocks having intermediate axis diameters of 12 cm (n = 1), 10 cm (n = 2), 8 cm (n = 3), and 6 cm (n =

4). This size distribution was representative of substrate sizes found in riffle and shallow run habitats of the Mae Ngao. We selected river rocks from a long-dried side channel and submerged them in a small stream just above the confluence with the Mae Ngao for approximately one month prior to deployment to allow for standardized biofilm accrual. Rocks were contained within a cage (20 cm × 30 cm × 10 cm) covered on the bottom and sides with plastic mesh having an aperture sufficiently large (1.4 cm) to allow settlement of drifting macroinvertebrates and access from above by fishes. At the time of deployment, we removed macroinvertebrates from each rock but left biofilms intact to provide food resources for colonizing macroinvertebrates. Two replicate cages of rocks were placed on the substrate of each 2-sided and 4-sided exclosure (n = 48).

Following 18-day (ML) and 19-day (SMP) deployments, we extracted cages of rocks by lifting them from the bottom above a fine net (363 μm) to capture any invertebrates that might have entered the draft when disturbed, then rinsing them well to collect all remaining macroinvertebrates. Invertebrates were preserved in the field using 80% ethanol. In the lab, all macroinvertebrates were removed from detritus, enumerated, and identified with sufficient taxonomic resolution (typically Family or Genus) to enable assignment to functional feeding groups using the best available taxonomic keys for the region (Dudgeon 1999, Yule and Yong 2004).

We also measured the biomass of attached algal biofilms from rocks in each cage. Biofilms were collected from the entire upward-facing surfaces of two rocks (12 and 10 cm size classes) from each cage using a nylon-bristled brush. Subsamples of the resulting slurry were filtered onto a GE pre-weighed filter for ash-free dry mass (AFDM). Filters were placed on ice in the field, then stored frozen until analysis. Following periphyton collection, we traced the entire scrubbed surface onto waterproof paper, the area of which was later measured using a LiCor LI-3100C leaf area estimator. In the lab, filters were dried for 48 hours at 60ºC, weighed, combusted in a muffle furnace at 500ºC for 48 hours and re-weighed to determine AFDM lost upon combustion. Areal mass of AFDM was calculated from rock surface areas.

Nutrient diffusing substrates

To test the relative strength of bottom-up (nutrient limitation) and top-down (grazing) controls on algal accrual, we used nutrient diffusing substrates (NDS) placed in both 4-sided and 2-sided exclosures. NDS were constructed from square clay flowerpot saucers measuring 82 mm per side and 17 mm deep. Clay pots were filled with approximately 115 mL of agar solution (20 g agar L^{-1}) containing either a joint nitrogen (1 M N as $NaNO_3$) and phosphorus (0.1 M P as NaH_2PO_4) amendment (N+P) or unamended agar as a control (C). Two nutrient amended and two control treatments were mounted in randomized positions on a sheet of acrylic plastic and secured to the bottom with rebar in every exclosure. NDS were collected after 25 (ML) and 20 (SMP) days, and comparisons between 2- and 4-sided exclosures were used to infer the effect of grazing. Algae was collected from NDS in the same manner as described for rock substrates, analyzed for AFDM, and $\log_{10}$-transformed prior to statistical analysis. To complement the primary NDS experiments, we also conducted a two-factor (nitrogen, phosphorus) randomized complete block NDS experiment at one mid-river location in each reserve using the same clay pots and nutrient amendment concentrations. These ancillary experiments were used to determine the nature of nutrient limitation during the main experiment, following deployments of 22 (ML) and 15 (SMP) days. The identity of the limiting nutrient was assessed based on AFDM accrual using 2-way ANOVA with a random block term (Tank and Dodds 2003).

Our ancillary NDS experiments showed that algal growth was N-limited throughout our experiments. Algal accrual on C and P treatments was significantly lower than both N and NP treatments (Figure S1). N had a significant effect on AFDM at both villages (2-factor ANOVA $p < 0.005$), but neither P nor the N×P interaction term were significant for either village (Table S1), indicating primary N-limitation . These results match those found previously from this river basin (Koning et al. 2017).

Fish Surveys

Local communities strictly enforce restrictions on fishing within reserves, and no exceptions are granted for research. Therefore, we were prohibited from using traditional methods of assessing fish communities (e.g., seining, electrofishing). Instead, we estimated fish densities using three different visual survey methods. In each case, we were able to identify fish to the species level for nearly all taxa. First, where depth allowed efficient snorkeling (>60 cm), we conducted three longitudinal snorkel surveys at each site by counting fish while drifting downstream for 50 m at approximately 25, 50, and 75% of stream width. All fish observed within 1 meter to the left and right of the researcher during a passive drift were identified and enumerated (i.e., survey area of 3 transects × 2 m width × 50 m length = 300 m^2 per site). Second, where depth was <60 cm, we conducted six lateral (bank-to-bank) transects at randomly selected distances upstream and downstream of the exclosures by placing a chain demarcated every meter across the river perpendicular to the direction of flow. After waiting 20 minutes to allow the river to return to pre-disturbance conditions, we identified all fish within a 2-meter band along the length of the chain, including manually disturbing the bottom to reveal cryptic fish (i.e., survey area of 6 transects × 2 m width × river width). Third, we counted the number of fish visible from above the water within 6 randomly-placed quadrats ($4m^2$) demarcated with painted rocks. Researchers climbed a folding ladder adjacent to each quadrat, waited 5 minutes for fish behavior to return to normal, and then counted every fish initially present in the quadrat, entering the quadrat during the next 5 minutes, and hiding within the quadrat (revealed by disturbing the substrate at the conclusion of the observation period) (i.e., survey area of 6 quadrats × 4 m^2 = 24 m^2 per site). Together, these three fish counting methods captured the major habitats within our study system: snorkel surveys targeted benthopelagic fishes (e.g., *Neolissochilus stracheyi, Tor spp., Hybsibarbus salweenensis*) in deeper areas (> 60cm), while lateral transects targeted cryptic benthically-oriented fishes (e.g., *Schistura spp.*) and quadrat surveys targeted both highly mobile and surface-oriented fishes (e.g., *Mystacoleucas argenteus*, *Barilius ornatus*) in shallower waters (< 60cm). Initial quadrat counts were nearly always zero and the time-integrated nature of the extended quadrat counts made them difficult to combine with near-instantaneous counts from longitudinal and lateral transects. Thus, we did not include quadrat data in the final analysis, but overall patterns from the 5-minute quadrat observations qualitatively mirrored the results from transect counts.

Human fishing effort

We measured human fishing effort at sites inside and outside riverine reserves by counting fishing gears present along the river. Observations were made along the full length of the river between the uppermost and lowermost exclosure pair. We performed these counts only once per village between the hours of 17:00-18:00 because the relevant gears (gill nets, trotlines, bamboo poles) are affixed to semi-permanent moorings every afternoon, then harvested and removed in the morning.

Statistical tests for trophic cascades

We used the strong contrast provided by harvested areas and reserves to test for cascading effects of human harvest on lower trophic levels (i.e., fish, macroinvertebrates, algal biomass). We further tested the top-down effect of fish as consumers in these systems by limiting their access to experimental substrates using exclosures under both harvested and reserve conditions. Finally, we tested the strength of top-down effects compared to bottom-up effects using nutrient diffusing substrates within exclosures. To test for statistical differences in harvest effort (i.e., fishing gears), fish densities and biomass (total, size-based, and trophic position), we used generalized additive models (GAM) with zone (i.e., upstream, reserve, and downstream) as a fixed effect and village (Sop Mae Peh; Mae Louie) as a smooth effect. We used negative-binomial distributions for fish count data offset by the log(area) of the observations to get density estimates. We modeled biomass data using Tweedie distributions, which have the characteristics of Poisson distributions at zero, but allow for continuous variables like the Gamma distribution above zero (Jorgensen 1987). We tested differences among means using a Bonferonni correction for multiple comparisons by zones and evaluating the 95% confidence intervals (Fig 2B). GAM were constructed using the ‘mgcv’ package (Wood 2004) in R.

Our experimental manipulations were designed as replicated split-plot, randomized complete-blocks. For macroinvertebrate and ash-free dry mass (AFDM) densities collected from experimental substrate cages, we constructed two-way

ANOVAs, with zone and exclosure treatments as fixed effects with an interaction term. To test the effect of zone, exclosure treatment, and nutrient amendment on algal biomass (AFDM) from nutrient diffusing substrates, we used a three-way ANOVA model with fixed main effects and all interactions. When interactions were not significant, we refit models with only main effects. Finally, we tested for differences among groups for all variables using Tukey-Kramer tests of least squares means (reported as grouping letters in Fig. 2). Model results are presented in Table S1. Models were fit using the 'lme4' package in R, and significance tests were conducted using Satterthwaite estimated degrees of freedom in the 'lmerTest' package (Kuznetsova et al. 2017). Tukey-Kramer least squares means tests of group differences were conducted using the 'lsmeans' package (Lenth 2016).

Top-down versus bottom-up effect size calculation

To test the relative strength of top-down versus bottom-up effects on algal biomass we used Hedge's *d* calculation of effect size (Gurevitch et al. 2000) as detailed in (Hillebrand 2002). Hedge's d_N is the standardized difference between treatment (nutrient addition) and control means divided by the combined standard deviation for both treatments. Hedge's d_G is the standardized difference between 4-sided exclosures and exclosure controls (2-sided). Positive values of d_N indicate increased algal biomass in nutrient enriched treatments, and positive values of d_G indicate increased algal biomass in the absence (or reduction) of grazers (i.e., when fish were present; 2-sided controls). The greater effect size of d_G indicates a greater overall effect of grazer removal than nutrient addition (Hillebrand 2002).

Trophic position and biomass estimates

Trophic positions for nearly all recorded fish species were determined using nitrogen stable isotopes from individuals sampled outside of reserve zones. Dorsal muscle tissue was dissected to remove all skin, scales, and bones, stored frozen in glass vials, oven-dried (60º C, 48 hours), ground to a homogeneous powder, and weighed into tin capsules. Stable isotope analysis was conducted at the Cornell Stable Isotope Laboratory using a Finnigan MAT Delta-Plus mass spectrometer, and showed high analytical precision (standard deviation = 0.06‰, n = 133). Trophic position estimates were based on Post (2002): trophic position = $\lambda + \delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}} / \Delta_n$, where λ is the trophic level of the organism used to calculate $\delta^{15}N_{\text{base}}$ (in our study $\lambda = 2$ for snails and mussels), $\delta^{15}N_{\text{secondary consumer}}$ is measured directly, and Δ_n is the trophic fractionation factor 2.24 (Blanke et al. 2017).

For those species we were unable to collect (2 of 24 species counted in surveys), we used trophic position estimates from FishBase (Froese and Pauly 2017), which generally matched our estimated trophic positions for other species in this system. Species-specific maximum body lengths were taken from literature values summarized by FishBase (Froese and Pauly 2017).

To estimate fish biomass, we estimated the average length of observed individuals for each fish species. Because two species, *Sperata aor* and *Folifer brevifilis*, had substantially different average sizes dependent on being observed within or outside of reserves, we used two different average lengths for reserve and non-reserve observation. Only one *S. aor* individual was observed outside of reserves, having length of 25cm, while the 3 individuals observed inside reserves each exceeded 80cm. Similarly, *F. brevifilis* were 20cm on average inside reserves, and 10cm outside reserves. Together these two species accounted for only ~1.5% of the total estimated biomass. All other length estimates were identical for reserve and non-reserve fish species.

We estimated the weight of fish of average length using standard length-weight regression methods. We used length-weight relationship for fish species for which we were able to collect a representative size distribution during stable isotope analysis. For species that we were unable to collect a representative size distribution (e.g., many large-bodied reserve species), we used length-weight relationships reported from the literature. Finally, to calculate total biomass, we multiplied density estimates for each species at each site by the weight of the average-sized individual estimated as described.

Redundancy Analysis

We performed transformation-based redundancy analysis ordination (tbRDA) (Legendre and Legendre 2012) on Hellinger-transformed density data using the 'vegan' package in R (Oksanen et al. 2017). For each site, we constrained the community matrix data by a matrix of density-weighted trophic position estimates and average maximum body lengths for each site. The overall test was significant ($F_{(4,19)} = 2.16$, p = 0.001, $R^2 = 0.31$). Density-weighted average maximum length and density-weighted trophic position were both highly significant in the model (p = 0.001) and strongly correlated with canonical axes 1 and 2 ($R^2 = 0.85$ and 0.83), explaining 17% and 8% of the total variance, respectively.

Non-consumptive effects

Algal biomass collected from rocks within experimental substrates showed a significant interaction of zone and exclosure (two-way ANOVA, $F = 3.99$, p = 0.033), where downstream exclosure controls had significantly higher AFDM and upstream exclosure controls had intermediate biomass relative to all others (Least Squares means, $\alpha = 0.05$; Fig 1G). This differing AFDM response pattern, despite comparable macroinvertebrate densities, suggests the presence of additional non-consumptive effects of fish on algal accrual. Unlike tile substrates, from which macroinvertebrates are easily predated, rock substrates are complex and offer refugia from fish predators. Refuge-seeking behavior has been shown repeatedly to reduce time spent foraging (McIntosh et al. 2002), suggesting prolonged exposure of macroinvertebrates to fish predators increases the strength of indirect non-consumptive effects of fish on algal biomass. Accordingly, we observed highest algal biomass from treatments exposed to fishes (i.e., exclosure controls) in upstream and downstream zones. While reserves were dominated by large-bodied, pelagic-oriented fishes, upstream and downstream zones contained higher densities of small-bodied, benthically-oriented invertivorous fishes co-occupying the river bottom with their macroinvertebrate prey. The AFDM data suggest this spatial proximity likely reduced macroinvertebrate foraging effort in both upstream and downstream zones, with a stronger effect downstream as a result of slightly higher small fish density or possibly the accumulated predatory scent cues delivered from the reserve upstream.

Fig. S1

Bankside view of a riverine reserve with inset of close-up of reserve fish including *Neolissochilus stracheyi*. Photo credits A. A. Koning.

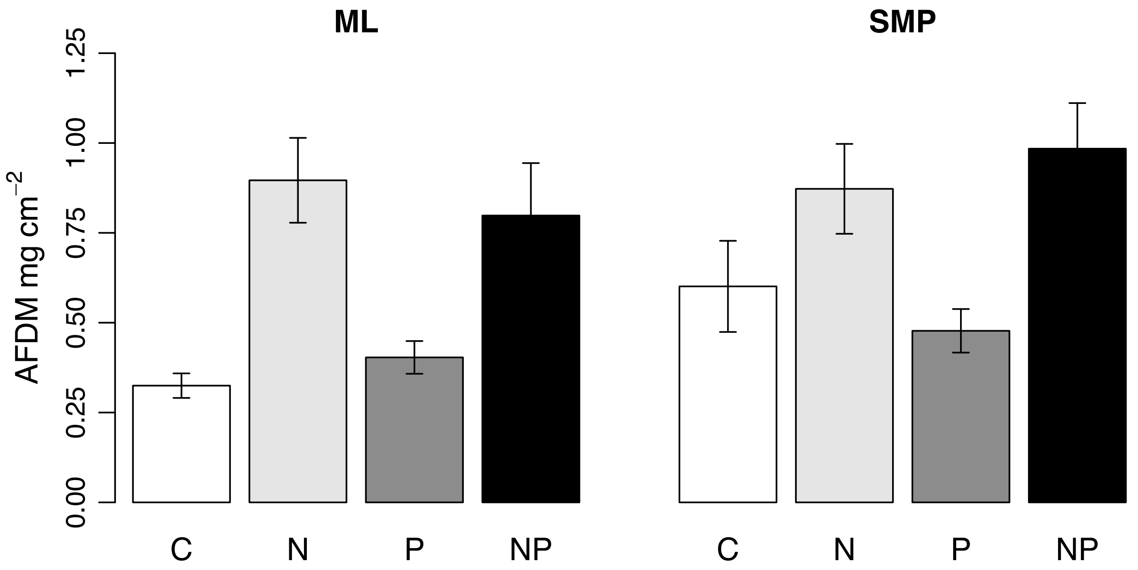


Fig. S2.
Ash-free dry mass (AFDM) from full factorial nutrient amendments at each village. Bars are means (± SE) of control (C), nitrogen amendment (N), phosphorus amendment (P), and N+P amendment treatments.

Table S1. Final fitted models and results for individual response variables presented in Fig. 2.

Response Variable	**Final Model**	**Results**			
		Zone	**Exclosure**	**Zone:Exclosure**	**Nutrient Treatment**
Fishing gears	$= \boldsymbol{zone} + (1 \mid village)$	$F_{(2, 21)} = 17.28$ **$p = 0.000037$**			
Large Fish	$= \boldsymbol{zone} + (1 \mid village)$	$F_{(2, 21)} = 9.66$ **$p = 0.0011$**			
Small Fish	$= \boldsymbol{zone} + (1 \mid village)$	$F_{(2, 24)} = 4.26$ **$p = 0.026$**			
Macro-invertebrates	$= (1 \mid village) + zone + (1 \mid village{:}zone) + (1 \mid site{:}village{:}zone) + exclosure + (1 \mid site{:}village{:}zone{:}excl)$	$F_{(2, 20.99)} = 1.00$ $p = 0.38$	$F_{(2, 22.29)} = 5.30$ **$p = 0.031$**		
AFDM (natural rock)	$= (1 \mid village) + zone + (1 \mid village{:}zone) + (1 \mid site{:}village{:}zone) + exclosure + zone{:}exclosure + (1 \mid site{:}village{:}zone{:}excl)$	$F_{(2, 20.03)} = 1.58$ $p = 0.23$	$F_{(2, 20.97)} = 11.86$ $p = 0.0024$	$F_{(2, 20.97)} = 3.99$ **$p = 0.033$**	
AFDM (NDS)	$= (1 \mid village) + zone + (1 \mid village{:}zone) + (1 \mid \mathrm{site}{:}village{:}zone) + exclosure + treatment + (1 \mid site{:}village{:}zone{:}excl)$	$F_{(2, 20.03)} = 1.40$ $p = 0.27$	$F_{(2, 22.85)} = 41.16$ **$p = 1.6\mathrm{x}10^{-6}$**		$F_{(2, 141.12)} = 148.04$ **$p < 0.000001$**

Table S2.

Two-factor ANOVA table for AFDM measured from replicated full-factorial bioassay.

Village	Nitrogen Treatment (N) *F*, p-value	Phosphorus Treatment (P) *F*, p-value	Nitrogen + Phosphorus Treatment (NP) *F*, p-value	Block *F*, p-value	Limitation Status
Mae Louie (ML)	**42.69, $4.2\text{x}10^{-5}$**	0.05, 0.83	2.16, 0.17	**11.34, <0.01**	N
Sop Mae Peh (SMP)	**14.62, 0.0016**	0.02, 0.88	1.21, 0.29	2.80, 0.11	N

Table S3.

Species label ID (Fig. 3), taxonomic name, maximum reported length, and estimated trophic position.

Label ID	Taxonomic Name	Maximum Length (cm)	Estimated Trophic Position	Reported Diet	Diet Reference No.
Q	*Hara filamentosa*	5.0	3.01	NA	
V	*Pethia stoliczkana*	5.0	2.79	NA	
W	*Danio albolineatus*	6.5	3.40	Invertivorous: insects, zooplankton	*27*
B	Nemacheiline loach	8.0	2.91	Invertivorous: aquatic insect larvae	*48*
F	*Parambassis vollmeri*	8.0	3.48	Invertivorous: invertebrates, worms, crustaceans	*27*
E	*Barilius ornatus*	10.0	3.23	NA, but likely invertivorous	
U	*Poropuntius sp.*	12.3	3.20*	NA	
D	*Mystacoleucus argenteus*	15.0	3.13	Invertivorous: worms, insect larvae, crustaceans	*27* (for *M. atridorsalis*)
X	*Crossocheilus burmanicus.*	16.0	2.96	Omnivorous: algae, aquatic invertebrates	*27*
A	*Garra salweenica*	20.0	2.95	Omnivorous: benthic algae, aquatic insects	*27* (for *G. micropulvinus*)
S	*Scaphiodonichthys burmanicus*	22.0	2.25	Omnivorous: insect larvae, detritus, periphyton	27
L	*Systomus rubriprinnis*	25.0	3.08	Omnivorous: algae, shrimps, aquatic insects, fish	*27* (for *S. sarana*)
P	*Glyptothorax sp.*	28.0	3.17	Invertivorous: aquatic insect larvae	*27* (for *G. laosensis*)
I	*Raimas guttatus*	30.0	3.89	Carnivorous: insects, small fishes	*27*
O	*Folifer brevifilis*	30.0	3.07	Omnivorous: algae, crustaceans, mollusks, insect larvae	*49*
R	*Hampala salweenensis*	30.0	3.56	Carnivorous: crustaceans, insect larvae, fish	*27* (for *H. macrolepidota*)
T	*Chagunius bayeli*	30.0	2.85	NA	
K	*Hypsibarbus salweenensis*	35.0	2.90	Omnivorous: algae, aquatic invertebrates	*27* (for *H. lagleri*)
G	*Xenentodon cancila*	40.0	3.78	Carnivorous: insects, small fishes	*48*
J	*Bangana devdevi*	50.0	2.20*	Herbivorous: periphyton, algae	27 (for *B. behri*)
M	*Neolissochilus stracheyi*	60.0	2.79	Omnivorous: terrestrial vegetation, aquatic animals	27

H	*Mastacembelus armatus*	90.0	2.86	Omnivorous: benthic insect larvae, worms, some plant material	27
N	*Tor c.f. tambroides*	150.0	2.98	Omnivorous, aquatic and terrestrial plants, aquatic inverts	27 (see also T. tor)
C	*Sperata acicularis*	180.0	3.56	Carnivorous: small fishes, worms	27 (for *Sperata aor*)

* denotes value from FishBase (Froese and Pauly 2017)

Chapter 3

Riverine reserves: adapting principles of reserve design to dendritic ecological networks

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Abstract

The efficacy of spatial protection for conserving species, habitats, and maintaining ecological processes has made reserves critical components of conservation efforts around the world. Inland waters likely benefit from terrestrial protected areas, but reserves targeted at conserving freshwater biodiversity are globally rare, especially within river systems. The creation of riverine reserves is challenged in part due to the inherent structure of rivers, which feature hierarchical network connections, polarized water flow, and narrow width within the broader landscape. In many regions of the world, riverine fisheries provide critical nutrition, but simultaneously face rapid biodiversity loss and uncertain harvest predictability as a result of over-exploitation. By adapting principles derived for terrestrial and marine reserves to the particular structure of riverine ecosystems we can provide guidance on how to achieve the dual goals of biodiversity conservation and support of critical riverine harvests. Here we review the theory that has guided conservation reserve design over the last four decades and evaluate how these concepts might be applied to reserve networks in rivers. We propose a set of 10 principles to be considered in the planning of riverine reserves aimed at maximizing their potential benefits. There is much yet to be learned about the potential for spatial protection to benefit riverine ecosystems, but by

building on lessons learned from years of reserve planning in other systems, it may be possible to accelerate the realization of similar conservation benefits for the world's rivers.

Introduction

Conservation reserves have often been imagined as refugia, untarnished landscapes or seascapes buffered from the effects of human development elsewhere (Phillips 2004). Yet, much has changed regarding our understanding of ecosystems and the scale of environmental degradation since the implementation of modern protected areas began (Naughton-Treves et al. 2005, Watson et al. 2014). Once considered "stable and infinitely resilient" (Folke et al. 2004), we now recognize ecosystems as complex adaptive systems (Levin 1998). This dynamic paradigm of ecosystems has resulted in greater realization that reserves must be incorporated into broader ecosystem-based management (EBM) strategies that focus on improving ecological resilience by maintaining the adaptive capacity of ecosystems (Pikitch et al. 2004, Gaston et al. 2008).

Reserves play important roles in achieving ecosystem-based management goals in terrestrial (Slocombe 1993) and marine systems (Pikitch et al. 2004), but their application for freshwater conservation has yet to be realized (Abell et al. 2007, Nel et al. 2009). To date, the application of EBM principles and spatial protection to riverine ecosystems has focused almost entirely on overlap with existing protected areas (Mancini et al. 2005, Nel et al. 2007, Lawrence et al. 2011), or as features of broader landscape conservation strategies (Abell et al. 2007). Rivers are inextricably linked to their watersheds (Likens and Bormann 1974, Hynes 1975, Allan 2004), and thus, terrestrial protected areas (PAs) have strong potential to confer benefits to freshwater biodiversity. This may be particularly true for regions where the dominant aquatic stressors are driven by landscape-based phenomena including nutrient pollution, sediment loading, and catchment disturbance (Vörösmarty et al. 2010).

There is, however, equivocal support for the idea that existing protected areas afford sufficient protection to freshwater biodiversity (Saunders et al. 2002, Herbert et al. 2010, Darwall et al. 2011). The few empirical tests of the benefits of terrestrial reserves to freshwater biodiversity that have been conducted reveal two general insights. First, terrestrial reserves do little to protect biodiversity in areas where the dominant threats apply throughout the catchment (Chessman Bruce and Cadotte 2013, Penha et al. 2014, Chu et al. 2018). This arises from the fact that most existing protected areas having been delineated without explicit consideration of watershed-scale factors (Abell et al. 2007). Consequently, protected areas located low in watersheds are subject to downstream conveyance of nutrient, chemical, or sediment pollution across reserve boundaries (Nel et al. 2007), as well as the negative impacts of upstream water diversion or abstraction (Chessman Bruce and Cadotte 2013). Second, protected areas have historically been sited based on terrestrial biodiversity patterns, which vary in their overlap with those of freshwaters, especially for rare and imperiled species (Abell et al. 2011, Lawrence et al. 2011, He et al. 2017, Frederico et al. 2018). In fact, areas of the world ranked in the top 15% for rarity-weighted richness, a metric of biodiversity value, overlap by less than 50% for terrestrial and freshwater biodiversity (Abell et al. 2010). There is evidence, however, that existing protected areas effectively confer protection to fish where overharvest is the dominant threat to fish diversity (Sanyanga et al. 1995, Abraham and Kelkar 2012, Britton et al. 2017). Taken together, these studies suggest that protecting freshwater biodiversity will require the addition of freshwater reserves to the existing mosaic of global PAs.

Overharvest of fish is a major threat to marine (Jackson et al. 2001, Worm et al. 2006) and freshwater biodiversity (Allan et al. 2005) and to sustained future harvests, particularly for lower income nations with high human nutritional dependence on fisheries (FAO 2016). While the fishery community has a long history of using stock-based management approaches to limit the pressure on wild fish, the role of protected areas as part of ecosystem-based management plans has advanced far more rapidly in marine than freshwater realms (Pikitch et al. 2004, Beard et al. 2011). Thus, there may be much to gain from additionally incorporating principles derived for marine protected areas (MPAs) into the discussion of freshwater protection. The reason for this is that unlike terrestrial reserves, in which biodiversity protection is typically the sole aim, marine reserve principles have been developed with the additional goal of stabilizing or augmenting harvest production. Like terrestrial reserves, marine protected areas (MPAs, in which human activities are restricted to varying degrees) and marine reserves (MRs, in which all extractive uses are prohibited) have been shown to successfully increase density, biomass, and species richness within their boundaries (Halpern and Warner 2002, Lester et al. 2009). In addition, growing evidence, both empirical (Roberts et al. 2001, Gell and Roberts 2003) and theoretical (Neubert 2003, Foley et al. 2010, De Leo and Micheli 2015), suggests that adjacent fisheries can benefit from spillover of fish (adults and larvae) from un-fished reserves. As a result, a considerable body of work has focused on determining optimum conditions to protect target species and sustain local fisheries (Botsford et al. 2003, Hastings and Botsford 2003, Roberts et al. 2003, Claudet et al. 2008, Gaines et al. 2010, Edgar et al. 2014).

Adapting the principles gleaned from terrestrial and marine reserve theory to freshwater ecosystems has great potential to improve conservation outcomes while buttressing local food security in areas of high fishery dependence. Large inland lakes that support commercial-scale fisheries, such as the Laurentian and African Great Lakes or Cambodia’s Tonle Sap, may offer the most direct transferability of MR theory (Sanyanga et al. 1995, Cooperman et al. 2012). However, small, hydrologically connected lakes (<1km^2) far outnumber large lakes (Messager et al. 2016), and lakes are generally rare in tropical latitudes where overharvest is a major threat to freshwater biodiversity (Allan et al. 2005). Additionally, at least half of the world's inland harvest comes from rivers (McIntyre et al. 2016, Deines et al. 2017), which differ profoundly in their physical structure and organismal life histories from the landscapes and seascapes where protected area theories were originally developed.

Thus, there is reason to suspect that directly applying existing reserve design guidelines to river networks would be unlikely to meet the pressing needs for conservation of inland fisheries and biodiversity.

Rivers have dendritic, or branching, network structure and strong hierarchical properties that define connectivity within their banks (Fagan 2002). Additionally, cross-basin connectivity is severely limited due to physical (watershed divides) and chemical barriers (oceans at river mouths). Rivers also experience unidirectional flow, which adds unique dispersal challenges of riverine organisms(Campbell Grant et al. 2007). Each of these properties sufficiently differ from terrestrial and marine systems to warrant thorough evaluation of the suitability of existing reserve design principles to the context of riverine ecosystems. Specifically, we evaluate the species–area relationship, the species–energy relationship, principles of habitat or reserve connectivity, and the role of reserves in supporting adjacent fisheries to develop nine principles for the design of riverine reserves.

Species–area relationships and reserve design

Reserve design theory has long been predicated on the “ecological law” (Dodds 2009) that species richness scales with area (Arrhenius 1921, Gleason 1922). Species-area curves (SAC) describe this relationship and are defined by a power function:

$$S = cA^z$$

where S is the number of species, A is total habitat area, c is a tuning coefficient, and z is a scaling coefficient on A. In a conservation context, reserves having larger size (A), or similarly, increasing the size of an existing reserve, should protect more species (Diamond 1975, Hastings and Botsford 2003). Additionally, reserves established in regions with high species density (larger estimates of z) will accumulate species more rapidly per unit of reserve area increase, while those with lower estimates of *z* will receive less return on investment regarding species gained per unit area increase. In a meta-analysis of 794 SACs covering a diversity of taxa, locations, habitats, and scales, estimates of *z* across independent sites varied systematically between terrestrial ($z = 0.31 \pm 0.03$ SE), marine ($z = 0.33 \pm 0.04$), and freshwater realms ($z = 0.21 \pm 0.03$)(Drakare et al. 2006). The low *z* value for freshwater habitats suggests that they accumulate species with area at a slower rate than in terrestrial or marine realms. Although this result would suggest that freshwater reserves must be disproportionately large to achieve similar levels of species protection as marine or terrestrial reserves, the parameter *c* is more important in determining the actual richness of a given habitat (Rosenzweig, 1995), where:

$$c = S/A^z.$$

We can estimate *c* for each global biological realm using reported *z* values (Drakare et al. 2006), the number of known vertebrate species (Grosberg et al. 2012), and their respective total areas (Raymond et al. 2013). While there is uncertainty in each of these parameters, their implications for *c* remain informative. We infer that *c* must be 13 and 40 times higher for freshwaters than for terrestrial and marine realms, respectively (Table 1). This is partly a reflection of low *z* values reported by Drakare et al. (2006), yet also accords with the fact that freshwaters support the same order of magnitude of vertebrate species richness as found on land or in the ocean, but in far less habitat area (Dudgeon et al. 2006, Strayer and Dudgeon 2010).

These species–area relationships provide two reasons to suggest that riverine reserves may not need to be as large as terrestrial or marine reserves. First, our estimates of *c* indicate that freshwaters are substantially more species-rich than either terrestrial or marine habitats *on a per-area basis*. Even small reserves should contain relatively high levels of the vertebrate biodiversity represented within a given river system. Second, the lower rate of species accumulation with area (lower *z*) suggests that increasing reserve area would have disproportionately small benefits for biodiversity coverage in rivers relative to marine or terrestrial realms. The implication of these inferences is that creating many small reserves is likely to capture more freshwater species than could be included in a few larger reserves of equivalent total area.

Investigating the dominant mechanisms hypothesized to drive species–area relationships provides further insights into how existing principles for reserve design can be best adapted to riverine systems in order to conserve biodiversity. It has been hypothesized that larger areas (or reserves) hold more species because they contain: 1) more individuals with larger genetic effective population sizes, thus a lower extinction probability (MacArthur and Wilson 1963, Soulé and Simberloff 1986); 2) increased colonization probability (Diamond 1975, Game 1980); 3) more habitat types, offering more niches for species to occupy (Williams 1964); and 4) more biogeographical provinces with differing environmental and evolutionary histories, thereby pooling ecologically and evolutionarily distinct biotas (Rosenzweig 1995, Gaston 2000). We will consider each of these mechanisms in turn, evaluating how they may inform reserve design within the dendritic structure and unidirectional flow that characterize river ecosystems.

The theory of island biogeography predicts that larger islands should contain more individuals of each species, which makes them demographically more likely to persist (MacArthur and Wilson 1963). Fundamentally, the necessity of reserves results from the inability of populations to maintain themselves under prevailing or expected conditions; that is, population losses (mortality and emigration) occur at higher rates than gains (recruitment or immigration). Therefore, for a single reserve to effectively maintain a given population, it must increase births relative to deaths within its boundaries (i.e., enhance self-recruitment) or receive sufficient immigrants to offset losses (i.e., recruitment subsidy; (Cowen and Sponaugle 2009). Assuming that negative growth rates were the motivation for establishing a reserve initially, it is unlikely that a single reserve will either benefit strongly from the recruitment subsidy mechanism or reciprocally enhance subsidies beyond its borders

(Hastings and Botsford 2006). Instead, for a single reserve to be effective, reserve size should be maximized to encompass populations large enough to sustain self-recruitment, which should roughly coincide with reserve dimensions that exceed the average dispersal distance of organisms of conservation concern (Botsford et al. 2001, Gaines et al. 2010).

Dispersal patterns vary broadly by habitat realm, resulting from differences in the life stage at which dispersal takes place. For example, terrestrial reserve dimensions are often based on large-bodied carnivores (Carroll et al. 2003, Kitzes and Merenlender 2013), which typically undergo their longest movements (10^1–10^4 m: (Whitmee and Orme 2012) as juveniles during natal dispersal from birthplace to first breeding site (Murray 1967, Greenwood 1980, Studds et al. 2008, Clutton-Brock and Lukas 2012). In contrast, most coastal marine species passively disperse during their larval stage and are sedentary thereafter (Botsford et al. 2001, Shanks et al. 2003, Guichard et al. 2004, Hastings and Botsford 2006). Consequently, design considerations for marine reserves have largely focused on distances of larval dispersal (10^1-10^7 m; (Kinlan and Gaines 2003, Shanks et al. 2003), which reflect both physical (e.g., advection and diffusion) and biological processes (e.g., planktonic larval duration, vertical swimming behavior; Mora and Sale 2002). Neither of these perspectives is readily applicable to river fishes.

Conceptualizing reserve size for rivers requires accounting for the dispersal distance of both larval and mature life stages. The polarized flow of rivers provides a single downstream vector of passive dispersal by small larvae. The maintenance of upstream populations requires this drift to be offset by upstream movement by adults or juveniles, the so-called “drift paradox” (Pachepsky, Lutscher, Nisbet, & Lewis, 2005). Riverine fish larvae are initially passive in relation to flow (Pavlov 1994, Lucas & Baras 2001), and become concentrated downstream along river thalwegs (Pavlov et al. 2008). Once larvae gain swimming ability, they are able to adjust their position in the water column and enter areas of reduced flow (e.g., flood plains, back eddies, deep pools; Hunter, 1972; Fortier &

Legett, 1983; Copp & Cellot, 1988; Reichard et al., 2001; Zitek et al., 2004a in Pavlov 2008). Larval drift distances for most freshwater fish are unknown, but are expected to reflect spawning location and rate of larval development. Studies using artificial propagules have shown modest drift distances for substrate-spawning (rithrophilus or phytophilus) species (10 m; Crisp 1989) and longer distances for pelagic-spawning (pelagophilus) species (~100 km; (Dudley and Platania 2007, Widmer et al. 2010). Therefore, for a single riverine reserve to self-recruit effectively, reserve lengths may need to extend for many river kilometers for pelagophilus species and in areas of high current velocity, but may be relatively short for species having alternative spawning habitats or occupying slow-flow areas. For adults, reserve size should reflect the home range of target species, which can vary from 10 m in small stream fishes (Hill and Grossman 1987) to 10 km in larger fishes (Woolnough et al. 2009). Home ranges typically scale with body size, but home ranges of fish in riverine systems are considerably smaller than birds, mammals, and lizards of comparable body mass (Minns 1995). Taken together, the lifelong spatial ecology of riverine fishes suggests that reserves may not require the same spatial extent as in terrestrial or marine ecosystems in order to be effective, even for the largest species.

The second hypothesis for the species area relationship states that larger islands increase colonization probability, thereby increasing species richness. Colonization rate is typically considered with regard to distances from mainland populations (MacArthur & Wilson, 1963; Simberloff & Wilson, 1969). However, while the proximity to a source population is one predictor of colonization rate, so too is the size and shape of the target. For example, the probability of a drifting raft of vegetation carrying a founding population of insects or plants to an island is a function of island perimeter, while the probability of persistence by these colonizing species is a function of island area. These factors together may explain the serial colonization successes of Madagascar, which has a long coastline paralleling the African continent, as well as sufficient size to allow for persistence and speciation (Ali & Huber, 2010). Likewise, reserves having longer perimeters are more likely to intercept colonists, but the likelihood of colonists persisting within a reserve is a function of its area (Game 1980).

The idea that larger reserves will facilitate higher rates of colonization applies well to marine and terrestrial systems, but not to most riverine organisms. This is due to differences in dimensionality of these ecosystems. Reserve planning for marine and terrestrial systems considers two dimensions, where increasing reserve area can be accomplished by increases in length or width. Theoretically, reserves could be designed to maximize colonization probability while supporting persistence (e.g., having elongated borders along dominant dispersal vectors; Game, 1980; Kunin, 1997), but in practice, marine and terrestrial reserves are typically designed to minimize negative edge effects (e.g., maximizing area-to-perimeter ratios; Ewers & Didham, 2005; Saunders, Hobbs, & Margules, 1991). In contrast, rivers are linear, one-dimensional systems; their depth and width dimensions are ecologically relevant but are fixed from a conservation planning perspective (Harvey & Gooseff 2015). Thus, increasing the aquatic habitat within most riverine reserves could be accomplished in only one dimension: length. In wide floodplain rivers it is conceivable that reserves would not extend entire river widths, thereby enabling reserve dimensions to be increased along two axes. However, given the majority of rivers on Earth are small, reserves would most easily encompass entire river widths to ease both delineation and enforcement. Longer reserves would likely facilitate higher population persistence by boosting census population sizes within reserve boundaries. Yet, because migration into or out of a riverine reserve occurs only across the fixed width dimensions of upstream and downstream boundaries, colonization probability remains largely unchanged by increased reserve length. From the standpoint of colonization, the conservation importance of riverine reserves lies in their network position and total number, as each new reserve creates two additional boundaries across which colonization may take place.

A third hypothesis for the SAR is also one of the oldest: larger areas should contain greater diversity of habitat types (Williams, 1964) and, by extension, species (Connor & Simberloff, 1979). More habitats allow for more niches, which through evolutionary time allow for diversification and increased richness (Rosenzweig 1995), a concept having broad empirical support across realms (Hortal, Triantis, Meiri, Thébault, & Sfenthourakis, 2009). In terrestrial and marine ecosystems, vertical structure is often provided by biogenic habitats (Angelini et al. 2011; Boström et al. 2011). For example, the positive relationship between local species diversity and vertical forest structure has been repeatedly shown (MacArthur & MacArthur 1961; Tews et al. 2004), and marine diversity is highest in regions having complex habitat structure built by corals (Buhl-Mortensen et al. 2010), kelp forests (Steneck et al. 2003), mangroves (Kathiresan & Bingham 2001), or seagrasses (Duffy 2006). This has led to the use of habitats as surrogates for biodiversity in siting both terrestrial and marine reserves (Ward et al. 1999; Margules & Pressey 2000).

However, relative to marine and terrestrial realms, river ecosystems have relatively low habitat diversity due to their physical structure. Riverine habitats typically lack a strong vertical dimension, which in other realms allows for more rapid species turnover leading to steeper SAC (Drakare et al. 2005). Rivers also generally lack habitats whose structure is biogenic. Instead, riverine habitat patterns are primarily the result of hydrogeomorphological processes (Leopold & Maddock 1953), and flow dynamics are the dominant factor in determining ecological patterns and processes (Vannote et al. 1980; Poff et al. 2006; Poff et al. 1997). In short, flow creates vertical mixing that both physically homogenizes the biota and inhibits stable vertical habitat stratification other than the distinction between benthos and water column.

Lacking vertical structure, the dominant axis of habitat change in rivers is longitudinal, from upstream to downstream (Leopold & Maddock 1953, Vannote et al. 1980). High gradient, flashy headwater streams create habitats prone to high levels of disturbance (Resh et al. 1988), whereas habitats in high discharge, low gradient lowland rivers show predictable temporal fluctuations in floodplain habitat linkages and extent while maintaining perennial main channel habitats (Junk et al. 1989; Power et al. 1995). At this broadest scale (10^4-10^6 m), patterns of riverine diversity largely match longitudinal changes in habitat types (Naiman et al. 1987; Ward 1998; Welcomme et al. 2006; Winemiller & Leslie 1992), and fish species richness is well-predicted by total discharge (Guégan et al. 1998; Xenopoulos & Lodge 2006). At the other end of the scale spectrum, local hydrogeomorphic processes create patches of discontinuous habitats at scales of 10^{-1}-10^2 m (e.g., riffle-pool-run; Pringle et al., 1988, Poole 2002). At intermediate scales (10^1-10^3 m), these discontinuous patch sequences are repeated every 5-7 river widths (Knighton 1984), resulting in habitat mosaics containing complete representation of all local-scale habitat types within a reach of modest length. This may explain why studies conducted at intermediate spatial scales in rivers have shown only marginal concordance of biodiversity with habitat (Heino and Mykrä 2006, Mykrä et al. 2008, Januchowski-Hartley et al. 2011), leading to low *z* values in SACs. Put more simply, there are greater differences in habitat types, and consequently local species assemblages, between a meandering lowland river and a flashy piedmont stream than there are within each setting. Thus, conservation planning guided by maximizing representation of habitat diversity would also suggest that creating multiple reserves spaced across stream orders would be more likely to incorporate additional new habitat types than increasing the size of any single reserve in a river.

The fourth hypothesis for the species area relationship is that larger areas contain more biogeographic provinces, each with its own evolutionary and disturbance histories resulting in unique conditions for speciation (Rosenzwieg 1995, Gaston 2000). Pooling provinces captures beta diversity, as well as effectively increasing alpha diversity by sampling a larger area. Again, this thinking applies most readily to marine and terrestrial systems where biogeographic provinces share permeable boundaries. On land and in the sea, ecoregional boundaries are often ecotones, combining habitats and species along a gradient of change (Olson et al. 2001). While this reduces endemicity in any one ecoregion, it allows for large reserves designed to incorporate ecotonal boundaries to encompass more species from adjoining ecoregions (Prendergast, Quinn, & Lawton, 1999).

Such logic applies less well to rivers, where watershed boundaries act as hard physical barriers, or chemical barriers in the case of the ocean, to freshwater organism dispersal (Olden et al. 2010). The geographic separation of river basins leads to rapid species turnover across short distances, where source populations and evolutionary histories are separated by a single topographic rise. As an extreme case, the aquatic flora and fauna of the Irrawaddy, Salween, Mekong, and Yangtze rivers have been divided for millions of years by only 3 mountain ridges spread across 70 km in their headwaters, whereas their outlets span >7,000 km of marine coastline (Rainboth 1996). At the global scale, such firm dispersal barriers result in over half of all freshwater fish species being endemic to a single freshwater ecoregion, despite their smaller average size than terrestrial ecoregions (Abell et al. 2008). Fully accounting for high levels of endemism in freshwater reserve networks would require creating PAs within each major river basin to encompass all species (Olden et al. 2010).

Application to riverine reserves

The species-area relationship (and its corollaries) has long been the central motivation for creating fewer, larger reserves. However, the parameters of freshwater SACs suggest that riverine reserves may conserve richness effectively at smaller relative scales than either terrestrial or marine reserves due to higher intercepts (c) and flattened accumulation curves (z). Based on this understanding of freshwater SARs, we propose the following principles for riverine reserve design:

1. **Individual reserve lengths should exceed the average downstream egg/larval drift distance of target species.** For example, reserves having lengths twice the drift distance of target species, at a

minimum, would retain larvae hatched from half of the reserve, ensuring the potential for self-recruitment.

2. **Multiple reserves facilitate colonization.** The boundaries over which colonization can occur increase in direct proportion to the number of reserves within a river basin. Thus, increases in the number of reserves of sufficient size to support viable sub-populations, rather than expansion of individuals reserves should enhance potential colonization of reserves from non-reserve areas.

3. **Reserve placement within a basin should be stratified by river size.** Hydrogeomorphic processes produce predictable variation in biodiversity within river networks (Poole 2002), hence multiple reserves will usually be required for complete representation of habitats within a basin. As a starting point, we suggest that one or more representative reserves be delineated within each stream order.

4. **Basin-specific riverine reserves are required to capture unique biogeographical provinces.** Limited movement of riverine organisms across watershed boundaries drives high rates of basin-level endemism. Complete faunal representation will require reserves within each river basin, especially where beta diversity among ecoregions is high.

Species–energy relationships and reserve design

A second major predictor for global distribution of species is the species-energy hypothesis (Wright 1983, Currie 1991, Evans et al. 2005). In its first formulation, Wright (1983) proposed that: "Species-energy theory should therefore prove more general than species-area theory, particularly by being applicable to islands which vary in their per-unit-area resource productivity, due to factors such as climate, topography, or soil chemistry." The species-energy relationship can be expressed by a similar equation as the species-area relationship,

$$S = kE^z,$$

where k is a constant related to the number of individual organisms supported by a unit of available energy, E is the total available energy, and S and z are the same as the SAC. Thus, c and k are closely related as constants related to population density per-unit-area and per-unit-energy, respectively (Wright 1983). The widespread recognition of the species–energy relationship has led for calls to use productivity measurements for siting reserves in both terrestrial and marine systems (Gaines et al. 2010; Cabral et al. 2016; Leslie et al. 2005; Bonn & Gaston, 2005).

Unlike species–area relationships, empirically derived species–energy curves show variable patterns, especially at smaller scales, complicating estimates of *z* and synthesis across ecological realms (Evans et al. 2005). However, estimates for net primary production (NPP) and vertebrate richness from each biological realm would suggest considerably higher k estimates for freshwaters, assuming *z* estimates across realms differed by less than a factor of three (Table 1). Analogous to our *c* estimates, these results suggest that freshwaters support substantially more species per unit of energy. It is important to remember, however, that estimates of energy (E) used in our calculations represents only autochthonous energy production (NPP) within freshwaters. Rivers receive substantial energy subsidies from allochthonous sources (Minshall 1967, Webster and Meyer 1997, Baxter et al. 2005), which would cause the estimate of *k* to be inflated. However, it is unlikely that allochthonous energy contributions account for the differences estimated at the global scale because terrestrial inputs are generally of lower quality than autochthonous production (Marcarelli, Baxter, Mineau, & Hall, 2011). Still, a loss of allochthonous energy contributions would reduce their carrying capacity for animal species.

Overall energy availability generally increases along the downstream length of a river (Vannote et al. 1980, Poole 2002). Based on richness–energy relationships, this would favor placing reserves in lowland river segments and large watersheds to maximize species representation. Indeed, lowland rivers have been repeatedly shown to harbor higher levels of fish diversity than headwaters (Naiman et al. 1987, Guégan et al. 1998, Winemiller and Jepsen 1998), suggesting initial protection of high-order streams should be prioritized. However, as described in Principle 3 above, achieving complete species representation will often require reserves across stream sizes to account for headwater endemics (Winemiller et al. 2016). Therefore, creating reserves in productive lowland river segments is necessary but not sufficient for basin-wide species representation.

Species-energy relationships may be particularly useful for guiding riverine reserve selection among sites of similar stream order within a river basin. To maximize energy availability within a reserve, four correlates of in-stream productivity should be considered: light, nutrients, hydrologic variability, and availability of organic matter (Bernot et al. 2010). All else being equal, in-stream energy availability increases with increased light reaching the stream bottom, which is positively associated with an open canopy, lower turbidity, more direct overhead solar angle, and shallower depth (Jones & Stanley 2016). Watershed size and discharge are positively correlated with in-stream production due to increased potential for nutrient delivery, increased stream width, and decreased light limitation (Lamberti and Steinman 1997, Hoellein et al. 2013). High gradient rivers are more likely to have lower production due to regular biofilm scouring (Stevenson 1997) and reduced light availability resulting from high turbidity (Davies, Bunn, & Hamilton, 2008). However, production in low-gradient rivers can also become limited by turbidity and sub-optimal attachment conditions for benthic algae on fine, shifting substrates. While each of these primary factors can limit river productivity when scarce, it can also become a stressor when over-abundant (Odum et al. 1979). For example, excessive nutrients can lead to harmful algal blooms, and UV-radiation and thermal stress may arise in areas that lack riparian vegetation.

Siting of reserves should also consider the importance of external subsidies of nutrients or energy to local in-stream production (Polis et al. 1997). Riparian vegetation provides important subsidies through the deposition of leaf litter (Likens and Bormann 1974) and fruits (Goulding 1980, Corlett 1998). For example, in streams having detrital-based food webs, reaches with intact riparian vegetation will likely provide higher energy availability for the local animal community by increasing the diversity of available energy flow pathways. Terrestrial animals ranging in size from insects to hippopotami can provide substantial nutrient and energy subsidies to riverine systems (Baxter et al. 2005, Menninger et al. 2008, Subalusky et al. 2014). Fish migrations can also increase ecosystem productivity due to nutrient subsidies via multiple pathways (Flecker et al. 2010). When possible, selecting river reaches known to receive substantial energy subsidies will ensure that the natural diversity of energy flow pathways and aggregate energy availability are available to support fish and other consumers within reserves.

While considering energy availability in reserve siting may require data unavailable to conservation planners, the prioritization of river segments at and immediately below confluences is an important starting point. Stream confluences are often more productive than main channels due to combined delivery of resources (nutrients, sediments, or organisms) from multiple upstream catchments (Ward and Stanford 1983, Poole 2002). Confluences are also areas of frequent, moderate disturbance (Benda et al. 2004), which may enhance the number of coexisting species (Levin and Paine 1974, Connell 1978, Kondoh 2001). Confluences also provide higher habitat heterogeneity than available in either individual channel (Benda et al. 2004, Kiffney et al. 2006, Jones and Schmidt 2016). Finally, migratory fish species often congregate at confluences prior to and during migrations (Pracheil et al. 2013). Reserves at these sites would serve to protect migratory species at these vulnerable (and often favored) harvest locations.

Application to riverine reserves

Species–energy relationships provide motivation for identifying river segments with high in-stream productivity and/or large subsidies to maximize species inclusion. Due to the patterns of riverine species richness described previously, species-energy relationships should provide additional guidance for selecting locations from among similarly sized river segments. We suggest the following principles:

5) **Reserves should be placed on channels having large upstream catchment area**. Selecting river reaches with higher watershed area should maximize instream productivity (wider streams with less canopy coverage, more watershed area nutrient input) as well as benefitting from downstream transport of autochthonous and allochthonous production.

6) **Reserves should target confluences**. Confluences are easy to identify, and can serve as target areas for capturing areas of high energy subsidy. Reserves including confluences will also benefit from moderated levels of hydrologic disturbance, increased nutrient flows, greater habitat heterogeneity, and provide protection for migrating species in areas of high vulnerability.

Connectivity and reserve network design

Social and economic realities are likely to have greater influence on the placement and spatial extent of reserves than range sizes or dispersal distances. This may result in reserves having dimensions too small for wide-ranging or long-dispersing species. Additionally, single reserves provide incomplete protection for animals that undergo long-distance migrations. Where single reserves are insufficiently large to provide long-term protection of target species, this end may sometimes be achieved by multiple, connected reserves (Diamond 1975, Beger et al. 2010).

Potential connectivity between reserves is strongly related to the dimensionality of terrestrial, marine, and freshwater systems. In terrestrial and marine realms, connectivity among reserves is two-dimensional and often represented by lattice graphs in which *nodes* (reserves) are connected by one or more *edges* (Urban and Keitt 2001, Minor and Urban 2008, Treml et al. 2008). Theoretically, in this type of network structure, movement from one node to any other can occur by multiple pathways. In marine ecosystems, reserve connectivity is facilitated by the fact that water provides a continuous medium for movement, but there is increasing recognition of the importance in connecting marine reserves, particularly for sedentary or sessile species (Carr et al. 2003, Beger et al. 2010, Brown et al. 2016). For terrestrial reserve networks, connectivity often requires the creation of habitat corridors through fragmented landscapes to facilitate animal movement (Chetkiewicz et al. 2006). Yet the two-dimensional nature of these systems allows for animal movement or corridor creation along multiple pathways to enhance connectivity in these lattice-type networks.

For fish and other water-bound organisms, connectivity pathways among riverine reserves are absolutely constrained by one-dimensional river network structure. River systems have dendritic network structure (Fagan 2002), which is differentiated from other ecological systems by having a hierarchical, linear organization through which movement between any two reserves can occur only along a single route (Campbell Grant et al. 2007, Campbell Grant 2011). While this limits the routes by which organisms may move, dispersal within dendritic ecological networks is facilitated by the fact that both branches and nodes are composed of continuous habitat (Campbell Grant et al. 2007). This continuity of habitat can encourage movement across reserve boundaries, thereby enhancing connectivity and increasing the likelihood of metapopulation persistence (Fagan 2002). Yet the constrained structure of dendritic networks makes them especially susceptible to connectivity loss. A single barrier can fragment entire river networks, completely isolating upstream and downstream habitats. River networks, however, are rarely impacted by a single barrier. In the Laurentian Great Lakes Basin alone, over 200,000 potential stream barriers strongly reduce system-wide connectivity (Januchowski-Hartley et al. 2013, Neeson et al. 2015).

Migratory species pose a significant challenge to the use of reserves to achieve adequate spatial protection across entire species ranges. Protecting areas necessary for critical life history stages like breeding grounds, nursery habitat, and seasonal feeding sites can still provide important protection (Roberts et al. 2003; McIntyre et al. 2016). For example, large-scale land migrations like the seasonal migration of wildebeest in eastern Africa require connectivity between dry- and wet-season feeding grounds (Morrison and Bolger 2014). Successful reserve planning for migratory species requires conserving these seasonal habitats as well as maintaining connectivity among them (Santini et al. 2016). The successful use of reserves for conserving freshwater migratory fish will likewise require targeting important spawning sites, nursery habitats, and feeding grounds (Bower et al. 2015).

Application to riverine reserves

The susceptibility of rivers to fragmentation due to their dendritic network structure is the primary challenge to maintaining connectivity. As such, reserve establishment should consider the potential impacts of future development of irrigation or hydropower infrastructure on the long-term success of reserves. In addition, we suggest that:

7) **Reserves should be sited to maximize effective connectivity**. This may be facilitated by establishing reserves in river segments with high connectivity, unfragmented by dams or other barriers. In highly fragmented systems, adding reserves to tributaries between mainstem barriers will increase their functional connectivity (Pracheil et al. 2013). Finally, connectivity metrics like “betweenness centrality” can facilitate prioritization of highly-connected river segments (Erős et al. 2011).

8) **Reserves should target critical habitats for life history events.** Reserves are unlikely to be large enough to encompass the entire range of migratory species, yet conserving feeding or spawning grounds can provide protection at the especially vulnerable locations.

Enhancing fisheries through reserve design

A hallmark difference between MPAs and conventional PA design theory has been the emphasis of MPAs on both protecting biodiversity in their core and encouraging biomass spillover to support harvest beyond reserve borders (Gell and Roberts 2003). For reserves to augment local fisheries, population growth within reserves must exceed carrying capacity such that density dependent factors cause individuals to cross reserve boundaries and enter adjacent fished waters (Rowley 1994). Thus, reserves must be sufficiently small to promote spillover of larvae and adults into fishable areas, yet large enough to maintain local persistence within the reserve (Botsford et al. 2008; Gaines et al. 2010). Spillover is highest along marine reserve boundaries (Abesamis & Russ 2005), and therefore harvest can occur along multiple boundaries. Additionally, reserve shape can be modified to either discourage or encourage spillover (Hastings and Botsford 2003). Striking the appropriate balance between supporting population persistence and providing sufficient spillover is a profound challenge (Walters et al. 2007). Due to the potential for demographic synergy resulting from two-dimensional spatial connectivity, networks of mid-sized and evenly-spaced marine reserves have been proposed to maximize both biodiversity conservation and fishery benefits (Gaines et al. 2010).

The ability for reserve geometries to promote or decrease spillover is largely predetermined in rivers. Potential spillover from riverine reserves can only occur at borders having dimensions fixed by river width. Additionally, the relatively small scale of rivers allows fishing effort across entire river widths, resulting in efficient harvest of large-scale fish movements across even major rivers. For example, the Cambodian 'Dai' fishery consists of 14 barrages of over 60 bag-nets, which together span the full width of Tonle Sap River above its confluence with the Mekong, and make it among the most valuable and intensive inland fisheries in the world (Sabo et al. 2017). Thus, for riverine reserves, the borders along which legal harvest is concentrated will be inherently small regardless of reserve area.

Reserve geometry also has important implications for effective enforcement of reserves. Effective governance and enforcement have been repeatedly recognized as a major predictors of protected area success (Nolte et al. 2013, Edgar et al. 2014). Just as minimizing perimeter-to-area ratios for reserves not only mitigates natural edge effects, it minimizes the perimeter over which poachers can access reserves. The scale of marine reserves provides a significant challenge to enforcement, but the conspicuous nature of fishing vessels provides the ability to monitor reserve boundaries by boat or even satellite. Enforcement at many terrestrial reserves occurs along roadways; while not totally effective, such transportation corridors serve as effective pinch points for regulation. In rivers, increases in reserve area require elongating protected reaches, which increases their perimeter to area ratios and thereby increases the effective edge length across which poachers can access riverine resources. Illegal harvest may be especially difficult to monitor within riverine reserves, especially across long reserves. Even in the widest rivers, such enforcement faces severe challenges, which is further hampered by the relatively small scale and diffuse nature of inland fisheries.

Application to riverine reserves

The geometry of riverine systems imparts strong limits on how to design reserves to maximize spillover. Thus, we suggest two additional principles for riverine reserves in order to enhance local fisheries.

9) **Small reserves maximize spillover and facilitate enforcement.** Increasing the size of a single reserve will maximize local community persistence, but spillover will remain limited by the largely unchanged width of upstream and downstream boundaries. However, creating additional reserves of sufficient size to maintain spillover should increase overall catch. Enforcement of riverine reserves is challenged by the inherently long perimeters of riverine reserves over which fish are easily poached. Therefore, creating multiple, smaller reserves in proximity to, and in coordination with, local communities will increase local enforcement, promoting regulated harvest at reserve boundaries.

Implementing Riverine Reserves

Integrating the lessons learned from the experience of terrestrial and marine PAs, we believe that networks of small riverine reserves could be a highly effective strategy for protecting freshwater biodiversity and fisheries. Species–area relationships suggest that small reserves will contain high representative species richness within a given area (large *c* values), and furthermore, that increasing reserves size will have lower return on investment (low *z* values). Dispersal distances and home range sizes for freshwater organisms are generally small relative to terrestrial or marine organisms, thus reducing the need for expansive reserves. The linear nature of rivers also suggests that demographic benefits associated with increasing the reserve size will have little to no effect on colonization probability but will inherently increase the difficulty in enforcing harvest prohibitions due to inevitable increases in perimeter-to-area ratios. Finally, implementing numerous small reserves will enhance stability of meta-populations, and, if stratified by stream order, may capture more complete species representation within river basins than the creation of fewer, larger reserves.

The demonstrated capacity of reserves to protect marine biodiversity has been widely hailed as a major conservation success. However, whether they can maintain or augment local fisheries is an area of active discussion and research. Despite ongoing debate, there is general consensus that marine reserves are important components of ecosystem-based management plans in conjunction with more traditional harvest regulation (Allison et al. 1998, Hilborn et al. 2004, Worm et al. 2009). Likewise, riverine reserves should not be seen as a panacea for protecting freshwater ecosystems. The pervasiveness and severity of ecosystem alteration and the nature of rivers as integrators of landscape-scale disturbance make this obvious. Yet, we suggest that riverine reserves can serve as integral components of broader integrated watershed management plans, especially where intensive fisheries pose a major threat to freshwater biodiversity and human food security.

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Table 1. Species–area and species–energy coefficient values by ecological realm.

Realm	**Vertebrate richness**[a]	**Area (km^2)**	**NPP ($Pg\ C\ yr^{-1}$)**	z[e]	c	k
Terrestrial	25,000	1.48×10^8	53.8[b]	0.31	73.3	7162
Marine	15,625	3.62×10^8	48.5[c]	0.33	23.4	4340
Freshwater	22,100	3.62×10^6 [d]	2.6[c]	0.21	927.0	25562

a (Grosberg et al. 2012)

b (Field 1998)

c calculated from (Dokulil 2009)

d (Raymond et al. 2013)

e recalculated from (Drakare et al. 2006)

Chapter 4

Riverine reserves increase richness, density, and biomass of inland fisheries

Prepared for Submission to *Science* with co-authors Martin Perales and Peter B. McIntyre.

Abstract

Intensive harvest has resulted in significant declines in biodiversity, abundance, and biomass of marine and inland fisheries worldwide. No-take reserves have become a critical component of marine ecosystem-based fishery management, yet the potential translation of spatial protection to freshwater systems, and specifically rivers, has not been fully realized. Several features of marine reserves have been found to predict reserve success regarding increasing fish diversity, density, and biomass relative to fished areas beyond reserve boundaries. These features include reserve size, age, enforcement, and isolation. Using a network of 23 community-based riverine reserves located in a tributary of the Salween River Basin, we test the potential for these same features derived from the marine reserve literature, as well as the network properties of our study river, to benefit riverine fish communities. We find that riverine reserves have strong positive impacts on overall fish richness, density, and biomass. However, different reserve features were found to predict richness, density, and biomass. Additionally, we found that the dominant predictors of reserve success varied further when we considered fish assemblages based on functional traits. The complexity of the responses to reserve protection strongly suggest that networks of reserves composed of independently designed reserve having

specific conservation targets will likely be required to achieve the full conservation potential of riverine reserves.

Introduction

Overharvest threatens fish diversity and continued provisioning of important nutritional resources around the world (Worm et al. 2009, McIntyre et al. 2016). In the past two decades, the creation of no-take marine reserves has become a critical management tool for conserving marine biodiversity, as well as sustaining local fisheries (Gell and Roberts 2003, Halpern et al. 2010, Edgar et al. 2014). Additionally, networks of reserves often show increased biomass or diversity incommensurate with individual reserve attributes, indicating that disproportionate benefits can emerge from well-designed reserve networks (Gaines et al. 2010). While much of the attention on overfishing has been focused on marine ecosystems, many inland fisheries arguably face even greater cumulative threats: globally high rates of biodiversity loss (Pimm et al. 2014), diverse stressors of both direct (fragmentation, flow regulation; (Winemiller et al. 2016) and diffuse origins (pollution, climate change; (Vörösmarty et al. 2010), and intensive and increasing human dependence on their resources (Allan et al. 2005, McIntyre et al. 2016). Though the potential benefits of creating reserves in inland waters are compelling, rivers and lakes are heavily underrepresented by dedicated protected areas (Abell et al. 2007a, Nel et al. 2009).

The success of reserves in enhancing the abundance, biomass, and body size of fish (Lester et al. 2009) has inspired theoretical and empirical analysis of principles for maximizing the efficacy of marine reserve networks (Roberts et al. 2003, Gaines et al. 2010, Edgar et al. 2014). In particular, exponential increases in benefits from marine reserves are associated with having 3 or more of the following key features: no-take status (vs. regulated harvest), vigorous enforcement, old age (years of protection), large size, and high degree of isolation (Edgar et al. 2014). Local human population size (Cinner et al. 2012) and network connectivity have been proposed as additional predictors of marine reserve success (Botsford et al. 2001, Botsford et al. 2003, White et al. 2010, Cinner et al. 2012).

The potential for these same attributes to reduce rates of species loss while supporting inland fisheries provides a forceful motivation for implementing networks of riverine reserves. To date, reserve-based conservation for freshwater ecosystems has largely been limited to addressing the potential for terrestrial reserves to confer protection to freshwater organisms (Nel et al. 2007, Darwall et al. 2011, Lawrence et al. 2011, Frederico et al. 2018). Terrestrial reserves generally are inadequate to represent freshwater biodiversity (Abell et al. 2011, Darwall et al. 2011), yet the adaptation of design principles from terrestrial and marine conservation to create riverine reserves could provide similar benefits, buffering freshwater biodiversity from a broad suite of current and future stressors (Koning Chapter 3).

Despite the appeal of transferring lessons from MPAs to inland waters, the physical structure of river networks has several important contrasts with the two or even three-dimensional connections among individual reserves in terrestrial and marine ecosystems. First, as dendritic ecological networks (Campbell Grant et al. 2007), rivers have a hierarchical structure in which connectivity between any two nodes (reserves) can only occur by one spatially constrained path: the river channel (Fagan 2002). In forests, grasslands, and oceans there are typically a variety of alternative paths between reserves, even when fragmentation has occurred (Santini et al. 2015, Brown et al. 2016). For example, seasonal movement within a network of 4 reserves from reserve A to reserve D can occur in any order (A-B-C-D, A-C-B-D, A-B-D, A-D, etc.). However, in a network of 4 riverine reserves, movement between reserve A and D can only occur by one path and will require passing through reserves B and C. Second, although river basins encompass whole continents, rivers as aquatic habitats are small landscape features. As such, the sizes of potential riverine reserves would likely be orders of magnitude smaller than either terrestrial or marine PAs (10^1–10^7 km^2), at least in terms of the channel area encompassed. Third, the natural habitat units in rivers—riffles, runs, and pools—tend to be small and predictably recurrent due to hydrogeomorphological processes (Leopold and Maddock Jr 1953). Thus, unprotected river segments between reserves generally provide physical habitat like that of reserves, differing from terrestrial or marine settings in which inter-reserve movement often requires negotiating degraded habitats within fragmented landscapes (Campbell Grant et al. 2007). These fundamental differences suggest that adapting design principles from terrestrial and marine reserve networks to rivers will be challenging.

Here, we test the effects of well-established reserve design principles and metrics of network structure on fish species richness, density, and biomass relative to non-reserves across a network of 23 riverine reserves. Specifically, we evaluate 4 key predictors of reserve success (reserve age, size, enforcement, and isolation) in conjunction with 3 network parameters that specify one-dimensional connectivity within riverine systems (distance from large-river confluence, distance to nearest reserve, centrality within reserve network). We then discuss the potential application of riverine reserve networks within an ecosystem-based management framework to conserve both biodiversity and sustainable fisheries in the world's rivers.

Methods

Fish Surveys

We surveyed fish assemblages within the Mae Ngao tributary of the Salween River, the largest free-flowing river in Southeast Asia (Fig. 1). We have identified 51 community-initiated reserves within the Mae Ngao basin. Between December 2017 and March 2018, we surveyed 23 individual reserves selected to represent a range in age (1-25 years), size (0.2 – 2 Ha), isolation from villages, size of nearest village, stream order (1st – 5th), and network position.

To estimate the potential for reserves to increase fish richness, density, and biomass, we surveyed 23 paired reserve and non-reserve locations. Non-reserve surveys were conducted immediately downstream of reserves at all locations except one, to which there was only upstream access. Where stream segments were sufficiently narrow and shallow (19 of 23 sites), two researchers wearing dive masks and snorkels censused fish by swimming/crawling 50 m from downstream to upstream. In the 4 large mainstem sites, one researcher conducted fixed-time (20 min) surveys from downstream to upstream throughout a 50 m reach. To account for benthic or cryptic species in shallow water (<80 cm) at these 4 sites, a second researcher conducted 4 lateral belt transects at each survey site. Belt transects were demarcated with a chain placed on the substrate perpendicular to flow. To allow disturbance effects to dissipate, we waited 15 min after chain placement to begin surveys. Fish were enumerated in a 2 m band centered on the chain for up to 20 m of stream width.

At each site, researchers recorded the number of individuals for each fish species observed, the total area surveyed, and the duration of survey effort. Counts of individuals and species were summed across researchers at each site and divided by the total area surveyed to calculate species-specific densities. For the 4 mainstem sites, we combined density estimates using count-weighted averages of both survey techniques for each survey location. To estimate site-specific biomass, we recorded total lengths of fish for each survey. When fewer than 10 individuals of a species were observed, lengths were estimated for each individual. For species with counts > 10, researchers estimated 10 lengths representing the size distribution observed at each site. Estimated lengths were cross-validated between researchers in the field using submerged measuring tapes.

We calculated species richness, fish density, and fish biomass for each reserve and non-reserve site. We used length–weight relationships developed from previous work in the Ngao River, supplemented with literature values, applied to site-specific length estimates to estimate biomass for each species at each location (Froese and Pauly 2017).

Habitat variables

We measured key aspects of reach habitats that could affect fish communities, then tested for habitat differences within (reserve vs. non-reserve) and among areas. We tested for differences in mean depth, maximum depth, mean width, median substrate particle size, and three metrics of substrate diversity: Simpson's diversity index of substrate types, and the loading scores for the first two axes of a principle component analysis of substrate types by site. At each transect location, we measured depth and substrate composition at 6 belt transects corresponding with 0, 10, 20, 30, 40, and 50 m marks. Depth and substrate were recorded at 10 equally-spaced locations across the stream. Substrate categories followed the Wentworth classification: silt (<62.5 μm); sand (62.5 μm–2 mm); pea gravel (2–8 mm); gravel (8–32 mm); pebble (32–64 mm); cobble (64–256 mm); boulder (> 256 mm); bedrock (>4000 cm). To calculate median particle size at each site, we used the median size of each particle class for each observation, then calculated the median across all 60 point-samples. For mainstem sites where both snorkel and benthic counts were employed, we conducted 5 lateral transects from the chain counts and snorkel surveys for a total of 10 transects and 100 benthic point samples. We also measured discharge at each reserve/non-reserve location to account for the effect of segment size on reserve outcomes using standard methods (Hauer and Lamberti 2007).

Reserve features

We interviewed community members to determine reserve features that could affect benefits for fish communities. For each reserve, we received a discrete estimate of age (years since reserve establishment), enforcement (penalty for illegal harvest; yes or no), size (reserve length × average width), local population (number of households in nearest village), and isolation (distance from reserve to nearest village and nearest road) from over 35 interviews. To calculate isolation metrics, we digitized the Mae Ngao River network from satellite imagery (Google Earth, 2017), and mapped all potential stream courses regardless of the presence of visible surface water. We used field observations to constrain the digitized stream network based on the presence of water in 20 tributaries during the height of dry season (early May, 2016). Using our digital river network and a digital elevation model, we extracted the upstream catchment area and average slope for each tributary, then modeled the presence of water using a logistic regression of area and slope estimates. We used this model to predict the upstream extent of perennial flows and trimmed our digitized network accordingly. We also delineated all roads and villages within the Ngao River Valley from satellite imagery. Finally, we calculated Euclidean distances between each reserve and the nearest road and village as metrics of reserve isolation.

Considering these reserves as a network, we also calculated three additional network parameters that could influence ecological responses: river distance to the nearest reserve, river distance to mainstem confluence, and betweenness centrality. Betweenness centrality is an index used in network analysis that describes the relative importance of each node (*i.e.,* reserve) to overall connectivity within the network. Specifically, the standardized betweenness centrality for a node *i* is calculated as:

$$BC_i = \frac{2 \times \sum_{j<k} g_{jk}(i)/g_{jk}}{(N-1)(N-2)}$$

where $i \neq j \neq k$, g_{jk} is the number of equally shortest paths between nodes *j* and *k*, $g_{jk}(i)$ is the number of these paths which include node *i*, and the denominator represents twice the total number of node pairs without node *i* (Jordán et al. 2006).

Data Analysis

To test the effects of reserve features on fish species richness, density, and biomass, we calculated a reserve response index for each outcome variable. For species richness (*S*), we calculated a reserve response index (R_s) as $S_{\text{reserve}} - S_{\text{non-reserve}}$ (Claudet et al. 2008). We calculated the reserve response indices for fish density (R_d) and biomass (R_b) as: $log_{10}({}^{reserve\ mean}/_{non-\ reserve\ mean})$. We also calculated the variance for each site-specific response index, which was then used to weight our regression models (Hedges et al. 1999).

In addition to testing the significance of overall reserve responses across sites, we used functional trait categorization of species to test for differential response by size and trophic position. Both the magnitude and direction (+/–) of organismal responses to reserve protection have been shown to vary based on life history and ecological traits (Claudet et al. 2010). We divided fish into large-bodied and small-bodied categories using a threshold of 15 cm maximum length based on the size frequency distribution across all species (Supplemental Figure 1). Fish were classified as top-predators based on trophic position estimates > 3.5 nitrogen stable isotopes (Koning Chapter 2) or literature data (10 of 38 species; Pauly and Froese, 2018).

We regressed the response indices for richness, density, and biomass against all predictor variables. Reserve area, distance to river mouth, distance to nearest reserve, discharge, distance to nearest village, distance to nearest road, and years of protection were $\log_{10}$ transformed prior to analysis. Log 10 transformation was used to account for an expected or observed saturating relationship with one or more response variables (e.g., response to protection is expected to decline with reserve age), and transformation improved the fit of models in every case. We evaluated all factorial models, and quantitatively compared the subset of models within 2 AICc units of the preferred model. We calculated Akaike weights (W_i) for each of the finalist models, and used them to estimate the relative importance of each predictor (Johnson and Omland 2004). All analyses and model fitting were conducted using the 'MuMIn' package in R (Bartón, 2018).

Results

Our results show the potential for reserves to have rapid and lasting effects on the species richness, density, and biomass of riverine fish communities (Fig. 2). Moreover, individual reserve features mediate the degree of benefits to particular functional groups (Tables 1–3). Thus, implementing effective riverine reserves will require designing a network whose features specifically support local conservation priorities (e.g., target taxa or functional groups, or biomass production for fisheries).

Richness response (R_s) to reserves

The single best predictor of the reserve effect on total species richness (R_s) was discharge ($W_i = 0.73$), indicating reserves in larger river segments had disproportionate benefits for local species richness (Figure 3A). R_s for large fishes was also best predicted by discharge ($W_i = 1.0$; Figure 3A;), but reserve area ($W_i = 0.25$) and nearest village size ($W_i = 0.21$) were also important. Interestingly, discharge was not among the best predictors of R_s for small fishes; instead, both the existence of a penalty and reserve age appeared in all of the best-fitting models ($W_i = 1.0$). Whereas R_s was higher for small fishes in reserves where illegal harvest was penalized, R_s actually decreased with the duration of protection (Figure 3B). Among the top predators, R_s was poorly predicted by any reserve attributes (e.g., the null model was the 4th best-fitting model; Table 1), but proximity to the Salween mainstem ($W_i = 0.71$), discharge ($W_i = 0.29$), and reserve area ($W_i = 0.29$) were included in some of the best models. The poor fit for predator richness may be due in part to having only 7 of 38 species whose trophic position exceeded 3.5.

The strong positive relationship between discharge and R_s for all fish, large fish, and predatory fish corresponds with general patterns of riverine species richness, which increases with discharge (Guégan et al. 1998, McIntyre et al. 2016), and thus the potential number of species gained within reserves. Likewise, species-area relationships suggest that larger reserves should confer greater benefit to R_s. In particular, the best predictors for predators, proximity to river mouth, discharge, and reserve area, are concordant with general patterns of increasing food chain length with ecosystem size across other freshwater systems (Sabo et al. 2010). Notably, the second-best-fitting model for total fish richness, and the sole model to not include discharge, contained only betweenness centrality ($W_i = 0.27$). This result suggests that connectivity among reserves may boost the richness of each, regardless of size. In summary, R_s for all fish, large fish, and predators was driven primarily by properties of the river network, while R_s of small fishes was associated with reserve features of such as enforcement and age.

Density response (R_d) to reserves

Density responses of all fish and large fish were strongly influenced by the feature associated with reserve isolation: proximity to nearest village (W_i = 1.0). Specifically, our results indicate that close proximity to a village increases reserve efficacy, or conversely, reserves further from villages are more easily harvested illegally (Figure 4). Small fish R_d, like R_s, was again best predicted by the existence of a penalty (Wi = 1.0) and decreased with reserve age (W_i = 0.48). Decreased R_s and R_d for small fish with increased reserve age likely results from competitive exclusion or consumptive/non-consumptive effects resulting from concurrent increases in densities of large-bodied and/or predatory fishes. Our previous work has shown similar inverse relationships between densities of large and small-bodied fishes across the boundaries of individual reserves (Koning Chapter 2).

Predator R_d was best predicted by two models that both included distance from river mouth, reserve area, and years of protection (W_i = 1.0 for all). No single predictor had a significant relationship with predator R_d when tested in isolation, yet each was strongly significant in combination ($p < 0.01$). Large reserves and those closer to the river mouth had higher predator R_d, while R_d values decreased with increasing reserve age (Table 2). We interpret these patterns as support for the importance of connectivity to external sources and reserve area for maintaining populations of roving predators, and that with time densities are increased sufficiently by connectivity and area to allow spillover of predators, which dampens R_d.

Biomass response (R_b) to reserves

Total fish R_b was best predicted by one model describing positive effects of distance to nearest village, reserve area, and distance from the Salween confluence. Similarly, R_b for large fish increases with reserve size (W_i = 1.0; Figure 5), greater distance from the river mouth (W_i =

0.44), and proximity to the nearest village ($W_i = 0.37$). Like R_d, increasing distance from nearest village decreased R_b, likely due to reduced enforcement resulting from greater isolation. Larger reserves and greater distance from the river mouth both increased the biomass response within reserves. One potential mechanism by which large reserve size may increase areal biomass is by reducing rates of spillover. Harvest is the greatest source of mortality for most fish within this system. However, larger reserves are more likely to include greater resource availability, thereby reducing the need to forage outside reserves and enabling fish to live longer and grow larger within them. The positive relationship between fish biomass and distance from the river mouth suggests that these patterns among reserves of the Ngao River are not dependent on source populations outside of the watershed. Although betweenness centrality was not included in the top model, within-network connectivity may be more important for maintaining reserve total fish biomass than connectivity to external sources. There were no reserve features that strongly predicted R_b for small fish; the best-fitting model was the null model and no single predictor had $W_i > 0.14$. Predator biomass response ratios decreased with increasing distance from the river mouth (unlike total and large fish R_b) and increased with increasing reserve area. Again, predator R_b decreased with increasing years of protection, suggesting spillover of predatory fish across reserve boundaries over time.

Discussion

Overall, we found that small riverine reserves (average length ~ 750 m) had strong effects on richness, density, and biomass of fish (Figure 2). While there is substantial theoretical support for the potential efficacy of even small reserves in riverine ecosystems (Koning Chapter 3), the overwhelmingly positive fish responses to reserves as small as 0.2 Ha is surprising. Despite the relatively small sizes of all reserves, key response variables still improved most in the largest reserves. This result contrasts with findings from marine systems, which suggest reserve size alone is often a poor predictor of response magnitude. Our best fitting models suggest that doubling reserve area results in increases of 150% and 450% in R_b for large and predatory species respectively. We did find that reserves begin to show benefits in relatively short periods of time, as has been shown repeatedly in marine reserves (Halpern and Warner 2002), albeit not instantaneously. We found negative response indices for all variables at the sole site having less than 1 year of protection, but positive R_b for all reserves receiving > 2 years of protection.

Comparing response to protection among functional groups of species underscores the role of community dynamics in mediating conservation outcomes. For instance, we found that richness and densities of small fishes decreased with increasing reserve age (Figure 3B), and further, that in large reserves located lower in the river network, predatory fish apparently reach high enough densities to spillover reserve boundaries as years of protection increase (Table 2). Together these results suggest that after an initial period following reserve establishment, in which small fish R_s and R_d are high, the lagged accumulation of large and predatory fish results in reductions in richness and density of small fish in reserves relative to outside. Additionally, although proximity to river mouth increased predator richness, betweenness centrality appeared in best-fitting models of R_s for all fish and small fishes. These patterns align with modeled meta-population dynamics for dendritic ecological networks (Fagan 2002). While persistence of smaller and more resident species is facilitated by bi-directional movement among multiple nearby source populations, the habitat requirements of large predators make their persistence more dependent on immigration from downstream sources in larger rivers (Campbell Grant 2011). Therefore, implementing reserves to protect targeted large predators may require networked mainstem reserves, but protection of smaller and more resident fish should incorporate highly connected reserves within the dendritic river structure. Indeed, encompassing the full spectrum of fish species within a reserve network may require inclusion of complementary low-order and high-order sites (Koning Chapter 3).

Interestingly, enforcement of penalties was only a significant predictor for conservation of small fishes, suggesting that surveillance by nearby communities is sufficient to deter harvest of most species (Tables 1–3). This undoubtedly reflects the structure of village societies within our study region, but also accords with widespread reports that empowering communities to manage local resources can achieve better conservation outcomes than top-down, centralized management structures (Agrawal 2003, Ostrom 2009, Nolte et al. 2013). Likewise, the apparent success of this reserve network has emerged through the largely uncoordinated actions of over 50 communities, including the people who create, enforce, and benefit most directly from protection of fish. This potential feedback may help to explain why fish density and biomass were strongly related to proximity to the nearest village (Fig. 5). Often, we found reserve boundaries were located within eyesight of the most upstream and downstream houses in a sponsoring village. This simultaneously reduces the work of surveillance and minimizes the distance required to access fishable river segments flanking each reserve.

Despite the apparent success of small riverine reserves to benefit species richness, density and biomass, important questions remain. First, if protected sub-populations become isolated by intensive fishing between reserves, long-term meta-population persistence is questionable (Hastings and Botsford 2003, Palumbi 2003, Gaines et al. 2010). This is especially true for large-bodied predators, which we found at very low densities within any single reserve. In highly seasonal ecosystems like the Salween River basin, connectivity between reserves likely occurs during the wet season, when seasonal rains increase water levels by several meters, simultaneously cueing spawning migrations by many species and decreasing the efficacy of local fishing efforts. This period of relaxed constraints on movement may enable a critical influx of individuals and alleles, avoiding bottlenecks and inbreeding that could doom the metapopulation (Palumbi 2003). Where movements are perpetually restricted or harvest effectively prevents reserve connectivity, effective conservation will require harvest management strategies as well (Hilborn et al. 2004, Worm et al. 2009).

It also remains to be seen whether riverine reserves can provide substantial biomass production to support local fisheries beyond their borders. To date, evidence of spillover from riverine reserves is anecdotal, yet fishermen regularly harvest large fish outside of reserves. While further research is warranted, the uncoordinated proliferation of reserves among communities lacking external incentive suggests the benefits of this conservation action are tangibly realized. Despite a general lack of coordination among communities, the emergent network of reserves has provided a model of effective grassroots conservation, and furthermore, has offered key insights regarding those features that contribute to greater reserve network success. Incorporating these insights with existing and future community-based reserve management plans may lead to even greater benefits to biodiversity and local food security (Gaines et al. 2010).

The transfer of reserve design principles from terrestrial to marine systems took decades, yet implementation and experimentation with marine protected areas has become pivotal in maintaining the resilience of ocean fisheries. Inland waters face similar threats, and the accumulated wisdom from marine and terrestrial reserve networks could be a springboard toward protecting freshwater resources upon which local communities depend heavily. Our results indicate that many of the same design principles are applicable, but also suggest that new ones may be required to suit the physical structure of river networks and life history needs of their species. In any case, the profound benefits of even small reserves for fish richness, density, and biomass in the Salween basin can serve as a model for further study and development of riverine reserve networks.

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Figure Captions

Figure 1. Reserve locations within the Mae Ngao River. Surveyed reserves are marked in blue; unsurveyed reserves are in red.

Figure 2. Response indices for species richness (R_s), density (R_d), and biomass (R_r). Gray bars give index means, red points are index medians, and black points are individual reserve responses. Scales differ based on index calculations found in the text.

Figure 3. Reserve response of species richness (R_s) by individual reserve features. A) R_s for all fish and large fish (max length >15cm) increased with $\log_{10}$(discharge). B) R_s for small fish (max length ≤ 15cm) decreased with reserve age ($\log_{10}$years of protection) and enforcement (penalty; inset).

Figure 4. Reserve response of species density (R_d) for all and large fish by $\log_{10}$distance to village.

Figure 5. Reserve response of species biomass (R_b) for all and large fish by $\log_{10}$reserve area.

Figure 1.

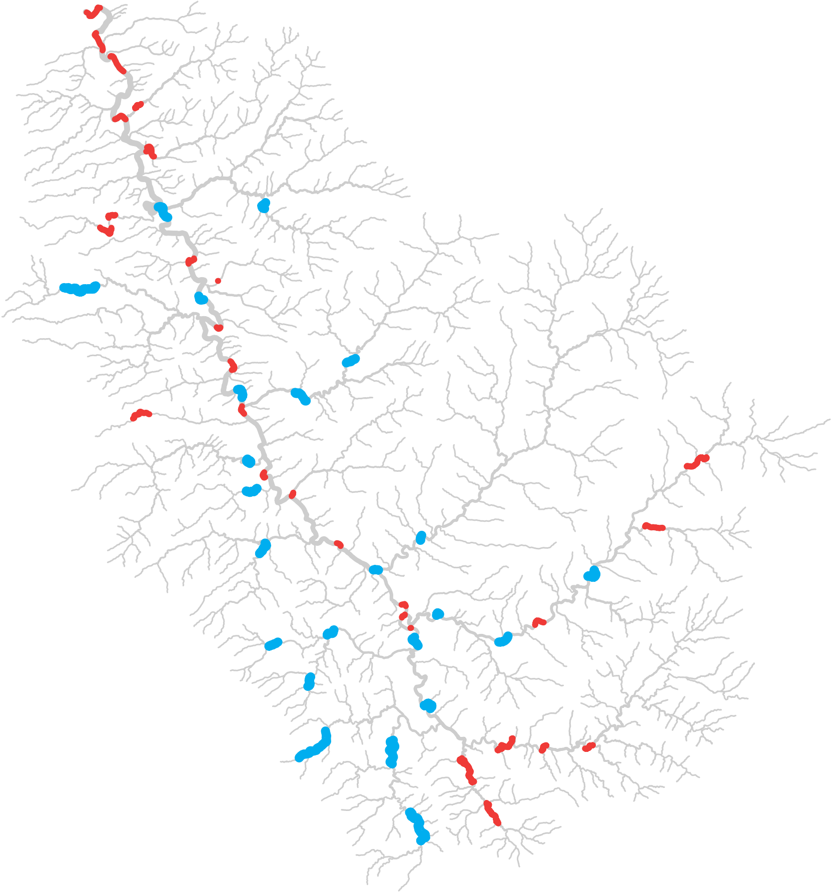


Figure 2.

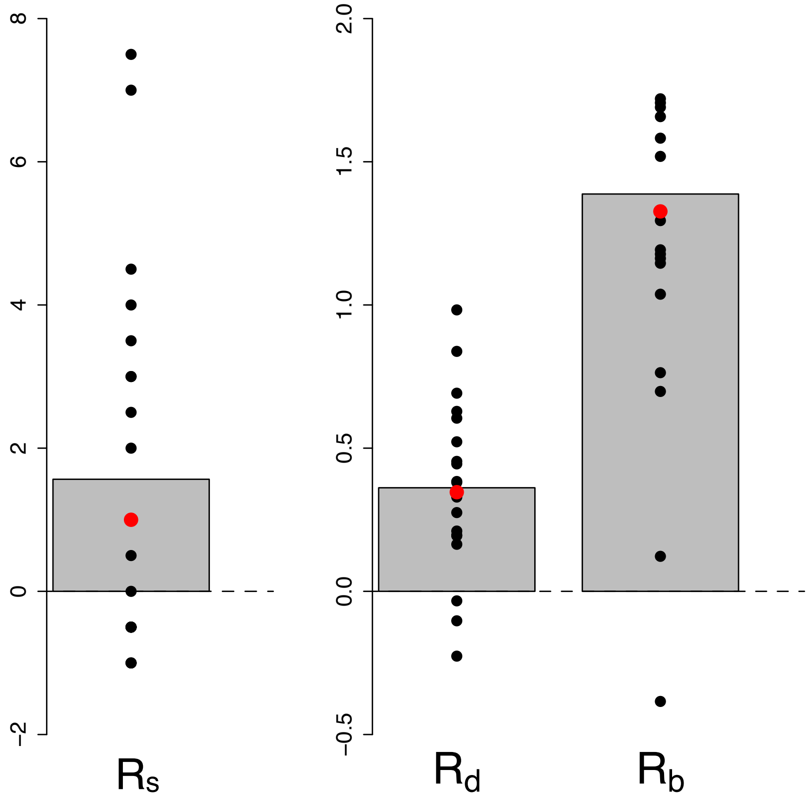


Figure 3.

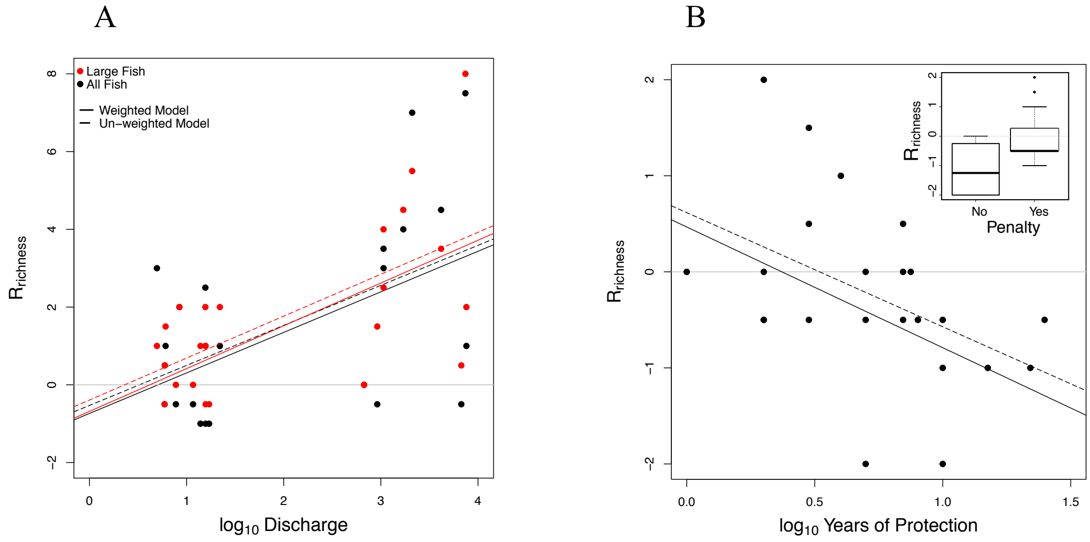

A
Large Fish
All Fish
Weighted Model
Un-weighted Model
$R_{richness}$
−2
0
2
4
6
8
0
1
2
3
4
$\log_{10}$ Discharge
B
$R_{richness}$
−2
−1
0
1
2
0.0
0.5
1.0
1.5
$\log_{10}$ Years of Protection
$R_{richness}$
−2
−1
0
1
2
No
Yes
Penalty

Figure 4.

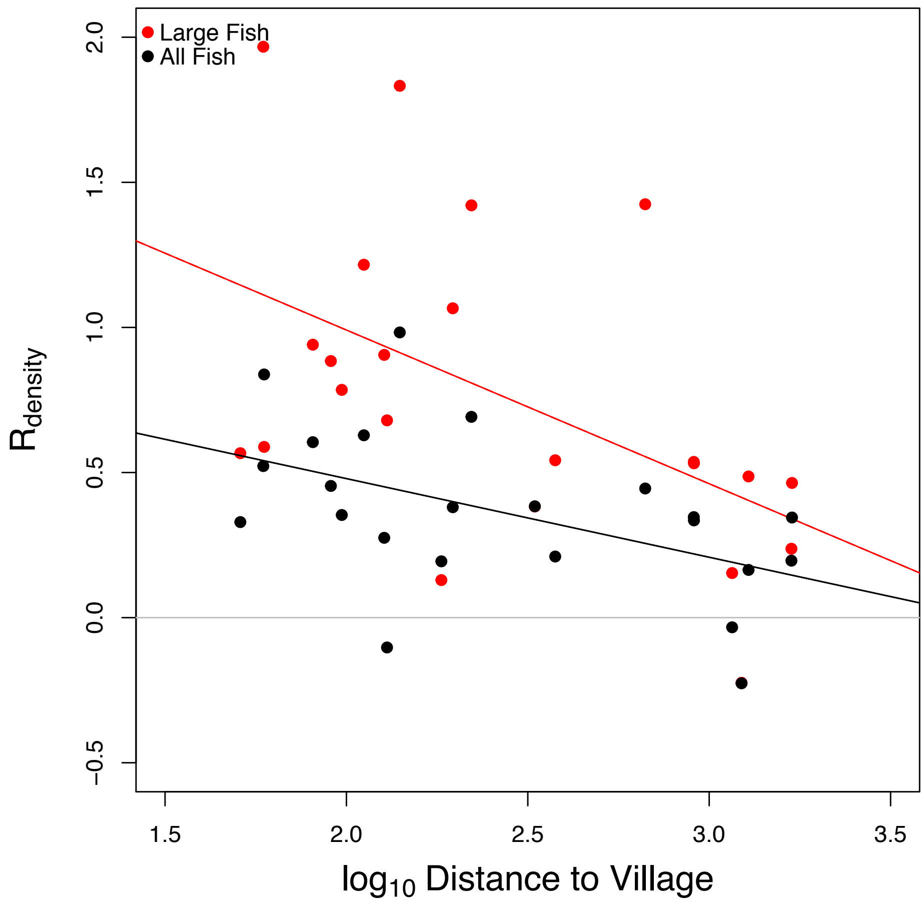
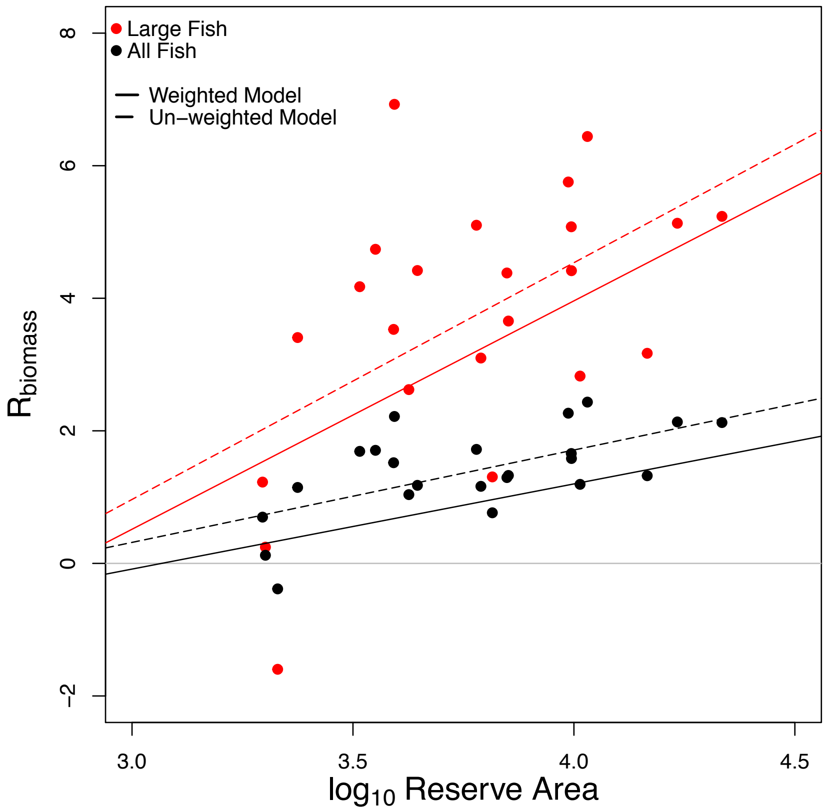

Large Fish
All Fish
$R_{density}$
$\log_{10}$ Distance to Village
2.0
1.5
1.0
0.5
0.0
−0.5
1.5
2.0
2.5
3.0
3.5

Figure 5.



● Large Fish
● All Fish
Weighted Model
Un−weighted Model
R$_{biomass}$
8
6
4
2
0
−2
3.0
3.5
4.0
4.5
log$_{10}$ Reserve Area

Table 1 Best-fitting model results for conservation outcomes predicted by reserve features.

				Size		**Age**	**Enforcement**	**Isolation**
		Model	**Intercept**	Reserve area	Discharge	Years protected	Penalty (+/-)	Distance to nearest village
R_r	All Fish	1	-0.74		**1.04**			
		2	0.49					
		3	-1.84		**1.04**		+ (1.33)	
		4	0.04		**1.14**	-1.36		
	Large Fish	1	-0.68		**1.10**			
		2	-6.08	1.53	**0.91**			
		3	-1.25		**1.17**			
	Small Fish	1	-0.45			**-1.74**	**+ (1.53)**	
		2	-0.50			**-1.90**	**+ (1.55)**	
		3	1.53			**-1.78**	**+ (1.52)**	
		4	-2.78	0.68		**-2.10**	**+ (1.55)**	
	Predatory Fish	1	6.38					
		2	7.60	0.96		-0.94		
		3	-0.25		0.15			
		4	0.06					
		5	5.03		0.11			
		6	4.56	0.35				

Table 1 (continued) Best-fitting model results for species richness predicted by reserve features.

Network Features			**Human Population**	**Model Selection**		
Distance to nearest reserve	Distance to river mouth	Betweenness centrality	Houses in nearest village	**logLik**	**AICc**	**Akaike weight**
				-49.5	106.2	0.39
		7.27		-49.9	107.0	0.27
				-48.8	107.8	0.18
				-48.9	108.0	0.16
				-43.5	94.2	0.54
				-42.8	95.7	0.25
			0.01	-42.9	96.0	0.21
				-22.5	55.2	0.43
		1.18		-21.5	56.5	0.22
-0.56				-21.7	56.9	0.18
				-21.8	57.1	0.17
	-1.37			-16.7	40.7	0.29
	-2.26			-14.0	41.6	0.18
				-17.3	42.0	0.15
				-18.8	42.1	0.14
	-1.12			-16.0	42.2	0.14
	-1.26			-16.2	42.6	0.11

Table 2 Best-fitting model results for fish density predicted by reserve features.

				Size		**Age**	**Enforcement**	**Isolation**
		Model	**Intercept**	Reserve area	Discharge	Years protected	Penalty (+/-)	Distance to nearest village
R_d	All Fish	1	1.02					**-0.27**
		2	1.69					**-0.25**
	Large Fish	1	2.05					**-0.53**
		2	2.21				+ (-0.24)	**-0.51**
	Small Fish	1	-0.50				**+ (0.55)**	
		2	-0.37			-0.27	**+ (0.62)**	
		3	3.37			-0.47	**+ (0.59)**	
	Predatory Fish	1	23.15	**2.22**		**-2.73**		
		2	22.82	**2.60**		**-3.02**		0.43

Table 2 (continued) Best-fitting model results for fish density predicted by reserve features.

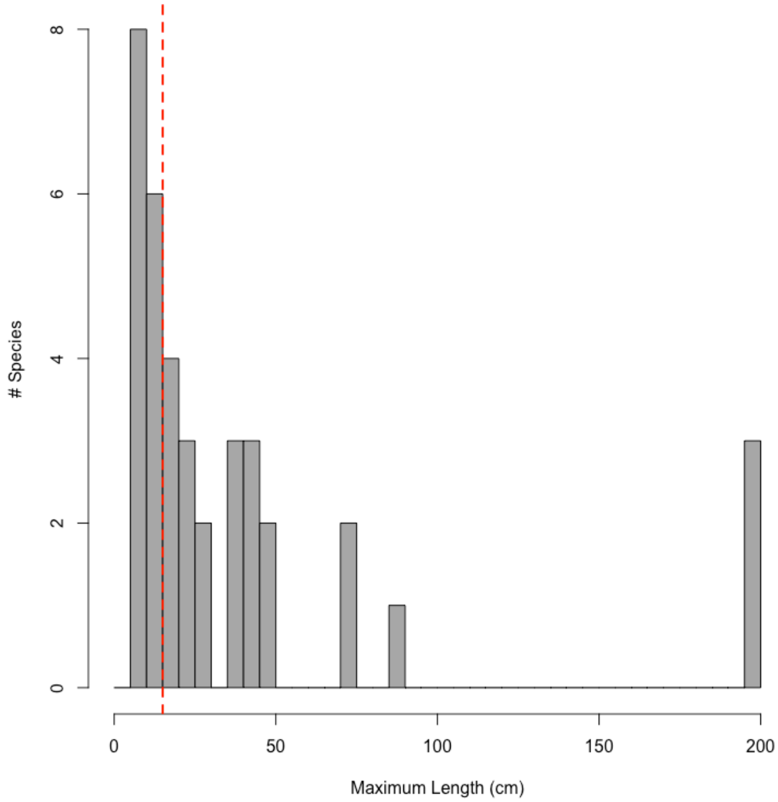
Network Features			**Human Population**	**Model Selection**		
Distance to nearest reserve	Distance to river mouth	Betweenness centrality	Houses in nearest village	**logLik**	**AICc**	**Akaike weight**
				0.3	6.6	0.67
-0.21				1.1	8.0	0.33
				-14.5	36.2	0.73
				-14.0	38.2	0.27
				-7.0	21.2	0.51
				-6.1	22.3	0.29
	-0.77			-4.8	23.2	0.19
	-3.50			-42.18	97.9	0.68
	-3.96			-41.09	99.4	0.32

Table 3. Best-fitting model results for fish biomass density predicted by reserve features.

		Model	**Intercept**	**Size**		**Age**	**Enforcement**	**Isolation**
				Reserve area	Discharge	Years protected	Penalty (+/-)	Distance to nearest village
R_b	All Fish	1	-9.60	**1.19**				**-0.48**
	Large Fish	1	-9.82	**3.45**				
		2	-5.24	**2.92**				-1.07
		3	-23.77	**3.70**				
		4	-20.82	**3.15**				-1.20
	Small Fish	1	-0.15					
		2	-4.21					
		3	0.14			-0.40		
	Predatory Fish	1	14.75	**3.90**		**-5.13**		

Table 3 (continued) Best-fitting model results for biomass density predicted by reserve features.

Network Features			**Human Population**	**Model Selection**		
Distance to nearest reserve	Distance to river mouth	Betweenness centrality	Houses in nearest village	**logLik**	**AICc**	**Akaike weight**
	1.56			-13.6	40.8	1.00
				-44.6	96.5	0.37
				-43.5	97.2	0.26
	2.81			-43.8	97.8	0.20
	3.25			-42.2	98.0	0.18
				-18.2	41.0	0.23
	0.88			-17.4	42.0	0.14
				-17.4	42.1	0.13
	-5.54			-37.2	87.9	1.00



Supplemental Figure 1. Frequency distribution for species observed by maximum reported length. Red dotted line is the 15cm threshold we used to split small- and large-bodied fishes.