SUMMARY

Null hypotheses concerning random distributions of species with respect to subregions and macrohabitats within the Rio Paraguay are tested with data from 131 species of macrocrustaceans and benthic invertebrates and 186 species of aquatic plants. The patterns are compared to the results for the distributions of fishes presented by Chernoff et al. (2004). The invertebrate data demonstrate the identical pattern among subregions as evident in the fish distributions. The results support the recognition of two zones: i) the Rio Paraguay zone containing portions of Rio Paraguay and Rio Negro, and ii) the Rio Apa zone containing Rio Apa and Riacho La Paz. For all data sets, the Rio Paraguay zone has higher species richness than the Rio Apa zone. The boundary between the two zones is abrupt, which is also supported by the plant data. Only 11 of 186 species of plants were found in both zones. There is no congruence of pattern among macrohabitats. The invertebrate and plant similarity matrices contain many values that are not different from mean random similarities among macrohabitats. The plant data set demonstrates a relationship among shore and sand habitats that experience greater currents than do other macrohabitats. The plants found in backwater habitats had little similarity to other macrohabitats. Based on these observations, we conclude that significant habitat within each of the zones must be preserved to maintain a large portion of the biodiversity.

Introduction

Freshwater ecosystems are home to tens of thousands of species and provide food and critical services for the health of humans and for the planet. Yet freshwater ecosystems are highly threatened and the organisms that live there are highly vulnerable to ecosystem modification (Naiman et al., 1995; Abramovitz, 1996; Stiassny, 1996; Folkerts, 1997; Pringle et al., 2000; Saunders et al., 2002). Water use, pollution, channelization, deforestation and dams are only a few of the threats facing aquatic habitats and their associated wetlands worldwide (Petts, 1990; Allan and Flecker, 1993; Boon et al., 2000). Our ability to manage these ecosystems requires knowledge of the organisms, their distributions, and their biotic and abiotic interactions. Integrated-use management or conservation strategies must take into account the patterns of spatial distri-
butions and habitat utilization within watersheds by various groups of organisms (Ward, 1998; Chernoff et al., 1999).

South American aquatic ecosystems are among the richest on the planet (Lundberg et al., 2000). However, information about species identities, phylogenetic relationships, natural histories and ecologies is vastly incomplete. For example, many benthic macroinvertebrates are immature semi-aquatic insects of unknown species (e.g. chironomids, mayflies, caddisflies), a situation that often limits taxonomic resolution of aquatic surveys to the genus or family level (Barbosa and Callisto, 2001).

A number of important studies are elucidating aquatic and riparian community structures (Ibarra and Stewart, 1989; Agostinho and Zalewski, 1995; Cox Fernandes, 1999; Marques and Barbosa, 2001; March et al., 2002; Rosales et al., 2002). Importantly, researchers are seeking to uncover the determinants of organismal distributions based upon biotic and abiotic parameters (Mérigoux et al., 1999; Lake et al., 2000; Pringle et al., 2000; Rosales et al., 2002).

From the standpoint of ecology and conservation, it is critical to understand the determinants of individual species distributions as well as correlated distributions among species or taxa. Chernoff et al. (2001, 2003) and Willink et al. (2000), and chapters therein, discussed general distributional patterns among aquatic and riparian plants (termed aquatic plants), plankton, benthos, macrocrustaceans, and fishes from four South American watersheds. For fishes, Chernoff and Willink (2000) established that fish distributions were significantly patterned, exhibiting either sharp faunal turnovers between adjacent regions or macrohabitats (e.g. Rio Orthon), or nested subset relationships (e.g. Pantanal). In contrast, the analyses of Takeda et al. (2000) did not reveal obvious spatial trends among benthic invertebrate communities in the middle and lower Rio Negro of the Pantanal. Because of inadequate sampling designs it was not always possible to quantitatively co-analyze the distributions among biological groups in the Orthon and Pantanal watersheds (Chernoff et al., 1999, 2001).

In a study of the northern Rio Paraguay watershed, Chernoff et al. (2004) rejected null hypotheses that the fishes were distributed either randomly or homogeneously with respect to sub-regions and macrohabitats. They discovered two broad sub-regions within which there is high faunistic similarity: i) the Rio Paraguay and the Rio Negro, and ii) the Rio Apa and the Riacho La Paz. The patterns of distribution associated with macrohabitats are congruent with those of the sub-regional analysis. Their results further indicate that the patterns are linked to the flooding cycle. In the Rio Apa and Riacho La Paz, the association among macrohabitats is due to terra firme, headwater conditions. These results were then used to construct a conservation plan to protect fishes and to evaluate the potential effects of environmental threats, such as Hidrovia (Chernoff et al., 2004).

Here the generality of the patterns discovered by Chernoff et al. (2004) is examined by analyzing distributions of benthos, plankton, macrocrustaceans and aquatic plants. The commonality or distinctiveness of the patterns is then used to propose a conservation plan that would protect the majority of the aquatic biodiversity within the portion of the Paraguay River watershed that was surveyed.
The collecting expedition took place in Sept 4 to 18, 1997, when the Río Paraguay basin was surveyed between Río Negro to the north and Río Apa beaches because the reduced invertebrate data set included 20 species of invertebrates and six macrohabitats.

The aquatic plant data set (Mereles, 2001) contains 186 species. The data do not allow a full sub-regional analysis but rather only a comparison of the flora of Río Paraguay (upper and lower subregions) with that of Río Apa. Presence-absence data were collected for the following macrohabitats: shores, flooded banks, semilotic, swamps, and sandy banks. The macrohabitat terminology of Mereles (2001) corresponds to that used for invertebrates and fishes as follows: flooded banks are referred to as backwaters, semilotic environments as flooded forests, and swamps as lagoons. Aquatic plants were collected along shorelines whether or not a beach (or clear area) was present. Thus, there is not complete concordance between the shoreline collections of aquatic plants with the beach collections of fishes and invertebrates. However, it is assumed that shoreline habitats for the aquatic plants function in the same way as the beach habitats for the invertebrates and fishes (e.g. the zone between deeper waters and areas exposed seasonally), and this assumption is used to estimate the congruence among the data sets.

The methods of Chernoff et al. (2004) are used to test the null hypotheses that distributions of invertebrates and aquatic plants are randomly distributed with respect to subregions or with respect to macrohabitats. The methods compare observed similarities against similarities generated at random for communities containing the same numbers of species. Simpson’s Similarity Index is used because similarity reflects the co-occurrence species not from joint absence. Our sampling methods cannot distinguish true absence from not present in sample. Samples containing different numbers of species are compared at the size of the smaller sample by rarefying the larger sample to the size of the smaller. Rarefaction is iterated 200 times and the mean similarity is used as the observed similarity.

If the null hypotheses are rejected, then branching diagrams are constructed as follows. For the subregional data, a Gabriel Network (Gabriel and Sokal, 1969) is used to represent the hydrological pattern of connectivity upon which the pattern of similarities are displayed. A dendrogram is also constructed using Camin-Sokal parsimony (Csp), which does not allow reversals. Csp only permits independent acquisitions. Thus, clustering summarizes similarities due to the shared presence of species, not the shared absence of species. PAUP* 4.0b was used to calculate Csp cluster analyses.
A Mantel’s test was used to determine if the similarities among subregions or among macrohabitats for the different data sets were correlated. The similarity matrices with mean, rarefied Simpson coefficients (S' of Chernoff et al., 2004) were converted to dissimilarity matrices by subtracting the values from 1.0 (Sneath and Sokal, 1973). The standardized Mantel coefficient, which is equal to the product-moment correlation between two dissimilarity matrices (Sokal and Rohlf, 1995) was calculated for each pair of matrices. The standardized Mantel coefficient was tested for significance using a random permutation test (Sokal and Rohlf, 1995) with 10000 iterations. The proportion of the permuted Mantel coefficients greater than the absolute value of the observed Mantel coefficient approximates the probability of obtaining the results at random (Sokal and Rohlf, 1995).

Results

Subregions

The invertebrate data base shows that the species richness of invertebrates was not distributed equally among all five subregions (Table II). That the fewest number of species was found in Riacho La Paz is partly an effect of effort, because the fewest collections were taken in that tributary (Table I). By far the richest subregion was the Upper Río Paraguay with 71 species. This region contained 54.2% of all the invertebrates collected.

The means of Simpson’s Similarity Indices are highly variable among subregions (Table II), ranging from 10% to almost 80% similarity. All indices among subregions differ significantly (P<0.01) from random distribution of similarities. The similarities of Río Apa or Riacho La Paz with Río Paraguay or Río Negro subregions are significantly lower than that expected at random. This indicates zones of marked faunal turnover; species are actively partitioning the basin into distinctive regions.

The pattern of similarities plotted on the Gabriel network of subregions (Figure 1) reveals two subregional zones of high similarity. The Río Paraguay subregional zone contains the Upper and Lower subregions of the Río Paraguay and the Río Negro. The Río Apa subregional zone contains Río Apa and Riacho La Paz. The high similarities within each of these subregional zones is due to large numbers of shared taxa within the zone but not due to uniquely shared taxa. For example, the Upper Río Paraguay shares 27 of 35 and 36 of 56 species with the Río Negro and the Lower Río Paraguay, respectively. However, only 11 and 6 species were found exclusively in the Upper Río Paraguay and the Lower Río Paraguay or Río Negro, respectively. Thus, each of the subregional zones is internally homogeneous and represents a different faunal assemblage with respect to the other subregional zone.

Between the Río Paraguay and Río Apa subregional zones there is a strong faunal turnover (Figure 1). From the Lower Río Paraguay into Río Apa there is a turnover of more than 35 species (62.5%).

The existence of the two groups of subregions is evident in the CSp cluster analysis (Figure 2). Notice that the order of joining within the Río Paraguay group reflects the hydrological connections. It is also important to note that the pattern (Figures 1, 2) is not due to a particular group of invertebrates but rather the signal is distributed across phyla and demonstrates the importance of broad taxonomic sampling.

These results are almost identical to those from the analysis of fish distributions (see Figure 6 in Chernoff et al., 2004). The similarity of pattern found in the invertebrate and fish data sets is manifest in the highly significant standardized Mantel coefficient (r=0.923, P<0.0001). The results indicate the following for both aquatic invertebrates and fishes: i) the Río Negro - Río Paraguay zone contains taxa associated with a flood-zone ecosystem, and ii) the Río Apa - Riacho La Paz zone contains taxa associated with terra firme, headwater habitats. Furthermore, the rate of faunal turnover between the zones is rather sharp. For both the invertebrates and the fishes, there is at least 60% turnover between the zones.

A total of 186 plant species was encountered from

![Figure 2. Camin-Sokal parsimony cluster analysis of subregions within the Río Paraguay based upon the full invertebrate dataset. APA: Río Apa, LP: Lower Río Paraguay, RLP: Riacho La Paz, RN: Río Negro, UP: Upper Río Paraguay.](image)

![Figure 3. Camin-Sokal parsimony cluster analysis of macrohabitats within the Río Paraguay based upon the reduced invertebrate dataset. BA: beach Río Apa, BP: beach Río Paraguay, BW: backwater, FF: flooded forest, FV: floating vegetation, LG: lagoon.](image)

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<td>MEAN SIMPSON’S INDEX OF SIMILARITY, S', AMONG MACROCRUSTACEANS AND BENTHIC INVERTEBRATES FOUND LIVING IN FIVE SUBREGIONS OF THE RÍO PARAGUAY BASIN</td>
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Larger samples were rarefied 200 times to the size of the smaller samples. Index values reported as percentages. All indices among subregions differ significantly (P<0.01).

APA: Río Apa, LP: Lower Río Paraguay, RLP: Riacho La Paz, RN: Río Negro, UP: Upper Río Paraguay.

n: number of species present, u: number of unique species, %u: percentage of unique species.
with respect to Río Paraguay

low similarity of Río Apa
tion on plant distributions, the
Thus, without more informa-
similarity (S* = 26.5%, stan-
t value is not significantly dif-
tween them is 22.5%. This
The reduced invertebrate data set was used to analyze faunal similarities among six macrohabitats. Only 6 of the
20 species were found in a single macrohabitat, while the number of species shared between habitats varied from 4 to 8 (Table III). There is a two fold difference in S* from just above 44% to almost 89%. Based upon simulations, observed similarities ≤50% could not be distinguished from random. Those >50% are significantly different from random (P<0.01).

The Camin-Sokal parsimony analysis cannot completely resolve relationships among the macrohabitats (Figure 3). The polytomy results because of the relatively large number of taxa shared among flooded forest, floating vegetation, backwater, and lagoon habitats. The close relationship between backwater and floating vegetation habitats results from their sharing uniquely two species: a crab, Valdivia cameroni and a gastropod mollusk, Marisma planogyna. The overall pattern is that there is high similarity among habitats that are seasonally inundated with the beach habitats being more dissimilar (Figure 3). The group of four inundated habitats share more species in common (6-8) than they do in general with either the Río Apa or Río Paraguay beaches (4-5; Table III). The exception is that 8 species were found in common between the Río Paraguay beaches and floating vegetation (Table III). This may result because floating vegetation habitats can extend to the shorelines. Nonetheless, the majority of the invertebrate biodiversity is found in less exposed, lentic habitats that are seasonally inundated. The overall pattern emphasizes a Río Paraguay group that communicates via a vis flooding cycles. The Río Apa beaches are the most distant from the Río Paraguay group.

The pattern of clustering among the macrohabitats for the reduced invertebrate data is basically congruent with the pattern found for fishes (compare Figure 3 with Figure 7 in Chernoff et al., 2004): the Río Paraguay beach and inundated habitats form a cluster separated from the Río Apa beach samples. However, in the case of fishes, the Río Paraguay beaches share the most species with backwater habitats. The differences in details of branching patterns among the inundated habitats for invertebrates and fishes result in the non-significant standardized Mantel coefficient among the sample similarity matrices (r=0.27, P>0.05). Thus, the similarity between the fishes and the invertebrates is due to the association among habitats that are created during the flood cycle along Río Paraguay. Beach habitats experience the effects of currents and many species of invertebrates may prefer quieter habitats with higher accumulations of decomposing organic matter. The invertebrates may not require access to deeper waters, thereby inverting the association among habitats from that demonstrated for fishes.

Information about six macrohabitats was collected from all 186 species of plants (appendices 5 and 6 in Mereles, 2001). There was more than twice the number of species in the richest macrohabitats (Río Paraguay shores, and la-
goons) as in the poorest (Table IV). Although Mereles (2001) noted that there was usually a high negative
correlation between species richness and degree of current, the Río Paraguay shoreline habitats were very rich, with 56 species present.

The plants exhibited a stronger degree of macrohabitat partitioning than the fishes or invertebrates. Out of 186 species, there were no species found in five or six macrohabitats. Only 5 species (Pistia stratiotes, Crataeva tapia, Combretum paniculatum, Polygonum punctatum and Mikania periplocifolia) were found in four macrohabitats. Fourteen species were found to occupy three macrohabitats. Furthermore, the number of unique species (Table IV) were high ranging from 41 to 86% of the species collected in any habitat. The number of unique species were significantly higher than random expectations (P<0.001).

The matrix of similarity coefficients (Table IV) shows that the coefficients range from 0 to 44.4% similarity. Only 7 of the coefficients are significantly different from random (P<0.001). The matrix shows that there are two main zones: i) a Río Paraguay zone that contained the Upper and Lower Río Paraguay subregions plus the Río Negro, and ii) a Río Apa zone that contained the Río Apa and Riacho La Paz subregions. Species within each zone were shared broadly and there was strong faunal turnover between zones. The subregional analyses of the full invertebrate data set displayed almost identical results to those for fishes (Figures 1, 2). Importantly, the invertebrate result was not due to any single taxon; rather, the evidence was scattered across a number of families, orders and phyla. The plant data were not collected in a way to support a full subregional analysis. Nonetheless, the aquatic plants demonstrated a strong floral boundary between the Río Paraguay and Río Apa zones; only 11 out of 186 species were collected in both. Thus, our conservation recommendations, presented below, emphasize that the Río Paraguay and Río Apa zones are highly distinctive and require separate conservation efforts.

The macrohabitat analysis of fishes demonstrated that within the Río Paraguay zone there was a non-random association of macrohabitats due to seasonal cycles of inundation (Chernoff et al., 2004). The Río Paraguay beach habitats were central from which most of the other interior

Figure 4. Camin-Sokal parsimony cluster analysis of macrohabitats within the Río Paraguay based upon the plant dataset. BW: backwater, FF: flooded forest, LG: lagoon, SA: shoreline Río Apa, SAN: sandy, SP: shoreline Río Paraguay.

Discussion

Conservation strategies for this part of the Río Paraguay basin should ideally be based upon as many groups of organisms as possible. Congruence of patterns among the components of biodiversity will enable us to derive the most effective conservation plan for the Río Paraguay basin between Concepción and the Brazilian border above the Río Negro.
habitats (e.g., flooded forests, backwaters, floating vegetation and lagoons) were basically nested subsets. The deeper waters of the main channel bore the closest faunal similarity to the Río Paraguay beaches but were distant from inland habitats. Another major finding of the macrohabitat analysis was that a different faunal assemblage was present in the habitats that characterize the Río Apa zone: beaches, rapids, and clear water. This zone contains habitats more associated with terra firme and headwater areas than lowland floodplains.

Unlike the subregional analyses, there was less congruence among the results for macrohabitats. For the reduced invertebrate data set, the majority of the observed similarities were significantly different from random but the pattern of similarities among macrohabitats was not significantly correlated with those for fishes. This lack of correlation is due to the close association in fishes between the Río Paraguay beaches and backwater habitats. Nevertheless, the clustering order of the nested sequence -lagoons, flooded forests, floating vegetation and backwaters-is identical in both fishes and invertebrates (Figure 3 and Figure 7 in Chernoff et al., 2004). Furthermore, for both the invertebrates and for the fishes, the Río Apa beaches are most different with respect to the other macrohabitats. These results must be regarded as preliminary, however, because only 23 species of invertebrates were scored for a subset of the macrohabitats for which the fishes were collected.

The patterns of similarities among macrohabitats are difficult to interpret for the plant data. Less than half of the similarity coefficients were significantly different from random. Given this limitation, two aspects of the plant data were not ambiguous. The first is that both sandy beaches and the Río Apa shores share a relatively large (>10) number of species with the Río Paraguay beaches. These three habitats are subject to relatively stronger currents than are other habitats and may accumulate similar species. The second is that backwater samples were very different from other samples, such that no species were found in common with flooded forests, lagoons and sandy habitats.

The results indicate that the distribution of species of riparian plants, aquatic invertebrates and fishes among the subregions is not random. Furthermore, the subregional congruence between the invertebrate and fish data sets was very high. Non-random spatial or subregional patterns within a watershed have been documented for lowland forests in the Río Caura, Venezuela, and the Río Negro, Brazil (Rosales et al., 2002, 2003), for invertebrates (Ramírez and Pringle, 2001; García and Pereira, 2003) and for fishes (e.g., Chernoff et al., 2004). The invertebrates add weak support for the flood-cycle relationship among macrohabitats exhibited by fishes (Chernoff et al., 2004). Similar results were obtained for macrohabitats by fishes and zoobenthos in the Southern Pantanal, Brazil (Chernoff and Willink, 2000). The lack of similarity between the fish and plant data is somewhat surprising given the strong association between “quiet-water” species of fishes and plants (Goulding, 1980; Lowe-McConnell, 1987; Goulding et al., 1988; Meschiatti et al., 2000). The fish samples contain many species such as Apistogramma commbrae and Hyphessobrycon eques that are usually collected in association with rooted aquatic vegetation.

Conclusions and Recommendations

Conservation plans must reflect departures from random distributions of the flora and fauna with respect to geography and macrohabitats. Geographic pattern can be interpreted from the full invertebrate data set and it is congruent with the non-random pattern exhibited by the fishes. The plant data provided a test that the Río Paraguay zone is different from the Río Apa zone, a finding congruent with both invertebrates and fishes. There is weak confirmation by the reduced invertebrate data set of the flood-cycle relationship among macrohabitats that was displayed by the fishes. Though the plants are incongruent with the fish and invertebrate pattern, the plants have a somewhat non-random distribution that is affected by different underlying causes. These conclusions lead to the following recommendations:

1. The aquatic flora and fauna comprise two major zones within the Río Paraguay basin above Concepción to the Brazilian Border: i) the Río Paraguay zone containing Río Paraguay and Río Negro, and ii) the Río Apa zone containing Río Apa and Riacho La Paz.
2. Based upon fishes, invertebrates and plants the Río Paraguay zone contains more species than does the Río Apa zone.
3. Significant habitat within each of these zones needs to be preserved to maintain a large portion of the biodiversity.
4. There is some congruence among the fishes and invertebrates with respect to their distributions among macrohabitats but not with aquatic plants. As a result samples of all macrohabitats must be preserved to maintain the majority of species.
5. Elimination of habitats that require seasonal flooding, such as flooded forests, lagoons, and backwaters, would eliminate almost 50% of the plant species.

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