

Macrophyte islands as an experimental model: new perspectives and a step-by-step protocol to access assumptions of Island Biogeography theory and habitat fragmentation

Ilhas de macrófitas aquáticas como um modelo experimental: novas perspectivas e um protocolo para acessar os pressupostos da teoria da biogeografia de ilhas e fragmentação de habitat

Tiago Henrique da Silva Pires¹

Sergio Santorelli Junior^{2*}

Gabriel Henrique de Mendonça Cardoso³

Julia Tovar Verba⁴

Alexandro Cezar Florentino⁵

Thatyla Luana Beck Farago⁶

Received 11/28/2022 | Accepted 03/23/2023 | Published 03/28/2023 | Edited by Rodrigo Gonçalves

Abstract

To evaluate whether artificially built macrophyte islands could be used as a short-term and cost-effective experimental model for the study of island biogeography and habitat fragmentation, 20 islands of two different sizes were built and fishes were sampled. Habitat complexity and source-island distance were standardized. A macrophyte bank 200 times larger than the islands was used as a source of individuals. The results show

1. Programa de Pós-Graduação em Biologia de Água Doce e Pesca Interior, Instituto Nacional de Pesquisas da Amazônia. Avenida André Araújo, 2936 – Bairro: Aleixo – Cx. Postal: 478 – CEP: 69.060 – 001, Manaus, AM, Brasil. 2. Instituto de Educação, Agricultura e Ambiente, Universidade Federal do Amazonas – Campus Vale do Rio Madeira. Rua 29 de Agosto, 786 – Centro – Humaitá/AM – CEP:69800-000. 3. Programa de Pós-Graduação em Biologia de Água Doce e Pesca Interior, Instituto Nacional de Pesquisas da Amazônia. Avenida André Araújo, 2936 – Bairro: Aleixo – Cx. Postal: 478 – CEP: 69.060 – 001, Manaus, AM, Brasil. 4. Division of Evolutionary Biology, Faculty of Biology II, Ludwig-Maximilians-Universität München, Planegg-Martinsried 82152, Germany. 5. Programa de Pós-Graduação em Ciências Ambientais, Universidade Federal do Amapá, Rod. Josmar Chaves Pinto, km 02 - Jardim Marco Zero, Macapá - AP, 68903-419. 6.Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia. Avenida André Araújo, 2936 – Bairro: Aleixo – Cx. Postal: 478 – CEP: 69.060 – 001, Manaus, AM, Brasil. *Corresponding author: santorelli.jr@gmail.com.

that, in four days, several fish species colonized both small and large islands. After four days, larger islands bore significantly more species than smaller ones. We raise the caveats and new perspectives for studies using this methodology, which can be largely applicable due to its cost-effectiveness in terms of materials, short time required, and flexibility.

Keywords: Neotropical fishes, Ecology of fishes, Amazon fishes, Metacommunity ecology

Resumo

Para avaliar se ilhas de macrófitas construídas artificialmente poderiam ser usadas como um modelo experimental econômico e de curto prazo para o estudo da biogeografia de ilhas e fragmentação de habitat, 20 ilhas de dois tamanhos diferentes foram construídas e os peixes foram amostrados. A complexidade do habitat e a distância da fonte-ilha foram padronizadas. Um banco de macrófitas 200 vezes maior que as ilhas foi utilizado como fonte de indivíduos. Os resultados mostram que, em quatro dias, várias espécies de peixes colonizaram ilhas pequenas e grandes. Após quatro dias, as ilhas maiores continham significativamente mais espécies do que as menores. Levantamos as ressalvas e novas perspectivas para estudos com essa metodologia, que pode ser amplamente aplicável devido ao seu custo-benefício em termos de materiais, pouco tempo necessário e flexibilidade.

Palavras-chave: Peixes neotropicais, ecologia de peixes, peixes amazônicos, ecologia de metacomunidades

Introduction

Even half a century past from the release of the Island Biogeography Equilibrium theory by MacArthur and Wilson (1967), some questions remain open, and a great variety of methods have been used to test the hypothesis from these authors. The theory has been applied to a large diversity of fields in biology, including community ecology, population ecology, and evolution (Steven and Willing, 2022). Given the great resemblance that islands' systems have to natural reserves surrounded by altered habitats, the ideas generated by the island biogeography theory are also of great impact on conservation policies (see Diamond 1975). From this conservation approach emerged the SLOSS debate (Single Large or Several Small) in which is discussed what is more desirable to keep unaltered, many small fragments or fewer of larger sizes (Liu et al., 2022).

Although the great reach and direct importance of this theory, many questions that arose from it remain without empirical evidence, mainly because the basic assumptions include processes difficult to be directly measured (Johnson, 2000, Resetarits et al., 2022). Many experiments were idealized to show the viability of the theory (e.g. Simberloff & Wilson, 1969). However, in many cases, the experiments failed in a variety of forms, disrupting the environment, requiring high costs, possessing insufficient numbers of replicates, or

more often, failing in sorting out important variables (e.g. Carlson & Hartman, 2001, Fuller 2001, Golden & Crist, 2000, Hargis et al., 1999, Robinson et al., 1995, Summerville & Crist, 2001). Fragmented habitats due to human degrading activities are suitable to test the island biogeography theory, and studies have been showing success in applying it using cost-effective designs (Hill & Curran, 2001, Laurance, 2010).

An experimental model to comprehend questions about island biogeography and habitat fragmentation should have some basic elements like the presence of a source (= mainland), islands (= patches), and a matrix that provides some level of dispersal resistance for the organisms under study. It is also desirable that variables set in the theory be easily manipulated such as habitat complexity, size of the islands and distances (either island-source or between islands). In this sense, floating aquatic macrophytes are excellent for experimental designs because they form an important microhabitat for aquatic organisms (Junk 1973, Rozas & Odum, 1988, Vazzoler & Menezes, 1992, Casatti et al., 2003, Thomaz & Cunha, 2010, Brito et al., 2021), and are widely available. In the neotropical region, they are abundant in flooding areas, frequently forming large banks of more than 500m long and are habitats for a great variety of fishes and aquatic invertebrates, bearing a typical ichthyofauna (Junk, 1970). In the Eastern Amazon, this system is mostly formed by 16 species, and it was already shown that

the level of heterogeneity of each macrophyte bank can influence species occurrence, which is relevant when focusing on island biogeography studies (Nonato et al., 2021). Additionally, sampling in this environment rarely includes organisms that swim freely through the water column; therefore, samples include only potential residents (Sánchez-Botero & Araújo-Lima, 2001). Thus, it is expected that the water surrounding the banks could represent a semi-permeate matrix for those organisms. Furthermore, aquatic macrophytes are relatively easy to manipulate and capable of being reallocated by hand. Considering the characteristics of this system, the objective of this study is to show a step-by-step protocol for the use of aquatic macrophytes in lentic regions as an experimental model to access assumptions of the Island Biogeography theory and habitat fragmentation.

Material & Methods

Step 1: Selection of Study area

For the experimental islands not to be carried by water movement, the experiment should be conducted in a lentic environment. Our pilot experiment was carried out in the Catalão lake (Figure 1). This lake is located 10 km away from the city of Manaus (3°10'04"S, 59°54'45"W), close to the encounter of Negro and Solimões Rivers. Lake Catalão is periodically flooded by both rivers, and the connection with these different river systems varies annually and may have periodic isolation from both systems (Brito et al., 2014). In this study, the protocol was implemented in August, corresponding to the receding period of both rivers, and the season in which the lake has limnological characteristics mainly influenced by the Solimões River.

Step 2: Pre-Experiment Tests

A preliminary test should be conducted to establish a minimum size and the best macrophyte composition for the experiment. To do that, we sampled aquatic animals (focusing on fish) in natural macrophyte islands from different sizes and species composition. Fish were caught by seine nets (10m long, 3m high and 5mm mesh size). These preliminary data showed fishes were present in islands of 1m², pointing

to a minimum size to be used in the experiment. Since we also aimed to investigate initial colonization, another preliminary test should be conducted to avoid the transference of animals along with the collected macrophytes, which could bias the results. In this methodological test, macrophytes should be collected from the environment, taken to a water tank and washed intensively. After this procedure we assume no individuals were carried, and the sampled macrophytes are ready to be used on the experiment.

Step 3: Islands assembly

First, macrophyte species should be selected to make the island's brim, depending on local availability. Second, cut and use it to make bundles of similar size, which can be held together by PET bottle rings. Thereafter these bundles should be linked with plastic tape, forming the circumference which bordered each island (Figure 2). Third, the groups of bundles should be moved to the experiment region; and after release on water, the circumferences should be fulfilled with other previously collected macrophyte species proportionally. The goal is the habitat structure is similar among all islands (Figure 2).

Due to its high abundance in the environment in our study area, *Paspalum repens* P.J. Bergius was the only species used to make the islands' brim. Five linked bundles were used to build the small islands (1m²) and nine for the large islands (3m²) used for the experiment. The circumferences were proportionally fulfilled with *Eichhornia crassipes* (Mart.) Solms, *Salvinia minima* Baker, *Ceratopteris pteridoides* (Hook) Hieron, *Pistia stratiotes* L. and *Neptunia* sp. (Figure 2). In total, twenty islands were assembled: ten small and ten big islands.

Step 4: Islands placement

All islands should be positioned at a standard distance from the source (Figure 3). The source could be represented by a macrophyte bank whose size would be considered infinite (>200 times the size of the big islands). A weight attached to the plastic tape should be used to prevent the islands from moving away from or towards the source. The islands should be positioned randomly (e.g. Figure 3) and monitored daily in the morning and afternoon to certify their positions did not significantly change and that the structure was not altered.

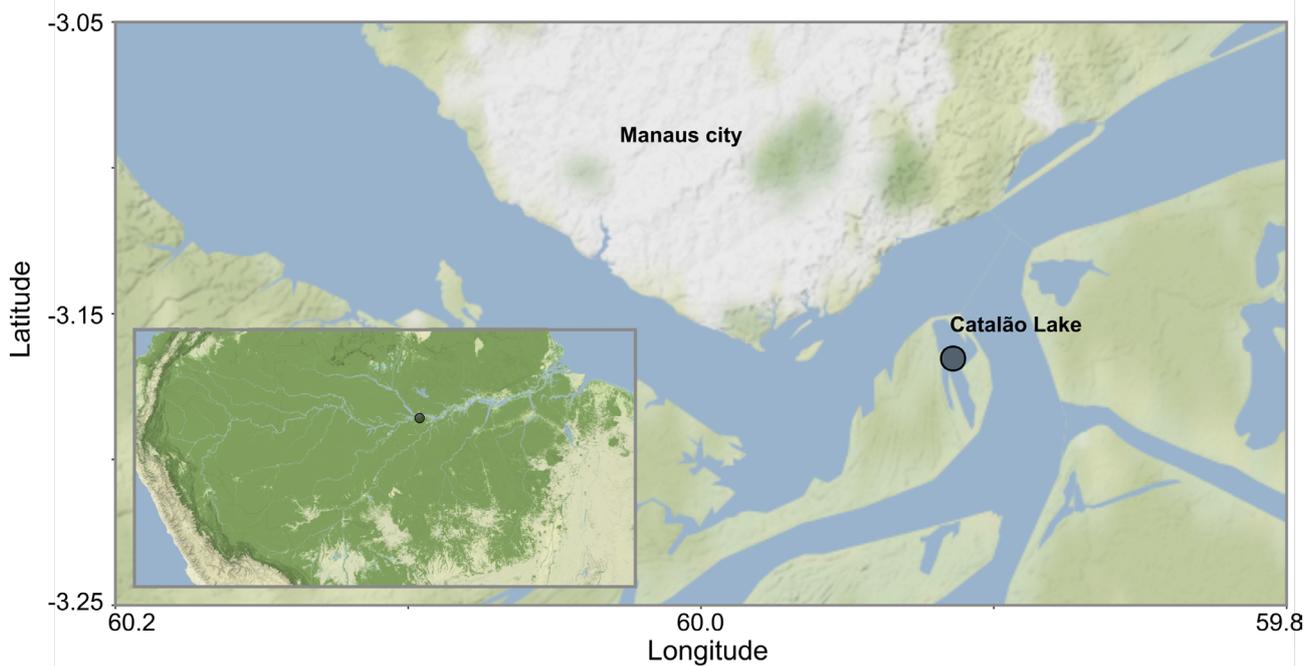


Figure 1. Map of the region where the experiment was performed (Catalão lake), Amazonas state, Brazil.

Step 5: Experimental islands survey

A seine net (5mm mesh size) can be used to sample fish, making a circle around the island and bringing the content inside the boat. The use of this fish net is suitable because it captures almost exclusively fish typically associated with macrophytes banks. These species can potentially be considered residents, in contrast to the fish fauna swimming freely along the water column and which is excluded by this fishing gear. The capture of pelagic fish could hamper the data analysis by contaminating the samples with matrix-related fauna. After the survey, fish can be packed in plastic bags, tagged and fixed in by using 10% formalin. In the laboratory, fish should be identified to the best possible taxonomic level using identification keys and the aid of specialists.

In our pilot, the fish fauna from each island was collected on the fourth day after setting the islands. In addition, during these four days, two other samplings were conducted. One was performed in a natural 30 m² bank of rounded format and predominantly composed by *P. repens* (named “control 30 m² island”). The second survey was conducted in a 3 x 10 m (30 m²) stretch from the same *P. repens* bank that was used as a source but located far away from where the experimental islands were set.

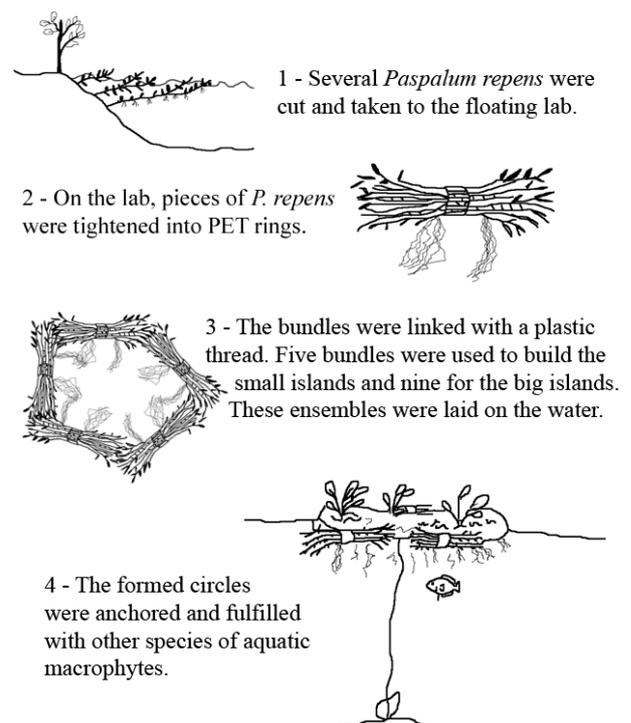


Figure 2. The procedure used in this study for the assembly and insertion of the islands.

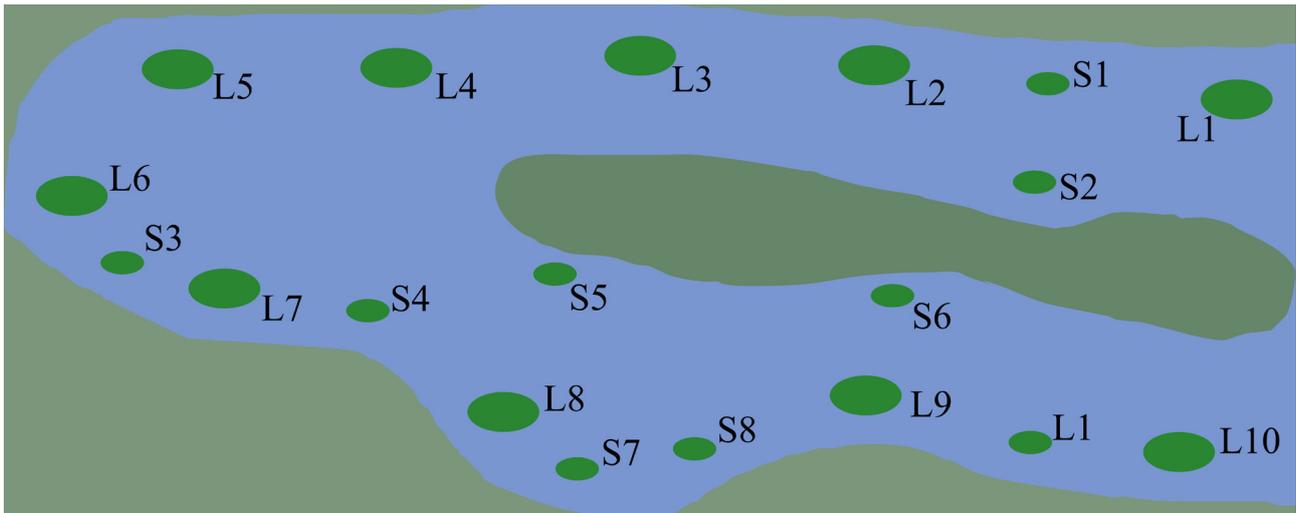


Figure 3. Sketch drawing (without scale) of the environment where the experiment was built. The L letters represent large islands (3 m²) while S indicates small islands (1 m²). The vegetation surrounding the islands was composed mainly by *Paspalum repens*, which constituted the species source. The matrix in blue was represented by water (space without macrophytes). The distance between the islands and the source was 4 m.

Step 6: Data analysis

To verify the differences in species richness between large and small islands in our pilot experiment, a student t-test was used. We expect that the difference would be significant, and that large islands would have significantly higher richness than small islands.

To show the similarity between islands, a two-dimension Non-Metric Multidimensional Scaling (NMDS) was carried out using the species composition (presence/absence of fish species) and abundance using the Jaccard and Bray-Curtis index, respectively. The grouping of islands that are geographically close could indicate that local fauna is affecting colonization on experimental islands. This could mean that the source of colonizers should not be considered homogeneous. All analyses were carried out in the R software (Development Core Team 2012).

Results

We carried out this pilot experiment during a short period - 4 days - and captured 122 individuals belonging to 12 species in the experimental islands: a total of six species in small islands and 11 in large islands (Table 1). The number of species in the large islands was significantly larger than in the small islands ($t = -2.9968$; $df. = 18$; $p = 0.007$) (Figure 4).

These results show a significant difference in species richness between large and small experimental islands, indicating a species-area relationship, the most well-established relationship in ecology. This shows the potential of this methodology in accessing other questions in island biogeography and habitat fragmentation. It is important to emphasize that this result was achieved in a short time, using a cost-effective approach, which constitutes positive points for the use of experimental macrophyte islands, especially in tropical regions.

The NMDS did not distinguish groups (Figure 5), indicating that the source in our experiment was homogeneous. These preliminary results led to the rejection of the null hypothesis that the distance between the islands influenced the species composition in the samples. This result is also important since it was desirable to isolate size as the only factor influencing colonization and establishment. With this, it is possible to conclude that the source (*P. repens* bank) could be considered homogeneous in the emigration potential of its fish fauna to the experimental islands. In the experiment, the open water environment in the lake seems to have worked as a semipermeable matrix, since the species captured by the seine net are regarded as not having a pelagic life cycle. Among the problems experienced in the field, the only variation in the position of the islands with time seems to be of major concern. Along with the water level decrease,

Table 1. Species and number of individuals samples in each island after four days of placement of experimental islands.

	Small Islands										Large Islands									
<i>Acaronia nasa</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>Anadoras grypus</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachypomus pinnicaudatus</i>	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1
<i>Cichla monoculus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Hoplias malabaricus</i>	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0
<i>Mesonauta festivus</i>	5	5	0	1	2	3	7	9	3	4	2	2	7	7	4	4	6	1	2	8
<i>Parauchemipterus porosus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Pyrrhulina brevis</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Schizodon fasciatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Serrasalmus maculatus</i>	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Synbranchus lamprea</i>	0	0	1	1	0	0	0	0	0	0	2	1	0	1	0	1	2	0	2	1
Abundance	11	7	2	4	3	3	8	9	3	4	4	5	7	8	5	9	9	3	6	12
Richness	2	2	2	4	2	1	2	1	1	1	2	4	1	2	2	6	3	3	3	5

fluctuation in the positions of the islands becomes greater and, to prevent the islands from changing the distance from the source, continuous monitoring is required, in which the experimenter adapts the length of the rope connected to the weight.

Fourteen species were sampled for both the 30 m² stretch of the source and in the “control 30 m² island”. Compared with the sum of the number of species found in the large experimental islands (10 x 3 m² = 30 m²), where 11 species were found, there was a slightly higher richness in the natural habitat.

Discussion

Perspectives for future studies on island biogeography

As indicated by the results, this approach is a promising tool for fast and cost-effective studies on Islands Biogeography theory focusing on colonization and extinction rates, which are predicted to vary accordingly to the distance to the source and size of the islands. Classical experimental works previously done under this context, such as Simberloff & Wilson (1969), Shoener (1974) and Molles Jr (1978) were under difficulties and none was sufficiently simple and manipulable as in the present study. We suggest that for future studies in colonization and extinction rates it would be necessary the settlement of a larger

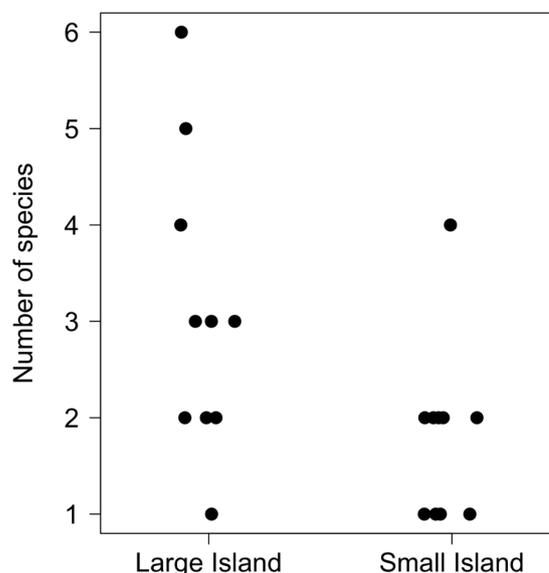


Figure 4. Total number of species found in large and small islands.

quantity of islands, which must be sampled at different moments. We suggest a daily survey performed until the reach of a colonization curve, as made by Simberloff & Wilson (1969).

The variable size and distance can be compared with other variables such as habitat complexity (Nonato et al., 2021). A question possible to be accessed is “how much bigger must an island with homogeneous habitat be to have a similar colonization rate as a smaller and more complex island?” This is one fundamental question in the island’s biogeography theory. For this purpose, habitat-homogeneous

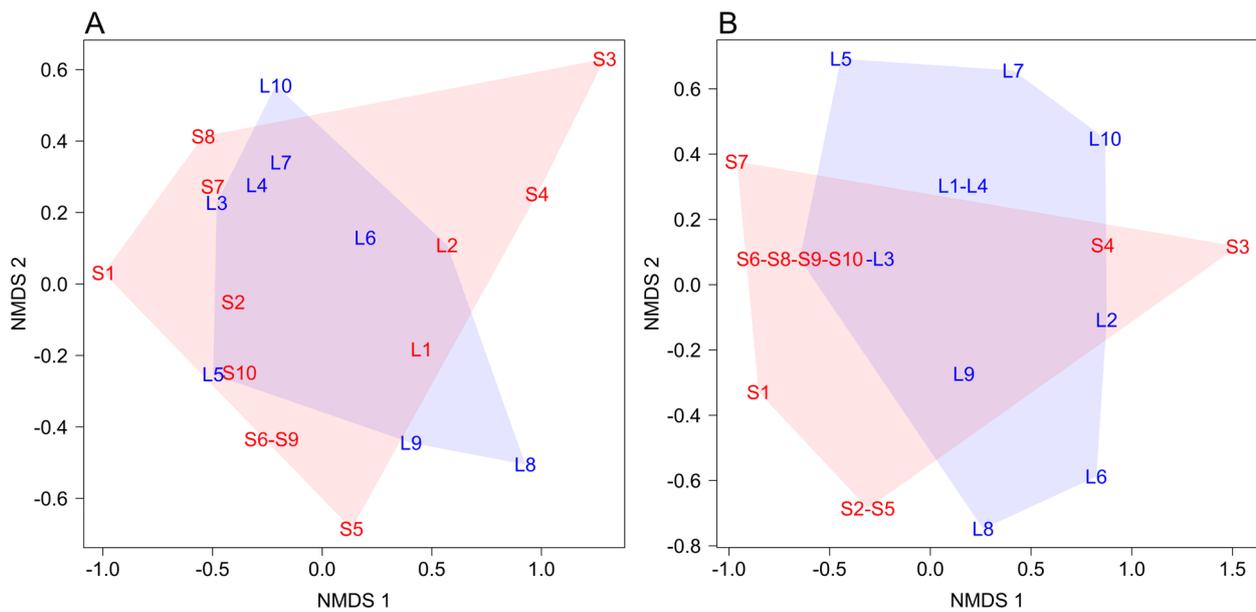


Figure 5. Non-Metric Multidimensional Scaling for absolute number of species by islands are separated into two categories: large (L) and small (S) islands, using a) Jaccard and b) Bray-Curtis indexes.

islands of different sizes should be built and their fauna should be compared to islands of more complex habitats, but of standardized size.

The “island effect” (whether smaller islands bear fewer species simply because they have a smaller area containing fewer habitats) could also be tested by this methodology once the habitat complexity is kept standardized. For this question, we suggest the use of a macrophyte species rather than *P. repens* as a source and islands. For a long-term study, the short lifetime of the macrophyte could become an undesirable variable. Therefore to assure the overlap of generations of fish (necessary, for instance, for the analysis of the local extinction effect), it is necessary to have constant maintenance.

Perspectives for future experiments on habitat fragmentation and habitat loss

The comparative data between the number of accumulated species in the larger islands versus fragments of the source and the “control 30 m² island” showed that the islands bore slightly inferior species richness. However, these data are not conclusive since they could be attributed to the short duration

of the experiment (which could be insufficient for the colonization of a higher number of species to take place) or to the difference in habitat complexity. Certainly, the number of species in a new experimental environment such as this should increase with time, but it is still unclear when it could reach a richness plateau. Furthermore, in the source stretch surveyed and in the “control 30 m² island” the roots grow deeper into the water, making a structured habitat that could potentially affect the number of species. To investigate if this affects the difference in species richness, long-term research is needed in which the habitat complexity would be controlled.

Besides the limitations found in this pilot experiment, the methodology is viable for the study of habitat fragmentation. With this experimental model, it is possible to access not only the effects of habitat loss and fragmentation, but also the border effect, by applying experiments alike by modifying other characteristics such as the number of surrounding islands, the presence/absence of corridors and different island formats. The facility of manipulation of macrophyte islands and the rapid response of its fish fauna shown in this study put forward countless forms in which future experiments can be performed in the ambit of habitat fragmentation, island biogeography and metacommunity.

Acknowledgments

The authors thank INPA / Graduate Program in Biology of Freshwater and Inland Fisheries and Dr. Jansen Zuanon and Dr. Rosseval Gadino Leite for logistical support in the discipline of Ecology of Wetlands. Sergio Santorelli Junior is supported by Programa de Fixação de Recursos Humanos no Interior do Estado from Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM/PROFIX-RH - Edital 009/2021, proc. 01.02.016301.00407/2022-94)

Conflicts of interest

The authors declare that this contribution was carried out without any conflict of interest.

References

- Brito, J. G. (2006). Influência do pulso de inundação sobre variáveis limnológicas de um lago de várzea da Amazônia Central, lago Catalão. Dissertação de mestrado. Instituto Nacional de Pesquisas da Amazônia – INPA/ Universidade Federal do Amazonas – UFAM. Manaus.
- Carlson, A. & Hartman, G. (2001). Tropical forest fragmentation and nest predation – an experimental study in an Eastern Arc montane forest, Tanzania. *Biodiversity and Conservation*, 10 (7): 1077-1085.
- Casatti, L., Mendes, H. F. & Ferreira, K. M. (2003). Macrófitas aquáticas como sítio de alimentação para pequenos peixes no reservatório de Rosana, rio Paranapanema, Sudeste do Brasil. *Brazilian Journal of Biology*, 63(2): 213-222.
- Development Core Team, R. (2012). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.
- Diamond, J. M. (1975). The island dilemma: Lessons of modern biogeographic studies for the design of natural reserves. *Biodiversity Conservation*, 7(2): 129-146.
- Dias, M. S., Toledo, J. L., Jardim, M. M., Figueiredo, F.O.G., Cordeiro, C.L.O., Gomes, A. C. S. & Zuanon, J. (2011). Congruence between fish and plant assemblages in drifting macrophyte rafts in Central Amazonia. *Hydrobiologia*, 661: 457–461.
- Fuller, D. O. (2001). Forest fragmentation in Loudoun County, Virginia, USA, evaluated with multitemporal Landsat imagery. *Landscape Ecology*, 16: 627-642
- Golden, D. M. & Crist, T.O. (2000). Experimental effects of habitat fragmentation on rove beetles and ants: patch area or edge? *Oikos*, 90(3): 525-538.
- Hargis, C.D., Bissonette, J.A. & Turner, D.L. (1999). The influence of forest fragmentation and landscape pattern on American martens. *Journal of Applied Ecology*, 36 (1): 157-172.
- Hill, J.L. & Curran, P.J. (2001). Species composition in fragmented forests: conservation implications of changing forest area. *Applied Geography*, 21: 157-174.
- Johnson, R.A. (2000). Seed-Harvester Ants (Hymenoptera: Formicidae) of North America: An Overview of Ecology and Biogeography. *Sociobiology*, 36(1): 89-122
- Junk, W.J. (1970). Investigations on the ecology and production biology of the “Floating-meadows” (*Paspalo-echinochloetum*) on the Middle Amazon. *Amazoniana*, 2(4): 449-495.
- Junk, W.J. (1973). Investigations on the ecology and production biology of the “floating meadows” (*Paspalo-Echinochloetum*) on the middle Amazon. II. The aquatic fauna in the root-zone of floating vegetation. *Amazoniana*, 4: 9-102.
- Liu, J., Macdonald, Z. G., Si, X., Wu, L., Zeng, D., Hu, G., Ding, P. & Yu, M. (2022). SLOSS-based inferences in a fragmented landscape depend on fragment area and species–area slope. *Journal of Biogeography*, 49, 1075– 1085.
- Macarthur, R.H. & Wilson, E.O. (1967). *The theory of island biogeography*. Princeton University Press, Princeton, NJ. 203p.
- Molles, J.R. & Manuel, C. (1978). Fish species Diversity on Model and Natural Reef Patches: Experimental Insular Biogeography. *Ecological Monographs*, 48: 289-305.

- Resetarits, W. J., Potts, K. M., Scott, R. C. (2022). Island Biogeography at the Mesoscale: Distance from Forest Edge Affects Choice of Patch Size by Ovipositing Treefrogs. *Ecology*, 103(9): e3766.
- Robinson, G.R., James, F.Q. & Maureen, L.S. (1995). Invasibility of Experimental Habitat Islands in a California Winter Annual Grassland. *Ecology*, 76: 786-794.
- Rozas, L.P. & Odum, W.E. (1988). Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. *Oecologia*, 77: 101-106.
- Santos, R.N., Ferreira, E.J.G. & Amadio, S. (2007). Effect of seasonality and trophic group on energy acquisition in Amazonian fish. *Ecology of Freshwater Fishes*, 17: 340–348.
- Sánchez-Botero, J.I. & Araújo-Lima, C.A.R.M. (2001). As macrófitas aquáticas como berçário para a ictiofauna da várzea do rio Amazonas. *Acta Amazonica*, 31(3): 437-447.
- Simberloff, D.E. & Wilson, E.O. (1969). Experimental Zoogeography of Islands: Defaunation and Monitoring Techniques. *Ecology*, 50 (2): 267-278.
- Schoener, T.W. (1974). Resource partitioning in ecological communities. *Science*, 185: 27-39.
- Summerville, K.S. & Crist, T.O. (2001). Effects of experimental habitat fragmentation on patch use by butterflies and skippers (Lepidoptera). *Ecology*, 82: 1360-1370.
- Vale, J.D. (2003). *Composição, diversidade e abundância da ictiofauna na área do Catalão, Amazônia Central*. Dissertação de mestrado. Instituto Nacional de Pesquisas da Amazônia – INPA/ Universidade Federal do Amazonas – UFAM. Manaus.
- Vazzoler, A.E.A.M. & Menezes, N.A. (1992). Síntese de conhecimentos sobre o comportamento reprodutivo dos Characiformes da América do Sul (Teleostei, Ostariophysi). *Revista Brasileira de Biologia*, 52: 627-640.