**HYDR-D-22-00442**

**Title:** Land use effects on the structure of trophic networks from Neotropical fish.

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To the editorial board at *Hydrobiologia*

Guest Editor *Dr.* *Franco Teixeira de Mello*

Dear Dr. Texeira de Mello,

Thank you for the opportunity to revise and resubmit our manuscript HYDR-D-22-00442.R1 entitled “Land use effects on the structure of trophic networks from Neotropical fish”. We very much appreciate the time invested by the referees and the editors to provide a thorough review, and we are excited about the overall positive reception during this first review.

We submit two versions of the manuscript—one clean version (as the Main Body) and one with track changes (as the Supporting Document). In our responses to reviewers, we detail the changes we made to the manuscript in response to each comment provided by the reviewers (in blue ink and in *italics*). Should this formatting be lost, we also precede each response with a ‘**Authors’ response**’. Please note that the line numbers in the response to reviewers document correspond to the document with track changes (uploaded as the Supporting Document), which make it easier to identify changes made during the revision process.

Once more, we appreciate such an insightful review and excellent editorial services provided by *Hydrobiologia*. We look forward to hearing back from you.

Yours sincerely,

Murilo S. Dias and co-authors

**Editor's comments, Dr. Teixeria de Mello**

**Editor’s comment#1.** *We have received the reports from our advisors on your manuscript, "Land use effects on the structure of trophic networks from Neotropical fish", which you submitted to Hydrobiologia. Based on the advice received, I feel that your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions, I recommend that you carefully consider reviewer 2 comments. When preparing your revised manuscript, you are asked to carefully consider the reviewer comments which are attached, and submit a list of responses to the comments.*

***Authors’ reply****: We appreciate the dedicated time and effort of the editor and reviewers in carefully reviewing our manuscript. We thank the reviewers and the editor for their valuable and constructive feedback. In response to reviewer #1's comments, we have added more details explaining why we expected a nested network. Additionally, we have addressed all of reviewer #2's concerns regarding the metrics considered in our analysis and have modified our discussion by removing speculation that was mentioned by the reviewer.*

**Reviewer 1’s comments**

**Reviewer 1’s comment#1.** *This is an interesting study about the anthropogenic effects on the trophic networks structure of Neotropical stream fish. Specifically, authors propose to evaluate the role of land use changes (pastures or cropland) on network structure (e.g., modularity, nestedness, links density).*

*I think the manuscript is well written and the conceptual framework is clearly developed. I have some minor comments, mainly about the analysis of the data, that I expect could improve the manuscript.*

***Authors’ reply****:* *We very much appreciate such positive feedback along with a summary highlighting the strongest points of our work. Please see below how addressing each of your comments improved the quality of our manuscript. Please note that line numbers refer to the manuscript version with inline tracked changes.*

**Reviewer 1’s comment#2.** *Line 43. "This nestedness pattern...." Why this? Which is the nestedness pattern that authors refer? Homogenization and reduction in network complexity*?

***Authors’ reply****: We appreciate the opportunity to improve our hypothesis and explain why we expected a modular network. Please see LXX-XX. “We hypothesize that increasing land use reduces biodiversity and modularity in fish web networks due to the loss of specialist species and faunal homogenization, resulting in nested networks”.*

**Reviewer 1’s comment#3.** *Link density and the average number of links per species is the same, not?*

***Authors’ reply****:* *We really appreciate this insightful comment. We now added some sentences to explain that these metrics are different. Please see LXX-XX. “We quantified five web network metrics based on the distribution of interactions (nestedness and modularity), trophic specialization, number of links per species (links density), and number of trophic links according to the number of nodes (number of links).”*

**Reviewer 1’s comment#4.** *About table the table S3 that has the classes of anthropic impact on land use, the rows should sum 100%?*

***Authors’ reply****: Thank you for the opportunity to streamline our supplementary tables. We checked all the values, and the sum of land use percentages is 100%.*

**Reviewer 1’s comment#5.** *I am not sure if Figure 2 support effect of land use on network metrics. There are a lot of dispersion in the data. Maybe a logistic regression between the probability of been nestedness (1) or modular (0) along the land use gradient could show the results more clearly. Complementary, a box-plot analysis showing the range of values of network structure along the gradient of land uses could be interesting.*

***Authors’ reply****:* *We appreciate the reviewer’s opinion, but our nestedness and modularity data are not probabilities. These values were estimates considering the null model and 999 permutations, resulting the values that varied among -2 to 2. Thus, we do not consider that use a logistic regression improve the tends observed. However, we added in significant regression the values R2 and p-value.*

**Reviewer 1’s comment#6.** *In addition, weighted matrices could be used to network structure estimations and then to the analysis.*

***Authors’ reply****:* *We appreciate the constructive suggestions aimed at improving the presentation of our work. However, the studies compiled during our systematic review diverge in their methods for quantifying consumed food items and sampling efforts. Such differences can introduce biases into the estimation of network metrics. Therefore, we minimized the potential effects of varying sampling effort across studies by focusing on the incidence, not the strength, of the cleaning interactions. That is, we converted all quantitative interactions into qualitative ones to focus only on the topologies of the network.*

**Reviewer 2’s comments****Reviewer 2’s comment#1.** *This study analyses how changes in land use affect the food web structure of different freshwater fish trophic networks among several sampling points in Brazil. To this aim, the authors gathered dietary information from 49 articles. From these studies, the authors estimated several network metrics related with the complexity and stability of the food webs. Additionally, the authors estimated the anthropogenic impact on riparian vegetation in each sampled site as the percentage of non-natural land-cover and evaluated its effect on food web structure and complexity. In general terms, I find that this study focuses on a relevant issue regarding the impact of anthropogenic activities on the stability and structure of ecological networks, and therefore has the potential to make a relevant contribution to the field of food webs and conservation. However, in its current form, the manuscript does not fulfil the requirements needed for publication. I detail below three main issues that the authors may want to consider. They range from writing to methodology. However, my major concern is that the discussion and conclusions are highly speculative and are based on results that lack statistical support.*

***Authors’ reply****:*

**Reviewer 2’s comment#2.** *Speculative discussion supported on results with lack of statistical support*

*The authors detected a negative and statistically significant relationship between network modularity and link density with land use gradient. These results are used to suggest that there is a reduction in the umber of specialist species and an increase in the number of generalist consumers. However, the authors tried to reinforce this hypothesis based on the relationships observed for nestedness and trophic specialization, which are not statistically significant and showed low effect size. It seems to me that the results are not robust enough to support the idea stated by the authors. In order to address this issue, the authors may consider: (i) analyzing if a nested pattern may also be detected among rows or columns since the metric NODF allows to perform these estimations; (ii) estimating the degree of turnover of specialist and generalist species using a beta diversity metric among sites. Finally, the reduction in modularity along the gradient of land use could also emerge because of changes in the structure of body size of both predators and prey. If, for example, the distribution of body size of prey changes from being aggregated to continuous, the diet of consumers is expected to change from modular to nested. I suggest the authors include to the discussion how land use could potentially affect the structure of food webs considering not only the diversity of prey but also their size.*

***Authors’ reply****:* *Thank you very much for the careful review highlighting the strongest points of our study and identifying the areas that needed improvement. We followed all your suggestions and addressed each comment, as explained below. Please note that line numbers refer to the manuscript version with inline tracked changes.*

**Reviewer 2’s comment#3.** *Not accurate estimation of the anthropogenic impact on each sampling site and lack of discussion of some methodological decisions and their potential limitations If I understood well, the authors estimate the anthropogenic impact only considering the loss of natural land-cover across sites. I find this criterion not properly correct, since there is an implicit assumption that the categories of land use identified by the authors have the same impact on the communities. For example, assuming two sites with 10% of non-natural cover, the impact on the food web structure should be higher if it is an urban area compared to a crop land. I think that including the relative weight of each anthropogenic impact to the estimation of the land use gradient may improve the results of the study. On the other hand, the authors decided to estimate the land use gradient considering a buffer area of 500m even though they tested different buffer lengths. This decision needs to be better justified and authors should discuss the potential consequences of it.*

***Authors’ reply****:*

**Reviewer 2’s comment#4.** *Awkward writing structure. Overall, I find that the structure of the introduction is messy, alternating between sections more related with network theory and the structure of ecological networks, and sections that describe the impacts of land use on ecological communities. I suggest the authors reorganize the introduction to make it more concise and straightforward to the point. Moreover, I detect several writing mistakes and style inconsistencies that could have been avoided with a more comprehensive review of the manuscript before its submission.*

***Authors’ reply****: We appreciate the constructive critique and endeavoured to reorganize this section.*

**Reviewer 2’s comment#5.** *L13-15: The hypothesis should be presented in a more comprehensive way, explicitly including the mechanisms by which land use intensity is expected to affect food web structure.*

***Authors’ reply****: We appreciate the opportunity to improve our hypothesis and explain why we expected a modular network. Please see LXX-XX. “We hypothesize that increasing land use reduces biodiversity and modularity in fish web networks due to the loss of specialist species and faunal homogenization, resulting in nested networks”.*

**Reviewer 2’s comment#6.** *L19-20: Define clearly what network complexity means (how was it estimated). Additionally, results are presented only for modularity and link density but those related with nestedness, trophic specialization and number of links should also be described.*

***Authors’ reply****: We follow the review suggestion and added the results found in other network metrics. Please see LXX-XX. “We observed that the web networks of freshwater fish are more nested than modular, thus supporting our hypothesis. However, we only observed a negative effect land use on species richness, modularity, and link density. Other network metrics, such as trophic specialization and the number of links, are not influenced by land-use”.*

**Reviewer 2’s comment#7.** *L28-38 I find that devoting an entire paragraph to introduce the concept of network, its representation, etc. is unnecessary in the context of this study. I suggest reducing these explanations to the minimum and devote more attention to the central point of the study.*

***Authors’ reply****:* *We understand the reviewer’s concern, but we have decided to maintain this paragraph in the introduction section because it defines the metrics that were used. This can promote an easy comprehension of our study for the reader. However, we tried simplifying this paragraph.*

**Reviewer 2’s comment#8** *L40-44: The connection between nestedness and human effects on network structure in not clear. Please, clarify. L44-46: This point should be clarified. L51-52: Please, clarify.*

***Authors’ reply****: Thank you for the opportunity to streamline our arguments. We clarified these sentences. Please see LXX-XX. “Recent research has been focused on the human actions on the dynamic and structure of networks. For instance, habitat degradation promotes homogenization and a reduction in network complexity, resulting in a network that is more nested (i.e., species interactions are hierarchically organized, with some species interact with many others) than modular (i.e., species interactions are compartmentalized with some species interacting only within specific subsets) (Bascompte, Jordano, & Melia, 2003, Pires & Guimarães, 2013, Olesen, Bascompte, Dupont, & Jordano, 2007; Dormann & Strauss, 2014). In trophic interactions, other network descriptors such as trophic specialization (metric that indicates the degree of species consumption on exclusive food items), number of links per species (links density), and number of trophic links according to the number of nodes (number of links given consumers and resources) also can be affect human actions. This is partly because human actions can destroy habitats and promote habitat fragmentation, disrupting the balance of species interactions within ecosystems. Since species within a community may not respond equally, and they have specialized roles which are altered due to remotion species (Staudacher et al., 2017). Thus, examining how the properties of networks vary according to anthropic stressors have the potential to provide insights into how the trophic networks are structure”*

**Reviewer 2’s comment#9.** *L69: What does "more complex networks" mean? This point should be*

*clarified. L83: Change "reduce" to "reduces" L84: Change "increase" to "increases" and "change" to "changes"*

***Authors’ reply****: We removed these sentences to avoid future confusion.*

**Reviewer 2’s comment#10.** *L85-91: It seems to me that this is the main idea that leads the study, connecting the expected impacts of land use on the structure of food webs. Therefore, it should be presented before in the introduction in a more direct way.*

***Authors’ reply****: We followed the reviewer’s suggestion and changed the order of paragraphs to present these ideas earlier in the introduction.*

**Reviewer 2’s comment#11.** *L92: Anthropogenic effects of what? Please, clarify.*

***Authors’ reply****: We clarified this sentence to specify that we evaluated the effects of land use on the structure of trophic networks.*

**Reviewer 2’s comment#12.** *L93-95: I find these questions well presented.*

***Authors’ reply****:* *We appreciate the reviewer’s opinion and positive feedback.*

**Reviewer 2’s comment#13.** *L93: Change "are" to "is"*

***Authors’ reply****: Done*

**Reviewer 2’s comment#14.** *L97: Change to "more complex ... and specialized ... networks"*

***Authors’ reply****: We removed the term 'more complex' from the entire manuscript to avoid potential misinterpretations, as this term can vary depending on the network metric. For instance, a nested network is not necessarily more complex than a modular network.*

**Reviewer 2’s comment#15.** *L92-101: In order to present the expected effects of land use on network structure, the authors stated two different hypotheses. This seems unnecessary since the set of predictions are complementary (i.e. along a gradient in resource diversity and supply, networks are expected to be more modular and less nested). Please, reduce the two hypotheses to a single one.*

***Authors’ reply****: We followed the reviewer’s suggestion and rephrased our hypothesis. Please see LXX-XX. “We hypothesized that locations with high vegetation cover could increase the supply and diversity of food resources (e.g., terrestrial insects, terrestrial allochthonous resources) to streams, resulting in a high diversity of specialized fishes that form a network more modular than nested”.*

**Reviewer 2’s comment#16.** *L107: Please, specify the other terms of interest included in the search*

***Authors’ reply****: We followed the reviewer’s suggestion and added other words considered during the search. Please see LXX-XX. “We conducted a systematic literature review of peer-reviewed articles from electronic databases and search engines, including Scopus, Web of Science and Google Scholar, covering the between 1982 and 2021. Our focus was on studies examining the diet of freshwater fish assemblages in Brazil. We followed the PRISMA protocol for reporting procedures of systematic reviews (Liberati et al., 2009 ). Our search focusses on several combination of keywords: (fish\*) AND (stream\*) AND (feed\*) AND (fish diet\*) OR (fish composition\*) OR (river fish\*) AND (land-use\*). The literature survey returned XXX studies, however we only considered studies (articles) with more than five species in studies that represent the local community, because we were interested in describing the local community network structure”*

**Reviewer 2’s comment#17.** *L125-127: Consider rephrasing to something like "...while the links between them represent trophic interactions" L132: Change to "The number of species (nodes), is defined..."*

***Authors’ reply****: We followed the reviewer’s suggestion and rephrased this sentence. Please see lines: LXX-XX. “The number of species (i.e., nodes) is defined by the total number of consumers and resources (May 1973; Tilman 1996).”*

**Reviewer 2’s comment#18.** *L174-177: If I understood well, the method used to standardize the metrics associated to network structure retains both the total number of nodes and links when the observed matrix is randomized. If this is the case, I wonder how a standard value for number of links can be obtain, since the algorithm does not change this parameter. This point requires more clarification.*

***Authors’ reply****: Thank you for the opportunity to clarify that we estimated the Standardized Effect Size (SES) of each network metric (e.g., NODF, Q, Link density, and Number of links) to control the difference associated with given the number of species and trophic links. Please see lines: LXX-XX. “Then, we quantified the Standardized Effect Size (SES) of each network metric (e.g., NODF, Q, Link density, and Number of links) as the difference between observed and random estimate values of network metrics using the following equation: (observed - μ) / σ, where ‘observed’ is the value of the focal network metric, ), μ is the mean value of focal metric over all null matrices, and σ is its standard deviation of all null matrices. Negative and positive SES values indicate observed values that are lower and higher, respectively, than the expectation given the number of species and trophic links. Empirical values of trophic network descriptors were considered to deviate strongly from the randomized food webs if these were outside the 0.05 to 0.95 quantile range of the null distribution. For each local network, the probabilities of the empirical values under the null model are presented in the Supplementary Material (Table S2).”*

**Reviewer 2’s comment#19.** *L178: Change "strongly" to "significantly".*

***Authors’ reply****: Done*

**Reviewer 2’s comment#20.** *L231: Change the comma for a period when reporting the number of total trophic interactions*

***Authors’ reply****: Done*

**Reviewer 2’s comment#21.** *L234: Remove "and".*

***Authors’ reply****: Done*

**Reviewer 2’s comment#22.** *L238: Change "then" to "than".*

***Authors’ reply****: Done*

**Reviewer 2’s comment#23.** *L241-250: The description of the relationship between each variable and land use should match the order of the plots presented in Figure 2. In addition, and following the coherence of figure 2, the plot corresponding to Number of species should not show the regression line since the trend is not significant.*

***Authors’ reply****: We followed the reviewer’s suggestion and re-ordered the plots in this figure.*

**Reviewer 2’s comment#24.** *L560-564: The order of the plots does not match the caption of the figure*

***Authors’ reply****: Thanks for mentioning this. We re-ordered the caption figure to follow the same order shown in the figure.*

**Reviewer 2’s comment#25.** *Table S1: What does "Network size" refer to? Clarification is needed.*

***Authors’ reply****:*

**Reviewer 2’s comment#26.** *Table S2: This table is confusing. Are the values p-values? If yes, I suggest the authors change them for the Z-score values. Table S5: Should the sum of each row not sum 1 since they are proportions?*

***Authors’ reply****:*

**Reviewer 2’s comment#27.** *Table S6: The authors define "number of links" as the total number of links observed in a local food web (L136-137 in the ms). Why does this variable have decimals? Should it not be a variable with positive integer values?*