Submission date. 27/06/2025

Associate editor's decision after peer review (18/08/2025).
Dear Dr. Jardim Jr:
Manuscript ID NI-2025-0114 entitled "Trophic response of an Amazonian catfish to a controlled flood pulse revealed by stomach content and stable isotope analyses" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended publication, but also suggest important revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
To revise your manuscript, log into https://mc04.manuscriptcentral.com/ni-scielo and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.
You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.
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You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text. Once the revised manuscript is prepared, upload BOTH versions (the tracked and a clean) and submit them through your Author Center.
When submitting your revised manuscript, you will be able to respond to the comments in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please reply POINT TO POINT all the suggestions of the reviewers and be as specific as possible in your response to the comments.
IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 18-Oct-2025. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.
Once again, thank you for submitting your manuscript to the Neotropical Ichthyology and I look forward to receiving your revision.
Sincerely,
Dr. Evelyn Habit
Associate Editor, Neotropical Ichthyology
ehabit@udec.cl

Anonymous reviewer #1
Recommendation. Major Revision
Comments. The manuscript entitled "Trophic response of an Amazonian catfish to a controlled flood pulse revealed by stomach content and stable isotope analyses" analyzed stomach contents and conducted C and N stable isotope analyses to describe the trophic ecology of the piscivorous/carnivorous fish Pinirampus pirinampu and its relationship with the hydrological cycle. The manuscript contains interesting results and was developed with scientific rigor; however, the approach is not original, since

the research group itself has already published several articles with similar questions and approaches at the same site and period:

1. Penha ICS, Jardim Jr AA, Prata EG, Seabra LB, Gusmão RR, Ferreira GT et al. Feeding ecology of a detritivorous fish in a controlled flood pulse area in the Amazon revealed by stomach content and stable isotopes analyses. Aquat Sci. 2025; 87(1):1–13. https://doi.org/10.1007/s00027-024-01147-x

2. Penha ICS, Seabra LB, Prata EG, Freitas TMS, Montag LFA. Unravelling a specialised diet of an Amazonian catfish in a controlled flood-pulse area by combining stomach-content and stable-isotope analyses. Mar Freshw Res. 2024; 75(6). https://doi.org/10.1071/MF23039

3. Prata EG, Seabra LB, Neres-Lima V, Montag LFDA, Freitas TMS. Diet composition and isotopic analysis unveil trophic dynamics of a fish in a controlled flood pulse area of the Amazonia. Hydrobiologia. 2025; 852(3):689–703. https://doi.org/10.1007/s10750-024-05716-x

4. Seabra LB, Huckembeck S, Freitas TMS, Lobato CMC, Penha ICS, Prata EG et al. Variation in basal sources contribution to the diet of a predator fish in an altered flood pulse area in the Amazon. Hydrobiologia. 2025a; 852(4):909–25. https://doi.org/10.1007/s10750-024-05736-7

5. Seabra LB, Tedesco PA, Oberdorff T, Winemiller KO, Huckembeck S, Freitas TMS et al. Exploring isotopic patterns of fish trophic guilds in the Volta Grande reach of the Xingu River, eastern Amazon, regulated by the operation of a hydroelectric dam. Aquat Sci. 2025b; 87(3):1–13. https://doi.org/10.1007/s00027-025-01187-x

Therefore, the authors should better highlight what is new in this manuscript or why they would expect different results from those already found in previous publications.

Although the authors found algae in the stomach contents, I found it somewhat odd to consider aquatic producers as potential food resources in the mixing model for a piscivorous/carnivorous fish species. I understand that the samples of aquatic producers are isotope results from samples of periphyton, phytoplankton, macrophytes, and algae pooled. Are periphyton, phytoplankton, and macrophytes truly potential food resources for Pinirampus pirinampu? Moreover, if a 200 μm plankton net was indeed used, the samples should contain more zooplankton than phytoplankton.

Please describe the mixing model in more detail in the Materials and Methods section, including and justifying the trophic enrichment factor used; clarify whether a concentration-dependent model was applied, since the resources have different %C and %N; and report the Gelman convergence index. Also, is there any reason for 500,000 iterations and 50,000 burn-in? Generally, the number of iterations and burn-in is increased from the package default when the mixing model has poor convergence.

I believe the authors need to better discuss the discrepancies between the results from stomach content (which providing an instantaneous measure of what the individual has just eaten) and those from stable isotopes in muscle tissue (which record assimilated diet over a longer time). I am struck by the high number of empty stomachs and by how a fish described in the literature as piscivorous (and capable of feeding on shrimp when prey fish are at low densities) has a digestive tract with an alimentary index of ~94% insects. This raises some questions for me:

1. Sampling: Was the collection time one when the species would be expected to have a stomach full of food? Seven out of 11 field campaigns had only 1–3 fish with stomachs analyzed. Is this number sufficient?

2. Prey availability: Were fish and shrimp prey of appropriate sizes for predation by Pinirampus pirinampu available? I think it would be interesting to present the CPUE and size distribution of prey fish.

In manuscript submitted, the authors conclude that the construction of a hydroelectric dam has noticeably altered the ecological dynamics of Pinirampus pirinampu due to the observed variation in assimilated carbon sources, along with subtle changes in diet composition between flood and dry seasons. They also conclude that the species' feeding plasticity promotes resilience, allowing it to maintain its functional role in the food web despite reduced resource diversity and environmental predictability. For me, it is unclear how the impact of the dam on the diet of Pinirampus pirinampu was detected without pre-dam data, and I believe the authors could present prey availability, at least for the fish collected (CPUE).

Specific comments:

L116–119 – If "flood pulse," "hydrological regime," and "hydrological periods" (L175 – "hydrological phase") refer to the same factor (with the two levels studied: high-water and low-water), please use one of these terms consistently throughout the manuscript.

L128–129 – For a piscivorous/carnivorous species, this prediction is rather trivial. Moreover, as I mentioned earlier, I question the use of basal resources as potential food sources in the mixing model. Except, if the authors are confident in the results of consumption of filamentous cyanobacteria in July and October. In this case, it would be the most surprising result of the study and would require further discussion.

L161–162 – High-water and low-water are not an analytical framework.

L170–171 – Could this sampling time somehow restrict the feeding period of Pinirampus pirinampu? The sampling effort was high; however, due to the issue of empty stomachs, the number of diet replicates per month was quite low in at least seven months.

L194 – Shouldn't it be 20 µm? What was done to avoid larger particles and zooplankton? Was any microscopic verification performed to ensure the purity of phytoplankton samples?

L199 – Were the stable isotope analyses performed at LABECO (UFPA) or at the Center for Nuclear Energy in Agriculture at the University of São Paulo (L215–216)?

L205–210 – Please clarify whether acidification with HCl was carried out to sterilize the Petri dishes or if the samples themselves were acidified. $\delta^{15}N$ values can be altered when samples are acidified to remove carbonates.

L222–223 – Was there no difference in stomach content and stable isotope values with fish size?

L228 – How were the weights of the different items quantified? Wet weight or dry weight?

L246 – Per day or per month?

L252 – Please cite the R software.

L258–259 – Standardize the font.

L261–263 – I know this is a recommendation from Post et al. (2007), but could you explain why $\delta^{13}C$ values of resources should also be corrected? Does the synthesis of predator lipids from prey fatty acids bias $\delta^{13}C$ values?

L265–271 – Given how you conducted the mixing model, I believe that a four-axis radar chart (for four resources) of the proportion assimilated would be more reliable than the niche area in $\delta^{13}C \times \delta^{15}N$ isotopic space, since the latter does not control for variation in resource isotopic values.

L279 – simmr

L286–290 – These results showing algae consumption with AI = 50% for only one genus of filamentous cyanobacteria in July and October are very unexpected for a piscivorous/carnivorous fish. Based on the diet in these months, Pinirampus pirinampu would have become omnivorous with a bias toward herbivory in October! These results were based on only 2–3 individuals. Is there any bias in the sizes of these individuals?

L315 – Please check this SD against that in Table 2.

L323–327 – There was a large variation in assimilation proportion values (Figure 4). Here, the authors only describe the mean (or median) results, but for fish assimilation, the proportion varies from almost 0% to 75% in both periods, and for crustaceans, it ranges from almost 0% to >80%. Therefore, this is a very inconclusive result. Mixing model packages have functions (e.g., compare_sources) to formally compare assimilation

proportions.
L331–332 – Refer to Figure 4 (proportion of item assimilated) instead of Figure 5 (biplot with the TDF = trophic enrichment factor). I suggest replacing Figure 3 with Figure 5, as the $\delta^{13}C$ distribution of Pinirampus pirinampu is repeated in Figures 3, 5, and 6.
L338–342 – The ellipses with only 40% credibility intervals represent the $\delta^{13}C$ and $\delta^{15}N$ distribution of Pinirampus pirinampu very poorly, since only three individuals from each period are contained within their respective ellipses. In fact, more individuals (5) from the low-water period are inside the high-water ellipse! I suggest replacing this approach with a four-axis radar chart using the mean/median assimilation proportions of the four resources.
L356–369 – I suggest showing resource availability over time—at least for the fish, which you should have—to support this discussion. Without this, the discussion becomes an attempt to explain the results without supporting data. I find it interesting to show monthly availability of food resources to help understand what happened in July and October to result in such high consumption of filamentous cyanobacteria.
L370–378 – It would be interesting to discuss the results in terms of Optimal Foraging Theory. With monthly abundance data for predators and prey of Pinirampus pirinampu, this discussion would be much more strongly supported.
Table 2 – Pseudanabaenaceae?

Anonymous reviewer #2
Recommendation. Major Revision
Comments. Thank you for the opportunity to review your manuscript. The study is relevant and timely, and it provides novel insights into the trophic ecology of Pinirampus pirinampu under regulated hydrological conditions. The general design and dataset are promising, but I believe the manuscript requires substantial revision before it can be considered for publication.
The main issues concern methodological transparency and ecological interpretation. Specifically, sample size limitations (especially for stomach content and isotopic data) should be more explicitly acknowledged; the isotopic mixing models require clearer justification and reporting (priors, discrimination factors, credibility intervals); and the mismatch between diet and isotopic signals deserves a deeper discussion, as it has important ecological implications. Improving these aspects, along with clarifying some methodological details and strengthening the figures and tables, will considerably enhance the rigor and impact of the study.
Overall, I find the manuscript promising and aligned with the journal's scope, but I encourage the authors to address these points thoroughly in a major revision.

Author's Rebuttal Letter (28/10/2025).
Dear Editor,
We sincerely thank the reviewers for their thoughtful and constructive comments on our manuscript entitled "Trophic response of an Amazonian catfish to a controlled flood pulse, revealed by stable isotope and stomach content analyses". We are grateful for the opportunity to improve our work and for its consideration for publication in Neotropical Ichthyology.
In response to the reviewers' observations, we have carefully revised the manuscript, incorporating all suggested corrections, clarification, and methodological justification. Where appropriate, we provided well-founded explanations to ensure transparency and rigor. We would like to note that we made minor revisions to the main text and adjusted the title to better reflect the study's findings. A detailed, point-by-point response to each reviewer's comment has been prepared, demonstrating how we addressed the reviewer's feedback. We are confident that these revisions have substantially strengthened the manuscript, enhancing both its clarity and scientific contribution.

We express our sincere appreciation for the reviewers' time and expertise, and we remain at the disposal of the editors and reviewers for any further clarification or additional information required.

Kind regards,

Jardim Jr,

On behalf of all authors

Reviewer(s)' Comments to Author:

Reviewer #1

General comment. The manuscript entitled "Trophic response of an Amazonian catfish to a controlled flood pulse revealed by stomach content and stable isotope analyses" analyzed stomach contents and conducted C and N stable isotope analyses to describe the trophic ecology of the piscivorous/carnivorous fish Pinirampus pirinampu and its relationship with the hydrological cycle. The manuscript contains interesting results and was developed with scientific rigor; however, the approach is not original, since the research group itself has already published several articles with similar questions and approaches at the same site and period:

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Answer: We sincerely thank the reviewer for the careful evaluation and constructive comments. We acknowledge that our methodological approach (stomach content and stable isotope analyses) has been applied by our group in the same area. However, the novelty of this study lies in its biological focus.

This is the first study to describe the trophic ecology of Pinirampus pirinampu in the Volta Grande do Xingu using this combined approach. This integration provides new insights into both short-term feeding patterns and long-term assimilated diet of this top predator under a controlled flood-pulse regime. Given potential population declines in

P. pirinampu due to long-term flow alterations, understanding its trophic dynamics is critical for interpreting ecosystem responses to hydrological change.

Thus, rather than replicating previous work, our study extends trophic investigation to a key predatory species, offering insights into how higher-trophic-level consumers respond to reduced hydrological connectivity. This contributes valuable information for food web modeling and conservation planning in dam-regulated Amazonian rivers.

Regarding the inclusion of aquatic primary producers in the initial mixing model, we appreciate the reviewer's observation. As noted, periphyton, phytoplankton, and macrophytes are not direct food sources for P. pirinampu, which is primarily piscivorous/carnivorous. They were initially included to evaluate potential isotopic overlap and carbon pathways through prey. Following the reviewer's suggestion, we refined the model by excluding these primary producers and retaining only fishes, crustaceans (shrimp and crab), and aquatic mollusks (gastropod and bivalves) as trophic sources.

This adjustment enhances the ecological realism of the model. In the revised analysis, aquatic insects did not appear assimilated resources during the high-water period, supporting their exclusion. The final model therefore reflects a more accurate representation of resource assimilation consistent with known feeding ecology of P. pirinampu.

Please describe the mixing model in more detail in the Materials and Methods section, including and justifying the trophic enrichment factor used; clarify whether a concentration-dependent model was applied, since the resources have different %C and %N; and report the Gelman convergence index. Also, is there any reason for 500,000 iterations and 50,000 burn-in? Generally, the number of iterations and burnin is increased from the package default when the mixing model has poor convergence.

Answer: We thank the reviewer for these suggestions. We have expanded the Material and Methods section to describe the mixing model in greater detail, including a justification of the trophic enrichment factors used.

Since primary producers and aquatic insects were excluded from the model, the remaining resources (fishes, crustaceans, and mollusks) have relatively similar %C and %N values, and therefore a concentration-dependent model was not applied. The Gelman convergence diagnostic is now reported in the text, confirming model convergence.

Regarding the number of iterations and burn-in, the model was reanalyzed using 10,000 iterations with a 10% burn-in, following the recommendations of Parnell (2021). This choice ensured stable posterior distribution and appropriate model convergence without unnecessary computational overhead.

References:

• Parnell AC. 2021. SIMMR: Stable Isotope Mixing Model in R. R package version 0.4. 5. https://github.com/andrewcparnell/simmr

• Parnell AC, Inger R, Bearhop S, Jackson AL. Source partitioning using stable isotopes: coping with too much variation. PLoS One. 2010; 5(3):e9672. https://doi.org/10.1371/journal.pone.0009672

I believe the authors need to better discuss the discrepancies between the results from stomach content (which providing an instantaneous measure of what the individual has just eaten) and those from stable isotopes in muscle tissue (which record assimilated diet over a longer time).

Answer: Thank you for these comments. We have added a paragraph to the discussion addressing the observed discrepancies between stomach content and stable isotope analyses. Specifically, stomach contents provide an instantaneous snapshot of recent feeding, whereas muscle tissue isotopes integrate assimilated diet over weeks to months. We emphasize that combining these approaches offers complementary insights, capturing both short-term feeding events and longer-term dietary assimilation.

I am struck by the high number of empty stomachs and by how a fish described in the literature as piscivorous (and capable of feeding on shrimp when prey fish are at low densities) has a digestive tract with an alimentary index of ~94% insects. This raises some questions for me:

1. Sampling: Was the collection time one when the species would be expected to have a stomach full of food? Seven out of 11 field campaigns had only 1–3 fish with stomachs analyzed. Is this number sufficient?

Answer: We appreciate the reviewer's concern. Although our standardized sampling methodology was applied consistently across the hydrological cycle, we acknowledge that the high frequency of empty stomachs in carnivorous/piscivorous fishes is a common feature that may introduce some informational bias. To address this, we have highlighted these aspects in the text, provided supplementary information (Figure S1), and expanded the discussion regarding the number of stomachs analyzed and the developmental stages of individuals, which were consistent between stomach content and stable isotope analyses.

Despite the relatively low number of stomachs containing food, the prey remains recorded still provide valuable insights into trophic habits. When combined with stable isotope data, which integrate diet over longer periods, our conclusions on feeding patterns and trophic plasticity are strengthened.

2. Prey availability: Were fish and shrimp prey of appropriate sizes for predation by Pinirampus pirinampu available? I think it would be interesting to present the CPUE and size distribution of prey fish.

Answer: We thank the reviewer for this suggestion. While data on prey availability (CPUE and size distribution of fish and shrimp) would indeed enrich interpretation, our sampling design was focused on assessing the trophic response of Pinirampus pirinampu through stomach contents and stable isotopes, without systematic prey sampling. Nonetheless, the integration of these two complementary approaches provides robust evidence of dietary plasticity across hydrological periods.

In manuscript submitted, the authors conclude that the construction of a hydroelectric dam has noticeably altered the ecological dynamics of Pinirampus pirinampu due to the observed variation in assimilated carbon sources, along with subtle changes in diet composition between flood and dry seasons. They also conclude that the species' feeding plasticity promotes resilience, allowing it to maintain its functional role in the food web despite reduced resource diversity and environmental predictability. For me, it is unclear how the impact of the dam on the diet of Pinirampus pirinampu was detected without pre-dam data, and I believe the authors could present prey availability, at least for the fish collected (CPUE).

Answer: We agree that, without pre-dam reference data, it is not possible to directly attribute changes in P. pirinampu diet to the Belo Monte Dam. Accordingly, we have revised the discussion to frame our findings in the context of flow regulation rather than as a definitive dam effect. The text now emphasizes seasonal and hydrologically driven variations in assimilated carbon sources, highlighting the species' trophic plasticity and resilience under altered environmental conditions, without implying a direct cause-and-effect relationship.

Specific comments:

L116–119 – If "flood pulse," "hydrological regime," and "hydrological periods" (L175 – "hydrological phase") refer to the same factor (with the two levels studied: high-water and low-water), please use one of these terms consistently throughout the manuscript.

Answer: Done.

L128–129 – For a piscivorous/carnivorous species, this prediction is rather trivial. Moreover, as I mentioned earlier, I question the use of basal resources as potential food sources in the mixing model. Except, if the authors are confident in the results of consumption of filamentous cyanobacteria in July and October. In this case, it would be the most surprising result of the study and would require further discussion.

Answer: We appreciate your comments and suggestions. Based on your observations and existing literature on the feeding habits of Pinirampus pirinampu, we have removed basal resources from the mixing model. This adjustment enhances the ecological realism of the analysis and provides a clearer representation of the species' trophic dynamics, focusing on prey types that are genuinely assimilated.

L161–162 – High-water and low-water are not an analytical framework.

Answer: Thank you for your comment. We agree that "high-water" and "low-water" refer to hydrological periods rather than analytical structures. We have clarified the text to indicate that these phases were used as categorical variables to structure our analyses.

L170–171 – Could this sampling time somehow restrict the feeding period of Pinirampus pirinampu? The sampling effort was high; however, due to the issue of empty stomachs, the number of diet replicates per month was quite low in at least seven months.

Answer: We appreciate this important observation. Sampling was conducted in the late afternoon and early evening (16:00–21:00) to standardize effort across months and sites, coinciding with the predominantly nocturnal or crepuscular feeding activity reported for Amazonian catfish. While this window may not capture the complete daily feeding cycle, it aligns with expected peak foraging. The high frequency of empty stomachs is common among carnivorous fishes, which feed intermittently, possess small gastrointestinal tracts, and digest prey rapidly. We have clarified this point in the Methods and Discussion.

L194 – Shouldn't it be 20 μm? What was done to avoid larger particles and zooplankton? Was any microscopic verification performed to ensure the purity of phytoplankton samples?

Answer: Thank you for the comment. After reexamining the dietary data, we excluded primary producers from the mixing model. Aquatic mollusks were retained instead, reflecting the predominantly piscivorous/carnivorous feeding habit of the species.

L199 – Were the stable isotope analyses performed at LABECO (UFPA) or at the Center for Nuclear Energy in Agriculture at the University of São Paulo (L215–216)?

Answer: Thank you. We clarified that sample processing (drying and grinding) was performed at LABECO (UFPA), whereas the stable isotope analyses of carbon and nitrogen were conducted at CENA, University of São Paulo.

L205–210 – Please clarify whether acidification with HCl was carried out to sterilize the Petri dishes or if the samples themselves were acidified. $\delta^{15}N$ values can be altered when samples are acidified to remove carbonates.

Answer: We appreciate the comment. The HCl solution was used exclusively to sterilize the Petri dishes; tissue samples were not acidified, avoiding any alteration of $\delta^{15}N$ values.

L222–223 – Was there no difference in stomach content and stable isotope values with fish size?

Answer: All individuals analyzed were juveniles, with standard lengths ranging from 11.8–52.0 cm SL, below the maturation threshold (C50 = 53.6 cm; Peixer et al., 2006). Ontogenetic variation related to sexual maturity is therefore unlikely to affect our results. We have included this information in the Methods and added a supplementary figure illustrating the size range.

Reference:

• Peixer J, Mateus LAF, Resende EK. First gonadal maturation of Pinirampus pirinampu (Siluriformes: Pimelodidae) in the Pantanal, Mato Grosso do Sul State, Brazil. Brazilian Journal of Biology. 2006; 66, 317–323. https://doi.org/10.1590/S1519-69842006000200014

L228 – How were the weights of the different items quantified? Wet weight or dry weight?

Answer: We used wet weight, following standard practice in fish diet studies. The aim was to quantify the relative contribution of each food item rather than absolute biomass.

L246 – Per day or per month?

Answer: Corrected.
L252 – Please cite the R software.
Answer: Done.
L258–259 – Standardize the font.
Answer: Done.
L261–263 – I know this is a recommendation from Post et al. (2007), but could you explain why δ13C values of resources should also be corrected? Does the synthesis of predator lipids from prey fatty acids bias $\delta^{13}C$ values?
Answer: We appreciate the comment. After revising the mixing model and removing primary producers, δ13C correction for resources was no longer necessary. Therefore, the correction was applied only to the muscle tissue of consumers, following Post et al. (2007).
L265–271 – Given how you conducted the mixing model, I believe that a four-axis radar chart (for four resources) of the proportion assimilated would be more reliable than the niche area in δ13C × δ15N isotopic space, since the latter does not control for variation in resource isotopic values.
Answer: Thank you for the suggestion. Our revised mixing model includes three sources (fish, crustaceans, and mollusks). We retained the isotopic niche area (δ13C × δ15N) approach because it is well-established and widely used in fish assemblage studies, providing an integrative measure of trophic niche width and overlap.
L279 – simmr
Answer: Done.
L286–290 – These results showing algae consumption with AI = 50% for only one genus of filamentous cyanobacteria in July and October are very unexpected for a piscivorous/carnivorous fish. Based on the diet in these months, Pinirampus pirinampu would have become omnivorous with a bias toward herbivory in October! These results were based on only 2–3 individuals. Is there any bias in the sizes of these individuals?
Answer: Thank you. After reviewing the data, we found inconsistencies in the five individuals that reportedly consumed algae. These individuals were removed, and analyses were redone. Most individuals were juveniles (below C50), and diet composition reflects opportunistic consumption rather than size-related feeding differences.
L315 – Please check this SD against that in Table 2.
Answer: Table 2 has been updated.
L323–327 – There was a large variation in assimilation proportion values (Figure 4). Here, the authors only describe the mean (or median) results, but for fish assimilation, the proportion varies from almost 0% to 75% in both periods, and for crustaceans, it ranges from almost 0% to >80%. Therefore, this is a very inconclusive result. Mixing model packages have functions (e.g., compare_sources) to formally compare assimilation proportions.
Answer: We acknowledge that the wide variation in assimilation proportions limits conclusions based solely on mean or median values. This variability reflects Bayesian uncertainty and ecological reality: P. pirinampu exploits different resources in varying proportions throughout the hydrological cycle, highlighting trophic plasticity.
L331–332 – Refer to Figure 4 (proportion of item assimilated) instead of Figure 5 (biplot with the TDF = trophic enrichment factor). I suggest replacing Figure 3 with Figure 5, as the $\delta^{13}C$ distribution of Pinirampus pirinampu is repeated in Figures 3, 5, and 6.
Answer: We appreciate the observation. Figures 3, 5, and 6 each represent distinct analyses: Figure 3 compares δ13C between periods, Figure 5 shows the isotopic biplot for the mixing model, and Figure 6 illustrates isotopic niche width/overlap. Therefore, all figures provide unique analytical value.
L338–342 – The ellipses with only 40% credibility intervals represent the $\delta^{13}C$ and $\delta^{15}N$ distribution of Pinirampus pirinampu very poorly, since only three individuals from each period are contained within their respective ellipses. In fact, more individuals

(5) from the low-water period are inside the high-water ellipse! I suggest replacing this approach with a four-axis radar chart using the mean/median assimilation proportions of the four resources.

Answer: We acknowledge the visualization limitation with small sample sizes. However, SEAc is robust for small datasets, reducing sensitivity to outliers and sampling bias, and is widely recommended (Jackson et al., 2011; Syväranta et al., 2013). It provides reliable comparisons even with limited individuals.

References:

• Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology, 80(3), 595-602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

• Syväranta, J., Lensu, A., Marjomäki, T. J., Oksanen, S., & Jones, R. I. (2013). An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. PloS one, 8(2), e56094. https://doi.org/10.1371/journal.pone.0056094

L356–369 – I suggest showing resource availability over time—at least for the fish, which you should have—to support this discussion. Without this, the discussion becomes an attempt to explain the results without supporting data. I find it interesting to show monthly availability of food resources to help understand what happened in July and October to result in such high consumption of filamentous cyanobacteria.

Answer: We added monthly variation in food consumption to the Results and Discussion. A table and figure on monthly consumption by species are now included in Supplementary Material (Figure S2).

L370–378 – It would be interesting to discuss the results in terms of Optimal Foraging Theory. With monthly abundance data for predators and prey of Pinirampus pirinampu, this discussion would be much more strongly supported.

Answer: We incorporated monthly variation in consumption into Results, Discussion, and Supplementary Material (Figure S2), and expanded the discussion of results in the context of Optimal Foraging Theory.

Table 1 – Pseudanabaenaceae?

Answer: Table 1 has been updated, and we remove algae of the food items.

Reviewer #2

General comment. Thank you for the opportunity to review your manuscript. The study is relevant and timely, and it provides novel insights into the trophic ecology of Pinirampus pirinampu under regulated hydrological conditions. The general design and dataset are promising, but I believe the manuscript requires substantial revision before it can be considered for publication.

The main issues concern methodological transparency and ecological interpretation. Specifically, sample size limitations (especially for stomach content and isotopic data) should be more explicitly acknowledged; the isotopic mixing models require clearer justification and reporting (priors, discrimination factors, credibility intervals); and the mismatch between diet and isotopic signals deserves a deeper discussion, as it has important ecological implications. Improving these aspects, along with clarifying some methodological details and strengthening the figures and tables, will considerably enhance the rigor and impact of the study.

Overall, I find the manuscript promising and aligned with the journal's scope, but I encourage the authors to address these points thoroughly in a major revision.

This manuscript examines the trophic ecology of the Amazonian catfish Pinirampus pirinampu under a regulated flood-pulse regime in the Xingu River, combining stomach contents and stable isotope ratios (δ13C, δ15N) across a full annual cycle. The question is timely and conservation-relevant given hydropower expansion in the Amazon, the temporal design is appropriate, and the manuscript is clearly organized and framed within established theory (Flood Pulse Concept; Optimal Foraging Theory). The dual approach is a strength, as it contrasts short-term ingestion with longer-term assimilation.

However, several limitations currently weaken inference. Only 45 of 139 fish had identifiable stomach contents, and isotopic inference relies on 23 muscle samples (11 high water, 12 low water). The striking mismatch between ingested items (insects dominant) and assimilated sources (fish/crustaceans) is central but remains underexplored in the discussion. In addition, key analytical choices require clearer justification and uncertainty reporting (e.g., confidence intervals for mixing-model contributions and sensitivity to trophic discrimination factors), and no size-based analyses are presented despite wide body-size variation. Some methodological aspects also warrant clarification in Methods (e.g., acidification of fish muscle prior to isotope analysis, the plankton mesh used to represent phytoplankton, and the exact lipid-normalization equations applied).

Overall, the study is promising and well aligned with the journal's scope. However, a major revision is needed to (i) strengthen methodological transparency and uncertainty quantification, (ii) explicitly acknowledge and accommodate the small sample sizes in diet and isotope data, and (iii) deepen the ecological interpretation of the diet–assimilation discrepancy and its conservation implications under regulated flows.

Answer: We sincerely appreciate the reviewer's insightful and constructive comments, which were invaluable for improving our manuscript. We carefully considered all suggestions and incorporated them wherever possible. In cases where certain recommendations could not be fully implemented, such as collecting additional pre-dam data or systematic prey availability measurements, we have provided clear explanations and justifications in the revised text.

Major comments

1. Sample size limitations

Only 45 of 139 individuals had stomach contents with identifiable prey items, and the stable isotope dataset included just 23 muscle samples (11 high water, 12 low water). Such small effective sample sizes constrain inference about dietary shifts across hydrological phases. The authors should explicitly acknowledge this limitation in the text and discuss potential biases (e.g., overrepresentation of insect prey in the limited stomachs analyzed). If possible, they could explore pooling samples by hydrological phase to increase robustness, or provide rarefaction curves/feeding diversity indices to show whether the sample size is sufficient to characterize diet.

Answer: Thank you for your comment. We have explicitly acknowledged in the discussion that the relatively small number of stomachs with identifiable prey and the limited number of muscle samples for stable isotopes may influence the robustness of our inferences. We also discuss potential biases, such as overrepresentation of insects in the stomach content dataset. Financial constraints limited the number of isotopic samples, as P. pirinampu was not the primary target of the broader community-level study.

2. Mismatch between diet and isotopic assimilation

The main ecological signal of this study is the discrepancy between stomach content (insect-dominated) and isotope-based assimilation (fish/crustaceans). This is highly relevant but currently underexplored. The authors should expand the discussion by considering mechanisms such as differences in digestion and assimilation rates, opportunistic insect feeding during high water, or methodological biases in prey identification. Comparative references from unregulated systems of P. pirinampu (e.g., diet dominated by fish/crustaceans) could help determine whether this mismatch is typical or a conseq uence of hydrological regulation.

Answer: We appreciate this comment. The discussion has been expanded to explore the discrepancy between stomach content (insect-dominated) and stable isotope assimilation (fish/crustaceans). We considered multiple mechanisms, including differences in

digestion and assimilation rates, opportunistic insect feeding during high water, and potential methodological biases in prey identification. Comparative references from unregulated systems were included to contextualize whether the observed mismatch is typical or may be influenced by flow regulation.
3. Hydrological regulation and ecological context
While the introduction highlights the impact of Belo Monte, the discussion does not fully connect the empirical results to the expected dynamics under natural vs. regulated flood pulses. A stronger contrast with historical or regional studies (including pre-dam dietary records where available) would help clarify whether regulated flows alter trophic strategies. At minimum, the authors should acknowledge the lack of baseline data as a limitation, and ideally, frame their results in terms of predicted consequences for energy pathways in regulated vs. unregulated systems.
Answer: Thank you for the suggestion. We clarified in the discussion that, in the absence of pre-dam baseline data, direct cause–effect conclusions are not possible. We reframed the results in the context of flow regulation and compared our findings with regional and historical studies. We also highlighted potential implications for energy pathways under regulated versus unregulated flood-pulse regimes.
4. Methodological transparency in isotopic analyses
Several aspects of the isotope methodology require clarification:
Were fish muscle samples acid-treated to remove carbonates? If not, justify why.
Answer: We clarified that HCl was used exclusively to sterilize Petri dishes, with no acidification of tissue samples.
The "phytoplankton" fraction was obtained with a 200 µm mesh, which likely retained mainly zooplankton and detrital particles. The authors should re-label this source appropriately (e.g., "planktonic POM") and discuss how this might affect mixing models.
Answer: Thank you for your comment. We chose to remove primary producers from the mixing model and, considering that the species is classified as piscivorous/carnivorous, we included aquatic mollusks (bivalves) in addition to the sources already present in the model. However, based on the mixing polygon, we observed that aquatic insects during the high-water period were not assimilated by P. pirinampu. Therefore, we decided to exclude aquatic insects from the mixing model, retaining only fish, crustaceans, and mollusks as dietary sources.
Lipid correction: specify whether %C was directly measured or estimated, and provide the exact equation applied.
Answer: Done. Lipid correction procedures were detailed, specifying measured %C and the equations applied.
Trophic discrimination factors: explain why Vander Zanden & Rasmussen (2001) was used for Amazonian catfishes, and consider a sensitivity analysis using alternative values (e.g., Post 2002).
Answer: We appreciate the reviewer's comments and suggestion. We justified the use of Vander Zanden & Rasmussen (2001) TDF values, noting their prior application to Amazonian catfishes, and discussed alternatives (e.g., Post 2002) while retaining the selected values for consistency with comparable studies.
References:

• Penha ICS, Seabra LB, Prata EG, Freitas TMS, Montag LFA. Unravelling a 606 specialised diet of an Amazonian catfish in a controlled flood-pulse area by 607 combining stomach-content and stable-isotope analyses. Mar Freshw Res. 2024; 608 75(6). https://doi.org/10.1071/MF23039
• Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia. 2007; 152:179–89. https://doi.org/10.1007/s00442-006-0630-x
• Prata EG, Seabra LB, Neres-Lima V, Montag LFDA, Freitas TMS. Diet composition

and isotopic analysis unveil trophic dynamics of a fish in a controlled flood pulse area of the Amazonia. Hydrobiologia. 2025; 852(3):689–703. https://doi.org/10.1007/s10750-024-05716-x

• Seabra LB, Huckembeck S, Freitas TMS, Lobato CMC, Penha ICS, Prata EG et al. Variation in basal sources contribution to the diet of a predator fish in an altered flood pulse area in the Amazon. Hydrobiologia. 2025a; 852(4):909–25. https://doi.org/10.1007/s10750-024-05736-7

5. Uncertainty reporting in mixing models

SIAR/SIMMR and SIBER results are presented without credibility intervals or posterior distributions. This limits interpretability, since mixing models are inherently uncertain. At minimum, the authors should report 95% credibility intervals for dietary contributions, and provide SIBER niche area estimates with error margins. Including posterior density plots (as supplementary figures) would greatly strengthen transparency.

Answer: After reanalyzing the mixing model, we included 95% credibility intervals for dietary contributions and reported SIBER-derived niche area estimates with error margins. Posterior density plots and monthly consumption graphs were added to the supplementary material (FIGURES S2–S3) to enhance transparency and interpretation.

6. Ontogenetic effects and body size

P. pirinampu undergoes ontogenetic dietary shifts, yet no size-based analyses are presented despite a wide size range in the dataset. If the authors have length data, they could test for correlations between body size and diet composition or isotopic values. If such analyses are not feasible, the limitation should be explicitly acknowledged, since pooling all size classes may obscure important trophic patterns.

Answer: T All individuals analyzed were juveniles, below the estimated L50 (SL 11.8–52.0 cm; Peixer et al., 2006), minimizing ontogenetic variation in diet. This is now explicitly stated in the Methods, and a supplementary figure illustrates the size range distribution.

Reference:

• Peixer J, Mateus LAF, Resende EK. First gonadal maturation of Pinirampus pirinampu (Siluriformes: Pimelodidae) in the Pantanal, Mato Grosso do Sul State, Brazil. Brazilian Journal of Biology. 2006; 66, 317–323. https://doi.org/10.1590/S1519-69842006000200014

7. Integration of results and ecological implications

The conclusion suggests that dietary plasticity confers resilience to flow regulation, but this interpretation is not fully supported by the presented data. The authors should either temper this claim or provide stronger evidence (e.g., references showing long-term persistence of piscivorous catfishes in regulated systems). Alternatively, the discussion could be reframed to highlight that observed plasticity may reflect short-term opportunism rather than long-term resilience, with implications for ecosystem integrity and fishery sustainability.

Answer: We revised the discussion to temper claims of resilience. While observed dietary plasticity may reflect short-term opportunistic feeding rather than long-term persistence, we highlight its ecological relevance in maintaining trophic interactions under regulated flows. References to similar patterns in other Amazonian piscivorous catfishes were included to contextualize potential ecosystem-level implications.

Specific comments

Title: is clear, informative, and appropriately reflects the focus of the study, highlighting both the target species and the combined methodological approach. However, a slight grammatical adjustment could improve fluency in English.

Line 1: (Title) “Trophic response of an Amazonian catfish to a controlled flood pulse revealed by stomach content and stable isotope analyses”. Suggested revision for smoother phrasing: “Trophic response of an Amazonian catfish to a controlled flood pulse, revealed by stable isotope and stomach content analyses”.

Answer: Done.

Abstract

Line 25–26: The number of stomachs (101) vs. isotopic samples (23) is reported, but note that only 45 stomachs had identifiable contents (as stated later in the manuscript). Suggest clarifying this already in the Abstract to avoid overestimating sample robustness.

Answer: Done.

Introduction

Lines 85–87: The phrase "together these methods offer..." is repetitive of the previous paragraph. Suggest condensing.

Answer: Done. Thank you for the suggestion.

Lines 92–98: When mentioning hydropower impacts, the references are adequate, but the link to trophic ecology could be stronger. Suggest explicitly stating how flow regulation may increase empty stomachs, reduce isotopic differentiation among sources, or shift prey availability.

Answer: Done. Thank you for the suggestion.

Line 99 ("approx. 80% downstream"): This is a striking number—cite the exact source more precisely (Zuanon et al. 2019 is secondary; check if ANA or dam monitoring data could be cited).

Answer: Thank you for your comments. We added a reference that reports this alteration and clarified that the ~80% value represents an annual mean discharge reduction, not an absolute figure.

Lines 115–120: The research questions are clear, but they read more like objectives than hypotheses. Recommend rephrasing into explicit hypotheses (e.g., "We hypothesized that stomach contents would show higher insect feeding during high water, whereas isotopes would indicate piscivory in both periods").

Answer: Done. Thank you for the suggestion.

Lines 122–129: Predictions are well formulated, but sound repetitive. Suggest condensing by merging points (diet stability + resource availability + isotopic consistency) into a shorter paragraph.

Answer: Done. Thank you for the suggestion.

Lines 130–134: The final sentence is too broad ("providing essential information for conservation strategies"). Recommend making it more specific to trophic pathways in regulated Amazonian rivers.

Answer: Done. Thank you for the suggestion.

Material and methods

Study area

Line 141–143: "hydrological regulation, with flow regimes controlled according to environmental guidelines..." → Suggest clarifying whether these guidelines attempt to mimic natural seasonality or are fixed thresholds, since this is critical to ecological interpretation.

Answer: Done.

Line 147 ("dense ombrophilous forest"): Consider changing to "dense evergreen ombrophilous forest" for accuracy, or cite vegetation classification reference.

Answer: Done.

Line 158–160: The reported discharge range (8215–707.8 m3/s) is striking. Recommend clarifying whether these values are daily averages, maximums, or minimums.

Answer: Done.

Line 161–162: Suggest explicitly linking the high-water (Dec–May) and low-water (Jun–Nov) phases to biological sampling design to reinforce the logic of comparisons.

Answer: Done.

Sampling procedures and processing the biological material

Lines 167–168: Provide justification for the selected gillnet mesh sizes (2–18 cm). Could note that these cover a broad range of fish sizes but may bias against small prey fishes.

Answer: Thank you for your comment. We used gillnets with mesh sizes ranging from 2 to 18 cm to capture a broad spectrum of fish sizes, ensuring representation of both small and larger individuals. We acknowledge that smaller prey fishes might be underrepresented due to mesh selectivity; this limitation is now noted in the Methods. The 2 cm mesh was sufficient to capture the smallest individuals of P. pirinampu without causing harm.

Line 170: "three gillnet sets" → clarify whether replicates were pooled for analysis or treated as independent samples.

Answer: Thank you for your comment. We clarified in the text that each gillnet set was treated as an independent replicate for analysis; results were not pooled across sets.

Line 173–174: The minimum number of individuals per hydrological phase is 12, but the Abstract mentions 23 total isotopic samples. Please ensure consistency.

Answer: Thank you. Eleven muscle samples were collected during the dry season and 12 during the flood season, totaling 23 samples. We have revised the Abstract and Methods to ensure consistency.

Line 182: Stomachs preserved in 70% ethanol: note that ethanol can alter isotopic values; clarify whether tissues for isotope analysis were fixed differently (the text later suggests they were but specify clearly).

Answer: Thank you. Muscle tissues for isotopic analyses were collected fresh and never contacted ethanol or other preservatives. This point has been clarified in the Methods.

Line 205–206: Categories (7 groups) are well defined. Suggest indicating whether "aquatic insects" were separated into functional groups (e.g., Diptera vs. Ephemeroptera).

Answer: Thank you. Aquatic insects were analyzed as a single category, although each item was identified to the lowest feasible taxonomic level.

Line 210–212: Hydrochloric acid treatment may bias δ15N; recommend citing Post et al. 2007 as justification.

Answer: We clarified that HCl was used solely to sterilize Petri dishes and did not meet tissue samples, avoiding any bias in δ15N.

Line 218: Report the precision of isotope ratio mass spectrometry (e.g., ±0.2‰) to strengthen methodological rigor.

Answer: Done. Precision of ±0.2‰ for δ13C and δ15N is now reported.

Data analysis

Line 238–239: PERMANOVA and PERMDISP are appropriate. Suggest reporting whether data were transformed (e.g., log, square root) before analyses.

Answer: Done.

Line 254–256: Standards for δ13C and δ15N are adequately cited. Could report analytical replicates (e.g., run in duplicate).

Answer: Done.

Line 265–271: SEAc and SIBER use is appropriate. Suggest clarifying number of iterations used in ellipse estimation.

Answer: We clarified that 20,000 iterations were run, with 1,000 discarded as burn-in, a thinning factor of 10, and 2 independent chains, resulting in 3,800 posterior samples per ellipse.

Line 274–275: State the priors used for Bayesian mixing models (uninformative/Dirichlet). Important for transparency.

Answer: We clarified that Dirichlet priors were used for the Bayesian mixing models, with 10,000 MCMC iterations and 10% burn-in.

Line 278–281: Reporting 95% credibility intervals is correct. Suggest also reporting model diagnostics (convergence, Gelman–Rubin statistic if checked).

Answer: We report 95% credibility intervals and applied Gelman–Rubin and Geweke diagnostic tests to confirm model convergence (Parnell, 2010).

Reference:

• Parnell AC, Inger R, Bearhop S, Jackson AL. Source partitioning using stable isotopes:

coping with too much variation. PLoS One. 2010; 5(3):e9672. https://doi.org/10.1371/journal.pone.0009672

Results

Line 288–289: The statement "aquatic insects alone contributed 75%" seems inconsistent with Figure 2 (87% in high-water, 68% in low-water). Clarify that 75% refers to the overall mean across both periods.

Answer: Done.

Line 292–294: When reporting percentages of other categories (fish, crustaceans, etc.), explicitly link them to Table 1 for transparency.

Answer: Done.

Line 299–300: The description of the Repletion Index (RI) would benefit from reporting the sample size per period (n) to contextualize the comparison.

Answer: Done.

Line 309–313: δ13C differences are well reported. It would help readers to also provide the absolute difference (Δδ13C ≈ 1‰) for ecological interpretation.

Answer: Done.

Line 320–328: Resource contribution percentages (42%, 36%, etc.) should state whether they are posterior means or medians from Bayesian mixing models, since Figure 4 shows broad probability distributions.

Answer: Done.

Line 338–342: The overlap metric could be phrased more clearly, e.g., "corresponding to 19.13% of the combined niche area." Also specify that overlap was calculated using SEAc in SIBER.

Answer: Done.

Discussion

Line 346–350: The statement that no significant differences were observed between hydrological periods could benefit from a brief mention of the relatively small sample size of stomach content analyses (n = 45), as this might limit the detection of subtle dietary shifts.

Answer: Done.

Line 352–356: When discussing δ13C variation, specify whether the magnitude of the observed differences (≈1 ‰) is biologically meaningful in the context of Amazonian floodplain systems.

Answer: Done.

Line 358–360: The phrase "moderate isotopic niche overlap" would benefit from providing the actual overlap percentage (19.13% from Results) for clarity and consistency.

Answer: Done.

Line 364–368: The discussion of supporting studies (Prata et al. 2025) could be expanded with explicit comparisons to highlight whether those species exhibited similar stability in diet or stronger seasonal shifts.

Answer: Done.

Line 379–382: The contrast with studies in the Volta Grande do Xingu would be clearer if the authors emphasize how differences in methodology (e.g., sample size, prey availability data) may account for divergent findings.

Answer: We appreciate the reviewer's suggestion. However, we decided to remove this comparison to avoid overinterpreting differences arising from distinct sampling designs, prey availability datasets, and hydrological contexts. Methodological and environmental differences between the Volta Grande system and our study area could lead to confounding interpretations rather than clarifying ecological patterns.

Line 387–393: The contrast between stomach content and stable isotope analyses should clarify whether temporal integration differences (short-term vs. long-term feeding signals) explain the discrepancies.

Answer: Thank you for your comment. The Discussion was expanded to clarify temporal integration differences.
Line 408–410: The statement about "dietary plasticity" could benefit from citing specific values of SEA or overlap that demonstrate this flexibility.
Answer: Thank you for your comment. The SEA and overlap values were added to support interpretation.
Line 412–419: Consider rephrasing to make clear whether the changes in organic resource deposition downstream of the dam are inferred or directly measured by the authors, since it is presented somewhat assertively.
Answer: Thank you. We have revised this paragraph to clarify inferred versus directly measured impacts.
Line 425–434: The final paragraph could be made more concise by reducing redundancy (e.g., "subtle changes in diet composition" and "adaptive foraging response" overlap conceptually). A more direct take-home message would strengthen the conclusion.
Answer: Done. Thank you.
Tables and figures
Table 2
Units of δ13C and δ15N (‰) should be explicitly included in the column headers.
Answer: Done.
A short note should clarify that P. pirinampu values correspond to muscle tissue, as mentioned in the text.
Answer: We have clarified in the caption that the reported values for P. pirinampu correspond to muscle tissue, in addition to specifying that "n" indicates the number of tissue samples per period.
Figure 3
The figure only shows δ13C; the caption should clarify that δ15N did not show significant differences and is therefore not presented.
Answer: Done.
Figure 4
The Y-axis label should be more explicit: "Proportion of assimilated carbon sources."
Answer: Done.
Figure 6
The blue/green colors are acceptable, but a stronger contrast would improve accessibility for color-impaired readers.
Answer: Done.
The caption should also report the percentage of niche overlap mentioned in the text, to link figure and results more clearly.
Answer: Done.

Associate editor's decision after peer review (12/01/2026).
Dear Dr. Jardim Jr:
It is a pleasure to accept your manuscript entitled "Trophic dynamics of a carnivorous catfish under dam-regulated flood conditions in the Amazon" in its current form for publication in the Neotropical Ichthyology.
Congratulations for the acceptance of your article, and be aware on the following topics:
1. Publication Fee
NI will charge a publication fee if none of the co-authors is an active SBI member. This measure is essential to strengthen SBI and thus ensure the continuity of our journal, scientific society, and biannual meetings. SBI is not limited to Brazilians but is open to anyone interested in freshwater and marine Neotropical fishes. More details on SBI are available at https://www.sbi.bio.br/. Please email tesouraria.sbi@gmail.com to confirm whether any of your co-authors is a current SBI member and to activate your SBI membership if needed.

Otherwise, if you will cover the publication fee of R$1.000, please inform us at the same email. For authors outside Brazil, the fee will be converted to US dollars based on the official exchange rate on the date of payment. The only exceptions to this fee are invited articles.
2. Science Communication and Social Media
NI actively promotes published articles to both academic colleagues and the general public, including science journalists. To support this, we create social media posts and require images and/or videos of fish related to your work. If your article does not include such images, please send a photo of a representative fish species, preferably alive in its natural habitat. If you do not have your own photo, you may provide a link to an online image, along with the source, author, and, if applicable, authorization for its use. We also publish video summaries of articles in Portuguese on our Instagram (@neoichth). We ask you to designate one author to record a short video using a mobile phone, following the attached instructions. The video should include visual materials (photos, graphics) and a script for subtitles to enhance accessibility. Please email the completed material to neoichth@nupelia.uem.br within 30 days. For now, please provide the name and email address of the author responsible for recording the video.
Additionally, if your article is taxonomic in nature and has been submitted to Zoobank, it is your responsibility to update the manuscript's status on Zoobank once it has been published.
All of the above information and materials are mandatory for the publication of your article, including the scientific dissemination component, which is crucial in the current climate of science denial and misinformation. If you have any questions, please feel free to contact us at neoichth@nupelia.uem.br.
Please respond to this e-mail within five working days to let us know you are aware of all the important points mentioned above.
Thank you for your fine contribution. On behalf of the Editors of the Neotropical Ichthyology, we look forward to your continued contributions to the Journal.
Sincerely,
Dr. Evelyn Habit
Associate Editor, Neotropical Ichthyology
ehabit@udec.cl

Anonymous reviewer #1
Recommendation. Accept
Comments. I appreciate the careful and thorough revision of the manuscript. The revised version shows substantial improvements in clarity, methodological transparency, and interpretative rigor. The main and specific points raised in my previous review were adequately addressed, resulting in a more coherent and scientifically robust study. The integration between stomach content and stable isotope analyses is now clearer, limitations are appropriately acknowledged, and the interpretation of results is better grounded ecologically. Figures and tables were revised effectively and are now consistent with the text.
I have no further scientific comments at this stage and thank the authors for their careful and constructive responses to the review.

Neotropical Ichthyology
OPEN ACCESS

HOW TO CITE THIS ARTICLE

- **Jardim Jr AA, Prata EG, Silva LM, Moraes JPS, Oliveira LP, Montag LFA, Freitas TMS.** Trophic dynamics of a carnivorous catfish under dam-regulated flood conditions in the Amazon. Neotrop Ichthyol. 2026; 24(2):e250114. https://doi.org/10.1590/1982-0224-2025-0114

Official Journal of the
Sociedade Brasileira de Ictiologia