Submission date. 12/08/2025

Associate editor's decision after peer review (13/09/2025).
Dear Dr. Vega Retter:
Manuscript ID NI-2025-0141 entitled "Effects of pollution and watercourse type on trophic structure: using macroinvertebrates and the silverside Basilichthys microlepidotus (Jenyns, 1841) in the Maipo River basin as a model" 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 manuscript received two divergent reviews. However, reviewerers provided clear guidance on the necessary revisions. 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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https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=136bb646eb164260baea591cf22f5a67
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 13-Nov-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. Minor Revision
Comments. The manuscript presents an interesting and relevant study on the effects of pollution and watercourse type on the trophic structure of aquatic ecosystems, using macroinvertebrates and the fish Basilichthys microlepidotus in the Maipo River basin as a model. It highlights the need to consider not only the abundance of macroinvertebrates

but also their nutritional quality in environmental assessments, which constitutes an important contribution to future ecological studies.
Comments:
- Figure 1: I would suggest adding orange/blue circles directly to the figure as a visual reference to make it more visually appealing and easier to interpret, beyond it being described in the legend.
- Introduction: It could benefit from a more explicit review of previous studies on the impact of canalisation in fluvial ecosystems. For instance, it would be useful to include examples from other systems where canalisation has shown similar or contrasting effects.
- Discussion: While the study is robust, it would be helpful to acknowledge some limitations. For example, the use of a single year of sampling might not capture important seasonal variations. Additionally, not considering other potentially relevant factors (e.g., water temperature, dissolved oxygen) could limit the interpretation of the results.
In conclusion, with some minor revisions, the article has the potential to be a valuable contribution to the scientific literature. Congratulations!

Anonymous reviewer #2
Recommendation. Major Revision
Comments. In general, the study provides relevant information on the nitrogen isotopic signal of the species B. microlepidotus under conditions of stress caused by habitat modification and pollution, within the context of trophic structure. However, the manuscript requires modifications and additional information before it can be published.
General comments
The first suggestion to the authors is to change the focus, because the topic has been mainly addressed from the perspective of macroinvertebrates. However, the isotopic signal analyses are centered on the silverside B. microlepidotus. In this context, it is necessary to highlight this species as the study model, with macroinvertebrates treated as food items.
Check the used concepts because there are inconsistencies in their application throughout the manuscript. For example, macroinvertebrates, benthic macroinvertebrates, and zoobenthos are used as synonyms; system and ecosystem; pollution and contamination. Only one term should be used consistently for each concept in the whole manuscript.
Check the writing and the use of concepts in English, since some are incorrect. For instance, canalization and canal-type should be replaced with channelization and channel type.
Update bibliographic references, especially those referring to stable isotopes
Specific comments
Abstract
Include information about stable isotopes in the introductory sentences, as well as in the description of the methodology. The information is vague; for a reader not familiar with isotopes as a tool in trophic ecology studies, the purpose of the study is not entirely clear.
Include the study design and the main analysis. Neither is mentioned.
Line 4: change macroinvertebrates communities to macroinvertebrates assemblages.
Line 28: change $\delta^{15}N$ level to $\delta^{15}N$ signal (in the whole manuscript as well).
Keywords
I suggest changing to: channelization, water pollution, fish diet, macroinvertebrates, stable isotopes.
Introduction
Page 4, Line 6–13: Provide more detail in this paragraph, adding hard data to make it more objective and less ambiguous.

Line 16: Define trophic structure.
Line 27: Replace pristine by low-altered rivers or another similar concept; no river is now pristine.
Line 32–33: Replace stream by river, and use terms consistently.
Line 34–35: Replace Chironomidae with chironomids.
Line 38–56: Try to find an example of native fish; trout are invasive both in Japan and in Chile.
Page 5, Line 9–10: Define stable isotopes, indicate their use in trophic structure studies, and highlight their advantages compared with other tools such as stomach content analysis.
Line 39–40: Keep the concept of fish, replace ichthyofauna..
Methodology
Page 7, Line 29–30: Include a table with the main recorded water quality variables.
Page 8, Line 43: Why were only gastropods used as a baseline? Generally, to assess trophic position, at least two additional trophic levels beyond the study model (B. microlepidotus) are needed. In this case, gastropods would be the primary consumers (grazers). Why were secondary consumers and producers (algae or macrophytes) not included? It is essential to justify why only gastropods were used as the baseline.
This also justifies why only the nitrogen signal was analyzed and not the carbon signal, which is highly relevant to determining the feeding sources of silversides. Fish are mobile organisms that can move between sites to feed.
Page 9, Line 20–21: The reference is very old and should be updated, as new methodological approaches are currently available. I also insist that using a single organism as a baseline is insufficient.
Statistical analyses
Line 30–53: Condense the text into a single paragraph.
Line 30–37: Include at least one PERMANOVA as a significance test, since NMDS only provides a graphical representation of patterns without statistical support for the observed groupings.
Line 40–45: Ideally, analyze response variables with a GLM, including continuous water quality variables and at least one variable related to slope or flow water velocity. This would help eliminate potential biases in site differentiation caused by habitat-specific conditions rather than pollution and/or channel modification.
There is no information on trophic position assessment. How was it calculated? I suggest using the tRophicPosition or SIBER packages in R to conduct trophic position analysis.
Discussion
I suggest removing the subtitles, or at most leaving only two, since they hinder the integration of the interpretation of results.
Line 9: Add the main message of the study, integrating information from both the nitrogen isotopic signal of silversides and the macroinvertebrate results as a food source, and therefore as part of the isotopic response.
Line 14–25: To support this interpretation, it is essential to include data on the body condition of silversides, using the SMI index (Peig and Green 2009). These SMI indices should be plotted across sites (polluted vs. non-polluted) and correlated with the total lipid concentration data of macroinvertebrates.
Overall, the results lack sufficient information to support some of the interpretations. I recommend, whenever possible, including carbon isotopes, at least one additional trophic level (chironomids could be a good group, given their abundance at some sampling sites), water quality data in the analyses, stomach content, and the body condition data mentioned above. This last point is essential.

Author's Rebuttal Letter (13/11/2025).

Dear Birindelli,

Many thanks for the comments to the Manuscript ID NI-2025-0141, entitled "Effects of pollution and watercourse type on trophic structure: using macroinvertebrates and the silverside Basilichthys microlepidotus (Jenyns, 1841) in the Maipo River basin as a model". Please find in this message our answers to the comments performed by the referees.

Reviewer: 1

Recommendation: Minor Revision

Comments:

The manuscript presents an interesting and relevant study on the effects of pollution and watercourse type on the trophic structure of aquatic ecosystems, using macroinvertebrates and the fish Basilichthys microlepidotus in the Maipo River basin as a model. It highlights the need to consider not only the abundance of macroinvertebrates but also their nutritional quality in environmental assessments, which constitutes an important contribution to future ecological studies.

R.- Many thanks for the comment.

Comments:

- Figure 1: I would suggest adding orange/blue circles directly to the figure as a visual reference to make it more visually appealing and easier to interpret, beyond it being described in the legend.

R.- We apologize for the inconvenience, but we believe that what the reviewer is requesting is already presented in that way, since on the map the circles corresponding to the polluted sites are shown in orange and those corresponding to the non-polluted sites are shown in blue. We apologize if we misunderstand the reviewer's request.

- Introduction: It could benefit from a more explicit review of previous studies on the impact of canalisation in fluvial ecosystems. For instance, it would be useful to include examples from other systems where canalization has shown similar or contrasting effects.

R.- Many thanks for the comment, in this new version we incorporated more information about the canalization impacts.

- Discussion: While the study is robust, it would be helpful to acknowledge some limitations. For example, the use of a single year of sampling might not capture important seasonal variations. Additionally, not considering other potentially relevant factors (e.g., water temperature, dissolved oxygen) could limit the interpretation of the results.

R.- we agree with the referee, we incorporated a phrase about this in the discussion. However, we do not account the dissolved oxygen as limitation, because this variable is included in our physicochemical characterization.

In conclusion, with some minor revisions, the article has the potential to be a valuable contribution to the scientific literature. Congratulations!

Reviewer: 2

Recommendation: Major Revision

Comments:

In general, the study provides relevant information on the nitrogen isotopic signal of the species B. microlepidotus under conditions of stress caused by habitat modification and pollution, within the context of trophic structure. However, the manuscript requires modifications and additional information before it can be published.

R.- Many thanks for the comment. We have incorporated all the requested modifications, and we believe that the article has significantly improved thanks to the reviewer's suggestions.

General comments

The first suggestion to the authors is to change the focus, because the topic has been mainly addressed from the perspective of macroinvertebrates. However, the isotopic

signal analyses are centered on the silverside B. microlepidotus. In this context, it is necessary to highlight this species as the study model, with macroinvertebrates treated as food items.
R.- we agree with the referee. Our focus always was the silverside, we change a couple sentences in the introduction and the objectives to make this clear.
Check the used concepts because there are inconsistencies in their application throughout the manuscript. For example, macroinvertebrates, benthic macroinvertebrates, and zoobenthos are used as synonyms; system and ecosystem; pollution and contamination. Only one term should be used consistently for each concept in the whole manuscript.
R.- Thaks you for mention this, now the text makes only reference to macroinvertebrate, pollution and ecosystem.
Check the writing and the use of concepts in English, since some are incorrect. For instance, canalization and canal-type should be replaced with channelization and channel type.
R.- We made the changes requested; canalization was replaced by channelization and canal was replaced by channel.
Update bibliographic references, especially those referring to stable isotopes
R.- We have incorporated new references from recent publications.
Specific comments
Abstract
Include information about stable isotopes in the introductory sentences, as well as in the description of the methodology. The information is vague; for a reader not familiar with isotopes as a tool in trophic ecology studies, the purpose of the study is not entirely clear.
Include the study design and the main analysis. Neither is mentioned.
R.- Many thanks for the commentary, we improve the abstract as requested.
Line 4: change macroinvertebrates communities to macroinvertebrates assemblages.
R.- Amended
Line 28: change $\delta^{15}N$ level to $\delta^{15}N$ signal (in the whole manuscript as well).
R.- Amended
Keywords
I suggest changing to: channelization, water pollution, fish diet, macroinvertebrates, stable isotopes.
R.- Amended.
Introduction
Page 4:
Line 6–13: Provide more detail in this paragraph, adding hard data to make it more objective and less ambiguous.
R.- Amended.
Line 16: Define trophic structure.
R.- Amended.
Line 27: Replace pristine by low-altered rivers or another similar concept; no river is now pristine.
R.- Amended
Line 32–33: Replace stream by river, and use terms consistently.
R.- Amended
Line 34–35: Replace Chironomidae with chironomids.
R.- Amended
Line 38–56: Try to find an example of native fish; trout are invasive both in Japan and in Chile.
R.- Thank you for the suggestion, now we have changed the example for one of a native fish.

Page 5:
Line 9–10: Define stable isotopes, indicate their use in trophic structure studies, and highlight their advantages compared with other tools such as stomach content analysis.
R.- Amended
Line 39–40: Keep the concept of fish, replace ichthyofauna.
R.- Amended
Methodology
Page 7, Line 29–30: Include a table with the main recorded water quality variables.
R.- A supplementary table has been added to show the water quality variables recorded for this study. Additionally, for more information about water quality and its analysis please refer to Cortés-Miranda et al. 2024.
Page 8, Line 43: Why were only gastropods used as a baseline? Generally, to assess trophic position, at least two additional trophic levels beyond the study model (B. microlepidotus) are needed. In this case, gastropods would be the primary consumers (grazers). Why were secondary consumers and producers (algae or macrophytes) not included? It is essential to justify why only gastropods were used as the baseline.
R.- We agree with the reviewer's suggestion; however, this is not possible because at that time (2016) we only collected snail samples for the baseline, and we do not have samples from other individuals. We only used gastropods because according to Post (2002), snails represent a suitable baseline for quantifying the trophic position of secondary organisms in aquatic ecosystems. Taking this into consideration, Physa sp. and Heleobia sp. were used as baseline, since they present an isotopic signature like that of the primary producers (periphyton and detritus). This was explained in the original version, but we have now clarified it further, and we hope it is easier to understand. Nevertheless, we conducted a brief literature review and found several recent studies that used a single species or trophic level as a baseline (some of these studies are listed below as examples). In addition, in this revised version, the trophic position was calculated using the recommended R package, and the results are slightly different.
Post DM (2002). Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. Ecology 83(3):703–18. doi: https://doi.org/10.2307/3071875.
Reyes-Ramirez et al. (2025) Feeding ecology, trophic level, and isotopic niche among batoid species in the northwestern coast of the Baja California Peninsula, Mexico. Regional Studies in Marine Science 89: 104317
Schlick-Steiner et al. (2025) Human pressure can reduce genetic diversity and elevate trophic position: an arthropod case study. Basic and Applied Ecology 88: 52–58
This also justifies why only the nitrogen signal was analyzed and not the carbon signal, which is highly relevant to determining the feeding sources of silversides. Fish are mobile organisms that can move between sites to feed.
R.- In this new version we included the analysis of the carbon signal, many thanks for pointing this out.
Page 9, Line 20–21: The reference is very old and should be updated, as new methodological approaches are currently available. I also insist that using a single organism as a baseline is insufficient.
R.- Thank you for the suggestion, now we have changed the analysis using tRophicPosition package, and we added two complementary references in relation to the enrichment per trophic level value. As we mentioned in another comment, we do not have samples from other organisms to be considered as a second baseline.
Statistical analyses
Line 30–53: Condense the text into a single paragraph.
R: Amended.
Line 30–37: Include at least one PERMANOVA as a significance test, since NMDS only provides a graphical representation of patterns without statistical support for the observed groupings.

R.- Amended, we include the analysis requested.
Line 40–45: Ideally, analyze response variables with a GLM, including continuous water quality variables and at least one variable related to slope or flow water velocity. This would help eliminate potential biases in site differentiation caused by habitat-specific conditions rather than pollution and/or channel modification.
R.- We have the water quality variables and velocity measures, but we have a different number of replicates between these parameters and the response variables, thus the only way to try to analyze it is using an average of the variables. We try to perform the analysis in this way, but this produces an overfitted GLM model because of the low number of observations, so in this case we could not perform the GLM analyses.
There is no information on trophic position assessment. How was it calculated? I suggest using the tRophicPosition or SIBER packages in R to conduct trophic position analysis.
R.- In the first version, we estimated the trophic position of the silverside using the formula proposed by Anderson and Cabana (2007):
$Trophic\ position = \lambda + (\delta^{15}N_{(}C_{s)} - \delta^{15}N_{(}base_{)}/\ X_{n})$ (Equation 1 in the first version).
However, in this revised version, we followed the reviewer's suggestion and estimated the trophic position using the tRophicPosition package in R.
Discussion
I suggest removing the subtitles, or at most leaving only two, since they hinder the integration of the interpretation of results.
R.- Subtitles were removed as suggested.
Line 9: Add the main message of the study, integrating information from both the nitrogen isotopic signal of silversides and the macroinvertebrate results as a food source, and therefore as part of the isotopic response.
R.- We incorporated a brief summary of the more relevant results at the begin of the discussion.
Line 14–25: To support this interpretation, it is essential to include data on the body condition of silversides, using the SMI index (Peig and Green 2009). These SMI indices should be plotted across sites (polluted vs. non-polluted) and correlated with the total lipid concentration data of macroinvertebrates.
R.- Thank you for the suggestion, in this new version of the manuscript we included the proposed analyses, and no correlation was found between body condition and total lipid concentration.
Overall, the results lack sufficient information to support some of the interpretations. I recommend, whenever possible, including carbon isotopes, at least one additional trophic level (chironomids could be a good group, given their abundance at some sampling sites), water quality data in the analyses, stomach content, and the body condition data mentioned above. This last point is essential.
R.- Thanks to the reviewer's suggestions, some results have changed, and the newly incorporated data allowed for a more reliable interpretation of all the analyses. We greatly appreciate the reviewer's comments, as we believe they have considerably improved our work.
Many thanks for considering our manuscript in Neotropical Icthyology,
Dra Caren Vega Retter
Universidad de Chile

Associate editor's decision after peer review (13/12/2025).
Dear Dr. Vega Retter:
Manuscript ID NI-2025-0141.R1 entitled "Effects of pollution and watercourse type on trophic structure: using macroinvertebrates and the silverside Basilichthys microlepidotus(Jenyns, 1841) in the Maipo River basin as a model" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

Following this second round of review, one reviewer continues to recommend a major revision, while the other has accepted the paper in its current form. I am pleased to recommend an overall minor revision, though I ask that you carefully address all reviewer comments. 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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https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=f14438503f2a49aab7212bff30a9e5c8
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 13-Jan-2026. 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. This manuscript presents valuable information for the region and utilizes a comprehensive approach to the topic. However, a revision is necessary to enhance the clarity, rigor, and accessibility of the work. I noted some issues with the consistency of the writing and presentation that need to be addressed throughout the manuscript. The suggestions below focus on strengthening the methodology, results interpretation, and overall clarity. These revisions might be used as minor or major revisions, due to simplicity and quantity, respectively.
Since line numbers are not present, I used a track changes document to suggest changes and reorder paragraphs.
Some key concerns that required clarifications:
1) Hypotheses/Research Questions: A critical element that is currently missing is a clear

statement of the study's hypotheses or research questions. These must be explicitly stated, ideally at the end of the Introduction, to guide the reader through the subsequent methods and results.

2) Study Area/Methodology Description: The Study Area description needs to be significantly enhanced, especially for readers unfamiliar with the region.

Please incorporate specific details regarding the type and intensity of impact for each sampling site (e.g., urban, industrial, agricultural). Quantifiable metrics, such as population size, type of industries present, or agricultural activity, would be very relevant.

Please provide a brief description of the irrigation and central channels. Are they lined with concrete? Are they colonized by vegetation? Do the central channels also have concrete or other anthropogenic structures? This contextual information is crucial for understanding the environment.

3) I conducted a quick Principal Component Analysis using the provided data and observed that the MEL site appears distinct and spatially distant from the other two impacted sites. If the authors also noted this difference, it would be beneficial to include a brief discussion on this observation and its potential implications for the results.

4) NMDS Stress Value: In the Results section, when presenting the NMDS ordination, the stress value should be included. Without this value, the reader cannot assess how reliably the ordination spatially represents the original differences (dissimilarities) between the data points.

Specific Figure Revisions. Please address the following points regarding the figures:

1) Figure 1 (Study Area Map). It would be highly informative to overlay the map with geographic layers showing urbanized areas, industrial zones, or other sources of contamination. This visual aid would directly support the enhanced Study Area description requested above.

2) Figure 2 (NMDS): Add the stress value to the figure caption or within the plot area itself.

3) Figure 6 (Isotope Plot): Please carefully review the axis labels. It appears that both axes are currently labeled as d15N. I suspect that one of these axes should be labeled as d13C.

4) Figure 7 (Trophic Position Plot): The symbols used in the legend and plot are inconsistent and difficult to interpret. The combination of color (for central/channel) and shape (for condition) is not intuitive, making the figure confusing for the reader.

Please use a clear, consistent combination of a single symbol type and color for each unique condition (e.g., a specific shape/color combination for "Polluted Channel," and a different one for "Non-Polluted Central," etc.). I also noted the absence of "non-polluted channel" conditions in the plot. Please confirm and clarify in the legend and/or methods if these were not sampled.

Isotopic Baseline and Trophic Position Calculation

1) Baseline Choice (Gastropods): I have a concern regarding the use of gastropods to establish the isotopic baseline for trophic position calculations. I know and have studied the references for using it, but those circumstances were different.

Supplementary Table 2 suggests that the specific gastropod species used may not be consistently present across all studied sites. This variation could introduce confounding factors and potential artifacts into the d15N signals used for the baseline.

There is also evidence suggesting that primary producers and grazer (such as gastropods) baselines can shift in response to contamination. This should be thoroughly discussed, and a strong rationale for using the selected baseline should be provided, or alternative approaches considered.

2) Stomach Content/Selectivity: The Discussion section would benefit from a more detailed exploration of the relationship between the observed isotopic signatures, possible stomach content analysis, and any feeding selectivity noted in the species. This will strengthen the interpretation of the trophic links.

Anonymous reviewer #2
Recommendation. Accept
Comments. (There are no comments.)

Author's Rebuttal Letter (06/01/2026).
Dear Dr. Birindelli,
Many thanks for the comments to the Manuscript ID NI-2025-0141.R1, entitled "Effects of pollution and watercourse type on trophic structure: using macroinvertebrates and the silverside Basilichthys microlepidotus(Jenyns, 1841) in the Maipo River basin as a model". Please find in this message our answers to the comments performed by the referee.
Reviewer: 1
The changes requested in the pdf were made. Following, we will answer the question that was in the PDF and was not present in the other commentaries performed by the revisor.
Did you transport invertebrates in cold water, how long did it take to kill them, and to prepare the material? Did you use other macroinvertebrates for SIA?
R.- Macroinvertebrates were transported in cold water using a cooler and were frozen upon arrival at the laboratory, which occurred within 2–3 hours after collection from the sampling sites. Only gastropods were used for stable isotope analysis (SIA); no other macroinvertebrate taxa were included. A brief sentence clarifying this procedure has been added to the Methods section.
Recommendation: Major Revision
Comments:
This manuscript presents valuable information for the region and utilizes a comprehensive approach to the topic. However, a revision is necessary to enhance the clarity, rigor, and accessibility of the work. I noted some issues with the consistency of the writing and presentation that need to be addressed throughout the manuscript. The suggestions below focus on strengthening the methodology, results interpretation, and overall clarity. These revisions might be used as minor or major revisions, due to simplicity and quantity, respectively.
Since line numbers are not present, I used a track changes document to suggest changes and reorder paragraphs.
Some key concerns that required clarifications:
1) Hypotheses/Research Questions: A critical element that is currently missing is a clear statement of the study's hypotheses or research questions. These must be explicitly stated, ideally at the end of the Introduction, to guide the reader through the subsequent methods and results.
R. Many thanks for the comment. We incorporated a hypothesis in this new version.
2) Study Area/Methodology Description: The Study Area description needs to be significantly enhanced, especially for readers unfamiliar with the region.
Please incorporate specific details regarding the type and intensity of impact for each sampling site (e.g., urban, industrial, agricultural). Quantifiable metrics, such as population size, type of industries present, or agricultural activity, would be very relevant.
Please provide a brief description of the irrigation and central channels. Are they lined with concrete? Are they colonized by vegetation? Do the central channels also have concrete or other anthropogenic structures? This contextual information is crucial for understanding the environment.
R.- Thank you for the comment, we added more information about the sites following your suggestions, including number of inhabitants, type of activities on those sites and information about central-channel and channel sites.
3) I conducted a quick Principal Component Analysis using the provided data and

observed that the MEL site appears distinct and spatially distant from the other two impacted sites. If the authors also noted this difference, it would be beneficial to include a brief discussion on this observation and its potential implications for the results.
R.- Many thanks for pointing this out. We incorporated a sentence about this in the M&M section and in the discussion section.
4) NMDS Stress Value: In the Results section, when presenting the NMDS ordination, the stress value should be included. Without this value, the reader cannot assess how reliably the ordination spatially represents the original differences (dissimilarities) between the data points.
R.- Amended
Specific Figure Revisions. Please address the following points regarding the figures:
1) Figure 1 (Study Area Map). It would be highly informative to overlay the map with geographic layers showing urbanized areas, industrial zones, or other sources of contamination. This visual aid would directly support the enhanced Study Area description requested above.
R. Amended. A new map was produced showing the urban and agricultural areas surrounding the sampling sites.
2) Figure 2 (NMDS): Add the stress value to the figure caption or within the plot area itself.
R.- Amended. We incorporate the values to the figure caption.
3) Figure 6 (Isotope Plot): Please carefully review the axis labels. It appears that both axes are currently labeled as d15N. I suspect that one of these axes should be labeled as d13C.
R.- The figure was reviewed, and the axes were found to be correctly labeled. Figure 6 shows $\delta^{15}N$ levels according to pollution level and watercourse type for silverside fish and the baseline. Figure 7, in turn, presents both $\delta^{15}N$ and $\delta^{13}C$, with the axes labeled accordingly.
4) Figure 7 (Trophic Position Plot): The symbols used in the legend and plot are inconsistent and difficult to interpret. The combination of color (for central/channel) and shape (for condition) is not intuitive, making the figure confusing for the reader.
Please use a clear, consistent combination of a single symbol type and color for each unique condition (e.g., a specific shape/color combination for "Polluted Channel," and a different one for "Non-Polluted Central," etc.). I also noted the absence of "non-polluted channel" conditions in the plot. Please confirm and clarify in the legend and/or methods if these were not sampled.
R.- Thank you for the suggestion, now the figure 7 has a unique combination of shape and color for each one of the conditions. As you mentioned we do not have the combination of non-polluted channel, because the physical and chemical conditions changed in 2016 in IM site that was previously characterized as non-polluted channel. We have added it to the methods section.
Isotopic Baseline and Trophic Position Calculation
1) Baseline Choice (Gastropods): I have a concern regarding the use of gastropods to establish the isotopic baseline for trophic position calculations. I know and have studied the references for using it, but those circumstances were different.
Supplementary Table 2 suggests that the specific gastropod species used may not be consistently present across all studied sites. This variation could introduce confounding factors and potential artifacts into the d15N signals used for the baseline.
There is also evidence suggesting that primary producers and grazer (such as gastropods) baselines can shift in response to contamination. This should be thoroughly discussed, and a strong rationale for using the selected baseline should be provided, or alternative approaches considered.
R. Thank you for pointing this out. We reviewed Supplementary Table 2 and identified an error related to Physidae at SFM. This has now been corrected, and the accurate number of individuals is provided. We acknowledge the referee's concern regarding the

choice of baseline. However, previous studies have shown that the use of gastropods as a baseline does not significantly affect trophic position estimates when compared with other commonly used baselines, such as benthic organic material or mussels (Lake et al., 2019). In addition, the use of site-specific baselines has been recommended to account for spatial variability when stable isotope analyses are conducted in degraded environments (Braun et al., 2018; Qiu et al., 2023). We have incorporated a sentence in the Materials and Methods section to clarify the rationale for our baseline selection. Moreover, we noted an inconsistency in the manuscript regarding the baseline taxa. In one section, Helobia was incorrectly indicated as the baseline, whereas in another section Littorina was mentioned. We apologize for the confusion caused by this inconsistency. The correct baseline taxa are Physa and Littorina, and this has been corrected throughout the revised version of the manuscript.

References:

Braun DP, Phillips ID, Nanayakkara L, Wissel B. Diet characterization and a preliminary investigation into trophic niche placement for an endangered lake sturgeon (Acipenser fulvescens) population in the Saskatchewan River, SK, Canada. PLoS One. 2018; 13(11). doi: https://doi.org/10.1371/journal.pone.0206313.

Lake JL, Serbst JR, Kuhn A, Smucker NJ, Edwards P, Libby A 7, et al. Use of Stable Isotopes in Benthic Organic Material as a Baseline for Estimating Fish Trophic. Canadian Journal of Fisheries and Aquatic Sciences. 2019; 76. doi: https://doi.org/ https://doi.org/10.1139/cjfas-2017-0381.

Qiu L, Ji F, Qiu Y, Xie H, Li G, Shen J. Water-Level Fluctuation Control of the Trophic Structure of a Yangtze River Oxbow. Biology (Basel). 2023; 12(10). doi: https://doi.org/10.3390/biology12101359.

2) Stomach Content/Selectivity: The Discussion section would benefit from a more detailed exploration of the relationship between the observed isotopic signatures, possible stomach content analysis, and any feeding selectivity noted in the species. This will strengthen the interpretation of the trophic links.

R.- Thank you very much for your comment; we have added a sentence about it to the discussion. Like the reviewer, we believe this greatly improves this section of the article.

Many thanks for considering our manuscript in Neotropical Icthyology,

Dra Caren Vega Retter

Universidad de Chile

Associate editor's decision after peer review (13/01/2026).

Dear Dr. Vega Retter:

Manuscript ID NI-2025-0141.R2 entitled "Effects of pollution and watercourse type on trophic structure: using macroinvertebrates and the silverside Basilichthys microlepidotus(Jenyns, 1841) in the Maipo River basin as a model" which you submitted to the Neotropical Ichthyology, has been reviewed.

The Section Editor has recommended publication, but also suggested some minor revisions to your manuscript. Therefore, I invite you to respond to comments and revise your manuscript. Please, check all comments at the bottom of this letter.

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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e7c25278c0
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 13-Feb-2026. 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. Fernando Pelicice
Associate Editor, Neotropical Ichthyology
pelicice.editor@gmail.com
==========
Dear Authors
Thank you for submitting your revised manuscript to Neotropical Ichthyology.
At this point, I have assumed the editorial task of this manuscript. Please, contact me if you have any questions.
I have checked previous letters and decisions, and observed that authors addressed comments and problems raised during the previous review. I congratulate you for your kind attention and effort. Because authors responded diligently to reviewer's comments, I think a third external round is not necessary.
Instead, I have conducted my own detailed review. My fresh review raised a number of issues that deserve attention. They are minor, but important. Please, check carefully the annotated pdf.
I recommend "Minor Revisions". Please send a Rebuttal Letter explaining how you addressed each comment.
I look forward to receiving a revised version.
Yours sincerely,
Fernando M. Pelicice
Section Editor (Neotropical Ichthyology)
Universidade Federal do Tocantins
Núcleo de Estudos Ambientais (Neamb)
Porto Nacional - TO

Author's Rebuttal Letter (16/01/2026).
Dear Dr. Pelicice,
Many thanks for the comments to the Manuscript ID NI-2025-0141.R2, entitled "Effects of pollution and watercourse type on trophic structure: using macroinvertebrates and the silverside Basilichthys microlepidotus (Jenyns, 1841) in the Maipo River basin as a model". The changes requested in the pdf sent were made. Following, we will answer some questions/commentaries that were in the PDF.

1.- We change the title to "Effects of pollution and watercourse type on trophic structure: the silverside Basilichthys microlepidotus (Jenyns, 1841) and its food resources in the Maipo River basin as a model" and we also change the abstract as requested.
2.- When we used the term "aggregate extraction" we refer to the process of mining sand, gravel, and crushed stone from natural sources. To a better understanding we change to "extraction of aggregate" and incorporate an explanation.
3.- Comment: "You have two factors (channel x pollution), so I presume the correct test would be a factorial Anova. Please, check it."
R.- We agree with the editor that this will be the best way to perform the analysis. However, it is not possible to perform a factorial ANOVA because our experimental design is unbalanced, as shown in the following table.
Polluted Non-polluted
Central MEL SFM
Channel PEL-IM X
Since one of the factor combinations (non-polluted – channel) is absent, the interaction cannot be evaluated. For this reason, a statistical analysis that does not focus on interactions, such as contrast-based ANOVA, was used instead.
4.- Comment: You report t-values for one-way Anovas. Is that correct? I expected an F-value. Please, check it all.
R.- I apologize for the mistake, we change t for F.
Many thanks for considering our manuscript to publication in Neotropical Icthyology,
Dra Caren Vega Retter
Universidad de Chile

Associate editor's decision after peer review (06/02/2026).
Dear Dr. Vega Retter:
It is a pleasure to accept your manuscript entitled "Effects of pollution and watercourse type on trophic structure: the silverside Basilichthys microlepidotus (Jenyns, 1841) and its food resources in the Maipo River basin as a model" 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. Fernando Pelicice
Associate Editor, Neotropical Ichthyology
pelicice.editor@gmail.com
=======
Dear Authors
Thank you for submitting your revised manuscript to Neotropical Ichthyology.
I congratulate the authors for your kind attention and effort in addressing comments and suggestions provided by reviewers and the Editor during the review process.
I checked the revised version (R3) of your submission, and I have no further corrections or comments to provide. I thank you for all effort and patience during the review process, and congratulate you for this fine contribution.
I recommend publication.
Yours sincerely,
Prof. Fernando M. Pelicice
Associate Editor
Neotropical Ichthylogy
Universidade Federal do Tocantins
Brazil

Neotropical Ichthyology

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SBI Official Journal of the Sociedade Brasileira de Ictiologia