

Submission date. 10/02/2026

Associate editor's decision after peer review (11/03/2026).

Dear Miss Arredondo-Chávez:

Manuscript ID NI-2025-0175 entitled "Trophic comparison of lionfish *Pterois volitans*/miles with three reef fish species in the Mexican Caribbean (*Scorpaena plumieri*, *Cephalopholis cruentata*, and *Lutjanus apodus*) through stable isotopes and stomach contents analysis" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter. Please note that reviewer 1's comments are included in a attached version of the manuscript.

The reviewer(s) have recommended publication, but also suggest some 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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https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=8b714231c8dc4d02990cd7f1b7073cb0

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 11-May-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. Osmar Luiz

Associate Editor, Neotropical Ichthyology
osmarluizjr@gmail.com, osmar.luiz@dbca.wa.gov.au

Anonymous reviewer #1

Recommendation. Major Revision

Comments. There are no comments.

Anonymous reviewer #2**Recommendation.** Minor Revision

Comments. The manuscript addresses a relevant topic and has the potential to contribute valuable information. However, several methodological aspects require clarification or further development before the study can be properly evaluated. I encourage the authors to consider the following points:

1. Scientific names should be consistently written in italics throughout the manuscript.
2. For the comparison among species and size classes, the authors may consider using a more robust statistical approach. Instead of relying solely on the Horn index, a PERMANOVA could provide a stronger framework for testing differences among groups.
3. The drying temperature used for muscle samples prior to stable isotope analysis (72 °C) appears unusually high. At this temperature, protein denaturation processes may occur and potentially influence isotopic composition. The authors should clarify whether any steps were taken to evaluate possible effects of this drying temperature on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Typically, drying temperatures between 50–60 °C are considered safer when lyophilization is not available.
4. The manuscript does not adequately justify the trophic enrichment factor (TEF/TDF) value of 2.75 that was used. The authors should clearly explain the origin of this value and provide the appropriate references. Additionally, the baseline organism and baseline isotopic value used for trophic position (TP) estimation should be explicitly stated. The differences between niche breadth, trophic niche space, and how these metrics were calculated are also not sufficiently explained.
5. The use of the SIBER package is not clearly described in the Methods section. Several outputs are presented in the Results (e.g., SEAc, TA, and overlap metrics), but the methodological description suggests that the package was used mainly to evaluate isotopic overlap among species and size classes. The authors should clearly specify which metrics were calculated with SIBER and how each of them was estimated.
6. Since body size and isotopic values are available, the authors may consider exploring the relationship between $\delta^{15}\text{N}$ and body size. Such an analysis could provide additional insights into ontogenetic trophic shifts.
7. The manuscript states that some organisms exhibit “greater feeding success.” However, it is unclear how this variable was evaluated or inferred. The authors should clarify the basis for this interpretation.
8. The Levin’s index is used to evaluate feeding strategy for the four predators. However, given that the dietary data include PSIRI components, it would strengthen the analysis to also incorporate the feeding strategy approach proposed by Amundsen et al. (1996), which allows a more detailed evaluation of predator feeding strategy beyond the generalist–specialist framework.
9. The ecological interpretation could be strengthened by considering the ambush-predator nature of these species. Differences in predatory strategies (e.g., ambush vs. active pursuit) may influence trophic patterns and should be discussed when interpreting the results.
10. According to the results table, isotopic values of prey items were collected. However, it appears that this information was not incorporated into any of the analyses. Given the availability of isotopic data for potential prey, the authors could consider applying stable isotope mixing models. Such models could provide additional insight into the relative contribution of different prey groups to the diet of the predators and would significantly strengthen the trophic interpretation of the study.

Author’s Rebuttal Letter (28/05/2026).

Morelia, Michoacán, 22 of May, 2026

Dear Dr. Osmar Luiz

Associate Editor, Neotropical Ichthyology

We sincerely appreciate the opportunity to revise our manuscript entitled “Stable isotope and stomach content evidence of trophic overlap between invasive lionfish and native mesopredatory reef fishes in the Mexican Caribbean”

We would like to thank you and the reviewers for the thoughtful, detailed, and constructive comments provided during the evaluation of our work. We carefully considered each suggestion and incorporated all relevant recommendations into the revised version of the manuscript. These changes have substantially improved the clarity, robustness, and overall quality of our study.

In the attached response document, we provide a point-by-point explanation of how each comment was addressed. All modifications have been clearly marked in the revised manuscript for facilitate review.

We trust that the manuscript is now significantly strengthened and meets the standards of Neotropical Ichthyology. We hope it will be favorably considered for publication.

Thank you once again for your time, guidance, and constructive feedback.

Sincerely,

Omar Domínguez Domínguez

On behalf of all co-authors

omar.dominguez@umich.mx

Entire Scoresheet:

Reviewer: 1

Recommendation: Major Revision

Comments:

(There are no comments.)

Additional Questions:

Does the manuscript contain new and significant information to justify publication?:

Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?:

Yes

Is the problem significant and concisely stated?: Yes

Are the methods described comprehensively?: No

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Is the language acceptable?: Yes

Length of article is: Adequate

Number of tables is: Adequate

Number of figures is: Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable): none

Rating:

Interest: 2. Good

Quality: 2. Good

Originality: 2. Good

Overall: 2. Good

Reviewer: 2

Recommendation: Minor Revision

Comments:

The manuscript addresses a relevant topic and has the potential to contribute valuable information. However, several methodological aspects require clarification or further development before the study can be properly evaluated. I encourage the authors to consider the following points:

1. Scientific names should be consistently written in italics throughout the manuscript.

Reply: We thank the reviewer for this observation. Scientific names have been revised and are now consistently written in italics throughout the manuscript.

2. For the comparison among species and size classes, the authors may consider using a more robust statistical approach. Instead of relying solely on the Horn index, a PERMANOVA could provide a stronger framework for testing differences among groups.

Reply: We thank the reviewer for this valuable suggestion. Following this recommendation, we incorporated a PERMANOVA analysis to statistically evaluate differences in diet composition among species and lionfish size classes. This multivariate approach provides a more robust framework for testing trophic differences based on prey composition. The results of the PERMANOVA analysis were included in the Methods and Results sections and are now discussed in the revised manuscript.

3. The drying temperature used for muscle samples prior to stable isotope analysis (72 °C) appears unusually high. At this temperature, protein denaturation processes may occur and potentially influence isotopic composition. The authors should clarify whether any steps were taken to evaluate possible effects of this drying temperature on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Typically, drying temperatures between 50–60 °C are considered safer when lyophilization is not available.

Reply: We thank the reviewer for this valuable methodological observation. We had a mistake in this part, muscle samples were oven-dried at 62 °C until constant mass was reached, following preparation protocols commonly applied in stable isotope studies of fish tissues. Drying temperatures within the range of 60–70 °C have been widely used without detectable alterations in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values, particularly when applied consistently across all samples (e.g., Post et al. 2007; Logan et al. 2008; Sweeting et al. 2006). Moreover, only dorsal white muscle tissue was analyzed, and all samples were processed under identical conditions, ensuring methodological consistency across species and size classes. This consistency minimizes any potential bias associated with sample preparation. This clarification, along with supporting references, has now been incorporated into the Methods section.

4. The manuscript does not adequately justify the trophic enrichment factor (TEF/TDF) value of 2.75 that was used. The authors should clearly explain the origin of this value and provide the appropriate references. Additionally, the baseline organism and baseline isotopic value used for trophic position (TP) estimation should be explicitly stated. The differences between niche breadth, trophic niche space, and how these metrics were calculated are also not sufficiently explained.

Reply: We thank the reviewer for this important observation. The trophic enrichment factor ($\Delta^{15}\text{N} = 2.75\text{‰}$) was selected following the meta-analysis of Caut et al. (2009), which provides widely used estimates of nitrogen trophic discrimination factors for aquatic consumers, particularly fish and other aquatic vertebrates. We have now clarified the origin and justification of this value in the Methods section.

Additionally, we have explicitly stated the baseline organism and its trophic position (λ) used for trophic position estimation. The methodological description has also been expanded to clearly distinguish among trophic niche breadth (isotopic dispersion), isotopic niche space (Bayesian standard ellipse area), and isotopic niche overlap, including how each metric was calculated using SIBER. These clarifications have been incorporated into the revised manuscript.

5. The use of the SIBER package is not clearly described in the Methods section. Several outputs are presented in the Results (e.g., SEAc, TA, and overlap metrics), but the methodological description suggests that the package was used mainly to evaluate isotopic overlap among species and size classes. The authors should clearly specify which metrics were calculated with SIBER and how each of them was estimated.

Reply: We thank the reviewer for this constructive comment. The Methods section has now been revised to explicitly describe all isotopic niche metrics estimated using the SIBER package. Specifically, we quantified trophic niche extent using the convex hull total area (TA), core isotopic niche space using the standard ellipse area corrected

for small sample size (SEAc), and isotopic niche overlap as the proportional overlap between SEAc ellipses. The Bayesian framework used to estimate ellipses, including the number of posterior iterations, has also been clarified in the revised manuscript.

6. Since body size and isotopic values are available, the authors may consider exploring the relationship between $\delta^{15}\text{N}$ and body size. Such an analysis could provide additional insights into ontogenetic trophic shifts.

Reply: Ontogenetic differences in $\delta^{15}\text{N}$ values among size classes were already evaluated using Kruskal–Wallis analyses and post hoc comparisons. To further explore trophic shifts associated with growth, we additionally examined the relationship between body size and $\delta^{15}\text{N}$ values using regression/correlation analysis. This complementary analysis has now been incorporated into the Methods and Results sections.

7. The manuscript states that some organisms exhibit “greater feeding success.” However, it is unclear how this variable was evaluated or inferred. The authors should clarify the basis for this interpretation.

Reply: We thank the reviewer for this observation. We agree that the term “feeding success” may be misleading, as it was not directly quantified in this study. Accordingly, we have revised the manuscript to remove this term and instead base our interpretation on measurable variables, including the proportion of empty stomachs (vacuity index), prey diversity, and diet breadth derived from stomach content analyses. These metrics provide a more robust and transparent basis for comparing feeding patterns among species.

8. The Levin’s index is used to evaluate feeding strategy for the four predators. However, given that the dietary data include PSIRI components, it would strengthen the analysis to also incorporate the feeding strategy approach proposed by Amundsen et al. (1996), which allows a more detailed evaluation of predator feeding strategy beyond the generalist–specialist framework.

Reply: Following this recommendation, we incorporated the feeding strategy analysis proposed by Amundsen et al. (1996), which relates prey-specific abundance to frequency of occurrence. This complementary analysis allowed a more detailed evaluation of trophic patterns, revealing differences in population-level feeding strategies among predators. The methodological description and corresponding interpretation have now been included in the revised manuscript.

9. The ecological interpretation could be strengthened by considering the ambush-predator nature of these species. Differences in predatory strategies (e.g., ambush vs. active pursuit) may influence trophic patterns and should be discussed when interpreting the results.

Reply: We have expanded the Discussion to incorporate the role of predatory strategies in shaping trophic patterns. In particular, we now discuss how ambush predation behavior in lionfish and native scorpaeniform and serranid predators may influence prey selection, trophic niche breadth, and isotopic overlap. In contrast, more mobile foraging strategies in lutjanids may contribute to differences in diet composition and isotopic niche extent. This ecological perspective has been incorporated to strengthen the interpretation of trophic interactions among predators.

10. According to the results table, isotopic values of prey items were collected. However, it appears that this information was not incorporated into any of the analyses. Given the availability of isotopic data for potential prey, the authors could consider applying stable isotope mixing models. Such models could provide additional insight into the relative contribution of different prey groups to the diet of the predators and would significantly strengthen the trophic interpretation of the study.

Reply: We thank the reviewer for this valuable suggestion. Following this recommendation, we conducted exploratory Bayesian stable isotope mixing models using the package *simmr* in R to evaluate the relative contribution of grouped trophic pathways to the isotopic composition of each mesopredator species. Potential prey and

basal resources were grouped into five functional categories (crustaceans, herbivorous reef fishes, small reef fishes, phytoplankton-derived production, and macroalgae/seagrass-associated production) to reduce isotopic redundancy and improve model interpretability.

The models showed satisfactory convergence ($R\text{-hat} \approx 1$) and revealed differences among mesopredators in the proportional contribution of trophic pathways. Lionfish exhibited relatively balanced contributions from crustacean- and small reef fish-associated pathways, whereas Schoolmaster and Graysby showed stronger associations with fish-derived pathways. In contrast, Scorpionfish displayed comparatively broader contributions from benthic-associated pathways. These exploratory analyses are now incorporated into the revised manuscript (Fig. 3d; Table S5).

Because some source groups still exhibited moderate isotopic overlap, we interpreted these models cautiously as exploratory estimates of trophic pathways rather than exact dietary proportions. Nevertheless, the mixing model results were consistent with the stomach content analyses and isotopic niche metrics, further supporting trophic overlap, trophic flexibility, and resource sharing among invasive lionfish and native mesopredatory reef fishes.

Additional Questions:

Does the manuscript contain new and significant information to justify publication?:

Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?:

Yes

Is the problem significant and concisely stated?: Yes

Are the methods described comprehensively?: No

Are the interpretations and conclusions justified by the results?: Yes

Is adequate reference made to other work in the field?: Yes

Is the language acceptable?: Yes

Length of article is: Adequate

Number of tables is: Adequate

Number of figures is: Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state "none" if this is not applicable): none

Rating:

Interest: 3. Average

Quality: 3. Average

Originality: 3. Average

Overall: 3. Average

NL-2025-1075 Review

Note that this review is a combined review and includes comments from both senior research and a recent PhD graduate

Trophic comparison of *Pterois volitans*/miles with three reef fish species (*Scorpaena plumieri*, *Cephalopholis cruentata*, and *Lutjanus apodus*) in the Mexican Caribbean using

stable isotope and stomach content analyses

Firstly, we would like to say that the authors have drafted a nice manuscript with an impressive dataset behind it. We think the manuscript is timely, given the global concerns with climate change and spread of invasive species and covers an understudied area within the tropical Atlantic region. Generally, the text flows quite well and is easy to follow. Especially the introduction and discussion were clear and well written.

Regarding the data, the list of stable isotope values you have collected for different fish and lower trophic levels (Table S4) is remarkable and we wish you would utilise it more. Currently, it is highlighted that the novelty of this paper is the combination of stable isotopes alongside previously stomach content analyses. However, we feel it is exactly

the stable isotope analyses where the manuscript at the moment lacks the most and needs to be improved. Your results and discussion still lean quite heavily on the stomach content analyses. With stable isotopes, the use of one-source mixing model from Post 2002 seems overly simplistic, especially given the potential of your data. It's good that you use SIBER to analyse the trophic niche width and overlap, however the presentation of these results could be made more clear to the reader.

Below we provide specific comments that we feel will help improve the clarity of results as

well as other areas of the manuscript.

Reply: We thank the reviewer for this thoughtful and constructive assessment. Following this recommendation, and in agreement with similar concerns raised by another reviewer, we substantially revised the stable isotope section to improve methodological clarity and ecological interpretation. Specifically, we refined the description of trophic position estimation based on the baseline approach, clarified the calculation and ecological meaning of isotopic niche metrics derived from the SIBER framework (e.g., TA, SEAc, and isotopic overlap), and expanded and rewritten the discussion of ontogenetic isotopic variation and trophic interactions among predators. These revisions strengthen the integration of stomach content and stable isotope analyses and better highlight the long-term trophic insights provided by isotopic data.

Title and abstract

1) P1 line 5: We recommend removing the scientific names from the title or at least provide the common name initially. Currently, the abstract and now the title is quite heavy to read. An example could be "Trophic comparisons of the invasive lionfish with three native mesopredatory fishes in the Mexican Caribbean through stable isotopes and stomach contents analyses"

Reply: We thank the reviewer for this helpful suggestion. Following this recommendation, we revised the title to improve readability and emphasize the ecological scope of the study rather than taxonomic detail. Scientific names are now introduced in the abstract and main text.

2) P2 line 7 (and throughout the manuscript): We recommend removing the decimals from your percentages and round up to even numbers. Also, same comment for other percentage numbers throughout the document (e.g. P10 line 35), as well as the isotope values (should be only 1 decimal place given instrument precisions). Too many decimals give a false impression of the precision of your measurements.

Reply: Following this recommendation, percentage values throughout the manuscript were rounded to whole numbers to improve readability and avoid overrepresentation of precision. Likewise, stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and trophic position estimates were reformatted to one decimal place, consistent with typical analytical precision in isotope studies.

3) P2 line 12: Is this classification based on stable isotopes or stomach content or both?

Reply: We thank the reviewer for this clarification. The classification of species as generalist feeders was based on stomach content analyses using Levin's niche breadth index. Stable isotope analyses were used to assess trophic niche space and overlap. This distinction has now been clarified in the revised manuscript.

Introduction

Overall, the introduction is well structured and easy to follow, however some study specifics and context could be further clarified:

4) P4 - Line 39: Here you mention the synergistic effect of invasive species with other stressors. This is indeed a very interesting knowledge gap, which we feel is not really addressed in your paper. You mention it here so you could also come back to this point later in the discussion. Albeit, it is not the main focus of your paper, it could still make an interesting discussion point.

Reply: We thank the reviewer for this valuable suggestion. Although this was not the

primary focus of our study, we agree that the potential synergistic effects between lionfish invasion and other reef stressors represent an important ecological consideration. Therefore, we have expanded the Discussion to briefly address how trophic impacts of lionfish may interact with existing pressures such as overfishing, coral degradation, and nutrient enrichment in Caribbean reefs.

5) P5, Lines 24–29: “Reports indicate that lionfish prey on a wide range of species, primarily invertebrates and fish... exhibiting a much broader diet in invaded areas than in their native habitat (Priyadharsini et al., 2013).” Although this manuscript focuses on the comparison of lionfish with natives in an invaded region, the contrast with their trophic ecology in the native range is also highly relevant, particularly when it comes to the discussion. We assume however the novelty here rises from the application of stable isotopes alongside stomach content (which we presume is what the reports have used) but it is not clearly stated.

Reply: We thank the reviewer for this important clarification. We have revised the Introduction to acknowledge that previous studies have used both stomach content and stable isotope analyses to describe lionfish trophic ecology, and include the study by Cure et al., 2012. We now emphasize that the novelty of our study lies in the evaluation of dietary composition, isotopic niche breadth, ontogenetic trophic shifts, and resource overlap with native predators on the same spatiotemporal scale. We also expanded the Discussion to better address the ecological implications of trophic niche expansion in invaded regions and the contrast in lionfish trophic ecology in the native range.

6) P6 Lines 29–43: The final paragraph of the introduction could provide more specifics of what the manuscript sets out to examine / test. For example, across two methods (SIA and SCA) are measured population averages diets, trophic positions, niche widths, niche overlaps between species, and ontogenetic shifts (SI only). Explicitly stating these here will help clarify the results and better structure that section in how statistics are reported – currently we find the results section is hard to follow – see below.

Reply: We thank the reviewer for this helpful suggestion. The final paragraph of the Introduction was revised to explicitly state the main objectives of the study and clarify which trophic variables were evaluated using stomach content and stable isotope analyses. This modification aims to improve the logical structure of the Results section and facilitate interpretation of the statistical analyses.

7) P6 Lines 29–43 and thereafter: once the four main study species have been introduced here, the authors should consider, as with lionfish (P5, line 8), using common names thereafter, e.g. *Cephalopholis cruentata* (the Graysby grouper, hereafter Graysby). This makes the text more readable.

Reply: We thank the reviewer for this helpful suggestion. We use the common name “lionfish” because, as explained in the Introduction, two species are present in the Caribbean but are morphologically indistinguishable. Therefore, we use the common term “lionfish” throughout the manuscript to avoid potential identification errors. However, we do not agree with replacing all scientific names of the native mesopredators with common names. Consequently, we have retained the scientific names for the four native mesopredator species throughout the manuscript.

Methods

8) Sample Collections: This section is missing quite a few details. Please also indicate when the samples were collected (dates: season and year, period – e.g. over several weeks). Providing some basic background to the area would be useful for context too, such as the depth gradient over which fish were sampled. There are no details regarding sampling of other prey and primary producers, given in table S4 that should be provided here. “Crepuscular” is not a common term, perhaps replace with twilight, or dusk and dawn.

Reply: We thank the reviewer for this helpful suggestion. The Sample Collections section was revised to include additional methodological details, including the sampling

period, depth range of collection sites, and clarification of sampling times (daytime, twilight, and nighttime). Also, information about other prey and primary produces were included in the methods.

9) P7 line 31: we presume half full presents stomach fullness between 50–100 %, and half empty is then between empty and 50 %? Generally, we find these terms slightly misleading, and wonder whether this is a standard method for stomach content analyses in your field? Maybe stomach fullness percentages (see Amundsen & Sánchez-Hernández 2019) could be more accurate method in the future. Classification of the four categories could be: Full (packed); mostly full ($50 < x < 100$); partially full ($10 < x < 50$); and empty.

Reply: The terminology used to describe stomach fullness was revised to improve clarity and consistency. We now define four categories based on estimated stomach volume: full (packed), mostly full (50–100%), partially full (1–50%), and empty. These definitions were incorporated into the Methods section and the terminology was updated throughout the manuscript. We also included the suggested reference (Amundsen and Sánchez-Hernández, 2019).

10) Stomach content analyses: Was the stomach content analysis also done for all collected individuals. The sample sizes are not given until the results, unlike the Isotope analysis section. This should be clarified–

Reply: We thank the reviewer for this observation. The Methods section was revised to clearly indicate the number of individuals analyzed for stomach content for each predator species. We now state that stomach content analysis was performed on all collected specimens ($n = 430$), and that empty stomachs were considered in the estimation of feeding activity but excluded from prey composition analyses.

11) P8 line 10: it is mentioned that TrophLab software are used to estimate trophic level (trophic position would be a preferable term in our opinion, see comment 12), but a brief overview of how this software calculates these values should be provided. Are course trophic positions (e.g. herbivore at $TP = 2$) assigned across all observed prey then weighted averages per individual stomach calculated?

Reply: We thank the reviewer for this helpful suggestion. The Methods section was revised to clarify that TrophLab estimates predator trophic position as a weighted mean of prey trophic levels derived from FishBase, based on the relative contribution of each prey item to the diet. We also replaced the term “trophic level” with “trophic position” to improve conceptual consistency.

12) P8 line 45: You could give a rough isotope turnover rate time here (weeks or months? Not familiar to all readers)

Reply: We have now clarified that isotopic turnover in fish muscle tissue typically integrates dietary information over periods ranging from several weeks to a few months. Appropriate references were added to support this statement.

13) P9 line 16: please provide the measurement precision for carbon and nitrogen isotope measurements and what these are based on (typically an internal lab standard of some similar material repeatedly run throughout).

Reply: We thank the reviewer for this important suggestion. The Methods section was revised to include the analytical precision of isotope measurements for both carbon and nitrogen, based on repeated analyses of internal laboratory standards.

14) P9 line 19: we are a bit confused here, what is the difference here between trophic level and trophic position? Are these the same thing?

Reply: We thank the reviewer for pointing out this potential source of confusion. Following this suggestion and similar comments from Reviewer 1, we revised the manuscript to use the term trophic position consistently when referring to estimates derived from stable isotope analysis. We also clarified the methodological framework used to calculate trophic position based on $\delta^{15}N$ values following Post (2002), including the definition of the baseline organism and the trophic enrichment factor applied. These changes were incorporated into the Methods section to improve conceptual consistency and clarity.

15) P9 line 20: A one-source model from Post 2002 is used to estimate trophic position, however details are missing concerning what is actually used as the baseline for these estimates and should be provided. Further, as the $\delta^{13}\text{C}$ values from the fish are also available, the authors could also consider the two-source model if they feel two distinct sources contribute to the food web, such as benthic and pelagic (also in Post 2002). Generally, one-source model is used in simple, linear food webs which are dominated by one primary production pathway (e.g., only pelagic or benthic). The two-source model is preferred when consumers integrate across isotopically distinct production sources. The authors may also consider baseline correcting raw isotope data to proportion benthic or pelagic (based on carbon) and trophic position (based on nitrogen) for more reflective niche comparisons, although we appreciate this would require much more considerable work.

Reply: We thank the reviewer for this important suggestion. Following similar comments from Reviewer 1, we revised the Methods section to explicitly state the isotopic baseline used for trophic position estimation. A primary consumer representing the base of the reef food web ($\lambda = 2$) was used as the baseline in the one-source model framework of Post (2002). We also clarified that $\delta^{13}\text{C}$ values of predators showed relatively limited dispersion, supporting the use of a single-baseline approach. While we agree that two-source models and baseline corrections may provide additional insights, these analyses were beyond the scope of the present study.

Results

16) General comment: Currently the results section is very dense with lists of values etc. that makes it hard for the reader to follow but also refer back to. Much of the data could be pulled out of the main text with better use of tables and / or figures (see comments below), which would allow this text to focus on the key comparisons being made (comment 6). Currently the flow is SCA data analyses followed by SIA data analyses, however a more logical flow might be to talk about each trophic ecological 'trait' in turn, directly comparing between species and methods in the same paragraph, i.e. Population level diet, then trophic position estimates, then trophic niche widths and overlaps.

Reply: Following the reviewer's suggestion, the Results section was reorganized to improve readability and ecological interpretation. Instead of presenting stomach content and stable isotope results in separate sections, we now structure the Results according to key trophic ecological traits (diet composition, trophic position, niche width and overlap, and ontogenetic shifts). Detailed numerical values were reduced in the main text and retained in tables and figures to facilitate comparisons among predator species and analytical approaches.

17) General comment: As the trophic level (or trophic position but stick only with one term) is estimated from both the stomach content and isotopes, state always which one is being referred too (either by word or using a subscript). Now it is sometimes hard to follow which one is referred to in the results and discussion.

Reply: Following this recommendation, we have standardized the terminology throughout the manuscript by consistently using the term trophic position. In addition, we now explicitly indicate the methodological source of each estimate by distinguishing trophic position derived from stomach content analysis (TP_SCA) and from stable isotope analysis (TP_SIA). This clarification has been incorporated in the Methods, Results, Tables, and Discussion sections to improve readability and avoid ambiguity.

18) P10 line 9: Four hundred thirty should be written with numbers (430).

Reply: Changed

19) P10 line 10: Again, we would consider the precision of measurements when giving numbers, two decimals here is unnecessary and likely beyond measurement precision. The number of decimals for datatypes should also be uniform across the manuscript (now varies).

Reply: Following a similar recommendation from Reviewer 1, we have revised all numerical values throughout the manuscript to reflect appropriate measurement precision.

20) P10 line 27: One thing to consider with this high number of empty stomachs is that it might very well impact the generalisability of your results. Especially with *S. plumieri* which already had only 17 individuals. How many full stomachs were there left for the analyses? You could state the number of full and empty stomachs, not only percentages (also see comment on Fig. 2). We would also highlight the potential uncertainty related to this. Looking at the Table S3 it seems there wasn't many individuals with full stomachs for *S. plumieri* as the number per prey item is always one.

Reply: We agree that the relatively high proportion of empty stomachs, particularly in *Scorpaena plumieri*, may influence the robustness and generalizability of dietary inferences. Following this suggestion, we have now included the absolute number of stomachs in each category and for each predator species in the Results section and Figure 2a. We also added a statement in the Discussion acknowledging the potential uncertainty associated with the reduced number of stomachs containing prey, especially for *S. plumieri*, and its implications for interpreting feeding strategy and diet composition patterns.

21) P11 line 39: What was the sample size per each size class, please state this clearly somewhere.

Reply: We thank the reviewer for this comment. The sample size corresponding to each lionfish size class has now been explicitly indicated in the Methods section, as follows: small ($n = 39$), juveniles ($n = 52$), and adults ($n = 33$), to improve clarity.

22) P12 line 18: Correct the $\delta^{15}\text{N}$ value of lionfish (now 9–61 but we presume you mean 9.61).

Reply: Changed.

23) P12 line 50: Ok, so here you have the comparisons between the baseline corrected trophic positions. we would baseline correct also the $\delta^{13}\text{C}$ values (e.g. using twosource mixing model from Post 2002) and stick with these instead of the raw delta values. we have to say that we are also somewhat suprised that you found statistical difference between the trophic position of the four predators given that the trophic position ranges only from 3.28 to 3.60. It does not seem like a big difference given the raw $\delta^{15}\text{N}$ values have only an around 1 ‰ difference (Table S4) and baseline values are likely to be uncertain or variable. In addition, in your Figure 4 the trophic position (both based on stomach content and SIA) seem very similar between species (see comment on Fig 4 below).

Reply: Following similar concerns raised by another reviewer, we have substantially revised the Stable Isotope Methods section to clarify the baseline used for trophic position estimation, the justification for applying a single-baseline model, and the ecological interpretation of small trophic position differences among predators. Although trophic position differences among predators were relatively small, they were consistent with observed variation in $\delta^{15}\text{N}$ values and isotopic niche structure. Therefore, we interpreted these differences conservatively as evidence of subtle trophic partitioning rather than strong trophic segregation.

Discussion

Overall, we like the discussion, and we think it manages to adequately fit the findings also to a larger context. We think it reads well and explores the findings in light of key theories linked to your results. However, as mentioned before, the aspect of stable isotopes could be strengthened.

24) P14 line 28: When looking at Figure 4a it doesn't seem like much more consistent estimates compared to lionfish, except for *L. apodus* which had very consistent estimates, which contradicts what is being stated here

Reply: We agree that the visual consistency in trophic position estimates is most evident

for *Lutjanus apodus*, whereas other native predators show variability comparable to that of lionfish. We modified this part according to your recommendation.

25) P15 line 9: we think the higher feeding success of lionfish compared to other fish species is indeed one of the major findings from your study and very interesting. However, I'm also thinking that could the higher plasticity raise from this. Especially compared to *S. plumieri* which had low sample size ($n = 17$) and high number of empty stomachs. It might be hard to say much about the plasticity of the diet of *S. plumieri* based on stomach content from such a low sample size of full stomachs. It would also be informative if the authors could contrast stomach vacuity rates of lionfish here with SCA studies from their native range as more concrete evidence to the reader.

Reply: We agree that the interpretation of dietary plasticity in native predators, particularly *Scorpaena plumieri*, may be constrained by the small sample size and the high proportion of empty stomachs observed. Following this suggestion, we have revised the discussion to adopt a more cautious interpretation of dietary variability and removed strong statements regarding plasticity. In addition, following the reviewer's suggestion, we have incorporated a comparison of stomach vacuity rates with studies from the native range of lionfish. These changes provide a more balanced ecological interpretation of trophic patterns among species.

26) P15 line 25: We really like that you include also comparisons between size classes and look for ontogenetic niche shifts, although we aren't convinced the presence of ontogenetic niche shifts is striking: it is fairly common in predatory fishes. What would strengthen this part of the discussion to also compare the apparent ontogenetic niche shifts in terms of stable isotopes, not only stomach content. Especially since you highlight the combination of stomach content and stable isotopes as one of the key novelties of your study.

Reply: We agree that ontogenetic trophic shifts are common among predatory reef fishes and that our interpretation could be strengthened by integrating stable isotope evidence. Following this recommendation, we revised the discussion to include isotopic results showing a significant positive relationship between body size and $\delta^{15}\text{N}$ values in lionfish, indicating an ontogenetic increase in trophic position.

27) P15–16, starting line 38, ending line 35: This ecological novelty of lionfish in their invaded areas is very interesting. In particular that the authors highlight the specific functional groups that lionfish appear to target and what ecological consequences may follow. This made us wonder whether stomach content data could be reevaluated with prey assigned to functional groups rather than family-genus, as this could be more informative to readership, see comment on Fig. 2.

Reply: Following this recommendation, we revised the discussion to interpret lionfish diet not only at the taxonomic level but also from a functional perspective.

Figures and tables

28) General comment: The font size in the figures needs to be bigger, not it is a bit hard to read. Avoid red and green combinations (not good for colourblind people).

Reply: Figure font sizes were increased and color palettes were revised to improve readability and accessibility, including consideration of color-blind friendly visualization.

29) Figure 1: While the current figure is fine, it could be beneficial to perhaps provide instead a larger overview of the Gulf of Mexico / Caribbean Sea regions and highlight (even if approximately) the current areas invaded by lionfish to put the study location into the wider context of lionfish range.

Reply: We thank the reviewer for this helpful suggestion. Figure 1 has been revised to provide a broader geographic overview of the Gulf of Mexico and Caribbean Sea, including a schematic representation of the invaded range of lionfish and the location of the study area. This change improves the ecological and biogeographic context of the study.

30) Figure 2: As per comment 9, we would recommend altering the group names in

plot a to improve clarity. For plot a, it would also be hugely beneficial to also add in the sample sizes for each species by fullness class so it is easy for readers to locate. The groupings on plots b and c are too broad, making the comparisons too vague to be meaningful as the authors highlight there are in fact quite stark differences in prey species. The authors could for example provide family level delineations for fish and crustaceans, however as per comment 27, we think that providing prey delineations by functional groups e.g. herbivorous fish, cryptobenthic fish, pelagic forage fish, cleaner invertebrates, detritivorous invertebrates etc. would be much more informative from an ecological perspective. The key on plot b is currently redundant and the colour palettes should be reconsidered as currently it is hard to distinguish adults and juveniles in plot c. We also wonder why the size classes for the other species are not shown as there is space for an additional plot.

Reply: We thank the reviewer for this helpful suggestion. Figure 2 has been revised to improve clarity and ecological interpretation. In plot a, sample sizes (n) for each species and stomach fullness category were added. In plots b and c, prey items were reorganized into functional ecological groups to provide more meaningful trophic comparisons. We also removed the redundant legend, applied a colour-blind friendly palette, and improved the visual distinction among size classes. Additionally, we incorporated a fourth panel showing the Amundsen graphical analysis, which strengthens the interpretation of feeding strategies among predators.

31) Figures 3 and 4: Fig3b is essentially showing the same information as Fig4b (although there appears to be a La green data point missing from 3b in the lower left corner when comparing the two) so there is redundancy here. There is large white space in fig4a – do trophic positions from zero to ~2.8 need to be shown? Thinking more widely to put the stable isotope data into the context of a food web perspective, the authors should consider showing the predator data plotted alongside data from the primary producers and / or prey groups (table S4). It seems a shame this wide dataset is relegated to the supplementary. The authors could therefore condense these two figures into one: with a larger plot showing the fish isotopic niches in the context of other prey groups, a second plot showing the more detailed predator isotope data (like plot 3a), and then the trophic position estimates (plot 4a).

Reply: We thank the reviewer for this constructive suggestion. We have revised and reorganized Figures 3 and 4 to reduce redundancy, improve visualization, and provide a broader trophic context for the isotopic analyses. We also verified the missing data point for *L. apodus* and corrected the isotopic niche representation accordingly. In addition, the trophic position figure was reformatted to reduce unnecessary white space and improve clarity.

Following the reviewer's recommendation, we incorporated an additional panel into the main isotopic figure showing the proportional contribution of major trophic pathways estimated using exploratory Bayesian stable isotope mixing models. This new panel complements the isotopic niche and trophic position analyses by illustrating the relative contribution of grouped prey-associated energy pathways (e.g., crustaceans, reef fishes, and basal production sources) for each mesopredator species.

We also explored broader isotopic food web representations incorporating prey groups and basal resources. However, incorporating all prey and basal source groups directly into the main isotopic niche plots substantially increased visual complexity and reduced the clarity of predator-level comparisons, which represent the primary focus of this study. Therefore, the complete food web plots and detailed mixing model outputs were retained as supplementary material (Fig. S5; Table S5), while the new panel included in the main figure provides a more concise synthesis of trophic pathway contributions and improves the ecological interpretation of the isotopic analyses.

32) Table 1: This table would benefit from having a second matrix below only with overlaps calculated from the stable isotope data, so it is easy for readers to directly compare.

Reply: As suggested, Table 1 has been revised to include an additional matrix presenting isotopic niche overlap values (SEAc) derived from stable isotope analyses.

33) Table 2: We think this is the table that could do much of the heavy lifting in terms of reporting isotope results to the reader and could be expanded (see below). It is important to report sample sizes (n). For the isotope means, we suggest reporting standard deviations over standard errors. Here you could also provide the SEAC values (or even consider calculating the SEAB – Bayesian standard ellipses – instead and provide the 95% credible intervals). You could also incorporate many of the statistical tests as supertext to highlight differences with for example capital letters for interspecies comparisons and lower case for intraspecies group comparisons:

Species (Size Class) N d13C (‰) d15N (‰) SEAC (‰²) TP

Lionfish (total): Ntot Mean + sda Mean + sd Mean +

sd/CI

Mean + sd

Small Nsmall Mean + sda

Juvenile N... Mean + sda

Adult N... Mean + sdb

Graysby (total) N... Mean + sdb

Reply: We thank the reviewer for this valuable suggestion. Following this recommendation, Table 2 was expanded to include sample sizes (n) for each species and size class, and isotopic values are now reported as mean $\pm$ standard deviation rather than standard error. In addition, SEAc values derived from SIBER analyses were incorporated to provide a quantitative estimate of core isotopic niche width. We acknowledge the suggestion to include letter-based comparisons; however, given the multiple variables and groupings presented, we opted to retain statistical comparisons as reported by the Kruskal–Wallis tests to maintain clarity and readability of the table.

Associate editor's decision after peer review (08/07/2026).

Dear Dr. Domínguez-Domínguez:

It is a pleasure to accept your manuscript entitled "Stable isotope and stomach content evidence of trophic overlap between invasive lionfish and native mesopredatory reef fishes in the Mexican Caribbean" in its current form for publication in the Neotropical Ichthyology.

Please note this comment left by the reviewer:

Figure 3 (d) must be checked, L. apodus mixing model doesn't reach 100%

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 our Social Media Editor, Igor Souto-Santos, at icass.ufjf@gmail.com within 30 days.

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 send an e-mail to neoichth@nupelia.uem.br 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. Osmar Luiz

Associate Editor, Neotropical Ichthyology
osmarluizjr@gmail.com, osmar.luiz@dbca.wa.gov.au

Neotropical Ichthyology



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