Submission date. 17/12/2025

Associate editor's decision after peer review (25/03/2026).
Dear Dr. Monzani:
Manuscript ID NI-2025-0213 entitled "Cyclosporine A administration in chemically sterilized triploid Astyanax altiparanae to prevent cell rejection for future interspecific transplantation of fish germ cells" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter. We kindly ask that you please refer to the attached file, which contains the report from Reviewer 1.
The reviewer(s) have recommended publication, but also suggest some 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.
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You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text. Once the revised manuscript is prepared, upload BOTH versions (the tracked and a clean) and submit them through your Author Center.
When submitting your revised manuscript, you will be able to respond to the comments in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please reply POINT TO POINT all the suggestions of the reviewers and be as specific as possible in your response to the comments.
IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 25-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. Claudia Martinez
Associate Editor, Neotropical Ichthyology
cbueno@uel.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments.
(There are no comments.)

Anonymous reviewer #2
Recommendation. Minor Revision
Comments.
This manuscript presents an interesting and relevant contribution to the field of fish reproductive biotechnology, particularly in the context of germ cell transplantation and conservation of neotropical species. The combination of chromosome manipulation, chemical sterilization, and immunosuppression is innovative and has clear potential applications.
The study is well aligned with the scope of the journal and addresses an important bottleneck in germline chimera technology, namely immune rejection in adult recipients. The integration of histological, hematological, and molecular approaches is a strength of the work.
While the manuscript shows good potential, some aspects could be further improved to enhance clarity, robustness, and overall impact. Most of the points raised below are intended to strengthen the manuscript and can likely be addressed with minor revisions.
1. Objective and Study Focus: The objective of the study could be stated more clearly. Since the manuscript includes both in vitro and in vivo approaches, it would be helpful to explicitly articulate how these components are connected within a single research question or hypothesis.
2. The experimental design is generally appropriate for a preliminary investigation; however, a brief acknowledgment of its limitations would strengthen the manuscript. In particular: the relatively small sample size and the absence of certain control groups (e.g., CsA-only treatment).
3. The observed mortality in CsA-treated groups is an important result and deserves a slightly more detailed discussion.
4. The Materials and Methods section is generally well described. Adding a few additional details would further improve reproducibility, such as: water used for fertilization, water source and basic quality parameters, histological fixation conditions and feeding regime.
5. The findings regarding CsA effects are interesting and promising. However, slightly moderating some statements, particularly those suggesting direct applicability, would make the conclusions more balanced.
5. The Discussion is well supported by the literature and provides good context. Minor refinements could improve focus by: reducing repetition of results, emphasizing key findings and limitations and slightly reducing speculative statements.

Author's Rebuttal Letter (08/06/2026).
Neotropical Ichtiology
Editor and Reviewer comments
Reviewer #1
Specific commentaries
Put the Keywords in alphabetical order
Line 44: the keywords were put in alphabetical order, from "Immunosuppressant; germline chimera; busulfan; fish conservation; chromosome manipulation." to "Busulfan; chromosome manipulation; fish conservation; germline chimera; immunosuppressant."
Introduction
Lines 72 - What is the germ cell transplantation biotechnology?
Lines 72-74: the following description about germ cell transplantation biotechnology was included: "Germ cell transplantation biotechnology consists of isolating and transferring undifferentiated germ cells from a donor into the gonad of a sterile recipient, where they proliferate and differentiate into donor-derived gametes (Majhi et al., 2009; Nóbrega et al., 2010)."
Lines 73-74 - Why is germ cell transplantation more beneficial for slow-maturing and endangered species?

Lines 77-81: the following explanation and further references were added to the text, explaining the importance of germ cell transplantation for slow-maturing and endangered species: "Particularly, for slow-maturing species, germ cell transplantation allows the use of faster-maturing surrogates as recipients, accelerating the production of donor-derived gametes (Nagasawa et al., 2019; Franěk et al., 2021), making it a potential technology for the maintenance and propagation of the competent germline of endangered species (Li et al., 2016)."
Line 77 - How should it be possible to generate hybrids?
Lines 84-86: the following sentence explaining how hybrids are produced from non-sterilized endogenous and donor-derived gametes fertilization was added, and, subsequently, relevant references for the explanation: "which could be generated from recipient and donor-derived gametes in non-sterile recipients (Brinster et al. 2003; Majhi et al. 2009; Ryu et al., 2022)"
Material and Methods
Line 116 - just one spike?
Line 126: the article "a" was deleted, since there's not necessarily one spike in the anal fin: "Breeders were chosen based on their secondary sexual characteristics - two females with a bulging abdomen and red papilla and two males with a narrow abdomen and spike in the anal fin."
Lines 136, 158, 184 - Since the words are in italics, you must sublicize the scientific name
Lines 148, 169, and 195: the words were put in non-italic form, and the scientific names were put in italic for a better presentation.
Line 147 - In which solution was it fixed?
Lines 160-161: the phrase was improved from "Gonad samples were collected from the fish and fixed for histological analysis." to "Gonad samples were collected from the fish and fixed in Bouin's solution (Cinetica, Laboratórios Adria, Brasil) for histological analysis." to specify in which solution the gonads were fixed.
Figure 1 - The authors must improve the legend, detail it more
Lines 672-677: The caption of Figure 1 was modified from "Figure 1 Experimental design illustration of chemical sterilization of juvenile triploids A. altiparanae" to "Figure 1: Experimental design illustration of the chemical sterilization protocol for juvenile triploid A. altiparanae. (1) Acclimatization period of male triploids (N=20) at 28°C for 1 week. (2) Gradual temperature increase (+1°C/day) to 35°C. (3) Maintenance at 35°C for 1 week. (4) Busulfan administration (50 mg/kg, once weekly for 3 weeks). (5) Temperature maintenance at 35°C for 1 week. (6) Gradual temperature decrease (-1°C/day) to 28°C. (7) Gonadal histology at 4 time points."
Statistical analysis - Did the author perform the presupposed tests for homoscedasticity and
homogeneity?
Due to the small sample size in some of the datasets, formal tests for normality and homogeneity of variances were re-evaluated and considered unreliable. Therefore, nonparametric analyses (Kruskal–Wallis followed by Dunn's post hoc test and Mann–Whitney U test for two-group comparisons) were applied when appropriate. For the in vitro experiments performed in triplicate under controlled conditions, ANOVA followed by Tukey's post hoc test was used. The Statistical Analysis section was revised accordingly.
Lines 267-272: Leukocyte viability and cell counting at different CsA concentrations were analyzed in vitro by ANOVA and the Tukey test for multiple comparisons ($p<0.05$), using the PAST 4.03 software. Cell density among the different treatments of in vivo CsA in the histological preparations of the head kidney and leukocyte proportions in the peripheral blood among fish treated with busulfan and CsA were assessed by Kruskal-Wallis followed by post hoc Dunn's test or Mann-Whitney U test

for two-group comparison ($p<0.05$) in the JASP 0.19.3.0 software.
Lines 737-741: The following correction was included in the caption of the Table 2: "Values expressed as mean ± SD. Statistical analysis was performed using the Kruskal-Wallis test (post hoc analysis: Dunn's test) or the Mann-Whitney U test for two-group comparisons. Different superscript letters indicate significant differences between groups within the same time point ($p < 0.05$). Only Basophils on Day 5 showed significant differences (Kruskal-Wallis $p = 0.039$; Dunn's test: 40 mg/kg vs. control with emulsion $p = 0.011$)."
Results
Lines 271-273 - nests? Do you mean cysts?
Lines 283, 284, 683 and 689: the word "nests" was replaced by "cysts" for an appropriate description.
Figures 3 and 6: First, the authors must provide an additional image for each figure. That blank space is not recommended for a publication. Furthermore, you should represent more structures in the gonad, such as the somatic Sertoli cells.
The requested considerations were addressed.
Figures 3 and 6 were revised to eliminate blank spaces and improve image presentation quality. In Figure 3, an additional image from after the third busulfan application containing more gonadal structures, including somatic Sertoli cells, were incorporated and properly indicated (Image F). In Figure 6, the image E indicating lymphocytes was deleted and these cells were indicated in Image B, together with eosinophil, since lymphocytes appeared more frequently in the smears. Consequently, the respective legends were improved as described below:
Lines 682-690: the legend "Figure 3: Testicles histology of triploid A. altiparanae chemically sterilized with busulfan at 50 mg/kg. A: testicle before temperature rises, with nests containing some spermatogonia (Sg), spermatocytes (Sp), spermatids (St), and lumen (L) of the seminiferous tubule without sperm. B: testicle one week after increased temperature. C: testicle one week after the first application of busulfan, confined by the columnar epithelium (filled arrow). D: testicle one week after the second busulfan application, with observed interstitial tissue (empty arrow). E: testicle one week after the third application of busulfan. A, C, and E: scale: 200 μm. B and D: 100 μm" was replaced by "Figure 3: Testicles histology of triploid A. altiparanae chemically sterilized with busulfan at 50 mg/kg. A: testicle before temperature rise, with germinal cysts containing spermatogonia (Sg), spermatocytes (Sp), spermatids (St), and lumen (L) of the seminiferous tubule without sperm. B: testicle one week after increased temperature. C: testicle one week after the first busulfan application, confined by the columnar epithelium (filled arrow). D: testicle one week after the second busulfan application, with observed interstitial tissue (empty arrow). E: testicle one week after the third application of busulfan. F: detail of the testicle one week after the third application of busulfan, showing Sertoli cells (Se) around cysts with a germ-free lumen (L); empty arrow indicates interstitial tissue. A, C, and D: scale: 100 μm. B and E: 200 μm. F: 50 μm" to include the sixth image with more details of the gonad and avoid the blank space. Additionally, the magnification scales were revised and corrected for all panels in Figure 3, as indicated: "A, C, and D: scale: 100 μm. B and E: 200 μm. F: 50 μm".
Lines 707-709: The legend "Figure 6: Blood smears from the different treatments of the experiment of in vivo administration of CsA, submitted to white blood cell count. The white arrow indicates: A: granulocyte. B: eosinophil. C: neutrophil. D: monocyte. E: lymphocyte. 200 μm scale" was replaced by "Figure 6: Blood smears from the different treatments of the experiment of in vivo administration of CsA, submitted to white blood cell count. The white arrow indicates: A: granulocyte. B: eosinophil; the black arrowhead indicates a lymphocyte. C: neutrophil. D: monocyte. 200 μm scale" to indicate the lymphocyte in the image B and avoid the blank space.
Lines 274 – 282 - This result must be shown. Even being small, the representation of the

testes is obligate. Histology allows to process even smaller structures.
Linhas 289 – 290 - We appreciate and agree with the reviewer's suggestion of including gonadal histological images. Tissue preservation in several gonadal samples was compromised, limiting our ability to acquire high-quality histological images suitable for publication (as shown below). Consequently, rather than including low-quality images, we revised the statement in the manuscript to reflect that no apparent gonadal alterations associated with CsA administration were observed under the evaluated conditions. The modification above was made:
Lines 493-494: The phrase "The gonads were not affected by immunosuppressant administration" was replaced by "No evident gonadal alterations associated with CsA administration were observed." to moderate the sentence.
The abstract was modified too – Line – 36 – 38 - CsA did not alter the cellular composition of the head kidney, and no evident gonadal alterations.
Line 329 – 333 – It was inserted in the results section: The histological gonads could not be effectively evaluated. The preservation of tissue in several gonadal samples was compromised, which limited the ability to obtain high-quality histological images suitable for publication. As a result, the gonadal histological analysis was not included in the results. However, the findings indicate no apparent gonadal alterations associated with CsA administration, as shown in Fig. S2.
Line 717 – 719 - Figure Supplementary S2 – Histological gonads analysis in chemically sterilized animals and treated with CsA. A – Control gonad 10 days after emulsion administration. B - Gonad 5 days after treatment with 20 mg/kg of CsA. C - Gonad 5 days after treatment with 40 mg/kg of CsA.
Discussion
Lines 357-358 - Isn't busulfan used for Chemical sterilization? Your statement is confusing.
Lines 373-375: the phrase was rewritten; "Combining chromosome manipulation, chemical sterilization with busulfan, and high-temperature treatment successfully sterilized triploid male A. altiparanae recipients, generating germ cell-free testis to receive transplanted germ cells." for a coherent explanation.
Lines 357-370 - There is a study from the authors' group that shows another combination to achieve a sterile host, combining hybridization and triploidization. You should also mention it
Line 383 – 385– The statement "Similarly, hybridization combined with triploidization via heat shock produced predominantly sterile hybrids of A. altiparanae and A. fasciatus (Piva et al., 2018)" was inserted to mention the recommended study.
Lines 450-451 - And what does it mean?
Lines 448-449 (in original draft): the statement "indicating that lymphocytes promoted the defense of the organism to the emulsion vehicle" was deleted, since it could not be stated with certainty.
Lines 452-467 - What about the high rate of mortality? Even in this scenario, the authors recommend the use of CsA?
Lines 462-466: the explanation "Although both concentrations showed about 40% mortality, suggesting similar nephrotoxicity, the high mortality rates observed in both CsA-treated groups may also reflect the cumulative physiological stress associated with triploidization and high-temperature exposure during busulfan treatment. Nevertheless, the 60% surviving recipients may still provide a sufficient yield for germline chimera production." was inserted to explain the possible reasons for the high rate of mortality and the recommendation of the use of CsA even in this scenario, since this immunosuppressor showed effectiveness in inhibiting the immune system.
Reviewer #2
Comments:
1. Study objective and focus: The objective of the study could be stated more clearly. As

the manuscript includes in vitro and in vivo approaches, it would be helpful to explicitly articulate how these components are connected within a single research question or hypothesis.
Lines 109–113: for a more complete statement of the objective of the study, the text "This study aimed to establish the concentration of the CsA that inhibits the differentiation and growth of T lymphocytes of the neotropical A. altiparanae, to find a balance between inhibiting the immune response of the transplanted cells and the lowest possible adverse effects on the recipients." was replaced by "This study aimed to determine whether different CsA concentrations could effectively suppress the immune response in the neotropical fish A. altiparanae while minimizing adverse effects on recipients. To address this hypothesis, in vitro leukocyte assays were used to identify immunosuppressive CsA concentrations, which were subsequently evaluated in vivo in chemically sterilized triploid recipients."
2. The experimental design is generally appropriate for a preliminary investigation; however, a brief acknowledgment of its limitations would strengthen the manuscript. In particular: the relatively small sample size and the absence of certain control groups (e.g., treatment with CsA only).
Lines 468–470: The following statement was inserted to recognize our limitations and offer the main contributions of our study: "Despite the relatively small experimental groups and the high mortality rate observed after CsA administration, the present study provides a useful preliminary framework to develop an optimized immunosuppression protocol for fish germ cell transplantation."
3. The mortality observed in the CsA-treated groups is an important result and deserves a slightly more detailed discussion.
Lines 462–467: the following explanation of the possible reasons for the high mortality was added: "Although both concentrations showed about 40% mortality, suggesting similar nephrotoxicity, the high mortality rates observed in both CsA-treated groups may also reflect the cumulative physiological stress associated with triploidization and high-temperature exposure during busulfan treatment. Nevertheless, the 60% surviving recipients may still provide a sufficient yield for germline chimera production. Therefore, a dose of 40 mg/kg CsA resulted in a greater reduction of interleukin-1β expression, although notable toxic effects were observed."
4. The Materials and Methods section is generally well described. The inclusion of some additional details would further improve reproducibility, such as water used for fertilization, water source, basic quality parameters, histological fixation conditions, and feeding regime.
Lines 116–121: The statement "Males and females of A. altiparanae were collected in fish farming tanks at the National Center for Research and Conservation of Continental Aquatic Biodiversity of the Chico Mendes Institute for Biodiversity Conservation (CEPTA/ICMBio), in Pirassununga - SP, Brazil." was replaced by "Males and females of A. altiparanae were collected in fish farming tanks filled with water from local hydrological sources from a tributary of the Mogi Guaçu river, at the National Center for Research and Conservation of Continental Aquatic Biodiversity of the Chico Mendes Institute for Biodiversity Conservation (CEPTA/ICMBio), in Pirassununga - SP, Brazil."
Lines 130–132: The statement "Fish were maintained in a 40 L aquarium at 28°C, filled with water from a local hydrological source, continuous aeration, and fasting conditions during the hormonal administration." was changed by "Fish were kept in a 40 L aquarium filled with dechlorinated water from the domestic water supply, using a closed recirculation system with continuous aeration, maintained at 28°C during hormonal administration.
Line 136: "aquarium water" was added to specify the origin of the fertilization water. A sentence of the phrase was rewritten.

Line 158-159: The statement "The fish were given commercial feed once a day throughout the experiment" was added to explain the feeding of the fish.
Lines 160-167: for a more detailed description of the fixation conditions in histological analysis, the paragraph was rewritten: Gonad samples were collected from fish and fixed in Bouin's solution (Cinetica, Laboratórios Adria, Brasil) for histological analysis. Histological processing, for inclusion in paraplast, was carried out by dehydration in a graded ethanol series, followed by immersion in xylene. The samples were embedded in paraplast. Once hardened, the samples were sectioned at 5 µm using a microtome (Leica Biosystems #RM2235, Heidelberger, Germany). Paraffin sections underwent dewaxing by immersion in xylene and rehydration in a decreasing alcoholic series, followed by staining with hematoxylin and eosin. The histological sections were observed under an optical microscope (Nikon-Eclipse Ni, Tokyo, Japan).
5. The results regarding the effects of CsA are interesting and promising. However, slightly moderating some statements, particularly those suggesting direct applicability, would make the conclusions more balanced.
Lines 392-393: The phrase "The use of adult triploid A. altiparanae could solve this problem" was replaced by "The use of adult triploid A. altiparanae may help minimize this problem."
Lines 395-396: The statement "These hybrids may be more effective for evaluating CsA in vivo, eliminating the need for chemical sterilization" was replaced by "These hybrids could represent an alternative model for evaluating CsA in vivo, eliminating the need for chemical sterilization."
Lines 497-500: The paragraph "The methodology applied can generate germ cell recipients for further interspecific transplantation in neotropical fish, with decreased cell rejection and rise of future germline chimera production, aiming at endangered ichthyofauna conservation actions." was replaced by "The methodology applied may contribute to the generation of germ cell recipients for further interspecific transplantation in neotropical fish, potentially reducing cell rejection and improving the efficiency of future germline chimera production, aiming at endangered ichthyofauna conservation actions."
6. The discussion is well-grounded in the literature and provides good context. Minor adjustments could improve the focus, such as: reducing the repetition of results, emphasizing key findings and limitations, and slightly decreasing speculative statements.
Lines 422-424: The statement: Hemolytic anemia can be caused by CsA administration in human patients (Pande et al. 2023). This condition may have also affected fish treated with immunosuppressants in this study. The decrease in white blood cell count is a known effect of the immunosuppressant (Azeez 2021). Differences in hematological patterns in fish can reveal immunosuppression, as was observed in O. niloticus treated with cyclophosphamide, which acts as an immunosuppressive drug by reducing erythrocyte and white blood cell counts (Elazab et al. 2021)." were replaced by "Such conditions indicate an immunosuppressive effect. Likewise, CsA administration in A. altiparanae may have affected hematopoietic tissues in the head kidney, contributing to anemia and leukocyte reduction. These effects have also been reported for other immunosuppressive agents in fish and humans (Azeez et al. 2021; Elazab et al. 2021; Pande et al. 2023)" to a better understanding and unification of the discussion, eliminating repetitions.
Lines 470-473 (in original draft): the paragraph "The proportions of eosinophils, which defend against inflammatory processes and parasites, monocytes, which combat infections and bacteria, and basophils, which aid in healing inflammation, were influenced by probable changes in the neutrophil-to-lymphocyte ratio." was deleted, since it was not the focus of the study and would extend the text unnecessarily.
Lines 456-457 (in original draft): the sentence "as a transplant immunosuppressant in juvenile triploid A. altiparanae recipients treated with busulfan, due to greater repression of relative expression of interleukin-1β." was deleted to avoid redundancy in the text.

Lines 471-475: The paragraph “The immunosuppression of transplanted fish treated with CsA was studied by Yoshinaga et al. (2021) using intracoelomic 20 mg/kg and 40 mg/kg administration in grafted O. mykiss with allogeneic testes. It was observed that the highest dose promoted more lasting immunosuppression by inhibiting the interleukin-2 expression for a longer time. In the current study, the administration of CsA at 20 and 40 mg/kg partially inhibited interleukin-1β expression on the 5th and 10th days post-injection. Although interleukin-1β expression was not completely suppressed, the application of these dosages of CsA in transplant recipients could increase the efficiency of transplants, reducing the immunological responses to transplantation.” was replaced by “The immunosuppression of transplanted fish treated with CsA was studied by Yoshinaga et al. (2021) using intracoelomic administration at 20 mg/kg and 40 mg/kg in grafted O. mykiss with allogeneic testes. The highest dosage resulted in longer-lasting immunosuppression by prolonging inhibition of interleukin-2 expression. Furthermore, these findings support the immunosuppressive effects of CsA in A. altiparanae recipients”, to give a more summarized and concise explanation.
Lines 487-494: The paragraph “According to the relative expression data obtained in this work, 20 and 40 mg/kg of CsA can immunosuppress recipients up to the 10th day after application. Despite the apparent efficacy of CsA as an immunosuppressant, blood smear analysis indicated that CsA doses administered cause anemia, decrease the number of leukocytes, and alter the consistency of the kidney on the 10th day after application. The combination of techniques of chromosome manipulation and chemical sterilization using busulfan at high temperature efficiently sterilized male juvenile triploid A. altiparanae recipients, ensuring free cell germinal space for germ cell transplantation. The highest concentration of CsA used in vitro caused the most immunosuppressive and cytotoxic effects on A. altiparanae leukocytes. Furthermore, in vivo administration of 40 mg/kg reduced the relative expression more efficiently on the 10th day after application. The mortality rates in fish treated with 20 and 40 mg/kg CsA were identical, and CsA nephrotoxicity observed was similar in both concentrations, suggesting that 40 mg/kg CsA can be initially used as a transplant immunosuppressant in juvenile triploid A. altiparanae recipients chemically sterilized. Despite the apparent efficacy of CsA as an immunosuppressant, blood smear analysis indicated that CsA causes anemia and a significant decrease in the number of leukocytes. The immunosuppressant did not change the cellular structure in the head kidney on the 5th day after administration, but the consistency was altered on the 10th day after CsA injection. The gonads were not affected by immunosuppressant administration.” was replaced by “The combination of chromosome manipulation and chemical sterilization using busulfan at elevated temperatures effectively promoted germ cell depletion in male juvenile triploid A. altiparanae recipients, thereby creating available germinal space for transplantation. Furthermore, CsA administration promoted immunosuppressive effects while reducing interleukin-1β expression; however, notable hematological and renal side effects were also observed. No changes were observed in the cellular structure of the head kidney on the 5th day after administration, but the consistency was altered on the 10th day after administration of CsA. No evident gonadal alterations associated with CsA administration were observed” to summarize and avoid repetitions of the results.

Associate editor’s decision after peer review (14/09/2026).
Dear Dr. Monzani:
Manuscript ID NI-2025-0213.R1 entitled "Cyclosporine A administration in chemically sterilized triploid Astyanax altiparanae to prevent cell rejection for future interspecific transplantation of fish germ cells" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended publication, but also suggest some minor 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=ebb22e325a53461682499daffd725e45
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 15-Oct-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. Claudia Martinez
Associate Editor, Neotropical Ichthyology
cbueno@uel.br
Your manuscript has cleared peer-review, but there are still some issues that need to be addressed prior to final acceptance. See the list below.
Lines 68-70. The authors just insert this paragraph “Germ cell transplantation biotechnology consists of isolating and transferring undifferentiated germ cells from a donor into the gonad of a sterile recipient, where they proliferate and differentiate into donor-derived gametes (Majhi et al., 2009; Nóbrega et al., 2010).” To explain what is the germ cell transplantation technology. However, it refers only to the methodology involving adult hosts. What about germ cell transplantation in embryos and larva?
Lines 80-81. The authors claim that this statement: “which could be generated from recipient and donor-derived gametes in non-sterile recipients (Brinster et al. 2003; Majhi et al. 2009; Ryu et al., 2022)” explains how a hybrid can be generated from donor germ cell transplantation in non-sterile host. However, the statement is not clear and a beginner reader will have difficult to understand it.
Figure caption 1: Thank you for improving the caption. However, step 7 is unclear. Did you use only TWO specimens for the analyses?
Statistical analysis: The authors should clearly state in the text why parametric tests were used for some datasets and non-parametric tests for others, including the criteria used to

determine which statistical approach was appropriate for each dataset.

Author's Rebuttal Letter (22/09/2026).
Neotropical Ichtiology
Editor and Reviewer comments
Lines 68-70. The authors just insert this paragraph "Germ cell transplantation biotechnology consists of isolating and transferring undifferentiated germ cells from a donor into the gonad of a sterile recipient, where they proliferate and differentiate into donor-derived gametes (Majhi et al., 2009; Nóbrega et al., 2010)." To explain what is the germ cell transplantation technology. However, it refers only to the methodology involving adult hosts. What about germ cell transplantation in embryos and larva?
Response: We thank the reviewer for this constructive suggestion. The paragraph has been expanded to incorporate germ cell transplantation across various developmental stages (embryos, larvae, and adults), rather than focusing solely on adult recipients. The text now reads as follows
Lines 68–76: Germ cell transplantation biotechnology consists of isolating and transferring undifferentiated germ cells from a donor into a recipient of the same species (intraspecific transplantation) or a different species (interspecific transplantation), where they proliferate and differentiate into donor-derived gametes (Majhi et al., 2009; Nóbrega et al., 2010). Different recipient developmental stages are suitable for germ cell transplantation. In fish, germ cells can be injected into the marginal region of the blastodisc of blastula-stage embryos (Saito et al., 2008), into the peritoneal cavity of newly hatched larvae (Yoshizaki et al., 2000; Takeuchi et al., 2003; Okutsu et al., 2006), or into the gonad of sexually mature sterile recipients (Majhi et al., 2009; Lacerda et al., 2010; López et al., 2024).
The corresponding citations have also been added to the References section.
Lines 80-81. The authors claim that this statement: "which could be generated from recipient and donor-derived gametes in non-sterile recipients (Brinster et al. 2003; Majhi et al. 2009; Ryu et al., 2022)" explains how a hybrid can be generated from donor germ cell transplantation in non-sterile host. However, the statement is not clear and a beginner reader will have difficult to understand it.
Response: The paragraph has been rewritten to better explain the necessity of host sterilization and the genetic consequences (e.g., hybrid progeny) when non-sterile recipients are used. The revised text reads as follows.
Lines 84–91: Germ cell transplantation in adult fish is a relatively simple technology (Yoshizaki et al., 2000). However, it requires the generation of sterile recipient fish, free of endogenous germ cells in their gonads, allowing the transplanted cells to colonize the depleted recipient gonad effectively and to prevent simultaneous production of donor- and recipient-derived gametes in non-sterile recipients, which could subsequently result in hybrid progeny (Brinster et al., 2003; Majhi et al., 2009; Ryu et al., 2022). Physiological and immunological compatibility with the donor, rapid growth, short reproductive cycle, and easy management in captivity are also key biological features sought in recipients to ensure transplantation effectiveness (Majhi et al., 2009)
Figure caption 1: Thank you for improving the caption. However, step 7 is unclear. Did you use only TWO specimens for the analyses?
Response: Yes, we used only two specimens (n = 2) for histological analysis at each of the four time points shown in Figure 1 (before the temperature increase and 7 days after each of the three busulfan administrations). This specification was added as indicated below:
Line 650: "N=2 per time point".
This sample size reflects a standardized sampling design at each time point, intended to monitor gonadal histological changes throughout the sterilization protocol.
Statistical analysis: The reviewers, including the criteria used to determine which

statistical approach was appropriate for each dataset.
Response: We have revised the Statistical Analysis section to explicitly describe the rationale behind choosing parametric vs. non-parametric tests, primarily driven by sample size (N) and experimental design balance. The revised text in Statistical analysis Line 253 - 259: We analyzed leukocyte viability and cell counts at different CsA concentrations in vitro using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons ($p < 0.05$) in PAST 4.03, as ANOVA is robust for balanced experimental designs. Conversely, cell density in histological preparations of the head kidney (in vivo CsA) and leukocyte proportions in peripheral blood (busulfan + CsA) were analyzed using non-parametric Kruskal-Wallis tests followed by Dunn's post hoc test, or Mann-Whitney U tests for two-group comparisons ($p < 0.05$) in JASP 0.19.3.0, due to the reduced sample sizes ($N = 2 – 3$ per group).
Furthermore, we added an explicit statement in the Discussion addressing the statistical limitations resulting from mortality.
Line 435 - 439: Despite the relatively small experimental groups and the high mortality rate after CsA administration, which limited the statistical power of the non-parametric tests used, this study provides a useful preliminary framework for developing an optimized immunosuppression protocol for fish germ cell transplantation.
Additionally, we have revised the writing of the manuscript, and the corrections are marked in red.

Associate editor's decision after peer review (22/09/2026).
Dear Dr. Monzani:
It is a pleasure to accept your manuscript entitled "Cyclosporine A administration in chemically sterilized triploid Astyanax altiparanae to prevent cell rejection for future interspecific transplantation of fish germ cells" 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.
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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. Claudia Martinez
Associate Editor, Neotropical Ichthyology
cbueno@uel.br

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