“*CORRECTION FORM*”

Dear Dr. Monge-Nájera

According yours instructions for the submission no. 18837, entitled: Cellular Development of the Germinal Epithelium during the Adult Reproductive Life History of the golden mussel *Limnoperna fortunei* (Dunker, 1857) (Bivalvia: Mytilidae), we are submitting the correction of the reviewers' comments below:

|  |  |
| --- | --- |
| Reviewers’ or Editor’s comment | How you dealt with the comment |
| **Revisor 1** |  |
| Suggestion 1 - I suggest the phrase gametogenic cycle instead of adult reproductive life history in the title and Bivalvia instead of mollusk in key words | As suggested by the reviewer, we have modified the title and key words. |
| **Revisor 2** |  |
| Evaluation 3 - Mytilidae must be included in the key words | As suggested by the reviewer, this was done. |
| Evaluation 4 - The first part of the introduction should be rewritten as it is reduced to a list of references. The introduction should include information that puts the reader on the subject, stating that type of organisms are (that is mentioned above). | As suggested by the reviewer, we have modified the first part of the introduction. **FROM:** “The evolutionary history, morphology, distribution, reproduction, larval cycle and other aspects of the life history of the golden mussel *Limnoperna fortunei* (Dunker, 1857) have been the object of several studies (Morton, 1973; Morton, 1982; Choi & Shin, 1985; Choi & Kim, 1985; Morton, 1991; Darrigran & Pastorino, 1995; Darrigran, Damborenea & Penchaszadeh, 1998; Darrigran, Penchaszadeh & Damborenea, 1999; Cataldo & Boltovskoy, 2000; Darrigran, Damborenea, Penchaszadeh & Taraborelli, 2003; Damborenea & Penchaszadeh, 2006; Darrigran & Damborenea, 2006; Mansur et al., 2012; Boltovskoy & Correa, 2015), and special attention has been paid to its environmental impact (Darrigran, Penchaszadeh & Damborenea, 2000; Darrigran, 2002; Brugnoli, Clemente, Boccardi, Borthagaray & Scarabino, 2005; Boltovskoy, Sylvester, Otaegui, Leites & Cataldo, 2009; Santos et al., 2012).”**TO:** *“*The golden mussel *Limnoperna fortunei* (Dunker, 1857) is a medium-sized [bivalve](https://en.wikipedia.org/wiki/Bivalvia) [mollus](https://en.wikipedia.org/wiki/Mollusc)k belonging to the family Mytilidae (Darrigran & Damborenea, 2006). Mytilidae is a family of small to large saltwater mussels (Soot-Ryen, 1955). Some of these species are edible and have high commercial value. However, *L. fortunei* is the only freshwater representative of Mytilidae (Darrigran & Damborenea, 2006). It is recognized as a successful invader of aquatic environments in Asia, South America and North America. An extensive revision was recently made by several authors about the biology, distribution, reproduction, ecology and other aspects of the life history of the golden mussel (Boltovskoy et al., 2015 for review).” |
| Evaluation 9 - The discussion is correct, but it could be improved if the results are compared with other Mytilidae species and with previous results in the topic (recently a review of the known information is found in Boltovskoy et al. 2015. Reproductive Output and Seasonality of *Limnoperna fortunei*. Pp: 77-103. In Boltovskoy (ed.), *Limnoperna fortunei*, Invading Nature - Springer Series). | As suggested by the reviewer, we have added this reference in the discussion (in Reproductive Phases – line 221).**FROM:** “In the mollusk, reproduction is a cyclical event during its life history. Thus, various scales categorizing gonadal alterations throughout the reproductive cycle are reported in the bivalve literature (see Lubert, 1959; Shafee, 1989; Saucedo & Southgate, 2008; Franco et al., 2011 for review). Despite reporting the same event, reproductive cycle scales vary depending on the author (see Darrigran & Damborenea, 2006; Callil, Gomes & Soares, 2012 for review). Searching for a type of reproductive scale based on the dynamic of the germinal epithelium, we found Brown-Peterson, Wyanski, Saborido-Rey, Macewicz and Lowerre-Barbieri, (2011). Herein, we have used the terminology of the scale from Brown-Peterson et al. (2011) to recognize phases over the reproductive cycle of *Limnoperna fortunei*, i.e. Developing, Spawning Capable, Regressing and Regenerating.”**TO:** “In the mollusk, reproduction is a cyclical event during its life history. Thus, various scales (i.e. inactive, developing, maturing, mature, and spent) categorizing gonadal alterations throughout the reproductive cycle are reported in the bivalve literature (see Lubert, 1959; Shafee, 1989; Saucedo & Southgate, 2008; Franco et al., 2011; Boltovskoy et al., 2015 for review). Despite reporting the same event, reproductive cycle scales vary depending on the author (see Darrigran & Damborenea, 2006; Callil, Gomes, & Soares, 2012; Boltovskoy et al., 2015 for review).Most authors (Boltovskoy et al., 2015 for review) consider the initial stage of the reproductive cycle as inactive or resting. However, in this stage there is intense cell proliferation and restocking of the gonad. Thus, the stage or phase previously called “inactive” becomes "regenerating" in this study. Herein, we proposed new terminology based on the dynamic of the germinal epithelium, to recognize phases over the reproductive cycle of *Limnoperna fortunei*, i.e. Developing, Spawning Capable, Regressing and Regenerating.” |
| **Revisor 2** |  |
| Line 14 - Niches has other meaning in ecology. Here can be used “área or regions or different habitats” | As suggested by the reviewer, this was modified **TO** “different habitats”. |
| Line 31 - An introductory sentence would help to the inexpert reader to know about what kind of species will the paper deal with. | As suggested by the reviewer, this was modified **TO**:*“*The golden mussel *Limnoperna fortunei* (Dunker, 1857) is a medium-sized [bivalve](https://en.wikipedia.org/wiki/Bivalvia) [mollus](https://en.wikipedia.org/wiki/Mollusc)k belonging to the family Mytilidae (Darrigran & Damborenea, 2006). Mytilidae is a family of small to large saltwater mussels (Soot-Ryen, 1955). Some of these species are edible and have high commercial value. However, *L. fortunei* is the only freshwater representative of Mytilidae (Darrigran & Damborenea, 2006). It is recognized as a successful invader of aquatic environments in Asia, South America and North America. An extensive revision was recently made by several authors about the biology, distribution, reproduction, ecology and others aspects of the life history of the golden mussel (Boltovskoy et al., 2015 for revision).” |
| Line 38 - Letter i - eliminated | This was corrected. |
| Line 48 - This reference seems to be wrong. The year is not coincident with the one in the references, is it mistaken with Oliveira et al 2014? | This reference was corrected. |
| Line 58 - pallial tissue | This was corrected. |
| Line 61 - Fujita & Fontes-Junior | This was corrected. |
| Line 70 - Or subtropical? | This was corrected **TO** “subtropical”. |
| Line 74 - Which are the aspects? | As suggested by the reviewer, this was modified **TO**:“Thus, in order to contribute to a better understanding of the reproduction of *L. fortunei*, we described the gonadal organization and the cellular dynamic of the male and female germinal epithelium during each reproductive cycle over its life history, using high resolution histology associated with quantitative data.” |
| Line 84 - What do you mean with “sexually active?” Do you mean mature? How do you determine this? | They are mature animals, which have entered into the reproductive cycle. This is determined by the size of the animals and the morphological characteristics of the gonad, such as disorganized tubules, residual sperm (in males) or degenerated oocytes (in females), as explained in the Tables 3 and 4. |
| Line 87 - or on aquatic macrophytes | Ok, the preposition on was inserted. |
| Line 88 - Which retractor muscles? Foot retractors? That is not needed for separate the valves | This was modified **TO** “opened and the visceral mass was removed by cutting the adductor and retractor muscles”. |
| Line 91 - For what? I don`t understand this. | This was modified **TO** “A sample of gonads from males and females was photographed using a digital camera (Fujifilm HD 14MP Finepix S2800) and processed for light microscopy”. |
| Line 113 - This is mentioned before. Please, check were is the best position for this | We would like to maintain it, since it relates to different methods using the same program. |
| Line 118, 125 - the pallial tissue | The letter “l” was inserted throughout text. |
| Line 165 - What do you mean? | The reproductive phases occur over a reproductive cycle. Each reproductive cycle consists of all types of phases. |
| Line 166- the evaluation of the type of germ cell | The possessive pronoun “their” was inserted. |
| Line 172 - the evaluation of germ cell | The possessive pronoun “their” was inserted. |
| Line 183 – space  | This was corrected. |
| Line 183 – space | This was corrected. |
| Line 201 – space | This was corrected. |
| Line 218 – scales (resaltar) | We added the scale normally used: “Thus, various scales (inactive, developing, maturing, mature, and spent) categorizing gonadal alterations throughout the reproductive cycle are reported in the bivalve literature…” |
| Line 223 - This paragraph must be rewriten. Why do the authors choose this terminology? | As suggested by the reviewer, this was modified.**FROM:** “Searching for a type of reproductive scale based on the dynamic of the germinal epithelium, 223 we found Brown-Peterson, Wyanski, Saborido-Rey, Macewicz and Lowerre-Barbieri, (2011). Herein, we have used the terminology of the scale from Brown-Peterson et al. (2011) to recognize phases over the reproductive cycle of *Limnoperna fortunei*, i.e. Developing, Spawning Capable, Regressing and Regenerating”.**TO:** “Herein, we proposed new terminology based on the dynamic of the germinal epithelium, to recognize phases over the reproductive cycle of *Limnoperna fortunei*, i.e. Developing, Spawning Capable, Regressing and Regenerating”.  |
| Line 269 - en varios nichos | This was modified **TO** “en diferentes hábitats” |
| Line 272 - la planicie de inundacion | This was modified **TO** “la planicie de inundación” |
| Line 276 - histología de alta resolución, basado | This was modified **TO** “histología de alta resolución basado.  |
| Line 332 - from Rio de la Plata | This was modified **TO** “Rio” (Capital letter) |
| Line 345 - *Research, 22*, | This was corrected. |
| Line 380 - This reference is wrong cited in the Introduction | This was corrected. |
| Line 415 - All the abbreviations used here are not used in other place, and are not the same used in the figures. I think they are not necessary. | The abbreviations were removed. |
| Line 431 - (Fig. 4 A, F, G.).  | This was corrected. |
| Line 431 - Vitellogenic stage  | This was corrected. |
| Line 440 - a lot of | “Many” was modified **TO** “a lot of”. |
| Line 440 - **Comentario [--14]:** Or phase? | This was corrected. |
| Line 440 - space | This was corrected. |
| Line 458- (in this cycle of reproduction,  | This was modified **TO** “in this phase of reproduction…” |
| Line 475, 477, 483 - pallial | This was corrected. |
| Line 483 - It will be easier to interpret, if the references are in an alphabetic order | This was corrected. |
| Line 484 - These are not indicated in the figura | The legends were added to the figure. |
| Line 503, 506, 507, 512 - pallial | This was corrected. |
| Line 511, 513, 514 - arrows | This was corrected. |
| Line 515 - cilia | This was corrected. |
| Line 536, 539, 545 - pallial | This was corrected. |
| Line 537 - This is not a general view  | This was modified **TO** “Detail of the testis…” |
| Line 445 - This is not indicates | The legend was added to the figure. |
| Line 569 - of a nest | This was corrected. |
| Line 601, 602, 603, 608, 610, 612 - pallial | This was corrected. |
| Line 612 remove ; | This was corrected. |
| Line 625, 626, 627, 637 - pallial | This was corrected. |
| Line 637 - These references have to be in alphabetically order | This was corrected in all figures. |
| **Text and illustrations format correction guide from associate editors**  |  |
| **TITLE**Do not include the taxonomic authority in this section. Note that capitals are not allowed within the title. | This was corrected.This was corrected. |
| **NAMES AND ADDRESSES**All authors postal and e-mail addresses should be updated and included. | This was corrected. |
| **ABSTRACT**Do not include the taxonomic authority here but in the introduction.This section is not complete because some results/findings and conclusions of the study are missing and should be included.Thousand should be written with a space instead of a comma or period. | This was corrected.The conclusion of the study was included: “The morphological data and reproductive parameters shown here confirm how informative the cellular dynamic of the germinal epithelium is for the understanding of the cyclic gonadal events during the adult reproductive life of the mollusk in general. These knowledge about the gametogenesis of invasive species can become a fundamental tool for the development of control strategies and implementation of programs to decrease their proliferation in natural environments.” |
| **KEY WORDS**Present the key words in lower case and separated by commas, as in this example:**Key words:** leaf senescence, nutriment absorption, trophic ecology, Oaxaca, soil recovery. | This was corrected. |
| **INTRODUCTION**This section has to be edited and improved in its justification. Since much research has been done on this species, your specific contribution should be highlighted here.Our new format for two authored citations is written with **&,** as in the example.The **et al.** particle should not be written in italics and its use follows APA (see left). | This was corrected according to the suggestions of the reviewers.The new format for two author citations was followed.The et al. particles were writing corrected. |
| **MATERIALS AND METHODS**References supporting your sampling and the analysis of alteration of the germinal epithelium methods have to be included.Our new format for two authored citations is written with **&,** as in the example.The **et al.** particle should not be written in italics.Thousand should be written with a space instead of a comma or period.A space between the numeric value and units should be included.Coordinates follow the next format: **(21°05’ N-76°00’23” W)** | This was corrected. The analysis of alteration of the germinal epithelium methods was made according to most authors, as Boltovskoy et al., 2015. The animals were collected in accordance with its availability in nature, giving preference to adults animals.The new format for two authored citations was followed.The et al. particles were writing corrected.Thousand was written with a space.A space between the numeric value and units was included and the coordinates format followed the format recommended. |
| **RESULTS**Figure and table calls should follow ascendent order. No figure or table insertion location is required in the text.This section is written in past tense.Refer to recently previous published documents for subtitles.A space between the numeric value and units should be included.Thousands should be written with a space instead of a comma or period. | The figures and tables were put in ascendant order.The location to insert figures and tables was removed in the text.The results were written in the past tense.This was done.A space was included between the numeric value and units.Thousand was written with a space. |
| **TABLES AND FIGURES**A total of 4 tables and 7 figures.Figure and table calls should follow ascending order.Fig. 7: follow our format with grey scales instead of patterns in this figure. Y axis scale could be written 0-20-40…. | The figures and tables were put in ascendant order.Fig.7 was corrected. |
| **DISCUSSION**Our new format for two authored citations is written with **&,** as in the example.The **et al.** particle should not be written in italics and its use follows APA format.Your results should be compared to findings in similar species.A space between the numeric value and units should be included.Thousand should be written with a space instead of a comma or period. | The author citations were corrected.The et al. particles were writing corrected.This was corrected according to the suggestions of the reviewers.This was corrected. |
| **ACKNOWLEDGMENTS**Eliminate titles such as Dr., Mr. and others: use only first and family name for each person; make acknowledgments as brief as possible | This was corrected. |
| **SPANISH ABSTRACT AND KEY WORDS**Should have the same information as the abstract. | This was corrected: “Los datos morfológicos y parámetros reproductivos que se muestran aquí confirman cómo informativo la dinámica celular del epitelio germinal es para la comprensión de los acontecimientos cíclicos gonadales durante la vida reproductiva de adultos del molusco en general. Este conocimiento sobre la gametogénesis de especies invasoras puede convertirse en una herramienta fundamental para el desarrollo de estrategias de control y aplicación de programas para disminuir su proliferación en ambientes naturales.” |
| **REFERENCES**Please double check your references in order to confirm that all of them are in the text and final list.Our new format for references and citations in the text follows APA6th edition.Do not write last names in capital letters. Family name is always followed by name initials in all cases. Name initials should have a space in between.  Before last author name the symbol & is included, preceded by a comma.Years are written within parentheses.Journals names are written in complete form and in italics as the volume. Pages are preceded by a comma and separated by a small hyphen. | This was corrected.  |
| **INSTRUCTIONS FOR GRAPHIC PRESENTATION**Fig.1-7 – Please enhance figures resolution for proper publication quality. | The figures are being sent separately, with resolution for proper publication quality. |
| **TABLES**Table 1-4 – Correct format. | **---** |
| **TEXT**The text must have single interline space.Times New Roman.12 ptsCheck for and erase blank spaces. | This was corrected.This was corrected.This was corrected. |
|  |  |