

September 4th 2019,

Dear colleagues,

We are submitting a new version of our original research article entitled “New insights into the population genetics of partially clonal organisms: when seagrass data meet theoretical expectations” to Peer Community in Evolutionary Biology (PCI Evol Biol). The new version takes into account comments made by the recommender Olivier Hardy.

Specifically, we included the edits suggested on the pdf (thanks for this detailed help), including the ones helping to tune our message and better emphasize the possible confounding effect of other components of the reproductive systems (such as the occurrence of selfing or inbreeding) on F_{is} . We also added other sentences along the discussion and conclusion as suggested.

We also added the analysis of Simpson index $D^*=1-L$ to check for its correlation with F_{is} . Adding it allowed us to observe that this relationship was lower than the one of R , likely due to the narrower range of values obtained with D^* . We however included the results and the rationale behind this test in the new version of the manuscript. We added the analysis in the manuscript and the figures illustrating the results in supplementary material. We briefly commented these results in the Results and Discussion sections.

We hope this revised version will have properly taken into account those two main comments.

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose. All authors have read and approved the final version of the manuscript. Thank you for your consideration, and we look forward to hearing from you at your earliest convenience.

Sincerely,

Dr. Sophie Arnaud-Haond, Solenn Stoeckel, and Dr. Diane Bailleul.

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Review by Stacy A. Krueger-Hadfield

Arnaud-Haond et al. perform a meta-analysis using four species of seagrass, that vary along a continuum of asexual to sexual with corresponding patterns of departure from Hardy Weinberg and genotypic richness, in order to validate the relationship between the inbreeding coefficient FIS and genotypic richness R . There were three main aims:

- (i) determining whether the estimate of genotypic diversity can provide informative information on clonal vs. sexual reproduction in species with a similar sampling scheme;
- (ii) does the departure from HWE reflect the distance from equilibrium and does the genetic composition of natural meadows provide a reliable estimate of relative clonal rates with species that invest differentially in sex vs. asex; and
- (iii) do the genotypic and genetic parameters provide congruent qualitative and quantitative estimates at both inter- and intra-specific levels?

R1.1: There is a frequent observation of apparently high genotypic richness, suggesting lots of sex, but systematic departures from HWE in the direction of an excess of heterozygosity (negative FIS). Importantly, the authors call for the use of FIS rather than R if the aim of a study is to understand the importance of clonal reproduction. R is extremely sensitive to sampling. While the authors focus on seagrasses, for which there are an abundance of data, I would argue many other partially clonal taxa from pathogens to algae warrant similar approaches. We need to have a better theoretical and empirical framework with which to understand population dynamics, connectivity, and the evolutionary trajectories of important crops, ecosystem engineers, invasive species, pests, etc. etc. This paper builds on the work of (Halkett *et al.* 2005; Arnaud-Haond *et al.* 2007) as well as the CLONIX consortium and is a necessary and important contribution to the literature on the population genetics of partially clonal organisms.

Action: Following the referee's advice, we added several additional comments in the discussion and a final sentence to the abstract: "*The results also emphasize the need to revise our appraisal of the extent of clonality and its influence on the dynamics, connectivity and evolutionary trajectory of partial asexuals in general, including in seagrass meadows, to develop the most accurate management strategies.*" We also added a sentence to the conclusion: "*Finally, this meta-analysis is the first attempt to revisit empirical data in light of recent theoretical developments (Stoeckel *et al.* 2019), calling for similar research on important clonal species such as other ecosystem engineers, important crops, invasive species or pathogens.*"

General Comments

R1.2: First, this article was a pleasure to read. It was well thought out and constructed. In short, there is very little that warrants critical attention. There are few points that I think could be made clearer and more approachable to a more widespread audience, that I think critically need to understand the population genetics of partially clonal organisms. I have also addressed specific grammar issues in the specific comments.

Comment: We thank the referee for this comment. We have included all suggestions and sent the MS for additional editing with a request for increased diligence (the company edited the manuscript before the initial submission, but the grammar was apparently still not completely correct).

R1.3: In the first paragraph of the introduction, the authors discuss the lack of clear theoretical predictions. I wonder if another brief thought is needed here in particular the lack of a standardized format for metrics to use, data reported? As part of a recent

metaanalysis, I was collating population genetic data from macroalgae, many of which are partially clonal, and this is indeed a problem across population genetic literature. I think this paper is *more* than just a meta-analysis and the relationship between *FIS* and *R*. Thus, I think at some point the lack of standardization across studies and across taxa is warranted. That could probably be a paper in and of itself, but I would suggest the authors acknowledge that that is also a problem that is on par with the lack of clear theoretical predictions.

Action: To answer this referee and a comment by the third referee on the introduction, in the introduction, we emphasized the results of Arnaud-Haond et al. 2007 and Goroskope 215 and the lack of standardization, as well as the issues detected with *R* and the sampling density.

R1.4: In the second paragraph of the introduction, the authors discuss the fact that *R* has been used a proxy to infer the relative influence of sex vs. asex. In the red macroalgae for which population genetics have been studied in detail, *Gracilaria gracilis* (Engel et al. 2004), *Chondrus crispus* (Krueger-Hadfield et al. 2011; 2013), *Agarophyton chilensis* (formerly *G. chilensis*; (Guillemin et al. 2008)), and *Agarophyton vermiculophyllum* (formerly *G. vermiculophylla*, (Krueger-Hadfield et al. 2016; 2017)), more than just *R* has been used to infer the influence of sex vs. asex. While the comparison of allele frequencies between free-living haploid and diploid stages is unique to haplodiplontic organisms, algal studies have used *Fis* (both single and multilocus estimates) and pairwise locus and multilocus estimates of linkage disequilibria. I admit that this work is my own and that of Myriam Valero and Marie-Laure Guillemin's lab. However, it might be worthwhile noting that partially clonal seaweed studies have not relied solely on *R*. I am curious as the introduction moves along in paragraph 4, do seagrass studies generally rely solely on *R*?

Comment: Our main point here was just that departures from HWE towards heterozygote excess were mostly considered when they were coherent with limited clonal diversity/richness and the possibility of nearly exclusive or at least recent and massive clonality. This may result from different mechanisms we prefer to avoid detailing here, as the subject is beyond the scope of the present article (from a mere assortment of alleles under the influence of nearly pure clonality –Balloux et al., 2003– to the Meselson effect –Welsh & Meselson, 2000– or to the clonal persistence of initially hybrid lineages –Delmotte et al 2004).

Action: We added (Introduction, page 3, last paragraph) several examples of such interpretations in this paragraph, including algae and aphids. We also added an account of recent theoretical developments in algal research as an example.

R1.5: When the four species of seagrass are introduced, I suggest the authors make a conceptual figure. As I mentioned above, this paper is incredibly important for conservation genetics, but also anyone doing population genetics. As a predominantly marine person, I think marine population genetics is in desperate need of these types of data. However, for non-seagrass researchers, it might be useful to have a conceptual figure in which there are sketches of the four species (also showing which ones grow faster, have higher shoot turnover) and below them is the continuum of sex to asex, *R*, *Fis*, etc. That would also make the paradox of high *R*, but significant departures from HWE also more intelligible. I suggest this figure also as a way for a new pop gen person, or someone who realizes partially clonality might be an important driver in their system or species, to be able to more easily wrap their minds around these concepts and metrics.

Action: We added a Box illustrating the biology and associated hypothesis. We hope these explanations and graphical illustrations will help.

Specific Comments

I don't know if it is just the citation style chosen, but the in-text references are not always consistent throughout the text.

- In order to avoid some confusion, I suggest you italicize *R*, genotypic richness.
- Done

All minor comments and editing suggestions were taken into account (before professional editing).

- Insert comma: "... coastal ecosystem, in terms ..."
- Insert comma: "... ecosystem services, but are ALSO declining..."
- Insert comma: "... sound management, conservation, and restoration..."
- Rephrase: "... of natural populations, severely limits our understanding..."
- Rephrase: "population genetic data" rather than population genetics data
- The sentence that starts with "Contrasting shoot life span" I wonder if this would make more sense to organize the seagrasses from *Z. noltii* as the sexual one with no or little departure from HWE and high genotypic diversity and move towards asexuality. That makes more intuitive sense as sex is what more population geneticists and evolutionary ecologists would be familiar.
- Insert comma: "... at the species scale, but..."

Introduction

- Insert comma: "...collapse, shift, or expansion..."
- Insert comma: "... dynamics, and evolution of..."
- Rephrase: "...The combination of sampling bias seriously limits the interpretation of *R*..."

Methods

- In the *Z. marina* data, the Becheler citations are strangely formatted; same for Diekmann and Serrão 2012
- In the genetic and genotypic indexes ... you should use the word indices to refer to more than one index
- Perhaps define the with and without replicates (ramet vs. genet level)?
- I think something is missing in the sentence describing the *R*² and the *F*-test. *R*² is being used to assess the quality of the model and the *F*-test?
- The *R*² is written as *R*² and *r*², be consistent

Results

- No specific comments

Discussion

- In the first paragraph, when using i.e. or e.g., these belong in parentheses
- The sentence that begins the third paragraph in the "Fitting empirical data to model predictions" is a run-on sentence. I suggest cutting up many of these sentences in this paragraph to short thoughts
- Insert comma: in the "Implications for understanding clonal vs..." insert a comma after selection on the second line; expansion on the 13th line

Conclusion

- This phrase is missing a word? "the mathematical hypothesis of greater deviations from HWE towards heterozygotes excess at higher levels of clonality" I think you mean greater deviation from HWE towards an excess of heterozygotes.

Figures

Figure 1 – I suggest making this a 'normal' boxplot and overlay the points from each of the studies. That is easier to visualize.

Figure 2 – If you make the conceptual figure suggested above, then you could use the line drawing or an image of each seagrass on this plot. It would be really helpful to keep all the species delineated in the reader's mind

Figure 3 – I do not know where you plan to submit this paper, but I would suggest color

(maybe it is color and I printed in black and white?) for the different species. You could even assign them a color in the conceptual figure and that would help to differentiate the tiny points. An alternative would be to make them transparent points that are all solid colors since there is a lot of overlap and the tiny points are hard to differentiate.

■ Figure S1, S2, S3 – Same comments as Figure 1, 2, 3 respectively

References

- Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology*, **16**, 5115–5139.
- Engel CR, Destombe C, Valero M (2004) Mating system and gene flow in the red seaweed *Gracilaria gracilis*: effect of haploid–diploid life history and intertidal rocky shore landscape on fine-scale genetic structure. *Heredity*, **92**, 289–298.
- Guillemin M-L, Faugeton S, Destombe C *et al.* (2008) Genetic variation in wild and cultivated populations of the haploid–diploid red alga *Gracilaria chilensis*: how farming practices favor asexual reproduction and heterozygosity. *Evolution*, **62**, 1500–1519.
- Halkett F, Simon J, Ballou F (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution*, **20**, 194–201.
- Krueger-Hadfield SA, Collen J, Daguin-Thiébaud C, Valero M (2011) genetic population structure and mating system in *Chondrus crispus* (Rhodophyta). **47**, 440–450.
- Krueger-Hadfield SA, Kollars NM, Byers JE *et al.* (2016) Invasion of novel habitats uncouples haplo-diplontic life cycles. *Molecular Ecology*, **25**, 3801–3816.
- Krueger-Hadfield SA, Magill CA, Bunker FSPD *et al.* (2017) When invaders go unnoticed: the case of *Gracilaria vermiculophylla* in the British Isles. *Cryptogamie, Algologie*, **38**, 1–22.
- Krueger-Hadfield SA, Roze D, Mauger S, Valero M (2013) Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed *Chondrus crispus*. *Molecular Ecology*, **22**, 3242–3260.

Reviewed by Ludwig TRIEST, 2019-04-03 09:29

This paper concerns a meta analysis with a clear hypothesis to test. The meta analysis was rendered possible due to a standardized sampling design and this at multiple locations and for multiple species. The testing of an excess heterozygosity as a proxy for clonality within sites (negative F_{is}) is valid and was compared to a regularly used easy metric R (clonal richness). The idea that H and F_{is} are less influenced by sample size and design than R is conceptually correct. The manuscript is well-structured and clearly written. Below I will give a series of minor advices to improve wordings/sentences encountered during first reading.

Title: reflects the study

Abstract: corresponds to aims and main findings

The sentence Line 4 -5 should be rephrased (verb missing ? “However, the effect of

clonality....”)

Action: Done

Introduction Paragraph 1 Line 3: the drastic decline of what ? of species diversity ?
Allele diversity? Gene diversity?

Comment: We meant the drastic decline of ecosystems themselves, which were reflected in or due to the decline of engineer species themselves.

Action: Changed to “for drastic decline OF ecosystems”

Paragraph 2 Line 5 : One could add (A and H) after “genetic” and (ML and MLL) after “genotypic”, for clarity towards reader. Paragraph 2 Line 8: i.e. the “multilocus” genotype ?

Action: We rephrased as follows: *“These improvements have allowed the identification in natural populations of clonal replicates (i.e., ramets) of distinct “genetic individuals” (i.e., genets) through the recognition of clonal lineages (multi-locus genotypes, MLGs, or multi-locus lineages, MLLs). This has permitted the estimation of not only genetic (i.e., allelic richness A and heterozygosity H) but also genotypic (i.e., indices based on the number of MLGs or MLLs) diversity. The simplest index of genotypic richness, G, corresponds to the number of MLGs or MLLs detected in a population.”*

Paragraph 2 Line 13: However, G naturally increases with sample size ... This is indeed an indirect effect of the increasing number of alleles (but not at all H).

Paragraph 2 Line 15: ... the sample size of ramets (add “ of ramets” for clarity)

Action: Here, we corrected the mention of R and added ramets: *“G naturally increases with the sample size in terms of the number of ramets (Ellstrand & Roose 1987; Dorken & Eckert 2001).”*

Paragraph 3 Line 4: ... interpretation of negative Fis values (heterozygous excess) “of remaining genets”, when not

Comment: We did not understand this suggestion.

Paragraph 4 Line 4: ... possibly partly due to “lowered” sampling density ... (I suppose here you mean lower and not higher density ?)

Action: We added low in front of sampling density as suggested.

Paragraph 4 Line 6: ... Values of genetic differentiation (Fst) “of populations when considering only genets” (I suppose you mean ‘genets’ and not ‘ramets’ here)

Action: As authors alternatively did one other or both, and this is not detailed in the review; we now phrased this area as follows: *“On the one hand, moderate to high levels of clonal richness, R, together with limited but significant values of genetic differentiation (F_{st}), whether at the ramet or at the genet level, have led some authors...”*

Paragraph 4 Line 9-13: ... Eventually rephrase this sentence (had to read it 3 times before understanding it ; this because of the used wording ‘...maximum value that Fst can reach, ... in combination with concept R and Ho or Fis)

Action: We split the sentence in two.

Paragraph 5 Last sentence : ... and moving towards null (or slightly “negative” if heterozygosity deficiency occurs...). I suppose this must be slightly “positive” because refers to Fis.

Action: Corrected (it was indeed meant to be positive because it referred to deficiency).

Paragraph 6 Aim 2 : ... does the genetic composition of natural meadows... What do you mean here exactly with “genetic composition” : MLG or gene diversity?

Comment: We tried to stick to a terminology where the terms gene or genetic would only relate to the genetic composition and descriptors of the population and not to the clonal architecture.

Action: We rephrased the second aim as follows: *“Does departure from HWE reflect the distance from equilibrium? Does the genetic composition (i.e., genetic descriptors)”*

Materials and methods

Studied species: Here, it would be interesting to mention the obligate outcrossing in case of sexual reproduction.

Done: “Finally, both *Zostera* sp. and *P. oceanica* are hermaphrodites, while *C. nodosa* is dioecious (Larkum & den Hartog, 1989).” And (next paragraph) “The fact that *C. nodosa* is an obligate outcrosser may, however, result in interferences by sexual reproduction and clonality on the distribution of heterozygosity (F_{is}), particularly in comparison with the long-lived *P. oceanica*.”

Genetic data sets : Because an unequal number of polymorphic loci (7, 8 or 9 loci) was used, it would be interesting to demonstrate that the probability of identity (PI and PI of siblings) was similarly low or at least sufficiently low to ensure detection of MLG repeats across samples (sites) and species. The methods section is clearly written and complete.

Comment: As we chose P_{sex} (over the strategy of P), and the number of loci was ascertained as discriminant for the four species, we would prefer not to add a new analysis of P (identity).

Results

Clonal richness R Paragraph 1, Line 1 : ...R increased regularly from Do you mean “gradually” instead of “regularly”?

Action: Regularly was replaced with gradually.

Relationship between R and F_{is} Paragraph 2, line 5 : When considering only the genets (i.e. no replicates)..... I suppose you mean “ramets” instead of “genets” because the legend of Figure S3 includes the ramets with replicates ?

Action: Clarified (adding “at the ramet level” first and “at the genet level” in the second part).

Discussion

My main advice would be to write a paragraph on the sexual reproduction of the considered seagrasses and that one expects outcrossing behaviour. This is an important assumption because many other plants (also aquatic plants) do have a mixed reproduction system ranging from selfing, partially selfing to outcrossing. The findings of this met analysis should make clear that the expected lowered F_{is} and correlation to R might not be that straightforward as presented here for the four seagrasses. A moderate level of inbreeding, due to the pollination biology of a species, might blur the presented relationship.

Comment: In fact, three of the four species are hermaphrodites, with the possibility for selfing, and, to our knowledge, no incompatibility has been reported in the literature. However, we acknowledge the interest in commenting on the possible interference of the sexual reproductive system with clonality and the possible consequences of this interference on F_{is} values. This subject was thus mentioned in the results section (see here above), and we added a comment here. Notably, *C. nodosa*, which is the only obligate outcrosser of the present panel, is also the species that slightly departs from expectations through its departure from HWE. This species tends to deliver more important heterozygote excess than the longer lived *P. oceanica*. We emphasize that this observation may be expected if the occurrence of selfing counters the effect of clonality, resulting in a lower excess of heterozygotes, yet we try to avoid being too speculative because we do not have replicates for this observation. Furthermore, thanks to the referee comment, we emphasize the fact that clonality may have a stronger influence on F_{is} than the sexual reproductive system.

Action: We addressed this comment in several parts of the discussion, particularly the following: “Moreover, although *C. nodosa* and *P. oceanica* were collected with an identical sampling strategy, the *C. nodosa* dataset contained more highly clonal meadows than the *P. oceanica* dataset, which may be partly due to its obligate dioecy (leading to lonely colonizers if relying on clonal growth), as well as the inclusion of sampling sites located at the limit of its distribution (Billingham et al., 2003)”. We also added the following paragraph in the discussion, as suggested by the referee: “Interestingly, three of the four species are hermaphrodites with no existing data

suggesting self-incompatibility. Nevertheless, all of the species show heterozygote excess at the ramet level, and this excess is maintained at the genet level to a different extent for two of the species. In the case where sexual reproduction significantly departs from random mating due to selfing or inbreeding, F_{is} is susceptible to be influenced (thus pulling it towards positive values reflecting heterozygote deficiency), and the influence of reproduction on the genetic composition of populations is blurred by the prevailing influence of clonality. In line with this hypothesis, the fourth species, *C. nodosa*, which is the only obligate outcrosser, is also the species that slightly departs from the expectations in terms of the comparison among species, with a distribution of F_{is} and extreme heterozygote excess similar to the values obtained for the longer lived *P. oceanica*. This observation supports, for the four species analysed here, clonality has a greater effect than sexual reproduction on the genetic composition of populations, particularly on F_{is} values.”

We also added a sentence in the conclusion: “The results, which were obtained for three hermaphroditic species and an obligate outcrosser, also suggest that the screening of F_{is} would greatly help assess the importance of clonal reproduction for many species, including depending on the importance of clonal reproduction and selfing, self-fertilizing species.”

Finding empirical data to model predictions

Paragraph 5, last sentence is unclear : ... The results are even clearer for ... What exactly do you mean here: the difference between genets and ramets or the fact of having interquartile F_{is} values =>zero ? Implications for understanding

Action: Rephrased as: “The tendency towards heterozygous excess for the first two species is even clearer when the data are considered at the genet (without replicates, Figure 2) rather than the ramet scale (Figure S2), with only interquartile F_{is} values equal to or greater than 0.”

Paragraph 2, line 6 : However, the prevalence of asexuality is associated with a diminution of the influence of drift...Bringing in “drift” here sounds confusing. Drift in small pops can cause both high and low F_{is} due to stochasticity ?

Comment: The results cited showed that clonality seems to buffer the influence of population size and the consequent incidence of drift. We tried to rephrase the relevant lines to make this clearer.

Action: “However, the prevalence of clonality is associated with a decrease in the loss of diversity due to the influence of drift ([Reichel et al., 2016](#)), which may partly explain the elevated levels of genetic diversity observed in well-established meadows.”

Referee 3

This manuscript seeks to apply new analytical approaches to an impressive collection of data synthesized for four species of seagrasses. Potentially this is a very interesting piece of work. However, the importance and novelty of the work are completely buried by the textual presentation where the introduction in particular needs to be completely re---written.

R3.A: The central problem is that the theoretical expectations need to be crystal clear without reading other (referenced) papers – what is the relationship between FIS and R and c? What will the R vs FIS slope show? What will be the expected effect of including all ramets vs. just genets? Perhaps the authors are modest in citing and profiling their own previous work, but failure to summarise this body of literature makes the current manuscript unapproachable to a fairly naïve reader. Simply put, in today’s age of data overload, one cannot expect their reader to look at five other papers simply to understand the paper that they are presently reading!

Action: See the action in the answer to R.1.3

R3.B: Also missing from the introduction is a sense of biology. Reworking some of the context currently sitting in the discussion and adding some conceptual diagrams will be helpful here.

Comment & Action: The conceptual scheme added to address a comment by referee 1 was also designed to answer this major point and the minor point 6 below.

Finally, the writing is very uneven both in terms of content and readability. Some very hard edits to pull it all together are required.

Minor points:

Please use line numbers to make commenting easier!

1. Abstract – Needs to provide some information on the theory being applied and the end goal of the analyses – as written I cannot tell what was really inferred and why it matters.

Action: We partly rephrased the abstract to make the bridge with theoretical findings clearer.

2. Introduction, 2nd paragraph – What is the main question or goal motivating this paragraph? To determine c ? State the problem in the beginning of the paragraph and the reason for wanting to know this so the reader can follow. R is not formally defined. “dynamics and evolution”/“evolution and dynamics” used in adjacent sentences.

Action: We rephrased part of the introduction, providing a common definition of R and P_d (the uncorrected and corrected ratio mostly used in early works).

3. Introduction, 3rd paragraph – Is the main point that FIS is an unreliable indicator of c ?

Action: Rephrased and took into account the comments from referee 1.

4. Introduction, 5th paragraph – What are the “recent modelling developments”? What are their innovations? A little on how they work.

Action: We added the following: “in light of recent modelling developments describing the effect of increasing c values on the genotypic and genetic descriptors (including R and F_{is}) of populations (Stoeckel, S. et al., submitted)”.

5. Introduction – it would be lovely to add a figure showing a picture of the four species and put the relevant life history traits onto the figure. This would help the reader evaluate the species metrics against life histories. Similarly a map of sampling sites and species ranges would be welcome biologists like to see biology!

Action: We merged this suggestion from referee 3 with the suggestions from referee 1.5 (see action above).

6. Introduction overall – The novelty of what is being done in this paper is not clear: much of this would be helped by discussing the new methodology and why/how it represents a distinct advance to the field. Perhaps a concept diagram modified from a previous publication can be included to set up the expectations for the reader.

Comment: We hope the rephrasing detailed in this letter and the conceptual scheme help address this comment.

7. Methods – no need to define R_2 and F tests: readers should be aware of basic statistics

Action: We removed those details.

8. Discussion lists expectations from theory --- make sure introduction adequately discusses each of these.

Comment: We hope the rephrasing detailed in this letter and the conceptual scheme help address this comment.

9. Discussion – with $N=4$ for species number, caution is advisable in terms of drawing conclusions regarding the influence of life history attributes.

Comment: We tried to take this comment into account when rephrasing parts of the discussion.

10. Discussion – midway through (starting with “Implications for understanding clonal versus sexual prevalence” the style and tone of writing shifts noticeably (=different authors writing different sections?”). This discussion is extremely

interesting but these ideas should first appear in the introduction and indeed would set up the motivation for this study. The discussion can easily revisit these themes in light of the new findings but much of this text needs to be moved.

11. Literature cited is inconsistently formatted as are in text citations. & 12.

Throughout there are spelling and grammatical errors.

Action: Professional editing was performed to ensure consistent formatting and avoid spelling and grammar mistakes.