June 7, 2016

*To*:

Dr. Stuart E.G. Findlay

Editor-in-Chief

Aquatic Sciences

*From*:

Dr. Marcus W. Beck

US Environmental Protection Agency

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We kindly thank both reviewers and the associate editor for providing constructive comments on our manuscript. We have provided a response below (in italics) to every comment and have modified the manuscript accordingly. Line numbers refer to those in the original submission.

Regarding comments from the first reviewer, we have significantly restructured the first section of the discussion for clarity and to provide better support for our primary conclusions. We have also addressed the many helpful comments throughout and have added color figures where appropriate. In particular, we have made a more careful distinction between dispersal limitation and habitat suitability. For the second reviewer, we have verified the information in table 2 and provided additional text describing *P. crispus*, snow cover, and the importance of lake depth.

To the editor, please note that we have now included color versions for figures 1-4. The color figures are to appear online. Black and white figures are included for print only. Figures 5-7 are unchanged.

We hope that our revisions are satisfactory to address the concerns of the reviewers. Thank you again for the opportunity to publish our work in Aquatic Sciences.

Respectfully,

Marcus W. Beck

*Response to reviewer comments, AQSC-S-16-00066, “Ecological determinants of Potamogeton taxa in glacial lakes: assemblage composition, species richness, and species-level approach”*

Comments for the Author:

Dear Dr. Beck

Thank you for submitting your interesting study to Aquatic Sciences. We have now received two reviews, both of which suggest minor revision. Please, thoroughly respond to all comments in order to improve the readability of the manuscript to readers.

Reviewer 1 provides thorough comments, I agree with the Reviewer that restructuring the Discussion would make it easier to non-specialists to pick up the most important results.

*We have edited the discussion to improve readability. See comments below in response to the first reviewer.*

Reviewer 2 was wondering

- the citation that P. crispus is light limited due to thicker snow depth on frozen lakes and

- why lake depth was more important determinant of species richness than lake size

I don’t see any conflict with these issues:

- Thick snow cover on frozen lakes can effectively decrease light penetration and

- Lake depth presumably better reflects important processes than lake size, e.g. greenhouse gas fluxes correlate better with lake depth than size. However, the availability of lake size data is much better than lake depth data, which might be reflected in literature, i.e. more citations to size than depth.

*We agree that our conclusions about snow cover and lake depth are justified. We have provided an appropriate response below to the second reviewer.*

Reviewer #1: This paper describes how environmental and spatial variables structure the assemblage composition and species richness of Potamogeton species in Minnesota and Wisconsin. Variance partitioning was used to examine the relative contribution of various local, climate and spatial variables. Individual distributions of common species were also analyzed. I found the paper to be well written and interesting, particularly in light of the current interest in using aquatic plant community "health" metrics as a measure of anthropogenic disturbance. Most of my comments involve the clarity of the figures and some points made in the discussion. My recommendation is to accept this manuscript pending minor revisions.

The biggest source of concern/confusion for me is the discussion of dispersal limitation. It may be that my understanding of the terminology is limited, but I understand dispersal limitation as describing a situation where suitable habitat exists for a given species, but access to that habitat is blocked because the species is unable to get to it. It seems to me that the authors describe species being unable to colonize certain areas due to unsuitable conditions (e.g. Northern Minnesota=too cold, long winters; Southern Minnesota = too eutrophic), rather than those areas being blocked to colonization by lack of dispersal. I was unclear to me whether there are lakes with suitable water quality in Southern Minnesota that also lack diverse Potamogeton communities, and that an explanation for this might be dispersal limitation due to the number of eutrophic systems in that region. I think this is an important point to clarify, because there is currently great interest in the importance of dispersal limitation in the upper Midwest, especially as it relates to repopulation of lakes with native species (both fish and plants) after successful efforts to control pollution and restore water quality. Another potential factor that could limit dispersal is the relatively low number of lakes in the south and west regions. It seems to me that lake density could affect many forms of dispersal, from floating downstream to being carried via road by recreational boaters.

*We agree that our use of the term ‘dispersal-limitation’ was inaccurate for instances when we have described habitat suitability issues as a limitation to Potamogeton diversity in our study lakes. The reviewer is correct in that our primary conclusion that species distribution is limited by climate or local factors is not related to dispersal limitation. Although we have not explicitly addressed recolonization in our study, we agree that contrasting dispersal limitation (via physical barriers, distance between habitats, etc.) vs. habitat suitability is an important management consideration. To address these concerns, we have verified and changed accordingly all instances in the text that have inaccurately described dispersal-limitation. Our restructuring of the beginning of the discussion (see manuscript) also included revisions that more clearly emphasize the potential reasons for differences in the results.*

*The following text was also added to emphasize the distinctions:*

*Abstract, line 23: ‘These results suggest that the distribution of Potamogetons in glacial lakes is limited by species tolerances to lake variation in local and climate characteristics across spatial gradients, whereas specific species may be more limited by dispersal barriers between lakes with suitable habitat.’*

*Added to first paragraph of discussion: ‘A contrast between dispersal-limitation and habitat suitability is necessary to understand potential mechanisms for species distribution of Potamogetons in glacial lakes. For example, dispersal-limitation occurs if a habitat is suitable for species colonization in regard to local or regional factors but transport to the location is prevented by physical barriers (e.g., distance, hydrologic connectivity, etc.). Conversely, species may also be absent from a location where dispersal is possible but environmental conditions may prevent colonization.’*

With that said, I think you do present convincing evidence for dispersal limitation playing an important role in structuring Potamogeton communities. The importance of the pure spatial effects could very well be due to dispersal limitation. The invasive P. crispus, which we would not expect to be fully dispersed throughout the study area like the native species are, gives us some good evidence to support this, since it has the highest fraction of variance accounted for by pure spatial effects even though it is not as limited by eutrophication as many other species appear to be.

*Agreed, the results for P. crispus seemed unique in that a large portion of its variation was explained by spatial variables. In this case, we present compelling results that suggest dispersal is mediated by transport in urban areas and its lack of presence in more rural areas suggests transport away from source populations is a limiting factor. At the same time, relatively high abundance in eutrophic lakes suggest higher tolerance to elevated nutrient levels.*

*The content in the conclusion describing P. crispus was expanded (page 18, line 10): ‘For example, the invasive species P. crispus was strongly related to both eutrophication and spatial variables. This suggests a higher tolerance to elevated nutrient levels and a mechanism for dispersal between lakes, respectively, to provide an explanation for the invasive spread of the species in the region.’*

I think that some of my difficulty interpreting the conclusions might be helped by restructuring the Discussion. The message that I took away from this paper, is that spatial effects, alone and through geographically structured local and climate variables, drive the distribution of Potamogetons in this region. I think that starting with the discussion of the pure effects alone contributed to my initial confusion. It seems contradictory to the reader, when you conclude on page 12 that geographic variation in environmental variables was unimportant, but on page 15 when discussing shared fractions of variation, you find that climate variables are geographically structured and the shared portion of the variance is substantial (which one would certainly expect to find). It is easier for my brain to work through how all of the variables (shared and pure) are related to Assemblage composition, and then move on to discussing Richness, etc. As I look back over the Results section, it might work better if the Discussion followed a similar order of topics, or if you used a similar structure to the Species Level discussion, which I found much easier to digest.

*We have revised the first section of the discussion (see manuscript) to provide better support for our primary conclusions. Specifically, the first paragraph was revised to first describe pure effects of spatial variables as a segue to describing share effects and geographical structuring of local and spatial variables. The original discussion, as noted by the reviewer, first describes pure effects followed by shared effects. The revised version places pure effects at the end of the first section and emphasizes geographical structuring of local variation as a means to understand local/climate effects on habitat suitability. We have also more carefully described pure effects of spatial variables and dispersal limitation as separate effects from geographical structuring of environmental variables.*

The Conclusion looks good overall. I wonder if you might want to bring up the dispersal issue again, in light of potential improvements in water quality that the we are working to achieve in the ag zone. Does this data make a case for stocking native species when water quality improves? All of our efforts to prevent the spread of invasive species will also hinder the re-establishment of native species. It may be a good point to add to your discussion of management implications.

*Agreed, some additional points were added to the conclusion.*

*Page 17, line 54: ‘…Further evaluation suggested that the pure effects of spatial variables potentially described dispersal limitations as lakes closer in space were more similar in species composition. More importantly, shared variation between spatial groupings and environmental factors described limitations in habitat suitability related to eutrophication…’*

*Page 18, line 5: ‘Accordingly, the geographic centers shown in Fig. 1 represent a tradeoff in habitat suitability related to geographical structuring of environmental variables.’*

*Page 18, line 29: ‘Dispersal limitation also has relevance for restoration efforts such that connectivity between lakes should be sufficient for colonization provided that habitat is suitable. Planting native species in suitable habitats may have minimal lasting effect if lakes are separated by large distances across the landscape.’*

I have attached some detailed comments in an Excel spreadsheet.

Page 6, lines 10-12: Were all of the surveys conducted in summer, versus early season surveys targeting P crispus?

*Added the following to Page 6, line 12: ‘Early season surveys that only targeted P. crispus were not used.’*

Page 9, lines 17: replace 'for which reason the different models can be' with 'which allows the different models to be'

*Done.*

Page 9, line 56: Here you mention 38 significant axes with positive Moran I scores

Page 10, line 58: Here you say 12 of 58 spatial variables were selected - should this be 38?

*Yes, corrected.*

Page 10, lines 25-37: To make this sentence easier to follow, I would suggest using semicolons to separate the important variables for each variable group. So I would change the comma to a semicolon after 'local variables' and 'climate variables'

*Changed*.

Page 11, lines 29-31: Use of the word 'numerous' - I'm not sure if there is a convention for when the threshold to numerous is crossed - it looks like 5 species had explained variance less than one for local effects and 11 for spatial effects. I think 11 seems like it could be 'numerous', but 5 seems more like 'several' to me. Or maybe the threshold for 'zero' explained variance was higher than 1, in which case you can ignore this comment.

*Zero was changed to < 1% for the inline text. All species less than 1% explained variance for local effects were noted.*

Page 12, line 27: You rarely refer to the tables in the discussion, but I found it helpful to refer back to them and would like at least an intial reference to the data being discussed.

*Citations were added for Table 3 on page 12, line 27, page 13, line 19, page 15, line 48 and for Table 4 on page 13, line 24, page 14, line 14.*

Page 12, line 27-31: I don't understand how the relative importance of pure spatial variables suggests minimal effect of geographically structured variables. Doesn't the relative lack of importance of pure local and climate variables combined with the substantial variance accounted for by the shared variables point toward important geographic structure that the statistics cannot separate?

*This sentence was in error. It was removed with our revisions to the discussion.*

Page 13, line 11-14: I agree that eutrophication appears to be limiting Potamogeton assemblage and richness in the southern region, but I'm not convinced that this is dispersal limitation. It seems like the habitat is unsuitable in that area for most species, although there is evidence of dispersal limitation for P. crispus and a comparision with P. pectinatus is interesting since both are tolerant of high TP.

*This section was revised based on the major comments above. For the specific sentence, we have removed ‘dispersal-limitation’ and rephrased as ‘…assemblage composition and species richness may be defined by habitat suitability related to eutrophication.’*

Page 13, line 24-25 Highlight that although only max depth and TP were correlated with richness, the amount of variance explained by these local variables was high.

*Added a follow-up sentence: ‘However, the variation in total richness that was explained by the pure local effects of depth and phosphorus was much higher than the pure effects of all local variables on assemblage composition.’*

Page 13: The discussion of depth and habitat heterogenity is interesting.

Page 14, lines 41-47: I'm not sure what you are getting at with the sentence about cold climate being an important filter for species. We just saw that species richness increased with colder temps.

*These sentences were revised to better explain the results: ‘The increase in Potamogeton species richness with decreasing winter temperature was unexpected given previous descriptions of temperature and aquatic plant richness (e.g., Pip 1989). However, Pip (1989) argue that temperature in itself is a poor predictor and the relationship with richness is likely related to interactions with other variables that influence macrophyte distribution. The relationship between temperature and richness may have also been poorly described with a linear model as the response is not monotonic across the gradient (i.e., species maxima at moderate temperatures). For example, Beck et al. (2014) used additive models to describe non-linear relationships between macrophyte indicators of community health and climate variables. Species richness showed a distinct modal response to increasing growing degree days measured at each lake. Therefore, we argue that macrophyte communities in lakes in the northern region of our study area are in fact limited by climate despite a positive association of richness with increasing minimum temperatures. Harsh winter conditions are known to restrict macrophyte growth…’*

Page 14, lines 48-56: I think that these conclusions are good ones, but they are better supported after including the individual species discussion about patterns in curly leaf and sago vs the rest of the species.

*These sentences were moved to the conclusions (page 18, line 14) and slightly modified: ‘These results provide support that the latitudinal gradient is partially based on climatic differences, whereas land-use changes along this gradient have further affected water quality in the southern parts of the states. A similar gradient that has been steepened by anthropogenic activities has been reported for wetland plant species in the Great Lakes region (Johnson et al. 2010).’*

Page 15, line 24: add "combined" after "three variable groups"

*Added.*

Page 15, line 34-37: This is not a request to redo your whole analysis, but did you explore other methods like boosted regression trees? I liked the presentation of a similar analysis in this paper by Dallas and Drake (2014,Ecosphere5(9):1-13): <http://onlinelibrary.wiley.com/doi/10.1890/ES14-00071.1/full>

*Interesting example, we will consider alternative approaches in further work.*

Page 15-16: I really like the species discussion - it flows better for me and is easier to follow than the first section.

*Thank you, we hope that our changes in response to the comments above have made the earlier discussion comparable to this section.*

Page 17-18, 58-4: This wasn't explained well enough to be clear to me (see the comment on line 13). The discussion needs to cover both the increase in species richness and we go north AND the lack of many species at the far northern edge of the study area. So we are seeing eutrophication pushing species back in the southern areas and extreme winters doing the same in the far north?

*Yes, those are the main conclusions – limitation in the northern edge by climate, limitation in the south by eutrophication. We hope that our changes to the discussion have provided a better foundation for our conclusions in this section.*

References: I tried and couldn't find a single mistake on the referenc list - nice job!

Figures (all maps): The map figures were difficult to read, especially on the version that I printed out. I think that the shade of the spots goes from grey to black when they overlap each other, but the contrast is not high enough for this to be obvious right away (I thought our printer needed toner at first). I think that the goal of these figures is to get an overview of the patterns, so the fact that the lake dots merge together into blobs is probably intentional, but it initially looks a little sloppy. I can't really say without playing around with these whether another way would work better - maybe it wouldn't.

*The lake points in all maps (figs 1 and 2) were changed for readability (white center, black outline). We have created color figures for the online version that help with visual contrast.*

Figure 1: The species geographic centers are weighted (sized) by species occurence: does that refer to occurence across lakes or the frequency from PI surveys, or a combination of both?

*The sizes and locations of the points were based on a weighted mean of the average lat/long for all lakes, where the weights were defined by the relative occurrence of a species at each lake. Occurrence was the number of surveys points with a species divided by total number of points at a lake. This was added to the caption for clarity: ‘…Geographic centers for each species were estimated as the average latitude/longitude weighted by species occurrence at each lake (number of points in a lake where a species was found divided by total survey points). Point sizes for the bottom plots are the average frequency occurrences across lakes….’*

Figure 2: Glad you included this figure, it really helps to understand the variance partitioning. I'm not sure if it would be possible with out using color, but it would be cool if you could establish a shading scheme on this figure that you carried through to figure 4.

*We have included this with our color figures – colors in figure 2 match those in figure 4.*

Figure 4: This one shows up ok (but not great) on my computer, but it didn't print out very well. I like the idea of how you chose the shading (giving each partition it's own shade), but without using colors the contrast level of the greys isn't high enough. It is really hard to distinguish the different sections of the bars in the lower two panels.

*Agreed, this was difficult with a black/white color scheme. We have changed the black/white version for better distinction and have also added a color version for online. The color version matches the color scheme in figure 2.*

Reviewer #2: In general this is a very good paper representing recent modern data analysis with very extensive and detailed data. As a macrophyte specialist, I cannot tell much of analytical methods and therefore my comments are mainly related to discussion and some minor things.

Table 2.

- can Perimeter of lake be 0.00 km ?

- Color cannot be 0.00 - there must be some missing value or other error?

- I'm really wondering Secchi value of 40,88 m - it is really near the world records in lakes

<http://www.secchidipin.org/index.php/monitoring-methods/the-secchi-disk/secchi-records/>

*The minimum value for lake perimeter and maximum value for secchi depth were in error and they have been changed. We have not changed the ‘0’ measurements for color after verifying the measured values in the original data. Table 5-3 in Wetzel 2001 confirms that zero color in lakes is possible for Pt-Co units. These values were observed in only two lakes. See Wetzel, R. 2001. Limnology. 3rd Ed. Academic Press, California.*

Discussion:

I would like see slightly more discussion of Introduced species of which Potamogeton crispus is the only one. It is mentioned that it is invasive, but its role as introduced plant should be emphasized.

*We have added content in the discussion to better describe the introduction of P. crispus to the region.*

*Page 12, line 48: ‘With the exception of introduced species (i.e., P. crispus), this suggests…’*

*Page 16, line 27: ‘Curly-leaf pondweed is the only invasive species in the genus and was likely introduced to the region in the early 1900s (Valley and Heiskary 2012). The species occurs in over 700 lakes in the region, although its abundance varies. P. crispus often dominates macrophyte communities in shallow, turbid-water lakes in southern Minnesota and is a nuisance species that affects recreation in heavily-used lakes near urban centers’*

I have not seen the paper by Valley and Heiskary (p. 16, l. 17) but the citation that P. crispus is light limited due to thicker snow depth on frozen lakes sounds really odd. Please explain the possible relationship.

*As noted by the editor, this mechanistic relationship between growth, snow cover, and light availability is expected. It is particularly relevant for P. crispus that begins seasonal growth from turions in late winter, unlike most Potamogetons that begin growth in the spring. Early growth of P. crispus in the winter provides a competitive advantage over other species. Therefore, light limitation from snow cover is a potential mechanism that may limit growth during the winter, which may improve growth of native species in the spring. We have provided additional text to explain this link.*

*Page 16, line 19: ‘The relationship between P. crispus and snow cover is important for understanding the competitive advantages of this introduced species. Unlike native Potamogetons, seasonal growth of curly-leaf pondweed begins before ice-off from turions in the sediment that were deposited by mature plants the year prior. Early growth provides an advantage over native species that begin growth later in the spring. Therefore, light limitation from heavy snow cover can reduce growth of curly-leaf pondweed early in the season and release native species from competitive pressures.’*

I'm also wondering why lake depth was more important determinant of species richness than lake size. Can you still check the results, because most international litterature highlights the importance of lake area.

*We agree this result is odd given expected species-area relationships. However, as the editor suggests, there are numerous explanations for why richness is better associated with lake depth and we have previously explained potential mechanisms (page 13, lines 24-41). We have provided some additional explanation as to why area was not included:*

*Page 13, line 58: ‘Moreover, lake area was not associated with richness, which is contrary to established relationships between the two (e.g., MacArthur and Wilson 1967). Lake depth is correlated with lake size for the study lakes and post hoc comparisons showed that depth and size were both positively correlated with richness, with the former having a stronger association. The variable selection procedure used in the analysis identified the most parsimonious model that maximized explanatory power and minimized redundancy among variables. Although lake area is related to richness, it was likely not selected given the relative increase in explained variability with maximum depth.’*