We would like to thank both reviewers for their constructive reviews and acknowledge their thorough evaluation. We are pleased to learn that the reviewers appreciate our innovative modeling study to identify assembly processes of species communities in a situation where knowledge and data regarding ecological requirements and morphological/anatomical features of the model species are limited.

We first summarize the five major critical points raised by the reviewers and demonstrate how we will account for them in our revised ms. In the second part, we then provide a point-by-point reply (in italics) to all comments.

Major criticism (MC) and reply:

- MC1: missing figure with the geographic position of Lake Ohrid
  We will include a map showing the location of Lake Ohrid.

- MC2: difficulties to see which and how certain variables were derived and used to infer the importance of community assembly processes.
  The inferred importance of all three assembly processes is based on only two variables. The hierarchical taxonomic classification of the gastropod species informed about the two selective processes environmental filtering and species interaction. The observed species prevalence is the only variable used for inferring the importance of dispersal limitation. No morphological measures parameterized the process-importance (see MC3). However, from reading the comments of referee #2 and talking to other colleagues we realized that our new analytical approach needs to be explained in more detail. In the revised manuscript, we will therefore include a flowchart sketching how this approach infers the processes of community assembly based on the two variables. Moreover, we will provide a clearer explanation of the stepwise community assembly (STEPCAM) approach in the Material and methods section.

- MC3: questioning the validity of translating morphology into a metric of gastropods' niche and therefore into the agent of species selection during the community assembly process.
  We used a taxonomic classification for inferring the importance of niche-based environmental filtering and species interaction (see MC2). We will modify the respective section to make this clearer to the reader. However, no matter whether morphological or taxonomical similarity is used, this concern is a serious criticism. It questions fundamental assumptions about using functional or phylogenetic metrics for estimating the relative importance of community assembly processes. This methodological revolution shifted community ecology from a correlative approach relating certain abiotic proxies with community composition to a process-based perspective and facilitated the finding of general assembly rules (reviewed in Emerson and Gillespie, 2008; McGill et al., 2006; Webb et al., 2002). The underlying assumption is that ecological selection (i.e., natural selection without any sexual selection) results in similarity of anatomical, morphological, and behavioral traits of species because environmental adaptations and traits are valid metrics of niche. Phylogenetic analyses of community assembly assume that closely related species do not differ much in traits because of their recent divergence and need to inhabit similar environment due to environmental adaptation of their ancestors. Therefore, phylogenetic measures, too, are a valid metric of niche (Pausas and Verdú, 2010). In this reply

letter (see below), we demonstrate that traits of closely related gastropod species of Lake Ohrid resemble each other more than expected by neutral divergence. We also show that taxonomic classification is a valid metric of niche.

- MC4: circularity in defining first eco-zones within Lake Ohrid (i.e., areas of similar species composition) and later use those areas to test whether they correlate with species distribution.
  First, we inferred the relative importance of community assembly processes. Then we delineated eco-zones based on similarity in gastropod community composition and their geographic and bathymetric location. Finally, we tested whether the importance of the assembly processes itself, and not the distribution, differed among eco-zones. Of course, the assembly processes eventually give rise to the species distribution but we do not consider both being equivalent and thus not committing circular reasoning.

- MC5: too far reaching implications of our actually inferred community assembly processes on modes of speciation.
  We do think that there is a sound theoretical basis for relating assembly processes with corresponding modes of speciation. However, in the revised ms, we will focus more on what we actually tested and less on their evolutionary implications. We will also modify the title of our manuscript accordingly as following: "Processes of gastropod community assembly in ancient Lake Ohrid: a metacommunity perspective"

Reviewer #1 (Thomas Neubauer)

General comments

The authors present a highly interesting study on the impact of community assembly processes on the structure of gastropod communities in Lake Ohrid. While most other studies focus on single processes, the authors apply an innovative and very comprehensive approach using up-to-date statistical analyses and simulation techniques (and even inventing new ones), in order to integrate as much information as possible. They convincingly demonstrate the importance of dispersal limitation, and to a lesser extent environmental filtering and species interactions, and that the specific impact of each process varies spatially. The results and conclusions on the non-exclusive roles of ecological and geographical speciation are important advances in the study of speciation in ancient lakes and speciation in general.

The hypothesis of the study is clear and the methods are sufficiently outlined and support the results. Title and abstract reflect well the content of the paper. The paper is well written and structured and the English is of a high quality. The authors give proper credit to published studies in the field.

I have only a few remarks, which rather are suggestions, and therefore recommend publication after minor revision.

Thank you!

Specific comments

I would like to see an additional figure showing the geographic position of Lake Ohrid (similar to the one you give in Föller et al. 2015 Biogeosc. Disc.). This would help readers not familiar with the lake and where it is located. The other figures are well made and essential to show sampling sites and eco-zonation and to highlight the relative importance of the three processes depending on the eco-zones.
We will include a map showing the location of Lake Ohrid (see MC1):

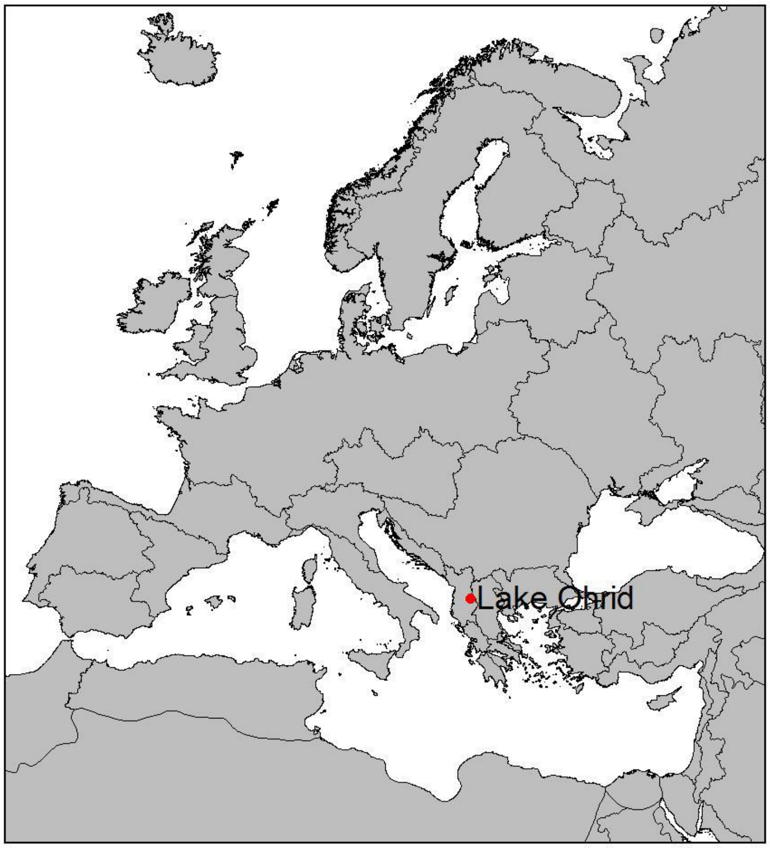

Figure 1. Location of Lake Ohrid and freshwater ecoregions.

The authors mention three times in the text (Abstract, Introduction, Conclusion) that the findings contribute to the goal of the SCOPSCO initiative. Maybe you could take it out in either the conclusion or the abstract.
True. We will therefore remove the explicit reference to the SCOPSCO initiative in the middle and at the end of the introduction.

Reviewer #2

General comments

The objective of this work is to use a largely endemic, diverse gastropod fauna to:

- Quantify and rank several ecological and evolutionary processes that are potential drivers of community assembly using a simulation model
- Test whether lake depth or additionally refined 'eco-zones' are better correlated with these quantified processes

This is the first application of this particular set of process-level ecological and evolutionary models to gastropod community data in an ancient lake. Other metacommunity analyses have been done in other systems, though the topic is in its infancy so the work is novel in its specific application. Its good to see a freshwater mollusc radiation being used for this kind of interesting process-level question, and gastropods are an appropriate focal group. Lake Ohrid is a fascinating, diverse model system.

The processes are stated to be neutral or selective. Dispersal limitation is considered a neutral process, whereas selection by physical agents (what the authors call environmental filtering) or by biological agents (here called species interaction) are active processes. The authors say they are looking for either a gradual or stepped change in the relative importance of these processes in the lake, as this might then help link biological and physical processes in the lake. They also mention that this informs on the role of speciation+extinction in community assembly, but I find that connection un- or minimally- addressed by the data in hand, as we don't yet have a phylogenetic context input into this analysis, nor do I see extinction data being considered. It might simply be that the text needs to be clarified on this.

I have a key concern with this paper - it is difficult to see how the variables (the basic data, traits or characters) going in to the analyses were derived and what they really reflect. Perhaps this just requires clarification in both the text and supplement.
We do understand that many readers may have difficulties to precisely understand our modeling approach. This might be due to the facts that the concepts applied are still in their infancy and that the actual approach used has been existing for few months only (also see MC2 and MC3). In the revised version of our ms, we will therefore include a more detailed description of our approach as well as a new flowchart for our community assembly model (Fig. 2). We will also add a description (Readme.txt) to the supplements with all steps for reproducing our analyses. All necessary files plus a script for running the analyses on one click were already included in the original version of the ms. Please note that we never spoke about extinctions in our ms. However, according to the reviewer's suggestion, our discussion will stays closer to our analyses of community assembly, and implications on speciation will be down-toned (MC5).

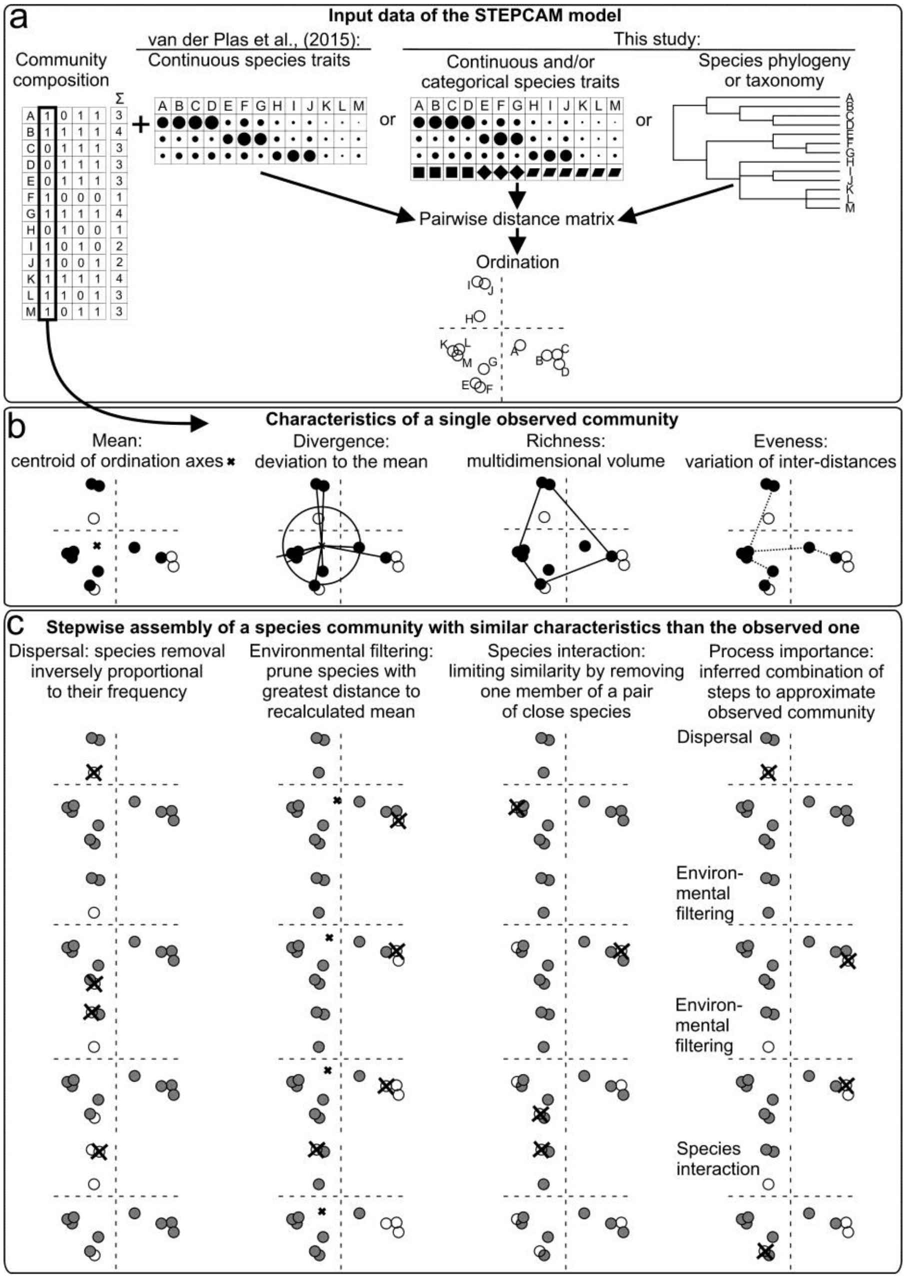

Figure 2. Illustration of the distance-based stepwise community assembly model (STEPCAM). (a) The model is parameterized by species' frequencies and either species traits or a species phylogeny or a hierarchical taxonomic classification, respectively. (b) For the focal species community, four, depending on the input, either functional or phylogenetic/taxonomic indices are calculated (for details see Villéger et al., 2008). (c) These index-values are the targets for the modeled community. A simulation starts with all species, stepwise removes species according to the three processes dispersal limitation, environmental filtering, and species interaction, and stops when the same number of species as in the focal community is reached. The process-combination that generates a species community with minimum differences between observed and generated index-values is inferred through Approximate Bayesian Computation within a Sequential Monte Carlo framework.

The ecological variables reflecting ‘niche’ are derived in an unspecified way from morphology. The authors say “these ecological requirements are assumed to be reflected by morphological features because the latter are often under selection”. I could not find an explanation of how morphology was translated to be a metric of niche. How was phylogenetic constraint (or signal, or history) removed from morphology so that it reflects ecological niche in a reliable, repeatable way? This seems essentially impossible at a broad scale – gastropods look quite different based on their evolutionary lineages. The argument that because selection can influence (at least some aspects of) morphology (in some gastropods), does not justify then using morphology as an overall metric of selection, and thus of an even more derived variable of ecological niche. Examples of selection changing gastropod morphology are of course numerous, but the transfer function is usually complex and lineage-specific, often working on quite fine scales. In addition, there are many examples of apparently non-adaptive differences in morphology, such as colour patterns on cone shells. Going from selection >>morphology is a black box that is a challenging puzzle within a single lineage; doing it as a blanket process doesn’t seem supportable. As the potential agents of selection on Ohrid snail shell morphology aren’t specified, it is not clear what part of the ecological system morphological differences might reflect. As an example, do these snails all have shell-crushing predators, and do these respond more to changes in shell thickness, sculpture or size? In addition, measuring molluscan morphology is an analytical challenge. Accretionary, spiral growth means one has to disentangle homologous and non-homologous changes in more explicit ways than for organisms with unitary growth. I could not find explanation of how this key variable for the analyses was derived, and I can’t readily imagine how it can be used for its stated purpose in the analysis. Perhaps this just needs clearer explanation.

Please note we translated our taxonomic classification into a metric of niche and did not use morphology. We fully agree with the reviewer about the power of morphological transfer functions. This is exactly the reason why we did not use morphology for the calculation of our similarity matrices but taxonomy. The latter appears to be much less prone to problems of homoplasy in gastropods from Lake Ohrid as the taxon has been studied exceptionally well in the past years (e.g., Albrecht et al., 2006; Föller et al., 2015; Hauswald et al., 2008; Schreiber et al., 2011; Wilke et al., 2007). Ricotta et al., (2012) showed that the conclusions of community assembly studies drawn by using phylogenetic or taxonomic distances are equivalent. However, in phylogenetic and functional community analyses both, phylogenies and morphological traits are shown to be valid metrics of ecological niche (see MC2; Pausas and Verdú, 2010; Webb et al., 2002). In fact, phylogenetic and functional community analyses often assess the equivalency of both metrics by testing for a phylogenetic signal in traits (i.e., closely related species resemble each other more than expected by neutral divergence) and do not aim to remove it. For this reply letter, we quantified the volume of our gastropod species through the Archimedes principle and found a strong phylogenetic signal in our taxonomic classification (Fig. 3). We could only use volume as a measure of body size because most other morphometric measures are not comparable among distantly related species (see the specific comment on non-homologous characters below). Body size is directly related to the ecological niche of the species because it restricts potential position in microhabitats. We will keep the valid point of species divergence along differential utilization of food resources in mind (see two other points of criticisms below, but also Sitnikova et al., (2012, 2014) for resource partitioning in ancient lakes gastropods and some analytical challenges related to it). Because we did not use shell morphology (see

MC2) we also did not specify any agent of selection on this phenotype. There are no major shell-crushing predators, only a small area in the western side of Lake Ohrid with few crabs.

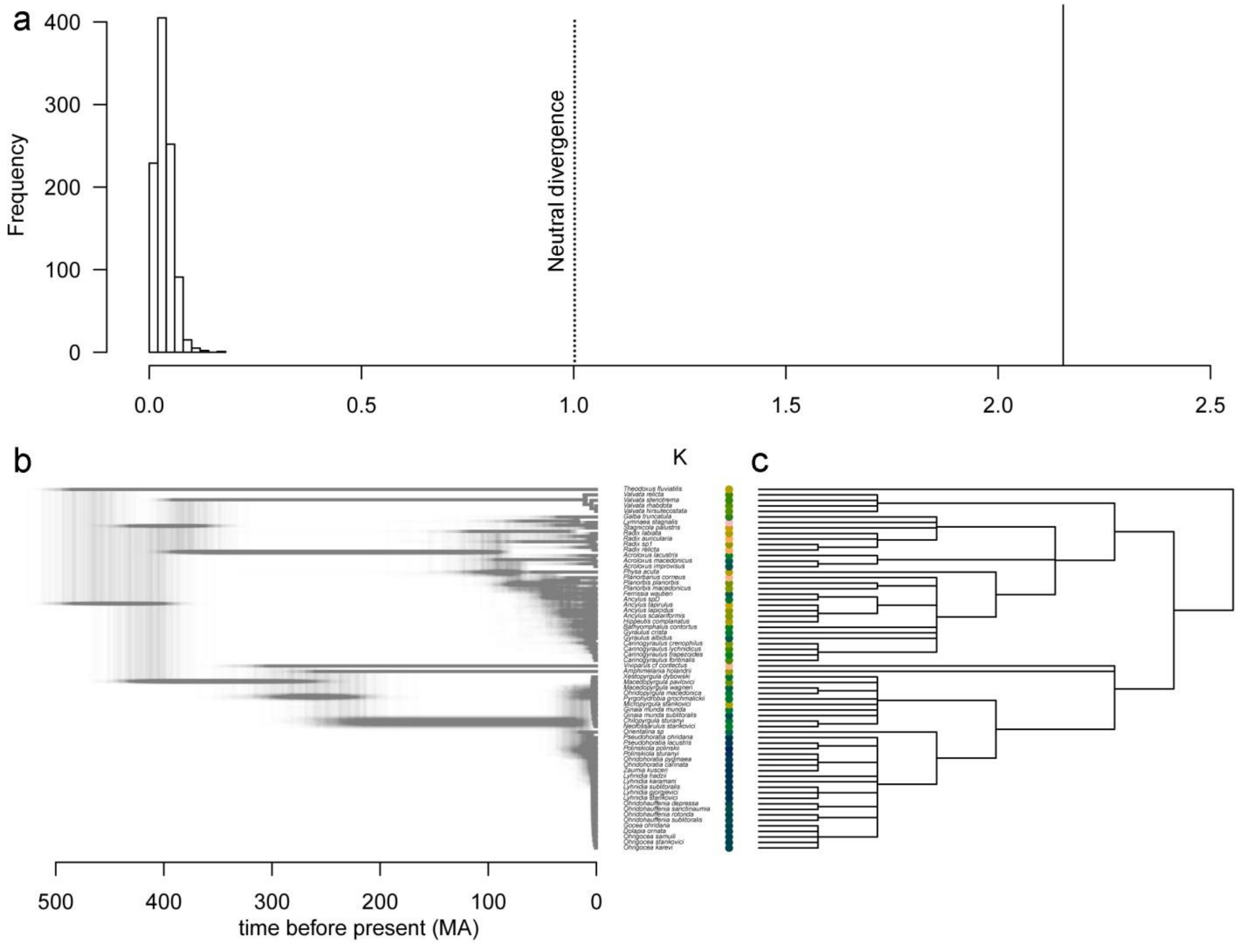

Figure 3. Closely related species show higher similarity in their body size than expected by neutral (i.e., non-adaptive) divergence. We found a (a) significant and stronger phylogenetic signal K (Blomberg et al., 2003) *of body size (colored dots) in our (c) taxonomic classification than in (b) 1000 hypothetical phylogenetic histories. Because no comprehensively dated phylogeny of freshwater gastropods including young and old splits exists, we inferred hypothetical phylogenetic histories based on published phylogenetic trees (Albrecht et al., 2007, 2008; Dinapoli and Klussmann-Kolb, 2010; Föller et al., 2015; Hauswald et al., 2008; Jörger et al., 2010; Wilke et al., 2007; Zapata et al., 2014). These known tree topologies, dated speciation events and genera memberships ('prior knowledge') were subjected to a Bayesian inference in BEAST 1.8.2 (Drummond and Rambaut, 2007) that simulates hypothetical phylogenetic histories and their uncertainties by sampling from these priors only. Uncertainty is shown by the overlay of the hypothetical phylogenies.*

Because of the stronger phylogenetic signal of an important niche metric in our taxonomic classification, we are confident that for our analysis of community assembly processes this classification is an even more robust variable than a dated phylogeny.

Similarly, the variables that were to reflect dispersal were apparently derived from current species distributions. This again seems many steps removed from what is intended to be the causal variable of limited ability to reach new habitats. I would expect that one couldn't comment on dispersal without some indication of gene flow, i.e., data from population genetics, and discussion of potential dispersal stages (e.g, if eggs, juveniles, or brooding mothers are likely to travel passively or actively. Reference in this section to adult movement rates for gastropods in mark-recap studies does not seem particularly enlightening as dispersal may well happen through passive means at other life stages.) While I have sympathy that population genetic data is not yet available for these taxa, I'm not convinced that current distributions are a contradiction-free proxy for dispersal. Again perhaps this just needs clearer explanation.

A principal assumption of the STEPCAM model is that current distribution patterns are caused by past dispersal processes (Janzen et al., 2016; van der Plas et al., 2015). In fact, in the absence of environmental heterogeneity, dispersal limitation is the only process that can cause differences in species' frequencies because environmental filtering and species interaction (e.g., competition for resources) cannot act. The species' frequencies are therefore used to parameterize the model (Fig. 2) without intermediate steps.

Dispersal itself can be limited by several abiotic and biotic factors. We have no indication that water currents are involved in creation of distribution patterns (and thus could act as dispersal mean). As for other life stages than adults as potential dispersal stages, we need to stress here that with the exception of one widespread species (Viviparus cf. contectus) no brooding occurs among Lake Ohrid gastropods (see Michel, (1994) for consequences of reproductive strategies on species distribution). Eggs are either laid on solid surfaces or might be carried by the adults. We therefore think that mark-recapture studies give an informative estimate of the mainly active dispersal capacity.

Of course it would be nice to have a population-genetics based background for each of the species living in Lake Ohrid for estimating gene-flow und thus (indirectly though) dispersal rates. As the reviewer admits, this is wishful thinking rather than reality for the time being.

Another variable used was a matrix of classification similarity – using taxonomy plus a level of common clade membership to derive a similarity metric. This looks straightforward enough, but needs to have the classification itself presented. The classification used to generate the data is referenced as a composite of three past works, but this composite needs to be provided somewhere in order to be repeatable. It should also be noted that of course classification similarity will have some redundancy on the morphological metric. Limpets are in their own family with a high 'dissimilarity score' from hydrobiids in this measure, but they will also have a great difference in the shape metric.

We respectfully disagree with the reviewer as our analysis, indeed, is reproducible: the pairwise classification similarity, together with all scripts were already included in the supplements of our original ms. Now, we also provide a supplementary figure (see Fig. 3c) and a separate "Readme.txt" for reproducing our whole community assembly analysis (See also criticism #15 in the editorial questions below).

Moreover, apart from gastropod community composition, the 'classification similarity' was the only variable used in our study (see MC2). In fact, we did not use morphology in the original manuscript. We only employed one morphological trait here in the reply letter to support an equivalency between 'classification similarity' and morphology, both assumed to reflect the niche of a species (e.g., Emerson and Gillespie, 2008; McGill et al., 2006; Webb et al., 2002).

Is this classification similarity also the same as the ‘relatedness’ variable that is mentioned in the ABC-SMC model run?
Yes, it is. We modified the respective sentence and now refer to ‘phylogenetic or taxonomic distances’, just as we did in line 6 of the same page (16087).

It seems that the variable of ‘ecozones’ are partly determined by depth, and then given added granularity by using species distributions to define them further, subdividing them in to an added lateral component. They then correlate better with data on distributions. Isn’t this circular? Aren’t the real environmental causal variables some combination of depth (linked with food availability, wave energy, oxygenation, other species presence, etc.), substrate, sedimentation, water chemistry, etc.?
See our reply to MC4. It is not circular reasoning, because we first inferred the relative importance of assembly processes and later tested whether those differ among eco-zones, which are characterized by similar gastropod community composition and space. The assembly processes are supposed to have caused the gastropod community composition but they are not the same. We do not search for ‘causal variables’ that are later typically interpreted as proxy for processes, but instead infer the process directly. In our previous study of causal variables (Hauffe et al., 2011) we showed that depth and nutrient related proxies correlate with gastropod community composition. Moreover, we identified a strong spatial autocorrelation in gastropod community composition. We interpreted these correlations as the processes of environmental filtering and dispersal limitation. However, in the current study we quantified these processes directly, as it is recommended for modern community analyses (McGill et al., 2006).

For each of these variables it seems that they are a highly derived, combined measure that is some distance away from what they are purporting to measure. I would feel much more comfortable with more clearly defined metrics in each case – things like the actual ecological variables, measures of gene flow, habitat descriptors. As it is, I’m not really sure what is being correlated with what, and if there is any explanatory power left after the underlying variables have been confounded in so many ways.
As explained above (MC2 and MC3), the variables are neither derived nor combined but the taxonomic classification informs about two different processes (but see also Figure 2). It is strongly believed that this approach has its merits because it makes the importance of processes directly comparable (Janzen et al., 2016; van der Plas et al., 2015). *If we would quantify gene flow among populations of individual species, we still a) would not know how dispersal influences community composition, and b) could not compare it with how strong environmental variables correlate with community composition. The STEPCAM model does not provide a direct measure of explanatory power like in such correlative studies (e.g., the coefficient of determination; r^2). We discussed with the developers (see our acknowledgments) such a possibility but there were good statistical reasons against it. However*, van der Plas et al., (2015) *showed indirectly the explanatory power of the STEPCAM model by comparing generated community composition with the observed one ($r^2 = 0.3$). Moreover, these authors provided a sensitivity analysis showing that the analysis infers correctly the processes, which were used to generate artificial test-data of community composition.*

It is also not clear to me how the reflexive nature of the model's analytical sampling is correlated to, or informs on, real-life processes. On this point the paper should be examined by a modeler (or statistician) who has extensive experience with these kinds of models in general.
We do think dispersal, environmental filtering, and species interaction are real-life processes assembling species communities. However, we do acknowledge that real-life processes are more complex. For instance, the developer of the model already suggested that the processes act on individuals and not species (van der Plas et al., 2015). We discussed methodological issues with the programmers of the model (see our acknowledgments).

I also felt that the paper would benefit from discussion of additional comparisons with research at the boundary of ecological and evolutionary processes in other lakes. For example, I would have expected discussion of the decades of results from Frans Witte and Ole Seehausen's labs on Lake Victoria cichlids. Sexual, trophic, habitat selection (etc.) all play in to diversification, extinction and community dynamics and have been ranked by their strength and timing of effects on the Victoria cichlid species flocks.
According to this suggestion, we will focus in the discussion of our revised ms on community assembly and down-tone the implications on speciation processes (see MC5). We refrain of discussing more evolutionary studies, especially of cichlids that diversify under strong sexual selection. However, we refer to a new study on cichlid community composition in Lake Tanganyika using the same model and showing the same tendencies in importance of assembly processes (Janzen et al., 2016).

Specific comments

In the Introduction the authors make a bold statement in a few starting sentences that changes in climate, geology, etc, only affected species abundances and community compositions, not speciation and extinction. However in the next set of sentences they say that abundance and composition "indirectly fostered species divergence …. Speciation, and extinction processes, in turn, also affect the spatial structure of extant communities". This reads like a 'yes-but-no-but-yes' bit of waffle, setting up the paper to be unclear throughout. My feeling was that the paper should be more modest in overall aims, but try to be clearer about what actually is being tested, what was shown, what it might mean, and how much confidence we can have in the results. It seems to a paper testing community assembly with fairly straightforward (but minimal) data on presence absence, but it gains its strength through having many sample sites and using simulation to generate statistical context.
We will rework the respective parts.

The Methods section lists:

Gastropod sampling and taxonomic classification –

This covers information on:

- Geography of sites – good and necessary – and supported by a map.
- A general statement on the classification similarity metric – but this needs to be supported by the actual classification. A further explanation on the classification/taxonomy/relatedness metric follows a full page later, but this should be moved up in the section on variables (traits). *We do not understand this comment because we explain the taxonomic classification directly after the geography of sites.*

And lacks:

- Details on how abundance data was taken (or was it? From the supplementary files all I can see is a P/A (presence/absence species/site occurrence list, but species richness is included in the output, which often includes an abundance measure, and is also mentioned in the ABC-SMC model text). E.g, were consistent surface areas sampled, time counts done, volumes from grabs picked, etc?
- Specifics on what kind of species richness metric was used
- Explanation of morphological/environment metric – this will require quite a lot of clarification, as it's a complicated thing and I'm not sure it works
- Explanation of dispersal metric (called a dispersal limitation metric, which is essentially an inverse). Isn't it simply an occurrence metric?

Correct. We used presence/absence data and the simple count of species as richness metric. Now we will mention these facts explicitly in the manuscript. Strong habitat heterogeneity requires different sampling strategies and species abundancies would not be comparable. In littoral stretches of approximately 50 m length, we always collected with 3-5 persons for an hour. We sampled depths > 5 m until a dredge was half-full or, in case of extremely rocky substrate, 10 min passed. Unpublished species accumulation curves for different habitats show that we reached an asymptotic relationship between species richness and specimens. This means, with an increasing number of sampled specimens we did not find more species and our simple metric of species richness is robust. More sophisticated richness metrics either include (e.g., Shannon diversity) or correct (rarefaction indices, e.g. Chao) for variation in species abundancies, but as mentioned above, number of sampled specimens depend on sampling strategy. In any case, our analysis of community assembly requires simple species counts.
For the issue of missing explanation of morphological and dispersal metric see above.

(note in this section the authors say “Species flocks likely evolved intralacustrine [sic] and are common in Lake Ohrid.” - but by definition a species flock evolved within the system. Better to just call them endemic clades that include several genera’.)

Done. Thank you for the suggestion.

Dispersal limitation – The paper says “STEPCAM simulates dispersal limitation by randomly removing species with a probability inversely related to their relative frequency in the regional species pool.” – is this a jackknife or a bootstrap resampling method (with or without replacement)? It is not clear to me how this informs us about dispersal limitation. I would have thought data on dispersal would need population genetic information on gene flow. Doesn’t this simply show that narrow-range taxa have a greater influence on differentiating communities? The narrow range could be due to other factors than lack of dispersal, for example, lack of appropriate substrate.

This concerns MC2 and MC3. STEPCAM *is neither a jacknife nor a bootstrapping method because the aim is not to evaluate the robustness of a result. See Fig. 2 and the additional explanations given in lines 200-204 for the purpose of species removal. Of course, narrow-range taxa do have a greater influence on differentiating communities because widespread species are present in most sites and cause high similarity among communities. If a narrow range would be determined by the availability of appropriate substrate, this should be reflected in a species community by the co-occurrence of closely related species of presumed niche similarity. Therefore, the relative importance of the environmental filtering over the dispersal limitation process shows the influence of abiotic variables.*

Environmental filtering – this seems to be an analytical pruning of species that are outliers, based on the metrics (traits) assessed and production of a comparison community by simulation. This assumes selection is for greater homogeneity, not for greater diversity. It is the classic debate about the relative roles of stabilizing, vs disruptive (or diversifying) vs directional selection. Intuitively, I would expect some diversifying selection if a habitat had diverse structure and roles, as is indicated by having elevated biodiversity. Just a bit more explanation of this would help the reader be less dubious, I’d think.

This relates to MC2 and MC3. Yes, the modeling step can be compared with pruning of extremes. According to the assumptions of phylogenetic community ecology for environmental filtering, co-occurring species should indeed be selected by homogeneity in their ecological requirements (Webb et al., (2002); see Fig. 2). Hubert et al., (2015) showed the importance of spatial and temporal scales in the corresponding metacommunity speciation model. A high within habitat diversity could exceed the habitat diversity of the whole ecosystem, which equals a homogenous landscape. This scenario predicts community assembly through dispersal and species diversification via geographical barriers and a neutral divergence of traits. In contrast, smaller variation within than among habitats leads to a community assembled by environmental filtering and ecological speciation with directional trait divergence (Hubert et al., 2015).

The Methods section on analysis has parts of the definition of the variables mixed in. This makes it harder to see exactly what the whole data set comprises and how it was derived. For example, the authors explain that: “The similarity between simulated and observed communities is the sum of absolute differences between four indices (i.e., richness, evenness, divergence, and mean distance to the community centroid) based on traits or relatedness." But there hasn't been a definition of these indices earlier, and the basic data does not seem to be available in the supplement.

This, again, refers to MC2 and MC3. We will clarify the meaning of the indices using a new flowchart (Fig. 2) and a revised method section. These are only a measure for evaluating how similar a simulated and the observed gastropod community composition are and were discussed in the original description of the STEPCAM model (van der Plas et al., 2015).

Often the term 'gastropod composition' is used. Shouldn't this be 'gastropod community data' or 'community species composition'? Gastropod composition would mean what the snails are made of, e.g., carbon, nitrogen, water, mucous, etc… Community composition is the smaller units that communities are made of, i.e., species. Data is a clearer, simpler word however, so might be preferable.

Correct. The term 'gastropod composition' is, indeed, misleading. We will therefore use 'gastropod community composition' in all cases where we refer to the gastropod species composition of the sampling sites.

While I also have done analyses on gastropod communities as an analytical topic, I will add that I think a more realistic portrayal of biotic communities should include other taxa. Snails might very well have much stronger interactions with their food, predator and parasite neighbours than with other gastropods. But I also agree that we have to start somewhere in figuring out how ecological communities are assembled, so limiting by taxon is a reasonable first pass. It might be a good idea to mention this early on, however. (Only in the end do the authors mention a comparison with diatoms).

We fully agree with the reviewer and in the revised ms, we therefore will stress that we performed a case study. Robert E. Ricklefs would certainly agree with the perspective of host-parasite interactions driving biodiversity, although acknowledging that this "community-level relationship…are only beginning to be analysed" (Ricklefs, 2015). Unfortunately, we are only at the beginning of creating an inventory of ancient lake parasites (Kmentová et al., 2016; Vanhove et al., 2015) or characterizing major features of trophic networks in ancient lakes, such as bacteria (De Wever et al., 2008), fungi (Čomić et al., 2010), or the complexity of trophic chains (Doi et al., 2012). According to a recent critical review of multiple definitions and actual use of the term 'community' (Stroud et al., 2015), our gastropod community composition falls in-between 'community' and 'assemblage'. However, the term 'community' is better known by a broader audience and adding the higher-level taxon (i.e., using 'gastropod community composition' as the reviewer suggested) elucidate the considered taxonomic scale of co-occurring species (Stroud et al., 2015).

The results show that things are all different in the SE of the lake - clearly the springs have a major effect, turning the processes around.

Exactly.

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List of relevant changes according to the points of major criticism:

- We included a map showing the location of Lake Ohrid (New Fig. 1).
- We completely rewrote the Material and methods section and included an additional flowchart sketching how the individual importance of community assembly processes was inferred (New Fig. 2). The Supplement of our reproducible analysis now contains a Readme.txt
- We modified the title of the manuscript, removed large parts of manuscript focusing on speciation, and included an additional comparison with a comparable study investigating cichlid community assembly in Lake Tanganyika.
- We reworked the English, removed passive constructions as much as possible, and included the suggestions of the reviewers.

Assembly processes of gastropod communities change with horizontal and vertical zonation in ancient Lake Ohrid: A metacommunity speciation perspective~~Gastropod diversification and community structuring processes in ancient Lake Ohrid: A metacommunity speciation perspective~~

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Abstract

The Balkan Lake Ohrid is the oldest and most ~~speciose~~ diverse freshwater lacustrine system in Europe. However, it remains unclear whether species community composition and the diversification of its endemic taxa is mainly driven by ~~neutral processes~~dispersal limitation, environmental ~~factors~~filtering, or species interaction~~s~~. This calls for a holistic perspective involving both evolutionary processes and ecological dynamics~~.~~, as provided by the ~~Such a~~ unifying framework ~~–~~of the ‘metacommunity speciation model’.

~~– considers how community assembly affects diversification and *vice versa* by assessing the relative contribution of the three main community assembly processes, dispersal limitation, environmental filtering, and species interaction.~~

The current study ~~therefore~~ used the species-rich model taxon Gastropoda to assess how extant communities in Lake Ohrid are structured by performing process-based metacommunity analyses. Specifically, the study aimed at i) identifying the relative importance of the three community assembly processes and ii) to test whether the importance of these individual processes changes gradually with lake depth or ~~whether they are~~ discontinuously with eco-zones shifts~~distinctively related to eco-zones~~.

Based on an automated eco-zone detection and process-specific simulation steps ~~for each of the three processes~~, ~~it~~ we ~~could be~~ demonstrated that dispersal limitation had the strongest influence on gastropod community ~~structures~~composition ~~in Lake Ohrid~~. However, it was not the exclusive assembly process but acted together with the other two processes – environmental filtering~~,~~ and species interaction. ~~In fact, t~~The relative importance of the ~~three~~ community assembly processes varied both with lake depth and eco-zones, though the processes were better predicted by the latter.

This suggests that environmental characteristics have a pronounced effect on shaping gastropod communities via assembly processes. Moreover,

~~T~~the study ~~thus~~ corroborated the high importance of dispersal limitation for both maintaining species richness in Lake Ohrid (through its impact on community ~~structure~~composition) and generating endemic biodiversity (via its influence on diversification processes). However, according to the metacommunity speciation model, the inferred importance of environmental filtering and biotic interaction also suggests a small but significant influence of ecological speciation. These findings contribute to the main goal of the SCOPSCO deep drilling

initiative – inferring the drivers of biotic evolution – and might provide an integrative perspective on biological and limnological dynamics in ancient Lake Ohrid.

1 Introduction

Ancient Lake Ohrid on the Balkan Peninsula (Fig. 1) is the oldest and most speciose freshwater lacustrine system in Europe (Albrecht and Wilke, 2008; Neubauer et al., 2015). Recently, an International Continental Scientific Drilling Program (ICDP; Wagner et al., 2014) has been conducted in the lake within the research initiative Scientific Collaboration on Past Speciation Conditions (SCOPSCO). One of the major goals of this collaborative project is to infer the drivers of speciation by linking the geological and biotic evolution of the lake over space and time. ~~In order to reach this goal, g~~Geological, limnological, and paleontological data from sediment cores are being complemented with phylogenetic and molecular clock data derived from DNA information of extant endemic species.

Though the sediment and molecular data are still being analyzed, first results ~~of the SCOPSCO campaign~~ indicate that climatic, geological, and/or environmental changes over time may have had little direct effect on speciation and extinction processes in selected endemic biota of Lake Ohrid (Föller et al., 2015). Instead, such changes ~~likely~~ potentially affected abundances of paleospecies ~~as well as~~and ~~species~~ community compositions over time (Albrecht et al., 2010; Belmecheri et al., 2009; Jovanovska et al., 2016; Reed et al., 2010; Wagner et al., 2014)~~, which, in turn may have affected speciation processes~~. ~~Similar effects could be shown for h~~Horizontal and vertical (bathymetric) environmental gradients~~. They~~ may not only have shaped the structure of extant Ohrid communities (Hauffe et al., 2011; Lorenschat and Schwalb, 2013), but also ~~indirectly~~ fostered species divergence (Schreiber et al., 2011; Wysocka et al., 2008). Speciation~~,~~ and extinction ~~processes~~, in turn, may have also affected the spatial structure of extant communities (Hauffe et al., 2011).

For Lake Ohrid, ~~T~~these results question the direct effect of climatic, geological, and/or environmental factors on diversification and call for a holistic perspective involving evolutionary processes (e.g., speciation) and ecological ~~dynamics~~ processes affecting community composition (e.g., dispersal limitation, species interaction) ~~(reviewed in Hubert et al., 2015)~~.

Such a mechanistic and unifying eco-evolutionary framework – the metacommunity speciation model – was recently proposed by Hubert et al. (2015). It considers how a metacommunity (i.e., a network of local communities linked by dispersal of multiple interacting species; Leibold et al., 2004) is affected by speciation and *vice versa*.

In principle, a local community is structured by three non-exclusive consecutive processes (e.g., Leibold et al., 2004; van der Plas et al., 2014): (1) ~~random~~ dispersal of individuals to a patch previously unoccupied by the species, (2) environmental filtering that permits the establishment of species whose ecological niche fits the local environmental conditions, and (3) species interaction including competitive exclusion among resident and colonizing species because of shared resources, predators, and/or pathogens.

From a theoretical point of view, all of these three processes might affect speciation (Hubert et al., 2015). However, their individual contribution and spatial dynamics in Lake Ohrid remain little understood. The latter is particularly true for the bathymetric structure of Lake Ohrid. The deep lake has a ~~large depth but also a~~ distinct horizontal eco-zonation (reviewed in Albrecht and Wilke, 2008). This leads to the question whether the individual importance of the three processes mentioned above gradually changes with lake depth or whether there are abrupt changes related to transitions between eco-zones. Conceptually, a differentiation between gradual and abrupt changes in structuring processes among eco-zones ~~is of high importance for understanding~~may shed light on how limnological complexity and stability affect community assembly and diversification processes.

Gastropods are ~~A~~a suitable model taxon to study community structuring processes ~~are the Gastropoda~~ as they represent the ~~largest~~ most species-rich animal ~~taxon~~ group in Lake Ohrid with 74 described species, 56 ~~of which being~~ endemic to the lake and its catchment (Albrecht et al., 2014; Albrecht and Wilke, 2008; Hauffe et al., 2011; Radoman, 1985). In the current study we therefore focus on the question how extant gastropod communities are assembled by performing process-based metacommunity analyses. Our working hypothesis is that, in general, dispersal limitation plays a crucial role in structuring communities.

Our specific aims are:

1. To identify the relative importance of the three community assembly processes (dispersal limitation, environmental filtering, and species interaction) in shaping the ~~lake's~~ lakes' gastropod community composition~~s~~. We simulated community assembly and measured the respective simulation steps corresponding to these three processes.
2. To test whether the importance of these individual processes changes gradually with lake depth or whether they are distinctively related to eco-zones. We first performed an automatic detection of eco-zones based on community ~~data~~ composition and then

inferred whether the relative importance of the three community assembly processes was better predicted by either lake depth or delineated eco-zones.

This study will ~~contribute to one of the major goals of the SCOPSCO deep drilling ~~help to infer~~ring~~ the drivers of community assembly and speciation in Lake Ohrid ~~—~~by providing an independent and unifying view ~~at ~~of eco-evolutionary processes and their relationships to ecosystem characteristics. The novel process-based model presented here may also serve as a basis for studies of the community ~~structures ~~composition and their underlying assembly processes in (paleo)species communities of Lake Ohrid, other ancient lake systems, or elsewhere.

2 Material and methods

2.1 Gastropod sampling and taxonomic classification

The current study is based on gastropod community compositions ~~information ~~taken from Hauffe et al., (2011), which were supplemented with additional field data obtained from 2010 to 2011. In total, we analyzed 264 localities of Lake Ohrid and its feeder springs~~ are analyzed~~. Gastropods were collected by hand picking, sieving, or dredging from small boats or the research vessel of the Hydrobiological Institute Ohrid. The latter enabled sampling depths of down to 70 m, thus covering the entire bathymetric range of gastropods in Lake Ohrid.

Though phylogenetic relationships among endemic gastropod taxa of Lake Ohrid are increasingly well characterized, a complete picture is still lacking. We classified ~~For critical taxa we here used the taxonomic assignments of Bodon et al. (2001), Hubendick and Radoman (1959), and Radoman (1983). T~~taxa ~~were classified ~~in five hierarchical levels: species, genus, species flock, family, and superfamily. Note that we ~~included ~~used the level ‘~~“~~species flock’~~”~~ for endemic clades that comprise more than one genus~~monophyletic groups of endemic species that may comprise more than one genus ~~(Föller et al., 2015; Wilke et al., 2007). ~~Species flocks likely~~Species flocks ~~-~~typically evolved ~~intralacustrine ~~within the lake and are common in Lake Ohrid. For critical taxa we here used the taxonomic assignments of Bodon et al. (2001), Hubendick and Radoman (1959), and Radoman (1983). We calculated pairwise taxonomic distances between the 64 collected gastropod species using the taxonomic classification above~~ with five hierarchical levels~~. This method ~~has been~~was described by

Clarke and Warwick (1998) and is implemented in the ~~R~~ package vegan v2.2-1 (Oksanen et al., 2015) for the R v3.1.2 statistical environment (R Core Team, 2015).

2.2 Simulation of individual community assembly processes

Processes of community assembly can be inferred by three distinct approaches. First, environmental parameters and measures of spatial autocorrelation are linked to community composition and may represent proxies for estimating indirectly the importance of processes such as environmental filtering (Sharma et al., 2011) and dispersal limitation (Legendre et al., 2005). Second, a comparison of the phylogenetic structure or trait distribution of a community with a null model of the expected structure or distribution informs about prevailing importance of environmental filtering or species interaction (Webb et al., 2002). Third, ~~van der Plas et al. (2015) suggested~~ a simulation method of stepwise community assembly (STEPCAM) infers the relative importance of all three processes (van der Plas et al., 2015). It prunes the regional species pool to the size of an observed community by sequentially removing species according to specific simulation processes for dispersal limitation, environmental filtering, and biotic interaction.

The simulation requires a presence-absence matrix of community composition and species characteristics such as traits or their taxonomic/phylogenetic relationship (Fig. 2a). We here extended the original STEPCAM approach, which only considers continuous species traits, and now allow a mixture of continuous and categorical species traits resulting in matrices of pairwise distance between species (Fig. 2a). This generalization also permits the use of phylogenetic or taxonomic distances. These distances are typically strongly correlated with trait divergence among species and used in analyses of community assembly (Emerson and Gillespie, 2008; Webb et al., 2002).

Trait or taxonomic characteristics of a community define the target of the STEPCAM model: if the values of trait/taxonomic indices of a simulated community approximate the observed ones, we assume that the performed species removal steps during the simulation reflect the relative importance of the three assembly processes for the observed community (see specific aim 1). The similarity between simulated and observed communities ($\text{Fit}_{\text{total}}$; van der Plas et al., 2015) is the sum of absolute differences between four indices (i.e., richness, evenness, divergence, and mean distance to the community centroid; Fig 2b) based on traits (Villéger et al., 2008) or phylogenetic/taxonomic distances (Helmus et al., 2007). However, calculating $\text{Fit}_{\text{total}}$ is problematic because the scale of the utilized indices differs: ‘evenness’, for example, is bounded between 0 and 1, whereas ‘richness’ lacks an upper limit. Therefore, van der Plas et al. (2015) standardized the trait indices by dividing them by the standard deviation (SD) of the respective index, calculated for all observed communities. For large differences in species richness or few sampled communities, this approach can be biased because some indices are not independent of species richness (Villéger et al., 2008) and the SD is less precisely estimated in case of small sample sizes. We estimated all SDs using an initial simulation of 500 communities based on observed species richness.

STEPCAM simulates dispersal limitation by randomly removing species with a probability inversely related to their relative frequency in the regional species pool (Fig. 2c). Dispersal limited species are therefore less likely present in the simulated community (van der Plas et al., 2015).

Environmental filtering and species interaction depend on the ecology of the species (i.e., its niche). These ecological requirements are assumed to be reflected by morphological features, ~~because the latter~~which are typically ~~often~~ under selection, or effected by the relatedness of species. Environmental filtering therefore predicts that species of a community show similar morphological traits or are closely related because environmental conditions only allow the establishment of species that are adapted to the specific ~~niche~~ habitat (Webb et al., 2002). ~~Environmental filtering is simulated by pruning~~ Pruning species ~~with trait values~~ most distant to the ~~trait~~ morphologically or taxonomically mean of the ~~simulated~~ generated community simulates environmental filtering (Fig. 2c).

Community composition driven by species interaction assumes that species with similar niches do not co-exist because they share the same resources, predators, or pathogens (Webb et al., 2002). Therefore, this process is simulated by removing one of two species from the regional species pool that -show the ~~most~~ highest similarity in their traits or taxonomy (Fig. 2c).

~~We here extended this original STEPCAM approach, which only considers continuous species traits, now to allow a mixture of continuous and categorical species traits based on pairwise distance matrices between species. This generalization also permits the use of phylogenetic or taxonomic distances, which are typically strongly correlated with morphological divergence among species (Emerson and Gillespie, 2008; Webb et al., 2002).~~

~~The number of species removed in each of the three simulation steps can be used as a direct measure for the relative importance of the three structuring processes (see specific aim 1).~~

Following van der Plas et al. (2015), we here utilized Approximate Bayesian Computation within a Sequential Monte Carlo framework (ABC-SMC) for inferring the number of species removals needed per simulation step, which results in a simulated community that approximates the observed community. ~~The similarity between simulated and observed communities (Fit$_{total}$; van der Plas et al., 2015) is the sum of absolute differences between four indices (i.e., richness, evenness, divergence, and mean distance to the community centroid) based on traits (Villéger et al., 2008) or relatedness (Helmus et al., 2007).~~

~~However, calculating Fit$_{total}$ may be problematic because different indices of community structure are bounded between 0 and 1, whereas others lack an upper limit. Van der Plas et al., (2015) standardized the diversity indices by dividing them by the standard deviation (SD) of the respective index, calculated for all observed communities. However, for large differences in species richness or few sampled communities, this approach can be biased because some indices are not independent of species richness (Villéger et al., 2008) and the SD is less precisely estimated due to the small sample size. Hence, we estimated all SDs using an initial simulation of 500 communities based on observed values for species richness.~~

Our distance-based community simulations built on the R package STEPCAM ~~package~~ v1.0 (Janzen and van der Plas, 2014) ~~for the R v3.1.2 statistical environment (R Core Team, 2015)~~. ~~However,~~ We improved the algorithm ~~was improved~~ for runtime and a commented, fully reproducible analysis including the taxonomic distance and ~~the community~~ presence-absence matrix of gastropod community composition is available in the Supplement.

~~We calculated pairwise taxonomic distances between the 64 collected gastropod species using the taxonomic classification with five hierarchical levels. This method has been described by Clarke and Warwick (1998) and is implemented in the R package vegan v2.2-1 (Oksanen et al., 2015).~~ The STEPCAM target for the assembly simulation for each of our 264 sampled communities was a final acceptance rate of ~~of~~ 1 in 10,000 simulated communities.

2.3 Testing for gradual vs. distinct changes among eco-zones

For testing whether the importance of individual structuring processes changes gradually with lake depth or ~~whether they~~ discontinuously with eco-zone shifts ~~are distinctively related to eco-zones~~ (see specific aim 2), reliable information for the spatial distribution of these zones is necessary.

Previous assessments of the vertical distribution of eco-zones in Lake Ohrid were based on analyses of habitat types, limnological features, and/or typical species assemblages (Albrecht and Wilke, 2008; Hauffe et al., 2011). In contrast, only a rough approximation of the horizontal dimension exists so far (Stanković, 1960).

Therefore, we first performed an automatic detection of eco-zones using a spatially constrained clustering of ecological networks (Miele et al., 2014). The analysis, implemented in the R package geoclust v0.2.0 (Miele et al., 2014), is based on the similarity of gastropod ~~communities~~ community composition and their geographical ~~adjacency~~ connectivity in horizontal (X and Y) and vertical (Z) dimensions. Community ~~Composition~~ composition similarity was calculated using Bray-Curtis distances and the vegan v2.2-1 package. Similarities were then arc-sin transformed to ensure normality, as required for ecological networks. The three-dimensional ~~adjacency~~ spatial network was based on ~~(i)~~ vertical (bathymetric) connectivity~~,~~ and ~~(ii)~~ the cost-distance between collecting points along isobaths. The latter approach had to be used because intralacustrine dispersal of benthic organisms is not well approximated by straight line distances (Heino et al., 2015b). We explored the robustness of our analysis by varying the number of directly connected neighbors from 15 to 264 with an increment of 10, ensuring a fully linked spatial network. For the vertical scale, a connectivity threshold of 8.2 m was determined by a multivariate community correlogram with 9999 permutations in vegan, showing that similarity between gastropod communities decreases significantly if their bathymetric distance exceeds this threshold.

In a final step, we used a Bayesian generalized linear model (BGLM; R package MCMCglmm v2.21; Hadfield, 2010) to test whether our multivariate response, i.e., the relative importance of the three community assembly processes, was better predicted by either lake depth or delineated eco-zones (see specific aim 2). As decision criterion between the two competing BGLMs, we utilized the deviance information criterion (DIC), a Bayesian measure of model fit (Spiegelhalter et al., 2002). In both BGLMs, we included species richness and its interaction with lake depth or eco-zones as additional predictor because process-importance may co-vary with species richness. Prior to model fitting, normality of the [0,1] scaled multivariate response was improved by an additive planar transformation, implemented in the compositions v1.4-1 package (van den Boogaart et al., 2014) for R.

3 Results

3.1 Relative importance of the three community assembly processes

Using our ABC-SMC-based STEPCAM approach, we obtained a posterior distribution for the relative contribution of the three community assembly processes (dispersal limitation, environmental filtering, and biotic interaction) in shaping the ~~lake's~~ lakes' gastropod community composition~~s~~. Averaging the posterior distribution over all 264 gastropod communities, dispersal limitation achieved the highest relative importance with 80.1% (95% bootstrapped confidence interval (CI): 77.0–83.0), followed by environmental filtering (mean 11.5%; CI: 9.4–13.8), and species interaction (mean 8.4%; CI: 7.2–9.7).

3.2 Testing for gradual vs. distinct changes among eco-zones

Prior to testing whether the importance of the three individual processes changes gradually with lake depth or ~~whether they~~ discontinuously with eco-zone shifts~~are related to distinct eco-zones~~, we performed an automatic detection of eco-zones using a spatially constrained clustering of ecological networks.

Applying a range of one to ten groups and different numbers of directly connected neighbors in the spatial network, our delineation of eco-zones consistently revealed that seven groups of gastropod communities showed the highest likelihood. These communities relate to seven eco-zones (southeastern (SE) upper littoral 1–3, ~~Non~~non-SE upper littoral, lower littoral, upper sublittoral, lower sublittoral). The horizontal and vertical ~~spatial~~ distribution ~~(horizontal and vertical)~~ of these eco-zones based on 65 neighbors is shown in Fig. ~~1~~ 3 (zone terminology roughly follows Stanković, 1960).~~ Clusters of communities across the different parameter settings were highly consistent, indicating a robust delineation of eco-zones.~~

Finally, we used two BGLMs to test whether the relative importance of the three community assembly processes was better predicted by either lake depth or delineated eco-zones. In general, assembly processes were explained both by depth and eco-zones~~ assumptions were supported~~. However, according to the rule of thumb of Bayesian model selection (Bolker, 2008), the difference in DIC ~~was~~ greater than 10 ~~and thus~~ decisively favored eco-zones over lake depth (DIC values of -861 versus -753, respectively). For a visual presentation of the eco-zone dependent relative importance of dispersal limitation, environmental filtering, and biotic interaction see Fig. ~~2~~4.

4 Discussion

In the current study, we addressed the question of how extant gastropod communities are assembled by performing a process-based metacommunity analysis. More specifically, we attempted to identify the relative importance of three previously suggested community assembly processes (dispersal limitation, environmental filtering, and species interaction) and tested whether their importance changes gradually with lake depth or ~~is ~~discontinuously with eco-zone shifts~~distinctively related to eco-zones~~. Given that geographical isolation has been stressed as important driver for speciation in Lake Ohrid (reviewed in Albrecht and Wilke, 2008) and adhering to the metacommunity speciation model, our working hypothesis was that dispersal limitation plays also a crucial role in structuring communities. We, indeed, could show that dispersal limitation had the strongest influence on gastropod community ~~structures~~ composition in Lake Ohrid. However, it was not the exclusive assembly process but acted together with the other two processes – environmental filtering~~,~~ and species interaction (Fig. ~~2~~4). In fact, the relative importance of the three community assembly processes varied both with lake depth and eco-zones (Fig. ~~2~~4), though the processes were better predicted by delineated eco-zones.

In the following, these principal findings are interpreted in the context of how ecosystem features influence community assembly. We also discuss the implications of our three community assembly processes for patterns and processes of speciation in endemic gastropods of Lake Ohrid. As structuring processes appear to be strongly related to eco-zones, we first address the result of our automatic eco-zones identification.

4.1 The eco-zones of Lake Ohrid

Previously, six horizontal and four vertical eco-zones have been suggested for Lake Ohrid (Stanković, 1960; Albrecht and Wilke, 2008). However, as they were largely based on an *a-priori* differentiation into horizontal and vertical features, we here automatically delineated eco-zones using a three-dimensional approach (i.e., involving the three spatial axes X, Y, and Z).

The zonation inferred (Fig. ~~1~~3) resembles, in large parts, the one previously proposed. However, it appears to be slightly more complex in terms of horizontal and vertical structuring. Moreover, the zones show a considerable degree of overlapping, reflecting the patchy nature of some habitats in the lake (see Albrecht and Wilke, 2008). The strong vertical structure largely corresponds to vertically arranged biotopes in Lake Ohrid, such as the ‘~~“~~*Chara* belt’~~”~~ in the lower littoral and the ‘~~“~~shell zone’~~”~~ in the upper sublittoral (Albrecht and Wilke, 2008). Note that in this study we could not identify a potential fifth vertical zone, the profundal, ~~could not be identified in this study~~ as the only species of gastropod previously reported from there has not been found in years~~; see~~ (Hauffe et al., 2011).

Interestingly, we do not see any significant horizontal structuring within ~~and among the~~ deeper vertical zones. However, within the upper littoral (i.e., a zone typically extending from 0 to 3 m water depth), a strong horizontal structure is evident. One eco-zone (‘~~“~~non SE upper littoral zone’~~”~~) comprises all shallow-water gastropod communities from the northeastern, northern, western, and southwestern parts of the lake. Three additional eco-zones can be found in the southeastern upper littoral (‘~~“~~SE upper littoral zones 1–3’~~”~~). This area, comprising major spring fields within the lake, has long been known for its high degree of point-endemism (Albrecht et al., 2006; Albrecht and Wilke, 2008; also see Hauffe et al., 2011, Fig. 3A for compositional similarities among these communities).

The emergent pattern of partly overlapping horizontal/vertical eco-zones, indeed, confirms that the gastropods of Lake Ohrid adhere to the metacommunity concept.

4.2 Relative importance of community assembly processes

Our results suggest that gastropod communities of Lake Ohrid are substantially structured by the dispersal limitation process (i.e., restricted specimen exchange; mean importance across all communities ~ 80%; see Fig. ~~2~~4). Environmental filtering and biotic interaction were of lower importance (12 and 8%, respectively). However, the individual importance of these three processes depends on lake depth and even more on individual eco-zones (Fig. ~~2~~4). The importance of dispersal limitation is, for example, relatively low in the SE upper littoral 1 (i.e., the shallowest and most species rich zone in Lake Ohrid) and in the lower sublittoral (i.e., a deep and relatively species poor zone in Lake Ohrid). The opposite is true for environmental filtering and, to a lesser extent, for species interaction (see Fig. ~~2~~4). This interesting relationship shows that the relative importance of the three community structuring processes does not depend on species richness or lake depth *per se*. The latter might also explain why eco-zones (i.e., three spatial ~~axes~~dimensions) have a higher explanatory power than lake depth (only one spatial ~~axis~~dimension).

Interestingly, the clearly demonstrated high importance of dispersal limitation for ~~both~~ maintaining species richness in Lake Ohrid (through its impact on community structure) ~~and generating endemic biodiversity (via its influence on diversification processes)~~ stands in contrast to some other studies on Lake Ohrid biota. In fact, previous investigations on gastropod (Schneider et al., 2014), ostracod (Lorenschat et al., 2014), diatom (Reed et al., 2010), and fungal communities (Čomić et al., 2010) suggested a strong environmental control (= environmental filtering) of ~~species~~ community composition. However, this is not surprising as other processes were not considered in these studies.

In studies that did consider more than one process, the picture was more complex and showed in addition to environmental filtering, also the influence of dispersal limitation and/or species interaction. This concerns gastropod communities in Lake Ohrid (Hauffe et al., 2011) and in other ancient lakes such as Lake Tanganyika (Meyer et al., 2011), but also communities in freshwater systems in general (Heino et al., 2015a).

In a comparable study, Janzen et al. (2016) investigated cichlid communities in Lake Tanganyika. Interestingly, the individual importance of the assembly processes inferred is very similar to ours, with dispersal limitation being the dominant process. In contrast to our study, they did not find a correlation of process-importance with environmental characteristics. The reasons for this difference remain unclear, though differences in the life-history of the study taxa and in the spatial extant of the study area (Janzen et al., 2016 performed a local study) may play a role.

As the importance of assembly processes often depends on spatial scale (Meyer et al., 2011) and resolution (Münkemüller et al., 2014), the sensitivity of the STEPCAM approach to spatial data aggregation should generally be considered. However, as the spatial resolution in our study is relatively high and as dispersal and movement rates of freshwater gastropods are generally low (Kappes and Haase, 2012; Michel et al., 2007), we here refrained from data aggregation. Note that the occurrence of invasive species, which often have high dispersal rates, could have affected the STEPCAM analysis. However, only two recent gastropod invasions of limited spatial extensions are known from Lake Ohrid (Albrecht et al., 2014) and should therefore not have biased our analysis.

4.3 Metacommunity implications for gastropod speciation

If we accept that species divergence and community assembly can be unified in an eco-evolutionary framework because both are based on similar processes (Hubert et al., 2015), then the here inferred importance of processes allows for a fresh look on the diversification mode of Lake Ohrid's endemic taxa. Research on species divergence, in general, and in ancient lakes in particular, has traditionally focused on the variant of geographic speciation (Cristescu et al., 2010; Schluter, 2001; also see Albrecht and Wilke, 2008 for a review on geographic speciation modes in Lake Ohrid). Adaptive diversification along environmental gradients, however, highlights the additional role of ecology in driving species divergence in ancient lakes (Schön and Martens, 2004).

Our results support this perspective of non-exclusive geographic and ecological speciation in Lake Ohrid. Though the inferred high importance of dispersal limitations in structuring gastropod communities implies a dominant role of geographic speciation in the lake (also see Albrecht et al., 2006; Kilikowska et al., 2013; Trajanovski et al., 2010; Wysocka et al., 2014), the ~~inferred~~ demonstrated importance of environmental filtering and species interaction suggests a small but significant influence of ecological speciation.

~~Interestingly, the relative contribution of geographic speciation (i.e., via dispersal limitation) and ecological speciation (i.e., via environmental filtering and biotic interaction) varies among eco-zones. However, as the total number of eco-zones inferred is relatively low, detailed studies that aim at linking the underlying biotic and abiotic drivers of community processes with modes of speciation are difficult to conduct.~~

~~Nonetheless, t~~The fact that ecological speciation appears to be more important both in the SE upper littoral 1 (i.e., a shallow and species-rich zone) and in the lower sublittoral (i.e., a deep and species-poor zone) potentially implies a bimodal contribution of ecological speciation in generating biodiversity over time.

4.4 Methodological implications and outlook

For many questions concerning processes in eco-evolutionary biology, such as dispersal- or ~~selection~~ selection-dependent ~~rates of~~ species diversification (Pigot et al., 2010) and community assembly (Rosindell et al., 2015), no analytical solutions are (yet) available. However, the outcome of processes can be simulated by utilizing a wide range of parameters values representing them. The set of parameter resulting in a simulated pattern that resembles the observed one best is then assumed to approximate the processes in question. Our newly developed distance-based STEPCAM approach is a relatively simple model that could be easily extended by making, for instance, dispersal limitation spatially or temporally explicit, but at the risk of overparameterization.

Moreover, we suggest further exploration of the STEPCAM approach using different taxa in Lake Ohrid, for instance in another ~~the~~ species-rich group ~~of~~, the diatoms. A combined study of extant and paleo-communities derived from multiple sediment cores would allow inferring the relative importance of dispersal limitations, environmental filtering, and species interaction over space and time.

5 Conclusions

Our study builds on a prediction of the metacommunity speciation model: if geographic speciation is the prevailing mode of species divergence, as suggested by many evolutionary studies, then dispersal limitation should also structure communities. The results of our analysis corroborate this hypothesis for gastropods in Lake Ohrid.

~~However, t~~The inferred importance of environmental filtering and biotic interaction in community assembly also suggests a small but significant influence of ecological speciation on diversification dynamics; an aspect which previously could not be quantified. Moreover, based on a novel automatic detection of eco-zones in Lake Ohrid, we show that ~~limnological characteristics such as lake depth and~~ these eco-zones do influence the relative importance of community assembly processes, whereas former studies could only show that these limnological features give rise to distinct ~~species~~ community composition~~s~~. These findings contribute to the main goal of the SCOPSCO initiative – inferring the drivers of biotic evolution – and provide an integrative perspective on biological and geological dynamics in ancient Lake Ohrid.

Moreover, our novel distance-based STEPCAM complements the growing eco-evolutionary toolbox that aims at ~~disentangles~~ disentangling patterns and processes.

Author contribution

TH conceived the study. CA and TH collected and identified specimens. TH performed the analyses. ~~The manuscript was written by TH and TW~~TH and TW wrote the manuscript with contributions from all co-authors. All authors gave final approval for publication.

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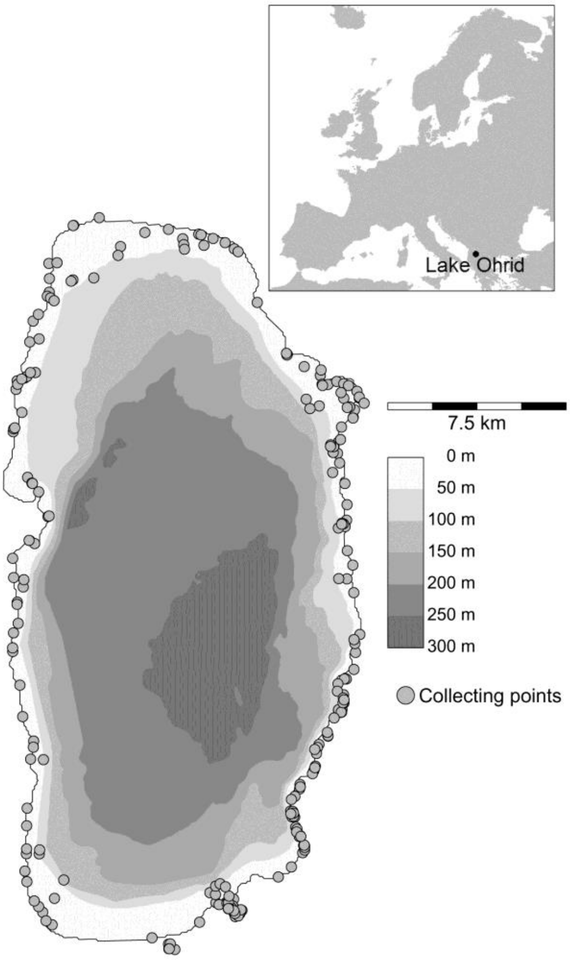

Figure 1. Bathymetric map of Lake Ohrid (Albania, Macedonia) with sampling localities of gastropod communities studied.

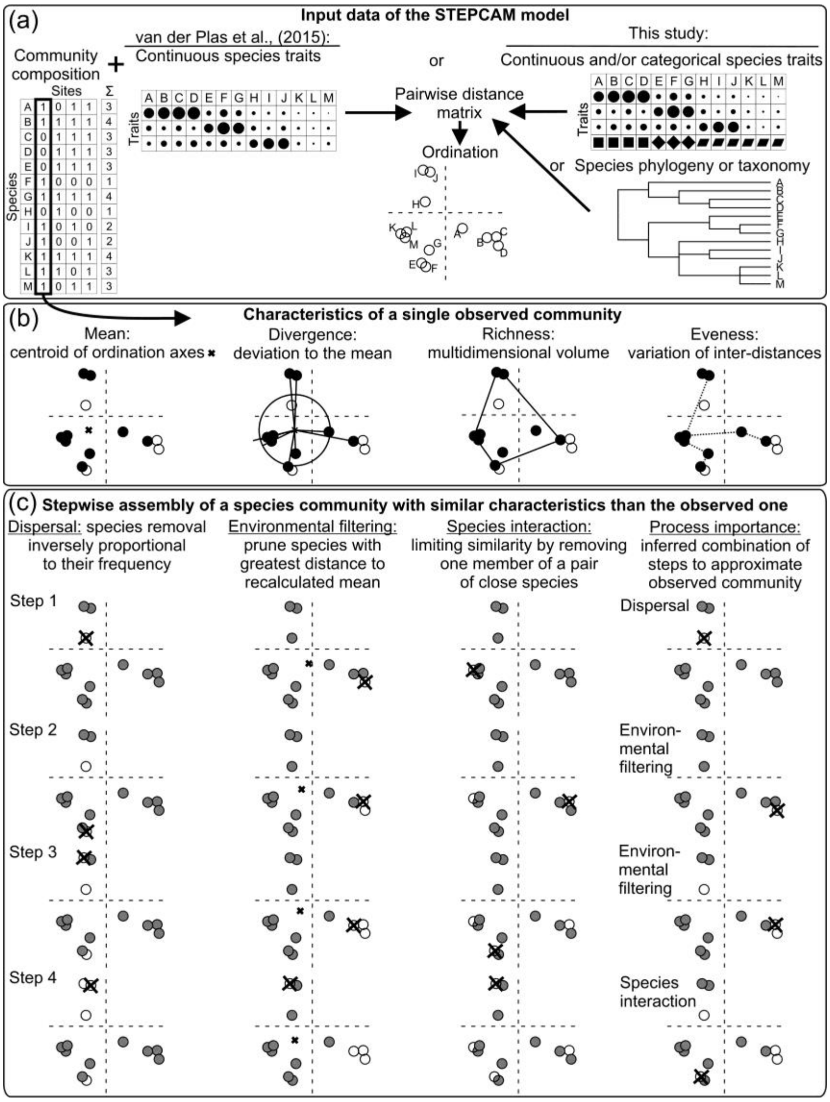

Figure 2. Illustration of the distance-based stepwise community assembly model (STEPCAM). (a) The model is parameterized by species' frequencies and either functional species traits, a species phylogeny, or a hierarchical taxonomic classification. (b) Functional or phylogenetic/taxonomic indices (for details see Villéger et al., 2008) characterize the focal species community. (c) These index-values are the targets for the modeled community. A simulation starts with all species of the regional species pool, removes stepwise species according to the three processes dispersal limitation, environmental filtering, and species interaction, and stops when the same number of species as in the focal community is reached. Approximate Bayesian Computation within a Sequential Monte Carlo framework infers the combination of simulation-steps that generates a species community with minimum differences between observed and generated index-values. The number of removal steps informs about the importance of the respective assembly process.

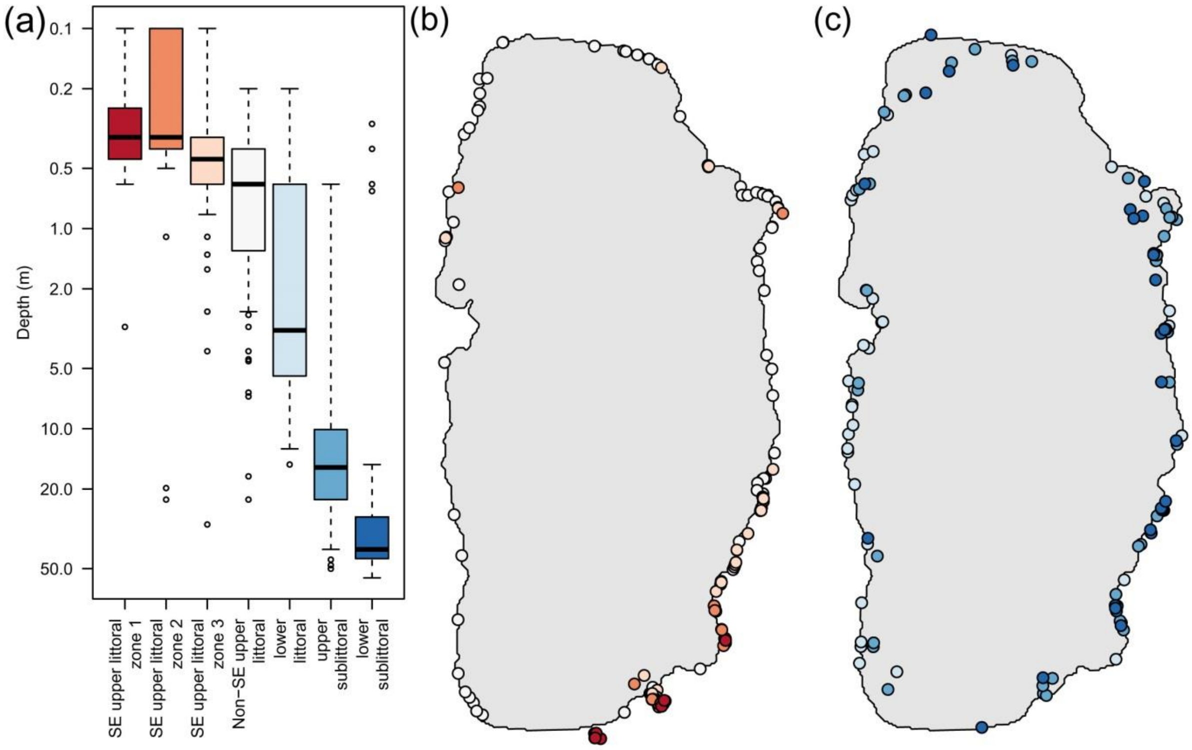

Figure ~~1~~3. Sampling sites and eco-zonation of 264 gastropod communities of ancient Lake Ohrid and its feeder springs. Eco-zones were detected by network analysis weighting links among sampling sites by their geographically proximity and ~~compositional~~ similarity in gastropod composition. (a) Vertical (bathymetrical) extension of the eco-zones. Due to the spatial density, plots of horizontal distribution of clusters were divided into (b) upper littoral and the feeder springs, and (c) littoral and sublittoral.

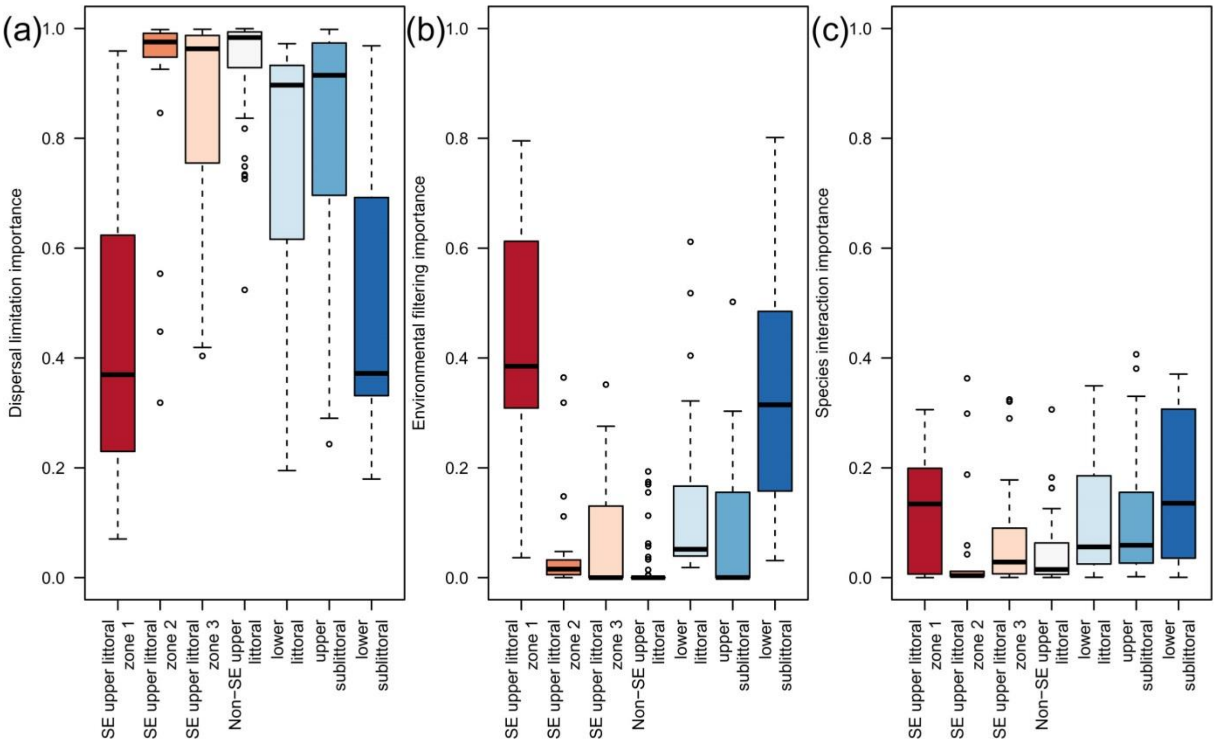

Figure ~~2~~4. Box plots showing the eco-zone dependent relative importance of (a) dispersal limitation, (b) environmental filtering, and (c) biotic interaction in structuring gastropod communities. Colors correspond to Fig. ~~2~~3. These differences in process-importance due to eco-zones are statistically supported by a multivariate Bayesian generalized linear.