Review of
Gastropod diversification and community structuring processes in ancient Lake Ohrid: a metacommunity speciation perspective

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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
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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.

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.

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.

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. Is this classification similarity also the same as the 'relatedness' variable that is mentioned in the ABC-SMC model run?

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.?

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.

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 modeller (or statistician) who has extensive experience with these kinds of models in general.

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.

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.

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).

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?

(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'.)

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.

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.

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.

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.

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).

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.

Technical corrections

A copy of the PDF with comment posts has also been sent.

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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 speci~~es~~ freshwater lacustrine system in Europe. ~~However, it remains unclear whether the diversification of its endemic taxa is mainly driven by neutral processes, environmental factors, or species interactions. This calls for a holistic perspective involving both evolutionary processes and ecological dynamics. Such a unifying framework – 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 distinctively related~~ to eco-zones.

Based on specific simulation steps for each of the three processes, ~~it could be demonstrated~~ that dispersal limitation had the strongest influence on gastropod community structures in ~~Lake Ohrid. How~~ever, ~~it was not the exclusive assembly process but acted together with~~ the other two processes – environmental filtering, and species interaction. ~~In fact,~~ 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.

The study thus corroborated the ~~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). 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~~

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 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, 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 endemic biota (Föller et al., 2015). Instead, such changes likely affected abundances of paleospecies as well as species compositions (Albrecht et al., 2010; Belmecheri et al., 2009; Jovanovska et al., 2015; Reed et al., 2010; Wagner et al., 2014), which, in turn may have affected speciation processes. Similar effects could be shown for 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, also affect the spatial structure of extant communities (Hauffe et al., 2011).

These results question the direct effect of climatic, geological, and/or environmental factors on diversification and call for a holistic perspective involving evolutionary pro-

cesses (e.g., speciation) and ecological dynamics (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., 2015): (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.

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 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 how limnological complexity and stability affect assembly and diversification processes.

A suitable model taxa to study structuring processes are the Gastropoda as they represent the largest animal taxon 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, 1983, 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 gastropod compositions. 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 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 – inferring the drivers of speciation – by providing an independent and unifying view at 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 community structures and their underlying assembly processes in other ancient lake systems or elsewhere.

2 Material and methods

2.1 Gastropod sampling and taxonomic classification

The current study is based on gastropod composition information taken from Hauffe et al. (2011), which were supplemented with additional field data obtained from 2010 to 2011. In total, 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. For critical taxa we here used the taxonomic assignments of Bodon et al. (2001), Hubendick and Radoman (1959), and Radoman (1983). Taxa were classified in five hierarchical levels: species, genus, species flock, family, and superfamily. Note that we included the level "species flock" for monophyletic groups of endemic species that may comprise more than one genus (Albrecht et al., 2008; Föller et al., 2015; Wilke et al., 2007). Species flocks likely evolved intralacustrine and are common in Lake Ohrid.

2.2 Simulation of individual community assembly processes

Van der Plas et al. (2015) suggested a simulation method of stepwise community assembly (STEPCAM). 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.

STEPCAM simulates dispersal limitation by randomly removing species with a probability inversely related to their relative frequency in the regional species pool. 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 are often under selection. Environmental filtering therefore predicts that species of a community show similar morphological traits because environmental conditions only allow the establishment of species that are adapted to the specific niche (Webb et al., 2002). Environmental filtering is simulated by pruning species with trait values most distant to the trait mean of the simulated community.

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 species from the regional species pool showing most similar traits.

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.

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 STEPCAM package v1.0 (Janzen and van der Plas, 2014) for the R v3.1.2 statistical environment (R Core Team,

2015). However, the algorithm was improved for runtime and a fully reproducible analysis including the taxonomic distance and the community matrix is available in the Supplement.

5   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 1 in 10 000.

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

10 For testing whether the importance of individual structuring processes changes gradually with lake depth or whether they 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 as-
15 semblages (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
20 of gastropod communities and their geographical adjacency in horizontal (X and Y) and vertical (Z) dimensions. 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 network was based on (i) vertical (bathymetric) connectivity, and (ii) the cost-distance
25 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., 2015a). We explored the robustness of our analysis by varying the number of 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 gastropod compositions. Averaging the posterior distribution over all 264 gastropod communities, dispersal limitation achieved the highest relative importance with 78.7 % (95 % bootstrapped confidence interval (CI): 75.5–81.7), followed by environmental filtering (mean 12.2 %; CI: 9.9–14.6), and species interaction (mean 9.1 %; CI: 7.9–10.5).

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 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 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-SE upper littoral, lower littoral, upper sublittoral, lower sublittoral). The spatial distribution (horizontal and vertical) of these eco-zones based on 65 neighbors is shown in Fig. 1 (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, both 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 −740 vs. −662, respectively). For a visual presentation of the eco-zone dependent relative importance of dispersal limitation, environmental filtering, and biotic interaction see Fig. 2.

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 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 a crucial role in structuring communities. We, indeed, could show that dispersal limitation had the strongest influence on gastropod community structures 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). In fact, the relative importance of the three community assembly processes varied both with lake depth and eco-zones (Fig. 2), 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 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) 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 a potential fifth vertical zone, the pro-

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fundal, 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 $\sim 80\%$; see Fig. 2). 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). 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). 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) have a higher explanatory power than lake depth (only one spatial axis).

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 investiga-
5 tions 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 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.
10 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., 2015b).

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
15 approach to spatial data aggregation should generally be considered. However, as the spatial resolution in our study is relatively high and as dispersal rates of freshwater gastropods are generally low (Kappes and Haase, 2012; Michel et al., 2007), we here refrained from data aggregation.

4.3 Metacommunity implications for gastropod speciation

20 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 geographic
25 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).

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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 importance of environmental filtering and species interaction suggests a small but significant influence of ecological speciation.

Interestingly, the relative contribution of geographical 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, 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 dependent rates of species diversification (Pigot et al., 2010; Rosindell et al., 2015), no analytical solutions are (yet) available. However, the outcome of processes can be simulated by utilizing a wide range of parameter values representing them. The set of parameters 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 the species rich-group of diatoms. A combined study of extant and paleo-communities derived from multiple sediment cores would allow

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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, 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, we show that limnological characteristics such as lake depth and eco-zones do influence the relative importance of assembly processes, whereas former studies could only show that these limnological features give rise to distinct species compositions. 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 patterns and processes.

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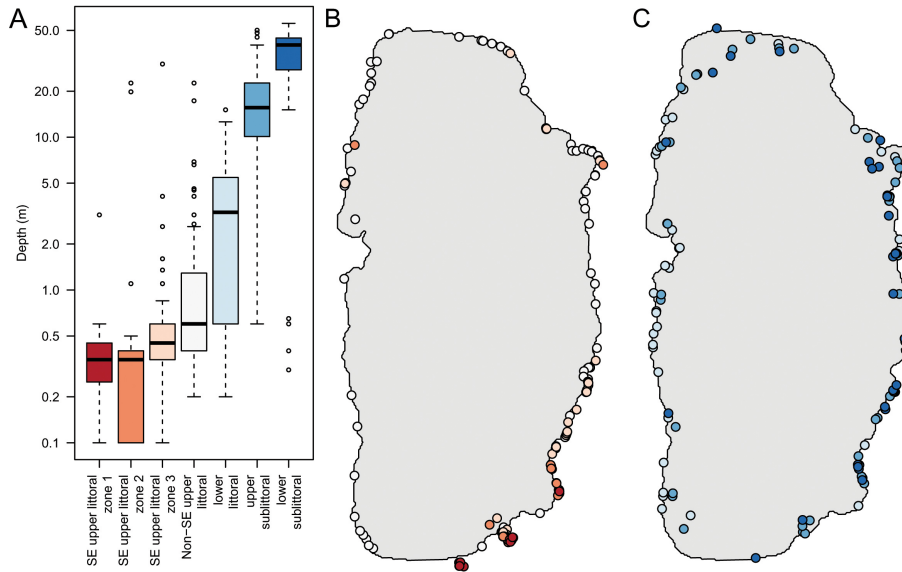
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Figure 1. Sampling sites and eco-zonation of 264 gastropod communities of ancient Lake Ohrid and its feeder springs. Eco-zones were detected by network analysis weighting links among sampling sites by their geographically proximity and compositional similarity. **(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 **(c)** lower littoral and sublittoral.

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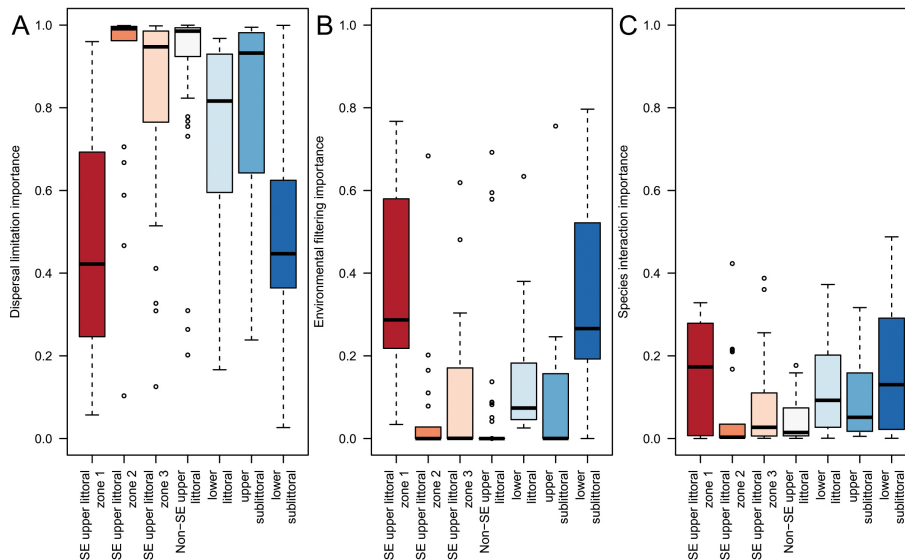
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Figure 2. Box plots showing the eco-zone dependent relative importance of **(a)** dispersal limitation, **(b)** environmental filtering, and **(c)** biotic interaction in structuring gastropod communities. Colors correspond to Fig. 1. These differences in process-importance due to eco-zones are statistically supported by a multivariate Bayesian generalized linear model.