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Spatially explicit analyses of gastropod biodiversity in ancient Lake Ohrid

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Abstract

Spatial heterogeneity of biodiversity arises from evolutionary processes, constraints of environmental factors and the interaction of communities. The quality of such spatial analyses of biodiversity is improved by (i) utilizing study areas with well defined
5 physiogeographical boundaries, (ii) limiting the impact of widespread species, and (iii) using taxa with heterogeneous distributions. These conditions are typically met by ecosystems such as oceanic islands or ancient lakes and their biota. While research on ancient lakes has contributed significantly to our understanding of evolutionary processes, statistically sound studies of spatial variation of extant biodiversity have been
10 hampered by the frequently vast size of ancient lakes, their limited accessibility, and the lack of infrastructure around them. The small European ancient Lake Ohrid provides a rare opportunity for such a reliable spatial study. The comprehensive horizontal and vertical sampling of a species-rich taxon, the Gastropoda, presented here, revealed interesting patterns of biodiversity, which, in part, have not been shown before for other
15 ancient lakes.

In a total of 224 locations throughout the Ohrid Basin, representatives of 68 gastropod species with 50 of them being endemic (=73.5%) could be reported. The spatial distribution of these species shows the following characteristics:

(i) within Lake Ohrid, the most frequent species are endemic taxa with a wide depth
20 range, (ii) widespread species (i.e. those occurring throughout the Balkans or beyond) are rare and mainly occur in the upper layer of the lake, (iii) while the total number of species decreases with water depth, the share of endemics increases, (iv) the deeper layers of Lake Ohrid appear to have a higher spatial homogeneity of biodiversity and related environmental factors, (v) biotic interaction due to possible spillover effects may
25 contribute to the establishment of hotspots, and (vi) eco-insularity within the Ohrid Basin occurs at two levels, at the level of the lake proper and at the level of the feeder-springs.

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It is also shown that large scale effects such as type of water body or water depth are mainly responsible for the distribution of biodiversity. In addition, small scale effects like environmental gradients or biotic interaction affect gastropod composition within a particular depth zone.

5 1 Introduction

Biodiversity, the variation among living organisms or ecosystems (UNEP, 1992), is known to be heterogeneous in temporal (e.g., Rohde and Muller, 2005; Weir, 2006) and spatial distribution (e.g., Diniz-Filho and Bini, 2005; Buckley and Jetz, 2008). Spatial variation of biodiversity, in general, is thought to be shaped by large scale factors such as latitudinal gradient, altitude or water depth, and peninsula or bay effects. Factors acting on smaller scales include habitat heterogeneity, barriers to dispersal, predation, competition or mutual stimulation (Gaston and Spicer, 2005). The prevailing state of these factors influences biodiversity patterns, but temporal dynamics are likewise important (Gaston, 2000). Several biodiversity measures have been proposed (Purvis and Hector, 2000) to account for biodiversity, though species richness (alpha diversity, Whittaker, 1972) is considered to be a good, easily accessible and intuitive proxy for ecological, genetic and organism diversity (Gaston, 2000; Vellend, 2005).

Unfortunately, our knowledge of biodiversity patterns is strongly biased towards marine or terrestrial habitats (Gaston and Spicer, 2005) and to conspicuous taxa like vertebrates (e.g., Abell et al., 2006; Strayer, 2006). Thus, our level of understanding of patterns in freshwater invertebrate distribution remains limited, raising the need for comprehensive studies (Hof et al., 2008).

To enable a spatial analysis of freshwater biodiversity some prerequisites should be fulfilled: (i) ideally the area studied should be well defined based on natural entities rather than geopolitical ones, (ii) the majority of the studied taxa should occur exclusively in the study area to reduce the impact of widespread species on biodiversity estimates and to avoid scaling problems (Gaston, 1997), and (iii) the taxon of inter-

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est should be characterized by high biodiversity and in-homogenous distribution, to diminish the correlation between local and regional diversity relationships (Gaston and Spicer, 2005).

The first prerequisite is typically met by ancient lakes, that is, water bodies that have continuously existed for at least hundred thousand years. They often show a high degree of biodiversity, and so they have attracted numerous studies of both fossil (Williamson, 1981; but see Van Boxclaeer et al., 2008; Harzhauser and Mandic, 2008) and extant lake biota (Wilson et al., 2004; Herder et al., 2006). Cohen (1994) emphasized that the species richness we see in ancient lakes today could be a temporary snapshot. Lake-level fluctuations and changes of basin morphology with considerable effects on lake biota, for example, are well documented for ancient lakes of the East African Rift Valley (e.g., Cohen et al., 2007; Scholz et al., 2007; Schultheiß et al., 2009), Lake Baikal (Kashiwaya et al., 2001), or the Caspian Sea (Reid and Orlova, 2002; Grigorovich et al., 2003). Comparable data about hydrological changes in the European ancient Lake Ohrid have been published only recently (Belmecheri et al., 2009; Lindhorst et al., 2010).

Lake Ohrid is a graben lake of bathtub shape with a maximum depth of 289 m, a surface area of 358 km² and a volume of 55 km³ (Matzinger, 2006a). Hence, it is one of the smallest ancient lakes of the world. Nevertheless, the Ohrid Basin has a complex geological and limnological structure. Different bedrock types, active tectonics (for details see Hoffmann et al., 2010), steep-sided mountain ranges and water-discharge by lake-side and sublacustrine springs (Matzinger, 2006b) cause the characteristics of different habitats (Fig. 1). Some workers have suggested that these complex features are partly responsible for a high number of ecological niches and an outstanding degree of biodiversity (Radoman, 1985; Albrecht and Wilke, 2008). Moreover, due to an exceptionally high degree of endemism, Lake Ohrid is quite distinct from other Balkan lake systems (Albrecht et al., 2009; Marková et al., 2010; Trajanovski et al., 2010).

While Lake Ohrid, indeed, appears to have a fairly isolated basin, thus fulfilling criterion (i) for spatial analysis of freshwater biodiversity, criteria (ii) and (iii), addressing

requirements of the taxon to be studied, are met by representatives of its gastropod fauna.

Compared to some other ancient lakes, the vast majority of Lake Ohrid's gastropod species is morphologically relatively easy to distinguish and gastropods probably constitute the best studied invertebrate taxon in the lake (e.g., Poliński, 1929; Hubendick and Radoman, 1959; Radoman, 1985; Hauswald et al., 2008; Wilke et al., 2009). Recently, even the first fossil molluscs have been reported from the Last Interglacial period (Albrecht et al., 2010). Moreover, there is growing evidence for vertical and horizontal gradients and barriers within the Ohrid Basin (Albrecht et al., 2006, 2008; Wysocka et al., 2008; Trajanovski et al., 2010), possibly promoting parapatric or even allopatric speciation events (Albrecht and Wilke, 2008). Despite these interesting findings, a comprehensive picture of the spatial distribution of gastropod biodiversity within Lake Ohrid is still missing. However, this is not a problem typical for Lake Ohrid. In fact, we have no knowledge of any ancient lake in the world where a species-rich invertebrate taxon has been sampled in a manner allowing for statistically sound studies of the spatial distribution of biodiversity. Reasons include the often large size of ancient lakes, their, in part, limited accessibility (both physically and politically), and the lack of infrastructure for lake-wide biodiversity studies.

In this regard, Lake Ohrid constitutes a rare exception. Not only does the relatively small size of the lake allow for comprehensive sampling but the lake is also readily accessible, and infrastructure for lake-wide biodiversity studies can be regarded as excellent. Given this unique opportunity for a comprehensive horizontal and vertical sampling of a species-rich taxon over a whole ancient lake basin, we are using information from 224 collecting points for gastropods from Lake Ohrid to:

- (i) provide the first comprehensive assessment of Lake Ohrid's gastropod diversity,
- (ii) identify potential faunal subdivisions of the Ohrid Basin utilizing a multivariate framework, and

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- (iii) provide a spatially-explicit description of biodiversity patterns within the lake proper.

2 Material and methods

2.1 Zonation of the Ohrid Basin

The basin of Lake Ohrid, that is, the intersection between paleoshorelines (N. Hoffmann, personal communication, 2010) and the conjunct surface watershed, has a size of 1605 km² (Fig. 1). It consists of the lake itself as well as surrounding lotic waters, pools, and springs.

2.1.1 Lake Ohrid

The vertical subdivision of worldwide lakes is typically reflected by the degree of benthic photic production with the littoral being the zone of macrophytic vegetation and the profundal being the layer without photic production. In deep oligotrophic lakes such as Lake Ohrid, it is difficult to infer an exact zonation based on biotic factors. Thus, we here adopted a strictly hydrological approach based on lake-level fluctuations and the location of the summer thermocline.

Surface Layer

The Surface Layer is the upper vertical zone in Lake Ohrid down to approximately 5 m depth. It is affected by wave action of up to 1.5 m height (Stanković, 1960), moderate intra-annual lake-level fluctuations of 1 m (Popovska and Bonacci, 2007), and high seasonal temperature variations (Stanković, 1960). The vegetation of the Surface Layer consists mostly of microalgae and extensive reed belts at the northern and southern shores (Watzin, 2002). Based on prevailing abiotic conditions, such as sub-lacustrine springs, substrate type and differences in geology (Hoffmann et al., 2010),

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a specific horizontal zonation of the Surface Layer has been suggested (Albrecht and Wilke, 2008).

Intermediate Layer

The Intermediate Layer is summer stratified by a relatively steep temperature decline and increase of dissolved oxygen. It consists of a partly continuous (Lindhorst et al., 2010) belt of *Chara* algae between 3–20 m water depth, which impedes the migration of benthic invertebrates (Albrecht et al., 2006; Sell et al., 2007; Wysocka et al., 2008). The shell zone below the *Chara*-belt is characterized by broad *Dreissena* beds (e.g., Wilke et al., 2010) and by sparse macrophytes (Stanković, 1960).

Deep Layer

The upper boundary of the Deep Layer is characterized by the end of the summer thermocline at ~25 m (Watzin, 2002) and higher oxygen saturation than upper zones (Stanković, 1960). As the first zone of definite fine-grained sediment deposition (Stanković, 1960), the substratum consists mainly of silt and mud (Vogel et al., 2010), and sporadic oxygen-depletion influences organic matter preservation (Holtvoeth et al., 2010). Below the 50 m isobath to the bottom of Lake Ohrid, there is no benthic photic production (Stanković, 1960).

2.1.2 Springs

Springs represent an important hydrological system in the Ohrid Basin as they are responsible for a considerable part of its water balance. They can be found within the lake (i.e. sublacustrine) or along the margins. The majority of them are located in the northwest and northeast of the lake, but the most powerful springs are the feeder spring complexes of Sv. Naum (Macedonia) and Tushemisht/Zagorican (Albania) in the south. They form spring lakes of up to 5 m depth (Kunz, 2006). Mainly supplied

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by neighbouring Lake Prespa, the feeder-springs are characterized by different water chemistry than the mainly precipitation-fed springs in the northeast and northwest (Matzinger, 2006b). Thus, we here distinguish between two different types of spring: The southern feeder-springs and the northeastern/northwestern springs.

2.1.3 Lotic waters and pools

Many of the northeastern and northwestern springs give rise to creeks and small, often artificial pools. Due to the steep-sided basin shape, the lotic waters are mainly short, shallow and often subject to seasonal desiccations. The two main lotic waters in the area are the effluent Crni Drim River and the affluent Sateska River (Fig. 1). The latter was artificially diverted into the lake in 1962 (Matzinger et al., 2006a).

2.2 Sampling gastropod fauna

Gastropods from the Ohrid Basin were collected during seven field trips carried out between May 2003 and September 2009. Individuals were obtained by hand collecting from hard substrata in shallow waters or from stones and rocks lifted when snorkelling. Soft substratum and plant material was sieved. Deeper parts of Lake Ohrid down to 60 m were sampled using a triangular dredge from small boats or from the Hydrobiological Institute Ohrid (HBI) research vessel. Data on sampling locations were recorded with a GPS device and stored in a database. Equal distance measurement for longitude and latitude degrees was obtained by transforming coordinates to UTM projection.

All materials are deposited at the permanent DNA, shell and tissue collection of the University of Giessen, Systematics and Biodiversity Group (UGSB). Species determination and nomenclature for gastropod samples follows Poliński (1929), Hubendick and Radoman (1959), Radoman (1983), Bodon et al. (2001), and Albrecht et al. (2006, 2008). A total of 284 samples were obtained during field work. Because of multiple collections at selected sites, 284 collecting events resulted in a total of 224 analysed collecting points. Non-parametric Wilcoxon tests showed no significant difference of species

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richness data between the pooled and unpooled datasets (Surface Layer: $W=5210$, $P=0.077$; northeastern and northwestern springs: $W=205.5$, $P=0.2446$; lotic waters: $W=182.5$, $P=0.9149$; feeder springs: $W=1153$, $P=0.4828$).

We calculated the normalized endemic species (N) area (A) index according to Eq. (1) (modified after Albrecht and Wilke, 2008).

$$I = \frac{\lg(N+1)}{\lg(A+1)} \quad (1)$$

2.3 Statistical analysis

2.3.1 Community composition and species richness

The number of species, proportion of endemics and species frequencies are important characteristics of ecosystems and local subdivisions, and may give clues as to the underlying abiotic constraints and/or different evolutionary histories (Cooper and Purvis, 2010).

First, species collecting frequencies were estimated and species were classified into rare, common and frequent. We used the rarefaction method for each species at each depth layer, because of unbalanced collecting frequencies in the three different depth zones. Species frequency and standard deviation was obtained for the lowest number of collecting points ($N=34$, Deep Layer) by random site accumulation of one layer with 4999 permutations. Calculation was performed with the vegan package (Oksanen et al., 2010) for the R statistic environment (R Core Development Core Team, 2009).

To test for faunal subdivision of the different layers within the Ohrid Basin, we compared gastropod community compositions. Species composition within a layer should be more similar than that of communities from different layers. Our hypothesis of distinct gastropod communities based on presence/absence data and Bray-Curtis dissimilarities was tested by a one-factorial, permutational analysis of variance (PERMANOVA, Anderson, 2001) utilizing the function `adonis` of the vegan package with

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4999 permutations. For explorative data analysis, we used non-metric multidimensional scaling (NMDS) to transform similarity of species composition into an illustratable low-dimensional figure. The applied vegan function `metaMDS` was stopped when two convergent ordinations with highest goodness-of-fit compared to original data structure were reached. The number of dimensions was selected in line with the suggestions of Kruskal and Wish (1978). 1000 null models were generated with the trial swap algorithm (Miklos and Podani, 2004), to check whether a better ordination structure could be achieved by chance. This technique preserves species richness per collecting point and species occurrence frequency. Water depth and species richness of the collecting points were fitted to the ordination of the lake proper and statistical testing was achieved by 4999 permutations.

Differences in mean species richness of distinct layers were tested by a one-factorial PERMANOVA with 4999 permutations.

2.3.2 Analyses of spatial patterns

In contrast to large scale differences in biodiversity, that is, between different zones of the Ohrid Basin, fine scale variations within a layer are more difficult to disentangle because of possibly co-varying environmental factors. This hampers, factor based a-priori classification and subsequent testing. However, spatial autocorrelation of environmental factors is likely (Legendre, 1993) and should be reflected by similar observations on species richness from nearby collecting points. An increasing number of studies benefits from straightforward eigenvector-based filters of, for example, phylogenetic information (Kühn et al., 2009) or, as initially suggested, small scale descriptors of spatial autocorrelation (Borcard and Legendre, 2002). A great advantage of these spatial filters, commonly called principal coordinates of neighbouring matrices (PCNM), is that they avoid grid based methods, in which a-priori selected grid sizes frequently influence results (Gaston and Spicer, 2005). As grid data often originate from merged sampling points, techniques that focus on analysing point patterns and associated processes are established (e.g., Ripley, 1977; Wiegand and Moloney, 2004). They enable, for

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ber of species exclusively restricted to a single depth layer. Whereas annual water level fluctuation and wave action restrict plant and bacteria growth in the Surface Layer (Stanković, 1960), the Intermediate Layer is relatively undisturbed, yet enough light reaches this layer (Stanković, 1960). Therefore, this photic zone is known to be the most bio-productive layer in Lake Ohrid (Tocko and Sapkarev, 1978). Similar patterns of elevated species richness in intermediate layers were observed in ancient lakes Tanganyika (Michel, 1994), Baikal (Sitnikova, 2006) and Titicaca (Dejoux, 1992). In contrast, the Caspian Sea shows a rather continuous decline of species richness and density with increasing water depth (Parr et al., 2007). However, elevated species richness in shallow parts is suggested to be a general feature of ancient lakes (Martens, 1997). No comprehensive species richness study of other taxa has been conducted in Lake Ohrid so far. But abundance data for e.g., phytoplankton or chironomids suggest a similar peak value at the Intermediate Layer, whereas Oligochaeta, Amphipoda and especially Ostracoda showed their highest densities in deeper waters (Stanković, 1960; Mikulic and Pljakic, 1970).

As proposed by Boss (1978), a common feature of ancient lakes is eco-insularity. Accordingly, well adapted ancient lake organisms may out-compete most invading species but are probably inferior outside the native lake. Hence, little faunal overlap is expected in the case of eco-insularity. The recent lake shore, however, may not necessarily constitute the “island” border. There are examples showing endemics range limits corresponding to the watershed border (Glaubrecht and Rintelen, 2008; Schultheiß et al., 2009). In Lake Ohrid, the situation appears to be more complex. Whilst we do see eco-insularity on a basin scale (Albrecht et al., 2009), we also see evidence for two patterns within the basin: eco-insularity of the lake proper and eco-insularity of the feeder-springs.

Interestingly, the species richness map (Fig. 4c) shows relatively uniform distributions of alpha diversity for the Deep Layer and Intermediate Layer. The Surface Layer, however, is characterized by a rather heterogeneous species richness distribution, with a hotspot inferred at the rocky southeastern shore (see below). The spatial descriptors

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inferred in our modelling of species richness variation of the Surface Layer (Fig. 5) are likely caused by co-varying environmental factors (sensu Borcard et al., 2002).

Indeed, spatial gradients of sediment features are shown (Vogel et al., 2010) and different substrata is likely to affect species distribution and biodiversity (Kershner and Lodge, 1990; Michel, 1994).

4.3 Hotspots of biodiversity

Of the three suggested biodiversity hotspots, the area around Veli Dab, appears to be outstanding in terms of point endemism. This hotspot, comprising an area of less than 1 km², harbours >10% of Lake Ohrids’ endemic gastropod biodiversity (Fig. 4a; also see Wilke and Albrecht, 2007). This is largely due to the fact that all point endemics of the Surface Layer were exclusively found in this particular area. These taxa are known to require a specific environment the so-called “littoral interlithon”, that is, a patchy system of porous stones or rocks often associated with sublacustrine springs (e.g., Hadžišće, 1956; Albrecht and Wilke, 2008).

Interestingly, an increasing number of widespread species are observed living in areas of the highest anthropogenic pressure in the southwestern (Lin Peninsula) and northeastern (Ohrid Bay, Sateska estuary) parts of the lake (Fig. 4d, also see Kostoski et al., 2010b).

One of the most surprising findings of our study, however, is the presence of mutual stimulation as indicated by the mark correlation study (Fig. 6). It clearly shows that alpha diversity at single points benefits from surrounding species richnesses. A spillover effect is likely, that is, the dispersal of specimens from the species’ range centres to more marginal parts.

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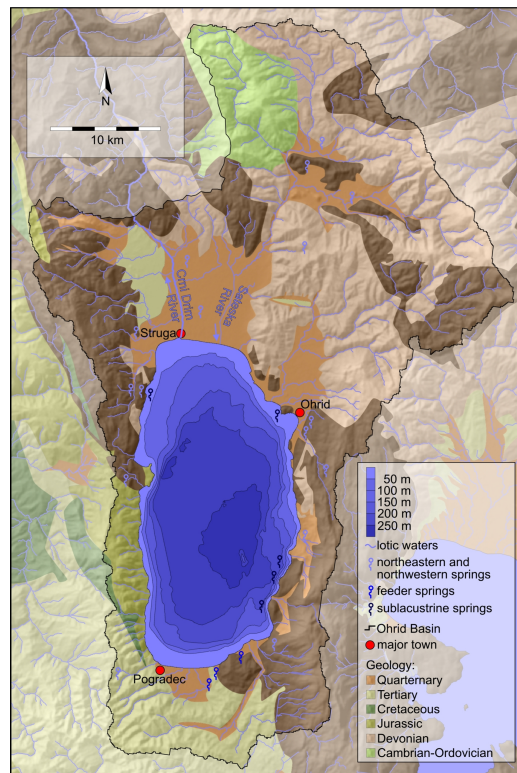


Fig. 1. Map of the Ohrid Basin indicating major hydrological and geological features. Map adapted from Hoffmann et al. (2010).

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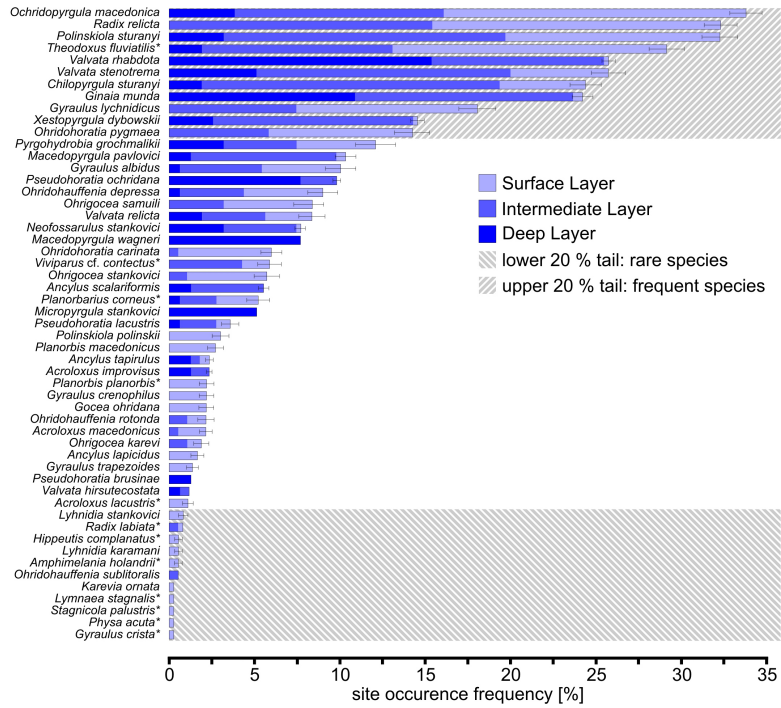


Fig. 2. Plot of frequency and standard deviation of species sites occurrence of gastropod species found in Lake Ohrid. Depth layers are colour-coded and non-endemic species are marked by asterisks.

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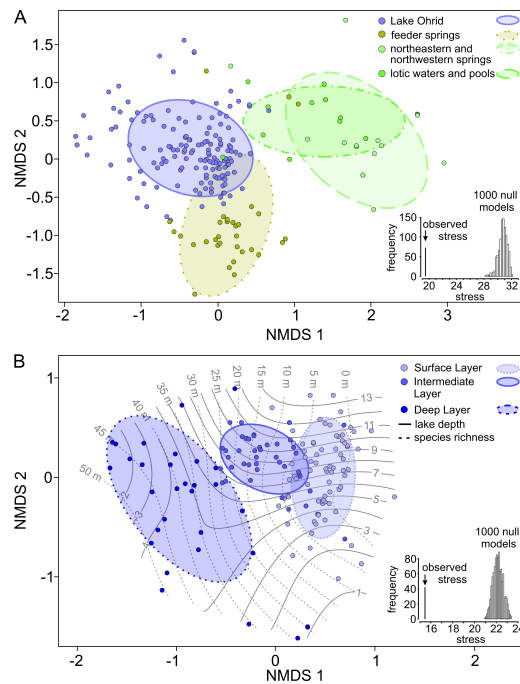


Fig. 3. NMDS plots of gastropod communities (Bray-Curtis dissimilarity). **(A)** Two-dimensional solution within the Ohrid Basin. **(B)** Vector fitting of sampling depth and species richness for three-dimensional solution within Lake Ohrid.

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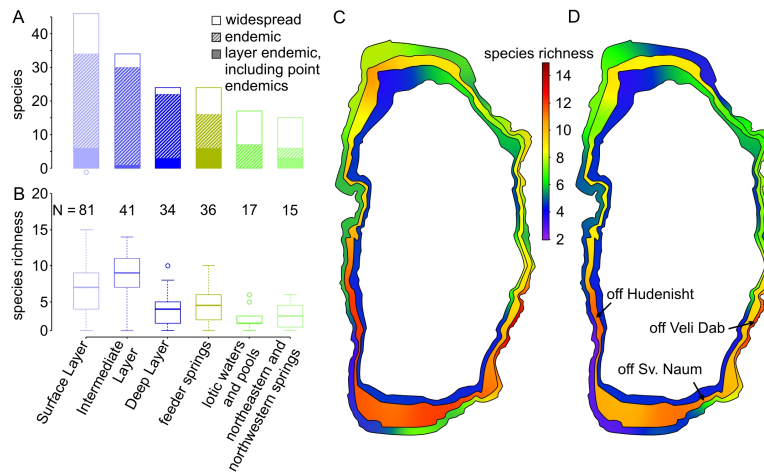


Fig. 4. Zonation-specific analyses of gastropod species richnesses in the Ohrid Basin. **(A)** Bar plots of species compositions. **(B)** Quartile box plots of species richness including median, upper and lower quartiles (boxes) and 1.5 interquartile range whiskers. **(C)** Species richness map based on all species. **(D)** Species richness map based on endemics. Candidate hotspots of endemic species richness are indicated by arrows. Because of the strong slope at the eastern and western tectonic faults, the depth layers could have a width of less than 10 m, and were thus stretched to ensure proper resolution.

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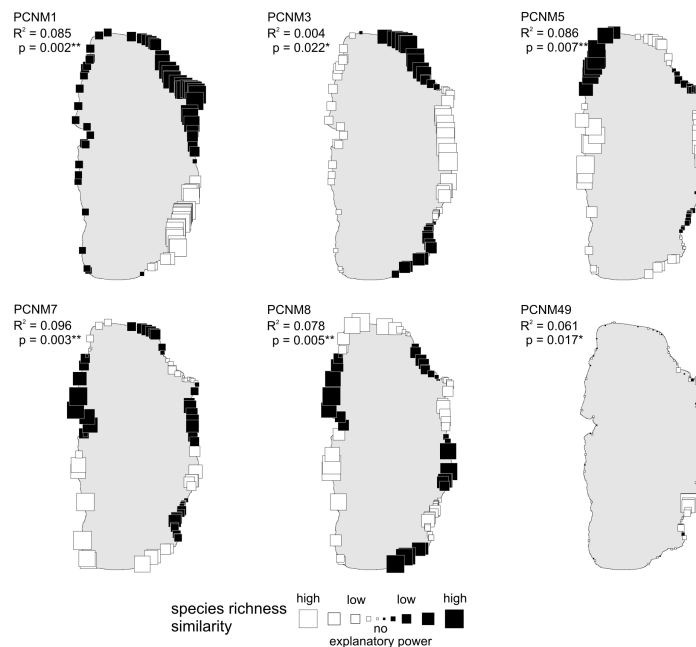


Fig. 5. Plot of PCNM eigenfunctions estimating gastropod species richness similarities within the Surface Layer of Lake Ohrid. Five broad and one fine scale vectors explain 40% of the spatial variation of alpha diversity. Within each eigenfunction, groups of equally-coloured squares indicate similar species richnesses. Increasing square size shows higher similarity in alpha diversity (note that small squares may not have sufficient explanatory power). Note that higher eigenfunctions represent increasingly smaller spatial scale predictions. Therefore, the frequency of alternating groups of black and white squares increases.

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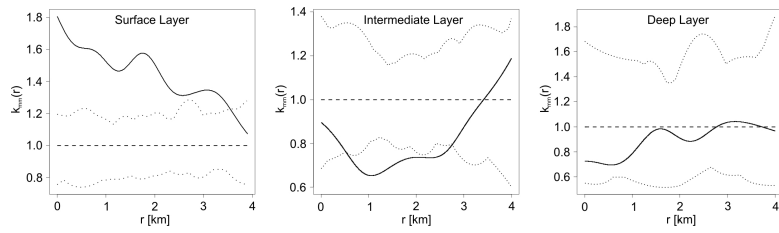


Fig. 6. Plots of mark correlation function (solid line) for the relationship between gastropod species richness and the distance between sampling locations within different layers of Lake Ohrid. No correlation is indicated by the horizontal dashed lines, the 95% confidence envelope by dotted lines. Values above and below the confidence envelope indicate mutual stimulation or inhibition, respectively.