SPATIAL DISTRIBUTION OF CYANOBACTERIA IN THE SPLASH ZONE OF THE VERUDA AND UGLJAN ISLANDS, CROATIA

Alžběta Hesounová, Jan Lepš, Pavel Fibich, Jan Kaštovský
Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic.
hesounova.alzbeta@gmail.com

Introduction

The splash zone is defined as the area of shoreline above the highest tide. The range of the zone varies geographically, but is generally between 0.4 and 4.5 meters vertically. Organisms living in this zone must endure physiologically challenging ecological conditions. Cyanobacteria protect themselves from high light intensity and UV radiation by producing pigments in their sheaths, or alternatively, by penetrating the substrate. Despite these stress factors, species rich communities of epilithic (on the surface of the rock) and endolithic (penetrating the substrate) cyanobacteria are often developed. There is a specific flora sometimes with endemic species – like *Adrianema adriaticum*, *Dalmatella* spp., *Tryponema endolithicum* or *Voukiella rupestris* which were described by Ercegović and were found only in the Mediterranean region or only on the Croatian coast. But also contains many species with cosmopolitan distributions as well (*Calothrix scopulorum*, *Entophysalis* spp., *Hyella* spp., *Mastigocoleus testarum*, *Solentia* spp. etc). Consequently, microbial communities develop in response to vertical gradient, and one can usually distinguish 3 to 5 zones which differ in species composition.

Study questions

Q1. Are there consistent differences in species composition of the cyanobacterial community in the splash zone of two remote islands (ca 140 km apart)?

Q2. What is the relative importance of the vertical gradient for cyanobacterial community composition?

Q3. Previous studies imply that geographic orientation (azimuth) of terrestrial substrate is important for species composition (different pattern of UV radiation, humidity etc.). Is the differentiation of the community composition of a more diverse system (i.e. of islands) the same?

Q4. What is the relationship of similarity of species composition to their distance on small spatial scales (in the range between 0.05m and 10m).How does species richness (and thus completeness of the survey) depend on the number of samples collected?

Q5. How does the species richness (and thus completeness of the survey) depend on the number of samples collected?

Material and methods

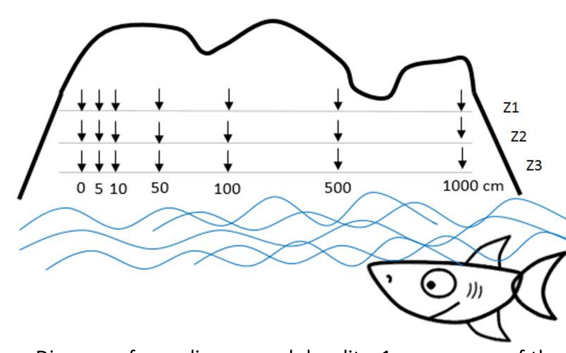


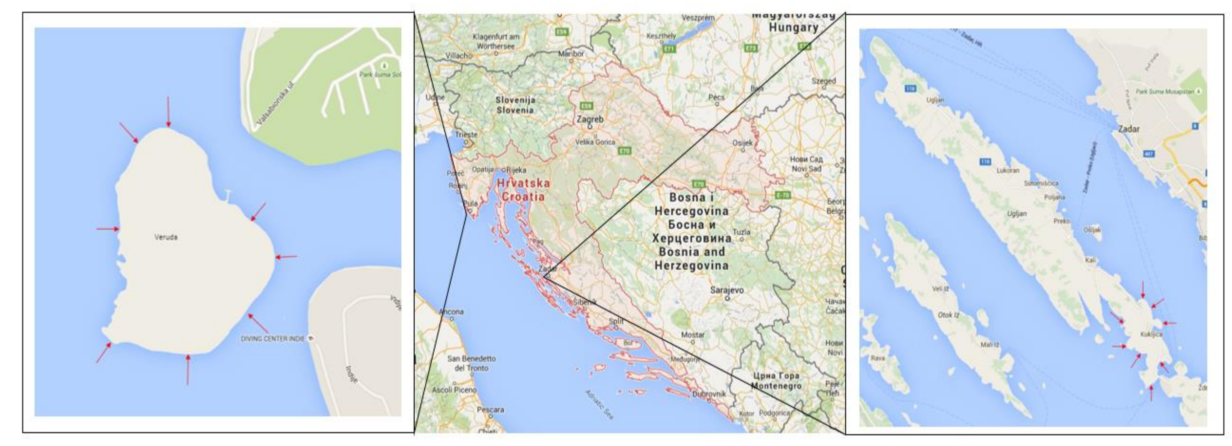
Diagram of sampling on each locality. 1 upper zone of the splash zone (Z1); 2 middle part (Z2) and 3 lower part (Z3). The arrows show the pattern of sampling in distance from the zero point to 1000 cm in each zone.

Sampling and Microscopy

Samples were clected from the splash zone of the rocky shore on two Croatian islands, Veruda and Ugljan, in June 2013. On each island a set of eight sites was established according to their geographical orientation (azimuth), including north, north-east, east, south-east, south, south-west, west and north-west orientations of the shoreline. At each locality the samples were taken acording the scheme on the left. This gave us a total of 336 samples from both islands. The fragments of the calcite rock were taken with a geological hammer, allowed to dry, marked, and transported to the lab. There were the samples microscoped using Olympus BX 51 microscope with digital camera Olympus DP – 71. Pictures were made with the software DP Controller 3.1 (Olympus corp.) and QuickPHOTO MICRO 3.0.

Statistical analyses

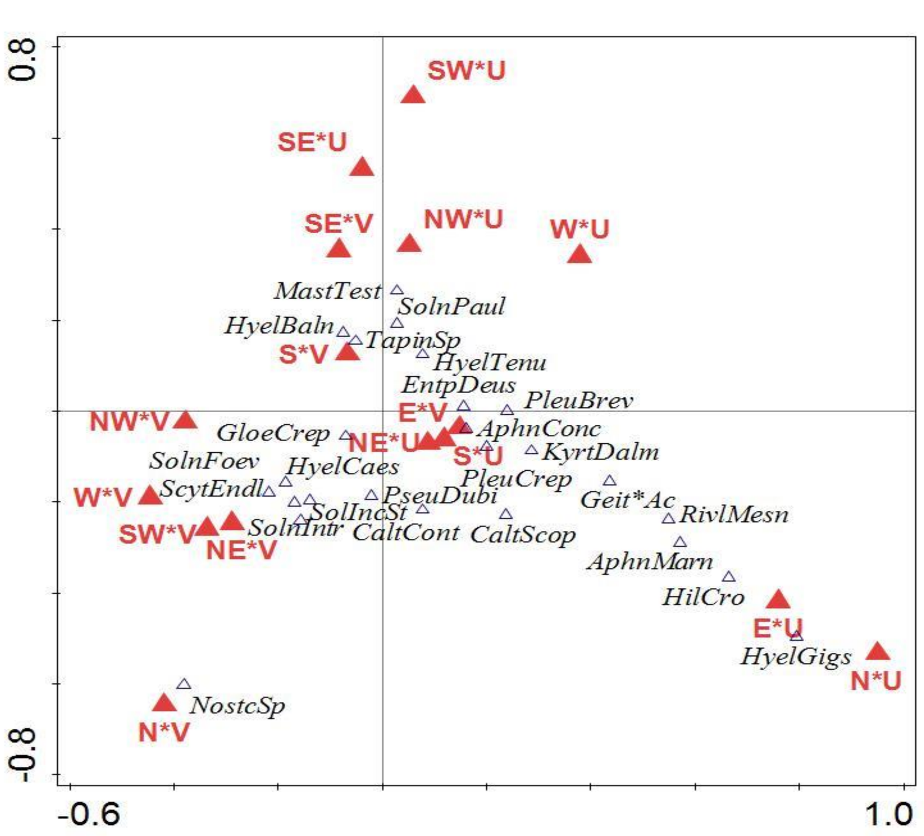
Q1 Two islands comaparison and **Q3 Geographic orientation** were analysed by canonical correspondence analysis (CCA), **Q2 Vertical gradient effect** was anylysed by partial canonical correspondence analysis in CANOCO program.
For **Q4 Spatial dissimilarity** the method of distance decay similarity was used, for **Q5 Sampling effort** was used the rare function method. Both tasks were analysed in R 3.1.3.



Sampling site locations on the Veruda and Ugljan island.

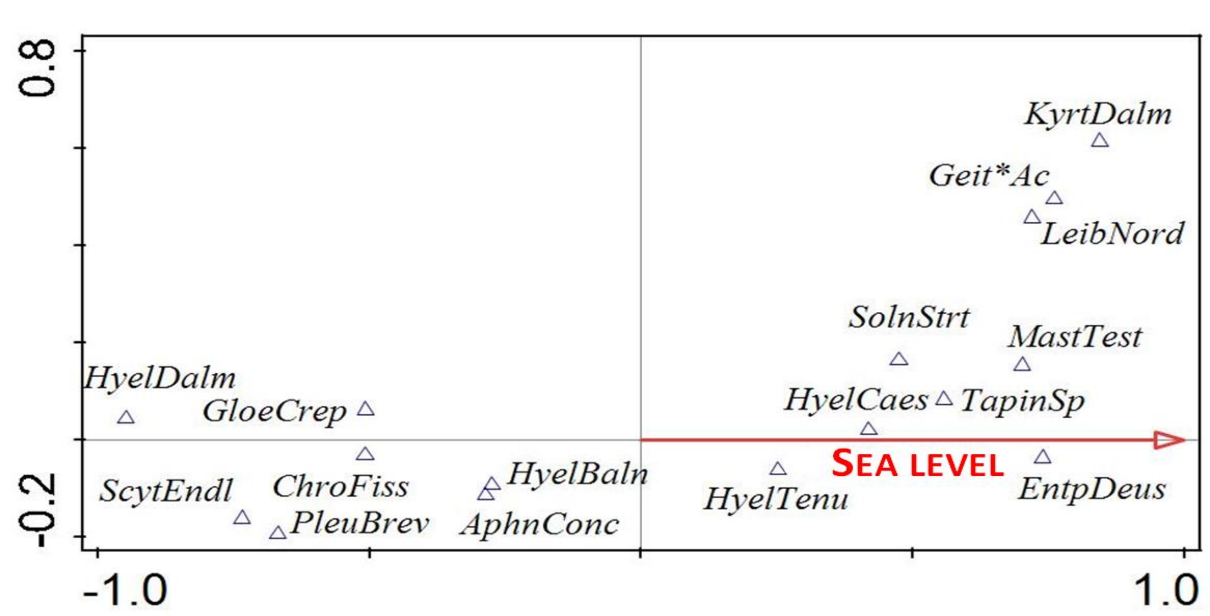
Results and discussion

Q1 Two islands comparison



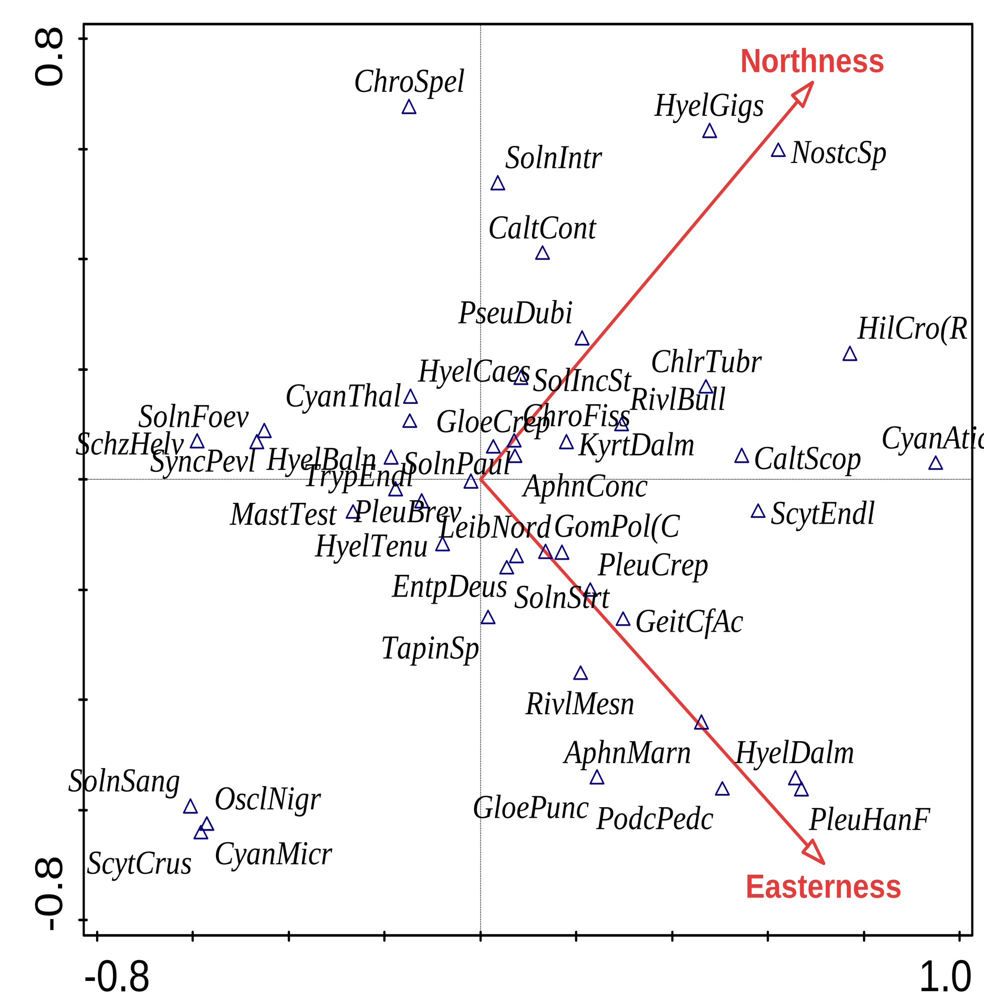
Results of CCA with interaction of site and position as a factor. The site labels are in the form geographic position * island identity (**U**glian / **V**eruda). Vertical gradient is used as covariable. The differences among localities explain 15.77 percent of variability after partialling out the effect of covariables. The first and second CCA axes explain 2.78 and 2.70 % of variability respectively, their efficiency is 44.65 % and 47.35 % respectively. The 23 best fitting species are shown. Results are highly significant (P=0.0002).
We have demonstrated small, but significant differences between the two, relatively close islands. The results show that the difference between islands explains two times more variability than the difference between sites within island. Concerning the difference in species composition of those two islands there was no difference between the islands in dominant species. The variation was caused only by the rarely species which were even not found in huge biomass.

Q2 Vertical gradient effect



The results of the CCA analysis with the vertical gradient as quantitative variable being the only explanatory variable. Note, that only the first (horizontal) axis is constrained, i.e. it corresponds to the vertical gradient, the second is the strongest gradient in the unexplained variability. Locality identity is used as covariable. The first axis explains 6.45% of variability, its efficiency 67.43 %. 16 species best fitting species on the first (i.e. the constrained) axis are shown. Results are highly significant (P=0.0002).
The vertical gradient is clearly the most strongest explanatory variable for the species composition. The comparison of the gradient as quantitative variable and as factor demonstrated that **there are probably no distinct zones, but rather continuous gradient**. This statement is supported by the observed occurrence of some cyanobacterial species.

Q3 Geographic orientation

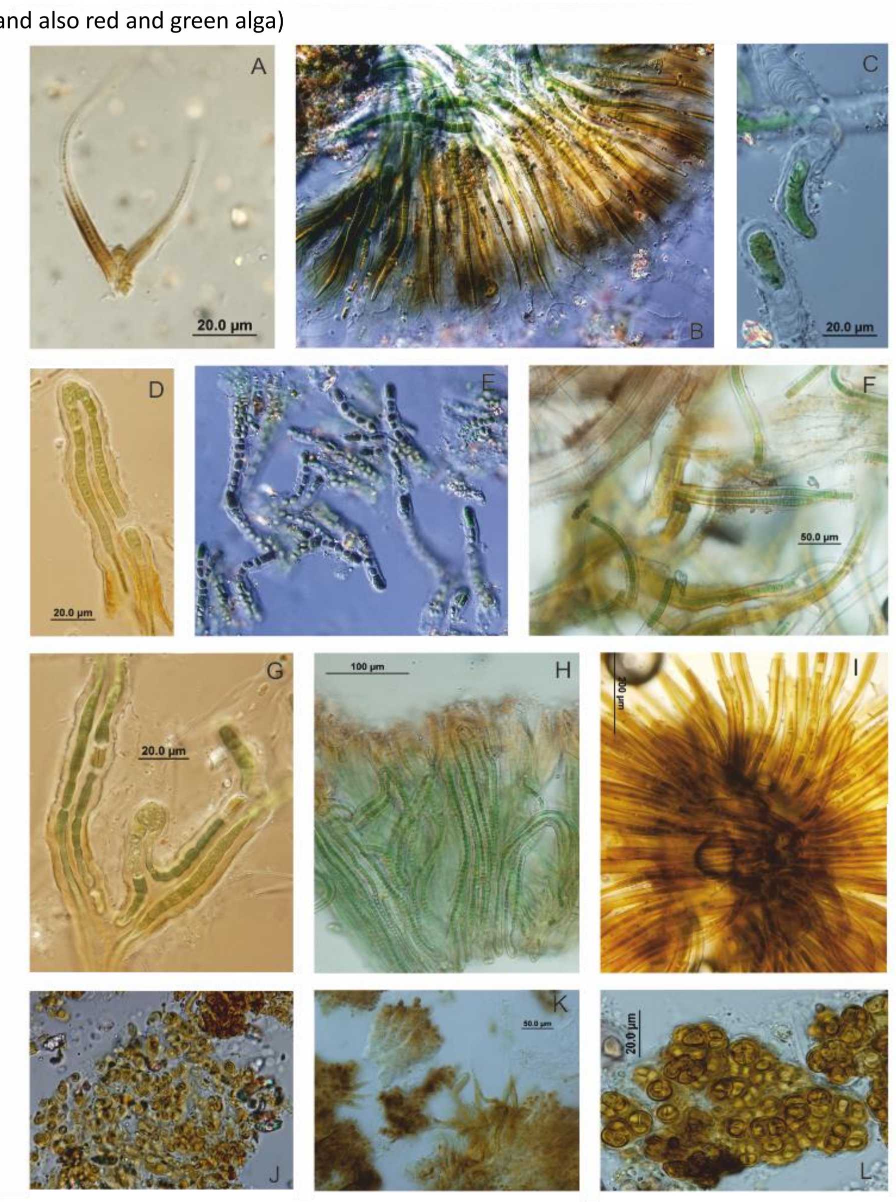


Results of the CCA analysis with Northness (cosinus of the azimuth) and Easterness (sinus of the azimuthe) as explanatory variables and island and vertical gradient used as covariables. The effect of explanatory variables is significant (permutation of the whole sites), P=0.021, the axes explain 1.72 and 0.99 % of variability after subtraction the effect of covariates. The best fitting species shown only.
The species composition is only loosely (if at all) related to the azimuth. But in general most of the species do not prefer any of the geographical position. Only few rare species occurred in specific geographical position. This occurrence might indicate species preference to azimuth to live. On the other hand, the occurrence might be caused just by the fact that those rare species were found too little to prove they also have any preference.

List of species recorded (cyanobacteria and also red and green alga)

Aphanocapsa concharum	*AphnConc*
Aphanothece marina	*AphnMarn*
Calothrix contrarenii	*CalCont*
Calothrix scopulorum	*CaltScop*
Cyanosaccus atticus	*CyanAtti*
Cyanosarcina thalasia	*CyanThal*
Cyanostylon cf. *microcystoides*	*Cyan*mi*
Entophysalis deusta	*EntpDeus*
Geitlerinema cf. *acuiforme*	*Geit*Ac*
Gloeocapsa salina	*GloeSal*
Gloeocapsopsis crepidinum	*GloeCrep*
Gomontia polyrhiza	*GomPol*
Hildenbrandia crouanii	*HilCro*
Hyella balani	*HyelBaln*
Hyella caespitosa	*HyelCaes*
Hyella dalmatica	*HyelDalm*
Hyella gigas	*HyelGigs*
Hyella tenuior	*HyelTenu*
Chlorogloea tuberculosa	*ChlorTube*
Chroococcidiopsis fissurarum	*ChrooFis*
Chroococcus spelaeus	*ChrooSpe*
Kyrtuthrix dalmatica	*KyrtDalm*
Leibleinia nordgardii	*LeibNord*
Mastigocoleus testarum	*MastTest*
Nostoc sp.	*NostcSp*
Oscillatoria nigro-viridis	*OscilNigr*
Pleurocapsa brevissima	*PleuBrev*
Pleurocapsa crepidinum	*PleuCrep*
Pleurocapsa hansgirgiana f. *rosea*	*PleuHans*
Podocapsa pedicellata	*PodoPedi*
Pseudocapsa dubia	*PseuDubi*
Rivularia bullata	*RivlBull*
Rivularia mesenterica	*RivlMesn*
Scytonema endolithicum	*ScytEndl*
Scytonematopsis crustacea	*ScyCru*
Schizothrix helva	*SchizHelv*
Solentia foveolarum	*SolnFoev*
Solentia intricata	*SolnIntr*
Solentia paulocellulare	*SolnPaul*
***Solentia* sp. initial stadium**	*SolIncSt*
Solentia stratosa	*SolnStra*
Synechocystis pevalekii	*SynchPevl*
Tapinothrix sp.	*TapinSp*
Tryponema endolithicum	*TrypEndl*

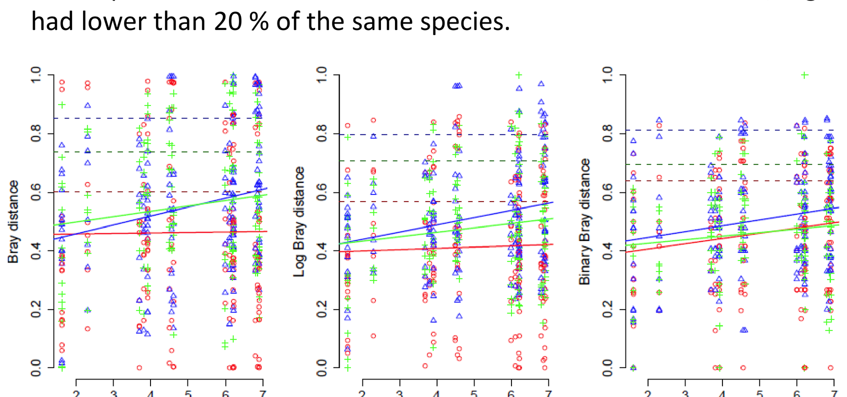
In the right column is a shortcut of the species used in graphs. Green are species found only on Veruda island, blue only on Ugljan island. In **bold** are dominant species.



A *Tapinothrix* sp., **B** *Rivullaria bullata* with *Geitlerinema* cf. *acuiforme* in its sheeet, **C** *Solentia stratosa*, **D, G, H** *Kyrtuthrix dalmatica*, **E** *Hyella tenuior*, **F** *Scytonema endolithicum*, **I** *Scytonematopsis crustacea*, **J** *Podocapsa pediculllata*, **K** *Entophysalis deusta* and *Tapinothrix* sp., **L** *Gloeocapsopsis crepidinum*

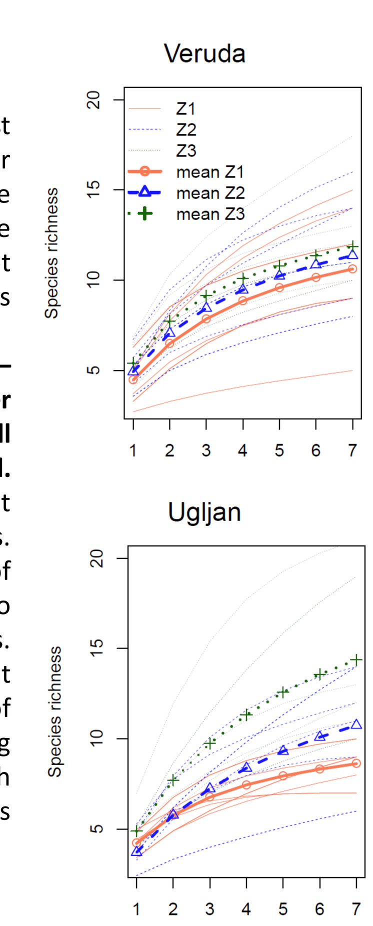
Q4 Spatial dissimilarity

On both islands, the lowest species similarities inside zones were observed with increasing physical distance in the top zone Z2 and Z3, and mostly the highest in the zone Z1. Also, there were lower similarities between zones than inside zones, with the lowest similarity between zones Z1 and Z3. Stronger dominance effect (similarity driven by highly dominant species) was observed on the Veruda Island, than on the Ugljan Island. Significant dissimilarity increase was observed in the Z2 for all ecological distances on the Veruda Island and for the transformed distances in the Z3 on Ugljan Island.
Even thought that we were able to demonstrate the increasing dissimilarity with the distance of samples within a site, the increase is very mild – this means that **two very close samples (as close as 0,05m apart from each other) have sometimes very different species composition.** Even stronger differences in composition were observed between zones on both islands, e.g. Z1 and Z3 had lower than 20 % of the same species.



Q5 Sampling effort

On both islands, Veruda and Ugljan, the highest species richness and probability to find another species (i.e. by resurvey of new plots) was in the zone Z3 and both gradually decrease for the upper to the lower zone. All species accumulation curves were not saturated, therefore higher sampling effort is necessary for the whole species spectrum.
Species accumulation curves do steadily increase – this suggests that even though we have rather intensive sampling in each site, further samples will very probably increase the number of species found. This suggests that any realistic sampling scheme is not able to provide exhaustive species list of the localities. It would be feasible to multiply the number of samples per locality, but it is definitely not feasible to carry out such intensive sampling in all the localities. The significant differences between sites suggest, that a sampling scheme concentrating on high number of samples within a locality would lead to undersampling of individual islands. Similarly, concentration of high number of localities would undersample the species richness of the whole surroundings.



Conclusions

- On two Croatian islands, Veruda and Ugljan, 42 cyanobacterial species were found. *Gloeocapsopsis crepidinum*, *Entophysalis deusta*, *Solentia* spp. *Hyella* spp., *Kyrtuthrix dalmatica* and *Mastigocoleus testarum* dominated the splash zone cyanobacteria flora and are typical species for this ecosystem. But there are also new records of some cyanobacterial species which have not yet been found in Mediterranean region or even in marine ecosystem.
- The splash zone might seem to be distinct into discernible zones. But in fact there are no strictly distinguished zones, the composition gradient only.
- In general, most of the species do not prefer any of the geographical position, only few species occurred in specific geographical aspect.
- In the richness species aimed studies of these habitats the most important is to cover sufficiently the vertical gradient.
- Each part of this gradient should be represented by many samples.
- Differences in the species composition slowly incerase with the distance between samples but even close samples can differ subtantially.

Acknowledgements

This study was supported by project of Grant Agency of the Czech Republic No. 15-11912S.

Přírodovědecká fakulta
Faculty of Science
Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Nadace
„Nadání Josefa, Marie a Zdeňky Hlávkových“