Lyons et al., (2010) Theory of island biogeography on a microscopic scale: organic aggregates as islands for aquatic pathogens

TL:DR Water collected for Knitting Mill Creek, rotated in 10l tank for 35 days. On days 1, 2, 7, 14 and 35, six aggregate samples and six aggregate-free samples taken on each day. Aggregates were sized. Biolog EcoPlate (microplate with 3 replicates of 31 carbon substrates) used to compare heterotrophic microbial communities. Each aggregate sample diluted to fill 1 set of 31 wells. Undiluted non-aggregate samples also inoculated into wells. Average well colour development used to compare community metabolic response (abundance of microbes) and functional diversity (species richness). Species turnover was assessed by comparing the specific substrates used by the aggregate-associated communities. Aggregate size did not vary much but there was a weak positive correlation between ‘island’ size and both community metabolic response and functional diversity. Aggregates = a greater number of more species for longer.

Intro

This experiment looks at ‘organic aggregates’ (material such as living, dead or inorganic particles suspended in aquatic systems) as islands for aquatic pathogens.

Method

1. Collected 15 litres of surface water in a sterile container from Knitting Mill Creek.
2. A 10 litre cylindrical tank was filled with source water and rotated at 1.5 rpm for 35 days at room temperature.
3. On days 1, 2, 7, 14 and 35 tank removed, placed horizontally and photographed.
4. Individual aggregates (n = 6) of similar size (2 to 4 mm long) were collected, one at a time, through the tank’s centre port with minimal water (<0.1ml) using sterile 1ml disposable pipettes.
5. Individual aggregates (islands) were transferred to labelled 15ml centrifuge tubes in which each aggregate was diluted to 10ml with sterile water.
6. A total of 30 samples (i.e. 5 sample days x 6 aggregates per day) were used for statistical analyses of aggregate data.
7. Water samples with no aggregates visible to the naked eye were collected from the centre port of the tank using sterile 10ml disposable pipettes.
8. The starting water (day 0 for both samples) included 1 sample based on 3 replicates.
9. On all other days, 6 samples (10 ml each) were collected for determination of community composition and concentration of culturable vibrios (gram-negative bacteria, aquatic pathogen).
10. A total of 31 water samples (5 days x 6 samples per day, plus 1 initial water sample) were collected and used for statistical analysis.
11. After sampling was completed, a similar volume of water (~90ml) was added back to the tank to maintain a constant volume.
12. With the tank in the horizontal position, individual aggregates were photographed and analysed using previously developed image analysis procedures for marine aggregates. Digital colour photographs were uploaded to an image analysis program and converted to 8-bit gray scale.
13. Aggregates were identified, numbered, counted and sized using a binary threshold and an automated particle counter. Results were manually verified against the original photographs.
14. Biolog EcoPlate microplates were used to characterize and compare the aggregate-associated and aggregate-free heterotrophic microbial communities.
15. EcoPlate consist of 3 sets of 31 carbon substrates (amino acids, carbohydrates, carboxylic acids) arrayed on a 96-well format. Each well contains a minimal growth medium and tetrazolium violet dye. The redox dye turns purple in electron transfer, indicating utilization by inoculated microbes.
16. A control well contains no sole-carbon substrate, thus any colour development indicates utilization of carbon sources inherent in the inoculated water or storage polymers of microbes.
17. Pooling aggregates prior to dilution yielded consistent results among replicate wells, but that diluting a single aggregate to fill all 96 wells did not. Consequently, individual aggregates were only diluted enough to fill one set of 31 wells per plate.
18. Samples of aggregates (diluted) and aggregate-free water (undiluted) were vigorously shaken and vortexed (30 s) before 150 µl samples were inoculated into each well.
19. Optical density of each well was determined immediately and after 3 d of dark incubation at room temperature with a BioTek plate reader. Bacterial concentrations were not determined.
20. Average well color development (AWCD, measure of average community metabolic response) was calculated in accordance with Garland & Mills (1991) after subtracting the starting values of each EcoPlate™ microplate from its 72 h readings (ΔAWCD) to account for intrinsic differences in the absorbance of the carbon substrates (Insam & Goberna 2004).
21. The average (n = 6 per time point) ΔAWCD (i.e. difference between times 0 and 72 h) was used to assess and compare community metabolic response and functional diversity of microbial communities in aggregates and aggregate-free water samples.
22. The number of substrates utilized by the microbial community of the sample (i.e. a measure of functional diversity, Zak et al. 1994) was determined by comparing the change in well colour development (adjusted for the control well) to a threshold value (0.250 optical density).
23. Readings greater than the threshold = substrates used by the community in the sample, whereas readings less than or equal to the threshold value were scored as substrates not used.
24. Species turnover assessed by comparing the specific substrates used by the aggregate-associated communities at Days 0, 1, 2, 7 when no significant differences in the number of substrates used (i.e. equilibrium, see section ‘Results’).
25. To do so, AWCD values of the 6 aggregates at each time point were averaged, then compared to the threshold to determine if the substrate was utilized or not utilized.
26. Finally, variance to mean ratios of the functional diversity (i.e. number of substrates used) of aggregate-associated microbial communities were calculated for each set of 6 aggregates collected at each of the 4 time points.

Results

Aggregate size – surface area weakly linked to average community metabolic response (average metabolic response (AMR) describes the average respiration of the C-sources by the microbial community and provides a single metric by which communities can be compared – how large is the community?) and functional diversity (community metabolic diversity (CMD) is represented by the number of substrates utilized by the microbial community and is analogous to community functional richness – how diverse is the community?)

Average community metabolic response – highly significant effects of day (decreased with time), this occurred more quickly in water without aggregates. Aggregates had a greater average community metabolic response (number of microbes).

Functional diversity – community diversity was lower in non-aggregate samples and declined after the first week for both treatments.

Species turnover – species diversity of aggregates remained fairly stable (state of equilibrium?) for the first week before declining.

Here individual ‘islands’ were isolated and the entire island sampled to determine the number of species.

Functional diversity was used as a proxy for species diversity to evaluate the temporal consistency of species number.

Analysis of the specific substrates utilized during this time period indicated a non-zero turnover of species (i.e. there were changes in species composition without changes in the number of species). The consistency of species number, coupled with non-zero turnover rates, supports the premise that a dynamic equilibrium within the aggregate-associated microbial communities was reached within 1 day and maintained for at least 1 week.

Species-area relationship – over the small size of aggregates the species-area relationship was confirmed with a weak yet significant relationship between aggregate size and functional diversity.

Peay et al., (2007) A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi

TL:DR Investigation into how ‘tree island’ size and isolation affect species richness of Ectomycorrihzal (EM) fungi. ‘Tree islands’ of the same age selected, sized, distance from source of EM fungal colonists calculated. Root tips and fruiting bodies sampled. Polymerase chain reaction used to amplify and then sequence samples. EM taxa identified using morphological molecular techniques. Richness estimates and species accumulation curves for each island were made with the program EstimateS. 14. To analyze the relationship between species richness, island area, distance from pre‐existing forest and soil type variability, simple regression and general linear models were used. Total species richness increased significantly with island area, distance had a small effect. They did not find good evidence linking the strong SAR for SM fungi in this system to increases in niche variety with habitat size. Lack of strong evidence for niche effect consistent with island biogeography and neutral community models, in which stochastic processes are key determinants of species richness and abundance.

Intro

While effects of habitat size and isolation have been successfully demonstrated for macro-organisms, there is currently strong debate about their usefulness in explaining richness patterns of microbial organisms. Because the Baas-Becking view predicts that microbial species will be ubiquitous in suitable habitats, one approach to testing it is by examining the degree of spatial turnover found in microbial species-area relationships (SARs). Molecular based studies have established microbial SARs, the degree of spatial turnover varies dramatically and it is unclear at what scale habitat size, isolation and dispersal should be considered in microbial ecology. In this study a ‘tree island’ approach is adopted for investigating SARs for Ectomycorrhizal (EM) fungi. Will investigate how habitat size and isolation affect species richness of EM fungi. Study derives one of the first quantitative SARs for EM fungi, and demonstrate levels of spatial turnover. Provide evidence that dispersal limitation can have both direct and indirect effects on EM assemblage composition.

Method

1. Conducted at Point Reyes National Seashore.
2. All ‘tree islands’ the same age as seed release activated by fires (1995).
3. Maps of the trees made based on manual classification using ArcGIS. Island area for newly mapped trees calculated in Arc GIS as the area of contiguous tree canopy as viewed from the aerial photo. ‘Island’ isolation calculated using pre-fire maps to calculate distance of each new ‘tree island’ from pre-existing patches of trees = closest possible source of EM fungal colonists on newly created islands. Soil types around trees also classified.
4. To ensure EM richness was sampled evenly across range of potential island size and isolation, islands divided into four size classes (1-10, 10-100, 100-1000 and >1000m^2) and three distance classes (0-100, 100-1000, >1000m). One island from each size class was then randomly selected from the population of islands in each distance class (4 x 3 = 12 islands).
5. Soil patches taken from around the trees showed ectomycorrhizal roots (microbes on the roots) were very rare after 1m and never seen after 4m.
6. To estimate species richness of EM fungi on each island, EM assemblage was sampled from both root tips and mushroom fruiting bodies.
7. EM root tips were sampled by directly removing soil using PVC cores. Because we expected more difficulty saturating sampling curves on larger islands, we took a variable number of cores depending on island size; six cores from 1 to 10 m2 islands, eight cores from 10 to 100 m2 islands and 12 cores from 100 to 1000 m2 and > 1000 m2 islands. After removal, cores ends were wrapped in Parafilm, brought back to the laboratory, and stored at 4 °C until processing.
8. To extract EM roots, soil from each core was washed through a series of soil sieves and the 500*μ*m fraction removed and spread evenly in a gridded, numbered Petri dish. Using the grid system and a random number generator, five individual root tips were randomly selected from each core for DNA extraction. Root tips were then flash frozen in liquid Nitrogen, lyophilized for 48 h, and stored at −40 °C until DNA extraction.
9. Mushroom fruit bodies were sampled 11 times on each island, in approximately 2 week intervals. Fresh tissue samples from each collected fruit body were removed and stored at −20 °C in CTAB for DNA extraction.
10. To identify root tips and unknown fruit bodies we used the polymerase chain reaction (PCR) to amplify and then sequence DNA from the internally transcribed spacer regions (ITS) of the ribosomal RNA genes (rDNA). Because this is the most commonly sequenced DNA region for fungi there is a large database of sequences and we know that it correlates well with current morphological species concepts for these fungi.
11. EM taxa were identified using a combination of morphological and molecular techniques. Fruit bodies collected in the field were identified first using standard morphological keys based on both macroscopic and microscopic characters. Most fruit bodies were identifiable to either species or genus using these keys. To improve identification of difficult taxonomic groups (e.g. *Inocybe*, *Hebeloma*, *Cortinarius*), rDNA sequences from fruit bodies were also compared with sequences available on the National Center for Bioscience Informatics (NCBI) website and assigned to a nearest taxonomic rank.
12. For root tips and unidentifiable fruit bodies, species were defined using a 95% DNA sequence similarity cutoff.
13. Richness estimates and species accumulation curves for each island were made with the program EstimateS, using a total species list (both fruit bodies and root tips) as well as partial species lists based on either root tips or fruit bodies. Each estimate was based on 500 randomizations of sample order without replacement.
14. To analyze the relationship between species richness, island area, distance from pre‐existing forest and soil type variability, we used both simple regressions and general linear models.
15. Ectomycorrhizal assemblage patterns were analyzed using the program BINMATNEST. This program was designed to measure the degree of ‘order’ in species occurrence patterns on fragmented habitat islands. To test whether EM colonization patterns were correlated with dispersal ability, we performed a regression to see if relative fruit body abundance was a significant predictor of the number of islands a species colonized. We also performed a regression between relative root tip and fruit body abundance to see if fruit body production was related to overall abundance within the EM assemblage.

Results

Total species richness increased significantly with island area.

Distance alone was a poor predictor of species richness, but a multiple regression including island area, distance and the interaction term significantly increased model fit and all effects were highly significant.

While island size explained approx. 70% of the observed variation in species richness, the interaction term indicated that the smaller effects of distance were size dependent, with distance reducing species richness only on the largest island sizes.

Island size also had a strong effect on EM assemblage structure. Species occurring on smaller islands were drawn from predictable subsets of assemblages of larger islands.

Varbiro et al., 2017 Functional redundancy modifies species-area relationship for freshwater phytoplankton

TL:DR Study investigates phytoplankton SAR in water bodies of large size scales. 312 samples taken from 64 water bodies (small ponds to lakes). Taxonomic identification carried out through investigation of traditional morphological features. Water body size, temperature, electrical conductivity, and pH recorded. Principal component analysis (PCA) used to explore the best combinations of variables. revealed the importance of spatial variables on lakes’ phytoplankton species diversity. Positively associated with log area of water bodies. Phytoplankton species richness showed characteristic right-skewed, hump shaped pattern along the spatial scale. Niche relations that are small-scale interactions are the most important determinants at the smallest spatial scale. High functional redundancy of the groups that prefer small-scale heterogeneity of habitats is responsible for the unusual humpback relationship. Species richness cannot be approximated by power or sigmoid models because, besides the small island effect, the ‘large lake effect’ also influences the shape of the curve.

Intro

Scale dependency of system attributes raises various questions in ecology. Evidence has demonstrated that predicted numbers of species increases with the area surveyed. Several models have been developed to describe SAR, including: the power, the exponential, the linear, and the logistic. Platonic algae form diverse assemblages that are maintained by niche partitioning, demographic stochasticity, dispersal limitation, and the physical disturbances of the aquatic environment. The aim of the study was to investigate phytoplankton SAR in a way that the area of water bodies studied covers large size scale, and the bias deriving from different richness estimations is minimized. It is hypothesised that the area of the water bodies has a significant effect on algal species richness, and the relationship can be described by power function.

Method

1. To avoid limitations and biases caused by heterogeneity of the water bodies and the large distance between them, we selected an area where water bodies of various sizes can be found. The Nagyiván puszta is an area in the Hortobágy National Park. Thousands of bomb crater ponds developed in the range of 100–102 m2. The larger water bodies are perennial, while the smaller ones are temporal. To increase size scale wider were also involved in the analysis of larger water bodies and two large lakes. Total 312 samples from 64 water bodies.
2. At least five water bodies from each size categories (in the range of 10−2–102 m2) are represented. Phytoplankton samples were collected in September 2011. Surface samples were taken from the center of each crater pond by a bucket and by a small plastic dish from the small pools. In case of larger lakes, the euphotic layer was sampled. In case of these water bodies, more sampling sites within the same lake were designated and sampled, then water samples were mixed in a large container, and half a liter from the mixed water was taken for the investigations. For the analysis of phytoplankton, the samples were fixed with formaldehyde solution at a final concentration of 4% and stored in darkness at 4°C until the analyses.
3. Water samples taken for chemical analyses were kept in coolers (0–4°C) during transportation to the laboratory.
4. Samples from Lake Balaton were taken with a tube sampler; thus, samples represent the whole water column.
5. Four lake morphometric variables were involved in the analyses: size, volume, depth, and index of basin permanency.
6. Taxonomic identification of algal taxa was based on light microscopic investigation of traditional morphological features. Phytoplankton subsamples were settled in 1‐ or 5‐ml settling chambers for 24 hr and counted with an inverted microscope at 400× magnification. For identifying morphologically closely related taxa, the algae were investigated at higher (630× and 1,000×) magnification with traditional upright research microscope (ZEISS Axioimager A2). To detect the rare, large‐sized taxa area of the whole counting chamber was investigated at 100‐fold magnification.
7. Temperature, electrical conductivity, and pH were determined immediately after sampling using the appropriate electrodes. Both pH and electrical conductivity values were temperature compensated (20°C). Total phosphorus was determined by colorimetric method after digestion with H2SO4.
8. 31 functional groups of phytoplankton were proposed by Reynolds, Huszar, Kruk, Naselli‐Flores, and Melo ([2002](https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.3512#ece33512-bib-0042)). In this study, each species was assigned to one of these groups.
9. Besides the truly planktonic organisms, the dataset contained several taxa that cannot be considered euplanktonic, for example, the benthic representatives of the diatoms and cyanobacteria groups.
10. The analyses were performed at both sample and water body level. For sample‐level analyses, actual environmental data and observed species richness values were used. For lake‐level analyses, means of the environmental variables and estimated richness values were applied. Richness estimations were based on Chao's sample‐based extrapolation curves. This approach is based on seamless rarefaction and extrapolation (R/E) sampling curves of three diversity metrics (richness, Shannon, and Simpson indices). To standardize the effort, estimated richness values belonging to identical sample coverage values (sc80 = 80%) were applied.
11. In case of lakes where the number of samples was smaller, short‐range extrapolation was applied. Rarefaction curves were drawn using the iNEXT package available in R. The applied extrapolated sample sizes exceeded less than twice the number of observed samples.
12. Principal component analysis (PCA) was used to explore the best combinations of variables in decreasing order of explained total dispersion and to assess the relationships between variables (size‐related and chemical properties of water bodies) and objects (sample‐based and lake‐based richness values) through optimal 2‐D graphical displays.

Results

Principle component analysis (statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components) revealed the importance of spatial variables on phytoplankton species diversity.

Phytoplankton species richness for samples and lakes showed characteristic right-skewed, hump shaped pattern along the spatial scale. The shape of the species area curve is influenced by niche relations, habitat diversity, mass effect, and ecological equivalency. Niche relations that are small-scale interactions are the most important determinants at the smallest spatial scale. At the largest spatial scale, the ecological equivalence is the most decisive factor i.e. an increase in functional redundancy is expected.

Functional richness (defined as the amount of niche space occupied by the species within a community) increased from smallest water bodies up to waters of 10^5 m^2 without characteristic breakpoint or critical area threshold that indicates the end of the so-called small island effect.

Functional richness showed its peak at ~10^5-10^6m^2 water body size. Habitat heterogeneity as one of the most fundamental diversity-maintenance mechanisms seems to be responsible for maintaining the highest phytoplankton richness at this size range.

Also decline in species at largest spatial scales, due to homogenizing impact of wind stress, decreasing the number of niches. Habitat heterogeneity can override SARs – “large lake effect” LLE.

Bell et al., 2005 Larger Islands House More Bacterial Taxa

TL:DR Bacterial genetic diversity increased with increasing treehole water volume (island size). Z slope of increasing diversity similar to that seen in larger organisms.

Intro

SARs is a generalisation in ecology, but some studies show the slope differs for microbes. This study shows the slope of SARs for natural bacterial communities inhabiting small aquatic islands is comparable to that of larger organisms. Number of taxa in an area is a balance between colonization and extinction. Size of area influences these mechanisms. Larger areas contain more habitats, therefore, more species. SARs relationship appears if more effort devoted to sampling, as number of taxa increases with sampling effort. S = cA^z – Species = taxon/location specific constant x area x slope of the line. Slope z for microbial taxa falls below that of larger organisms. Larger area = marginal increase in microbial species because they are ubiquitous. It is predicted that the slope of species-area relationships for insular bacterial communities would be similar to that found for communities of larger organisms.

Method

1. Measure water volume (island size) and bacterial genetic diversity (taxon richness) in 29 treehole islands, using denaturing gel electrophoresis.

Results

Bacterial genetic diversity increased with increasing island size according to the familiar species-area power law.

Slope z of the relationship indistinguishable from published values for larger organisms. Area size strongly influences diversity of microbial communities.

Bolgovics et al., 2016 Species area relationship (SAR) for benthic diatoms: a study on aquatic islands

TL:DR 217 samples was taken from 64 water bodies to determine the relationship between diatom species and habitat area. Diatom species were identified from the samples and divided into four functional guilds. The results show a small island effect, followed by increasing species richness with area. Although species richness of all guilds increased with the area of the water bodies, ratio of these guilds also showed differences in the various water body size categories.

Intro

The so-called “species-area relationship” (SAR) is one of the most general patterns in ecology. When small habitats are involved in the SAR studies, the relationships can be characterized at least by two distinct patterns (Lomolino, 2001). It has been demonstrated for several groups of organisms that the positive relationship between area and species richness does not exist below a certain area size threshold. This phenomenon is called the small island effect. Beyond the SIE range, toward larger sized habitats, is the range of the SAR in the traditional sense of the term. The large number of ponds and the large size differences among them make these habitats ideal objects for testing the various SAR models. functional diversity is also an important component of diversity because it is considered a useful metric that reflects the ecosystem complexity and processes. The aim of our work was to study the SARs for benthic diatoms. We hypothesized that in case of a wide range of spatial scale, the relationship between the number of diatom species and area can be described by the sigmoid model. Our additional hypothesis was that the small island effect can be shown for benthic diatoms.

Method

1. An unused shooting range with thousands of bomb crater ponds and other small aquatic pools in the middle of the Hungarian Great Plain was chosen as the sampling area.
2. Thirty-seven pools and ponds were sampled in this area such that all size categories were represented at least by five water bodies in the range of 10−2–102 m2.
3. To increase the size scale, late summer data of several nearby ponds and oxbows of the Tisza River and larger lakes were also involved in the analyses. The total scale covered a range of 10−2–108 m2. All together, 217 samples were taken from 64 water bodies.
4. Based on the measurements of chemicals all water bodies involved in the study were eutrophic. The exception is the large, shallow Lake Balaton, which is a meso-eutrophic system.
5. To study the diatoms, epipsammon and epipelon samples were collected from small pools of 10−1–10−2 m2.
6. In case of larger water bodies, diatoms were collected from the surface of macrophytes, mostly from reed stems. The samples were preserved with formaldehyde solution (final concentration 4%) and stored in dark bottles at 4°C until analyses. Geographical coordinates of the sampled pools (latitude and longitude) were recorded in the field with handheld a Global Positioning System (Garmine TrexH). Diameters of the bomb crater ponds and pools were also measured on the site by tape measure. The lakes and oxbows involved in the study were also sampled in the late summer period.
7. Organic matter of diatoms was removed by digestion using hot H2O2. To remove calcium carbonate, drops of HCl were added to the samples. After digestion, the material was washed by repeated sedimentation, and permanent slides were made using Cargille Meltmount mounting medium (refractive index = 1.704). Counting and identification of diatoms were done using oil immersion and DIC contrast at a magnification of 1000×. To equalize the counting effort, 400 valves were counted in each sample. Identification of diatoms was performed according to Krammer and Lange-Bertalot. Diatom species assigned to the four diatom guilds according to Rimet & Bouchez ([2012](https://link.springer.com/article/10.1007/s10750-015-2278-1#CR61)).
8. When microbial communities are studied, one has to face the problem of how to determine the exact number of species. When isolated islands are studied, the authors usually use others’ data and the efforts in these cases are not standardized. This implicitly results in high uncertainty of the results. To avoid these uncertainties when microbial diversity is investigated, use of species richness estimators is strongly recommended. For this reason, in our analysis the SARs were given for three data sets: the observed number of species, estimated number of species using the Chao2 estimator and estimated data where the sampling effort was standardized by rarefaction.
9. In the lower size categories (10−2–103 m2), preparation of the rarefaction curves was based on the five samples that belonged to the same size category. The curves were calculated as the average of 99 curves constructed from random permutations of the sampling order for each water body.
10. In case of the larger water bodies, more samples were taken; thus, the rarefaction curves could be prepared for each pond and lake respectively. The value of the smallest replicate was five; therefore, the species numbers belonging to the fifth replicates were considered later in the analyses. In case of larger lakes, more replicates were considered (Lake Velencei: 10; Lake Balaton: 15). The analyses were performed with the PAST software package.
11. Species area relationships were investigated in log-log space. The most frequently applied power model was used to describe the relationship. In log-log space the relationship can be described in linear form: log*𝑆*=log*𝑐*+*𝑧*×log*𝐴*, where *c* is the intercept and *z* the slope of the line.
12. The general additive model as an exploratory tool was used to reveal the general shape of the relationships. The GAM algorithm selects the best shape of given complexity (defined by the degree of freedom) using the Akaike information criterion (AIC).
13. In our model, the quasi-Poisson distribution and canonical log link function were used with the CANOCO 5 package. When the GAM algorithm indicated that the relationship could be better described by a nonlinear formula, we supposed that nonlinearity was caused by the SIE. The possible occurrence of the SIE and position of the break point on the shape of the species-area curve were investigated by using linear piecewise (breakpoint) regression. The method minimizes the sum of square of errors by fitting two lines to the data, and the position of the breakpoint is where one relationship shifts to the other. The software STATISTICA 8.0 was used to conduct the regression analyses. These analyses were done for the total number of taxa and also for each functional group of diatoms, respectively.

Results

Applying the breakpoint regression the relationships could be described by two linear sections with a breakpoint at 104 m2 water body area. The steepness of the lines in the lake area of <104 m2 size range was considerably lower. Above this point, the richness increased remarkably, which resulted in a greater slope of the lines. Asymptotes were not obtained; thus, sigmoid relationships could not be demonstrated.

Taxa numbers in all functional guilds showed increasing tendencies with the area.

We hypothesized that the SAR would be described by the sigmoid model if a sufficiently wide range of spatial scale is considered, but our results support that it does not hold true for benthic diatoms. We demonstrated that the relationship can be best described by breakpoint regression applying a single breakpoint at 104 m2, which means that a considerable increase in species number can be expected in large lakes.

We found that in small-sized water bodies (10−2–104 m2), the species richness did not increase considerably, and the variation in the number of taxa was remarkable. Thus, the SIE is a characteristic feature of benthic diatom SARs.

Our hypothesis that the proportion of diatom guilds varies at different size scales was supported by the results. Although species richness of all guilds increased with the area of the water bodies, ratio of these guilds also showed differences in the various water body size categories.

Henebry & Cairns 1980 The Effect of Island Size, Distance and Epicentre Maturity on Colonization in Freshwater Protozoan Communities

TL:DR Investigation into the effect of island size, distance and epicentre maturity on freshwater protozoan communities. Polyurethane foam (PF) substrates were placed in the benthic region of Douglas Lake and removed at different intervals. PF units put in colonization tanks with pasteurised lake water. Islands were harvested at 1, 3, 5 and 7 days, by lifting substrate from the water, inserting it into the mouth of a screw-cap pint jar, and squeezing as much liquid as possible from it. Each harvest was sampled by examining four slides of living material taken from the top and bottom, and the light and dark sides of each jar. Each slide was scanned fully and systematically with high dry power. For each sample, a species list was compiled using standard protozoological references. Closer islands accumulated species faster. Islands farthest from the epicentre both the lowest colonization rate and species richness. Islands of smaller area acquired species faster and had higher species richness than larger islands. Need larger range of island sizes and longer time to reach equilibrium to adequately test the island effect.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 | Experiment 5 |
| Colonization time | 1 Year | 1 Year | 2 Weeks | 3 Weeks | 4 Weeks |
| Size of islands (cm3) | 207 | 207 | 207 | 73 | 26 |
| Distance from epicentres (cm) | 38 | 19 | 9.5 | 9.5 | 9.5 |

Intro

Paper presents the results of several studies involving the colonization of initially barren artificial islands by natural” assemblages of protozoa. These “natural” assemblages came from artificial substrates that were allowed to be colonized for varying lengths of time. Previously, the relationship between island area and protozoan community development was examined and a positive relationship between size and equilibrium number of species was found for a certain minimal range of sizes. Phase 1 of this study was designed to (a) obtain baseline data on the colonization of islands by protozoa in a laboratory system for comparison with data collected in natural systems and (b) investigate the effects of island area and distance from a source pool of protozoan species on the process of colonization. Experiments in Phase 2 designed to test effect of epicentre community development on colonization of surrounding islands.

PHASE I

Method

1. Experiments were carried out in two closed laboratory systems, each consisting of plastic pans measuring 42 x 20 x 6 cm and covered by removable transparent tents which minimized contamination of the system by airborne propagules.
2. A 16-hr light:8-hr dark cycle was maintained throughout the experiments. Each tank was illuminated by two Sylvania F15T8-ww white fluorescent tubes mounted across the peak of the tent to reduce movement of phototrophic species in response to ambient light.
3. Temperature was uncontrolled, varying with the ambient (~ 18-22 G).
4. Sets of three 72 x 64 x 25 mm polyurethane foam substrates (PF units) were removed at weekly intervals from the benthic region of Douglas Lake and brought to the laboratory. Substrates used in the first two experiments had been in the lake for 1 year. Those used for the last three experiments had been in place for 2, 3 and 4 weeks, respectively. All substrates were assumed to be fully colonized.
5. At each collection, one PF unit substrate was harvested and examined by the method discussed below. This served as representative of the epicenter communities. Each of two PF units was placed intact in the center of one end of a colonization tank.
6. Approximately 16 liters of water were taken from, the lake at the site of epicenter collection and pasteurized by heating to 75-80 C for 10 min. Pasteurization was selected over autoclaving and filtering to avoid loss by volatilization of any essential micronutrients or detritus needed by the protozoa.
7. Colonization tanks were filled with the pasteurized lake water, covered and allowed to cool for 1 hr while being reaerated with an aquarium pump and air stones. Aeration was terminated then, and the epicenters were positioned.
8. Four sterile (protozoan-free) PF substrates of uniform size were placed in each tank at equal distances from the epicenter. These "islands" were harvested sequentially from both tanks at 1, 3, 5 and 7 days following establishment of the system.
9. Five experiments were carried out in duplicate, varying either distance or size of the island substrates from the epicenter. Distances of 38, 19 and 9.5 cm and PF unit islands of 207, 73 and 26.5 cm3 volume were used (in distance experiments all islands were 207 cm3).
10. Volume was chosen as indicative of substrate size since the PF units are porous, and protozoans can penetrate the interior of the cubes.
11. "Harvests" of epicenter and island PF units were accomplished by lifting a substrate from the water, inserting it into the mouth of a screw-cap pint jar, and squeezing as much liquid as possible from it.
12. Each harvest was sampled by examining four slides of living material taken from the top and bottom, and the light and dark sides of each jar.
13. Sample examination was completed within 36 hr after harvest to minimize loss of delicate species and changes in the structure of the community due to reproduction. Each slide was scanned fully and systematically with high dry power. For each sample, a species list was compiled using standard protozoological references.

Results

Distance effect – The number of species present on epicentre substrates was relatively constant. Distribution of the species present between the three classes of protozoans was also relatively constant. Islands located 9.5cm from the epicentre accumulated species faster and reached higher species number by the end of 7 days than islands of equal volume further away. The islands farthest from the epicentre (38cm) showed both the lowest colonization rate and species richness.

The reason that the final time to equilibrium may be longer for the nearest islands may be that the high invasion pressure from the epicentre forces a few more species to be added very slowly as new niches become available. Scanning electron microscopy studies have shown that the surfaces of the PF units become more complex through time.

Area effect – Islands of 26cm3 volume accumulated species faster and consistently had slightly higher species richness than larger islands during the 7-day observation period. Increasing size of experimental islands resulted in higher equilibrium species richness. In this experiment, species richness on the smallest (26cm3) islands was consistently higher than on the two larger volume islands. It may be that not enough time was allowed for all islands to reach equilibrium, so equilibrium species richness on islands could not be compared. With the 207cm3 islands, the observed and predicted values for equilibrium species diversity was greatly divergent. The size range between the largest and smallest islands used in these experiments may have been too narrow to get significant differences in species number on islands. It may still be possible to demonstrate the area effect in laboratory systems if a suitable size range of islands is allowed to be colonized for an adequate length of time.

PHASE II

Method

1. Laboratory experiments investigating the effect of maturity of epicenter communities on the colonization patterns and rates of nearby artificial islands were conducted. Methods were the same as in previous sections with the following modifications:
2. Each epicenter used in a microecosystem was one-half of a 76 x 64 x 51 mm PF unit which had been anchored in Douglas Lake for 1 day, 1 week, 3 weeks or 1 year.
3. The PF units were cut into halves; one-half was harvested and the species identified immediately, the other half was used as an epicenter in a microecosystem in an attempt to determine more fully the species composition of epicenters at the beginning of each experiment. There should be lower variation in the species composition of two halves of the same PF unit than between several PF units.
4. The epicenters were positioned in the center of each tank with two island PF units on either side at a 10-cm distance.
5. All four PF unit islands in each system were harvested on days 1, 3, 5 and 7 after initial set-up. Each island was removed using disposable surgical gloves, harvested, dipped in pasteurized lake water and replaced in its former position in the system. A control, consisting of an identical system, but with an uncolonized PF unit serving as a "false" epicenter, was run simultaneously with each experiment. The control provided evidence of nonepicenter invasion pressures.

Results

Epicentre maturity – The number of species present on epicentre PF units at the time of the initial harvest increased with length of time each had been anchored in Douglas Lake. The number of species on islands generally increased with time.

Eisenhauer et al 2013 Niche dimensionality links biodiversity and invasibility of microbial communities

Investigation testing the effect of two biodiversity indices, taxonomic richness and functional dissimilarity, on the invasibility of Pseudomonas fluorescens communities in microcosms of varying resource richness, herein used as a proxy for niche dimensionality. 21 communities set up by randomly assembling 8 strains of P. fluorescens, creating richness levels of one (x8), two (x8), four (x4) and eight(x4) genotypes (total of 24 replicate communities). Bacteria grow in a gradient of resource richness to modulate niche dimensionality – 14 combinations of glucose, mannose, fructose, sucrose and cirtrate (5 x single carbon sources, 4 x 2 carbon sources, 4 x three carbon sources, 3 x 5 carbon sources = total 16 treatments). 16 treatments applied to 24 replicate communities = 384 combinations. Invader success negatively correlated with diversity of resident community. For high niche dimensionality, functional dissimilarity increased community productivity and reduce invasion. For low niche dimensionality invasibility was driven by the presence of particular genotypes, rather than the biodiversity of the resident community.

Methods

1. Resident bacterial communities were assembled from eight P. fluorescens strains.
2. We setup 21 different bacterial communities by randomly assembling these strains, establishing richness levels of one (all eight monocultures), two (eight communities), four (four communities)and eight genotypes (four replicates; 24 treatments).
3. We used P. putidaIso as model invader, chromosomally tagged with green fluorescent protein.
4. Bacteria were grown in a gradient of resource richness to modulate niche dimensionality.
5. Microcosms contained 14 different combinations (16 treatments) of glucose, mannose, fructose, sucrose and citrate, mixed to a total of one (all single carbon sources), two (four combinations), three (four combinations) and five carbon sources (three replicates).Total substrate concentration was always 5 g substrate L -1.
6. To keep bacterial diversity and resource richness orthogonal, each bacterial community was grown in each of the resource treatments (24 bacterial treatments x 16 resource treatments, that is a total of 384 combinations).
7. We used two indices for the biodiversity of the resident communities: taxonomic richness was defined as the number of genotypes present at the beginning of the experiment.
8. Further, we estimated the functional dissimilarity of bacterial communities to account for niche differentiation among resident taxa.
9. First, each genotype was grown in minimal medium supplemented by one of the used carbon sources.
10. Growth was recorded as the optical density (OD600) of the culture after 24 h incubation at 26°C.
11. For each treatment, three replicates were set up. We used these data to compute pairwise Euclidian distances between all genotypes as
12. The functional dissimilarity of the community was then defined as the average pairwise distance between the genotypes present in the community.
13. Bacteria were pregrown overnight in Lysogeny Broth (LB), pelleted by centrifugation (10 000g, 1 min), washed twice in 0.85% NaCl and adjusted to an OD600of 0.5. Bacteria were grown at 25°Cin 384-well microtiter plates. Each well contained 40lL OS minimal medium supplemented with one to five carbon sources at a total concentration of 5 g L-1. We chose glucose, mannose, fructose, sucrose and citrate as carbon source because each of these sources supports the growth of at least one genotype and the invader.
14. The total OD600was 0.05 in all treatments. Resident communities were then grown either alone or in the presence of the model invader P. putida (5%of total start biomass).
15. Communities were grown for 24 h, a duration allowing all communities to reach the plateau phase under the tested conditions.
16. We measured the optical density (OD600, proxyfor total community biomass) and the green fluorescence (Ex:485 nm, Em: 520 nm, gain 80, as proxy for the biomass of theGFP-tagged invader) in a M200 Plate reader.
17. Invader success was estimated as the relative fluorescence(RFU, GFP/OD600). The fluorescence signal was blanked on the basis of the background signal from the invader-free treatment, and pure cultures of GFP-tagged P. fluorescens IsoF were used to calibrate the RFU to biomass relationships.
18. Note that resident genotypes and the invader were inoculated simultaneously.
19. As detailed above, the model invader was added at much lower density than the resident genotypes. We chose to use this approach because adding invaders at a later stage would have caused major disturbances of the microcosms which may have affected the growth phase of resident genotypes.