Introducing Microbiome Bioinformatics

Part 9.

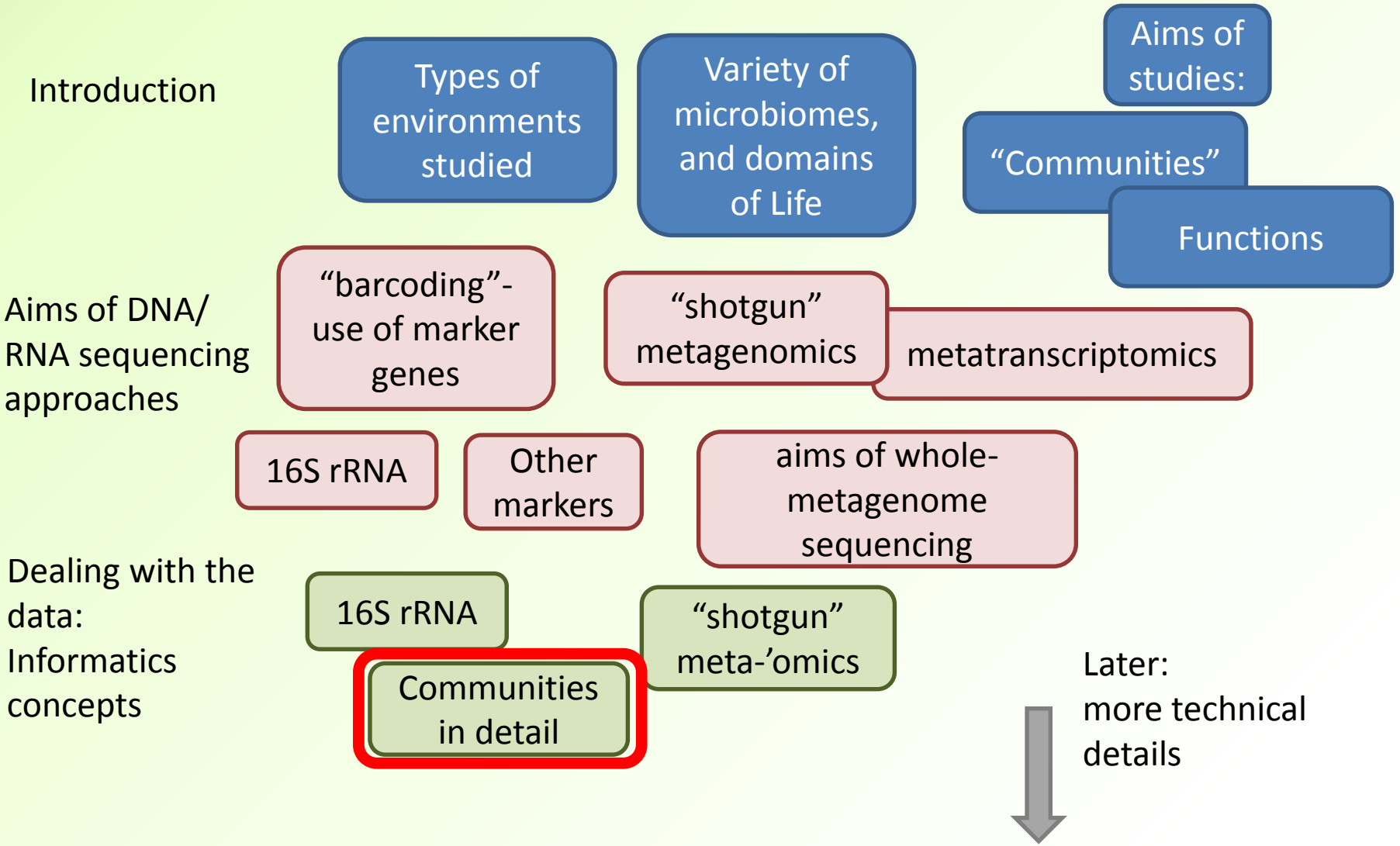
Microbial ecology –

Diversity (part 2)

Recap: Aims

- **Microbiome analysis**
  - with particular regard to **sequence informatics concepts**
- "Top down" – putting analysis tools and resources in context
- No highly detailed technicalities (yet)
  - No instructions on how to run particular programs
- Why you are using the bioinformatics approaches you use; pros, cons; alternatives

Topics, top-down



Series of talks

- 8 so far
- Open ended… as long there is demand
- Expected to be every 2 weeks
  - Notwithstanding some larger gaps for various reasons…
  - all dates will be confirmed in advance
  - *Please refer to:* ***Bite-size bioinformatics mailing list***
- Informal and flexible
  - Please interrupt and ask questions
  - **Suggestions for topics for further focus**

Series of talks

- Part 1: 27/1/2017
  - "Biological and Experimental Stuff that a microbiome bioinformatician needs to know"
  - Overview of marker gene sequencing for community analysis
- Part 2: 10/2/2017
  - Overview of whole-metagenome sequencing
- Part 3: 24/2/2017
  - Focus on metatranscriptomics
- Part 4: 10/3/2017
  - Different bioinformatics approaches to processing 16S read data
- Part 5: 24/3/2017
  - *De novo* OTU clustering: sequence identities and how thresholds have been determined historically; relationships to taxonomic levels
- Part 6: 7/4/2017
  - The clustering problem: different approaches, and what can go wrong; the influence of amplification artefacts, sequencing errors and sequence lengths; computational OTUs versus species
- Part 7: 21/4/2017
  - Introducing microbial ecology: using observed abundances of OTUs (or species, or functions) to estimate the richness of the community (number of different OTUs, species etc)
- Part 8: 2/6/2017 – continuing microbial ecology: community diversity : diversity indices
- Part 9: today – continuing microbial ecology: community diversity : true diversity
- Slideshows - http://ghfs1.ifr.ac.uk/ghfs/

Future talk(s)

- 30th June Barton
- TBC?
  - 14th July
  - 28th July
- None planned for August
- Topics?
- Format?

Today

- Today and recent sessions:
- Measurements/estimations of richness and diversity of a microbiome
- (21st April) : Richness : number of species (or OTUs or functions etc)
- (2nd June) : Diversity indices
- (Today) :
  - True diversity
  - α-diversity, β-diversity, γ-diversity (…being optimistic?)
  - ~~Phylogenetic Diversity~~

Recap

Measurement versus estimation

Richness

Diversity indices

“Amounts of different things”

- “Things”: different –
  - Species
  - OTUs
  - Some other taxonomic unit
  - Phenotypes
  - Molecular functions
  - Pathways

phylotypes (Species, OTUs, Some other taxonomic unit)

types of organism (Species, OTUs, Some other taxonomic unit, Phenotypes)

types of gene (Molecular functions, Pathways)

- Whichever we are interested in, we will benefit from a **simple metric**, instead of a large table
- Enables easy and direct comparison between samples
  - Disease/health states
  - Genotypes
  - Different time points for the same subject

You have a table like this:

SAMPLES …. ***…..***

OTUs

or ***species***

…. or other 'phylo-types'

…. or gene functions

	#1	#2	#3	#4	#5	#6	#7	#8
a								
b								
c								
d								
e								
f								
g								
h								
i								
j								
k								

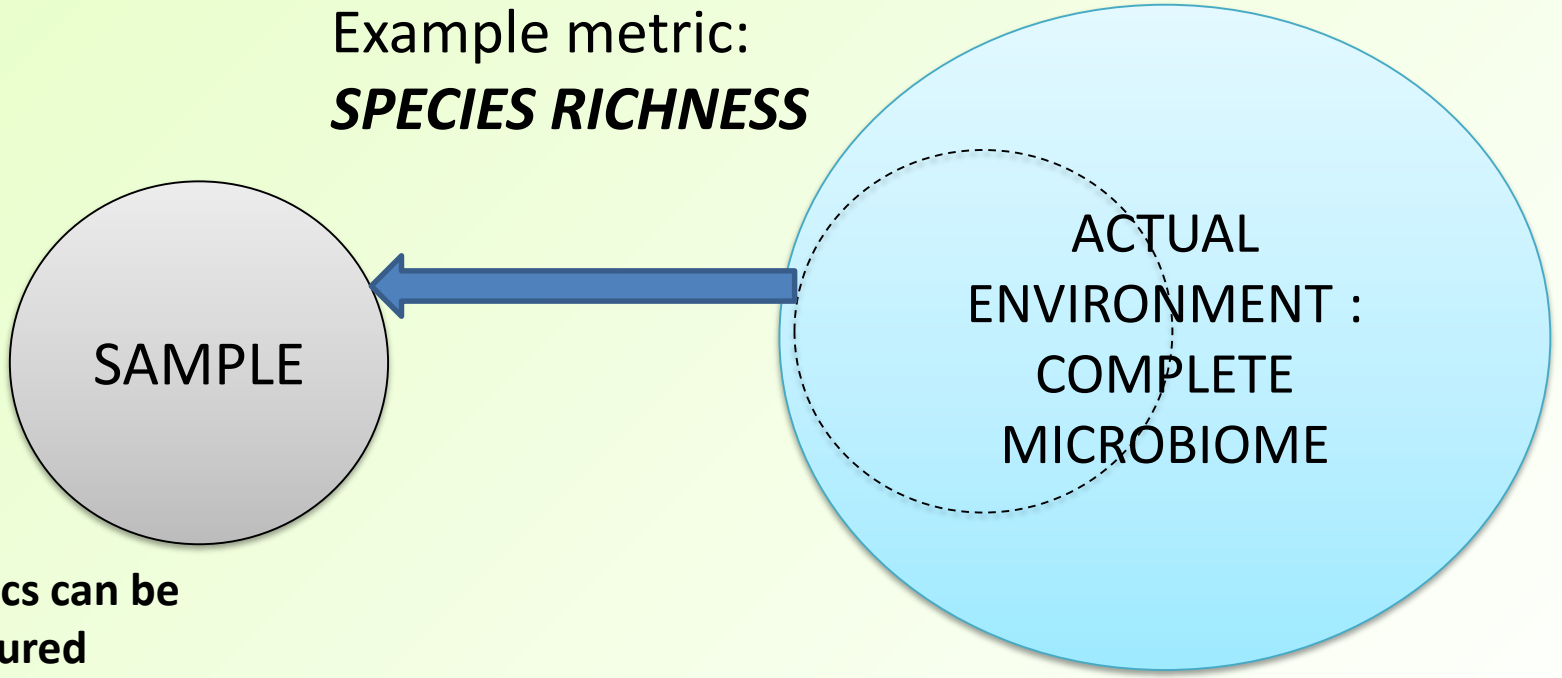
(relative) frequencies….

This could result from 16S rRNA gene sequence (16S rDNA) analysis, **or** metagenomics sequence analysis;

and from OTU-based approaches, and non-OTU based

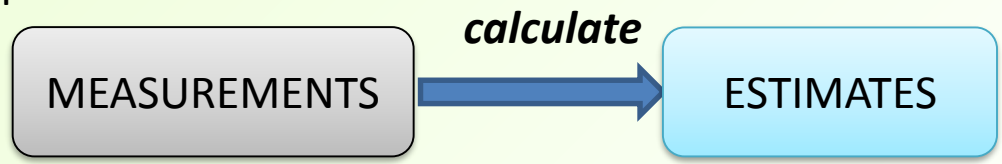
Measurement and estimation

Example metric:
SPECIES RICHNESS

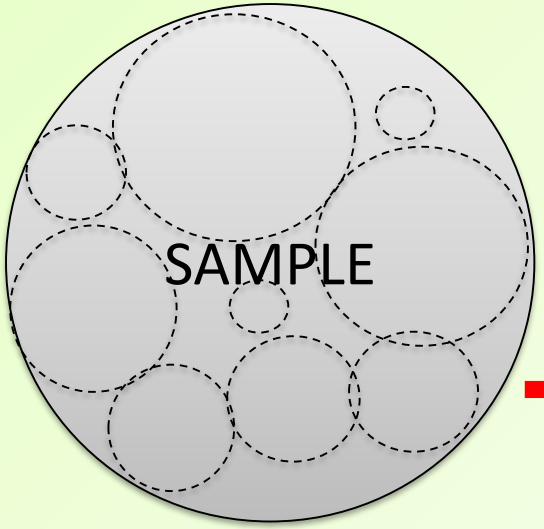


Metrics can be measured
E.g. Richness: count the number of different species present

Can't be 'measured' – unless we are able to observe literally every organism present



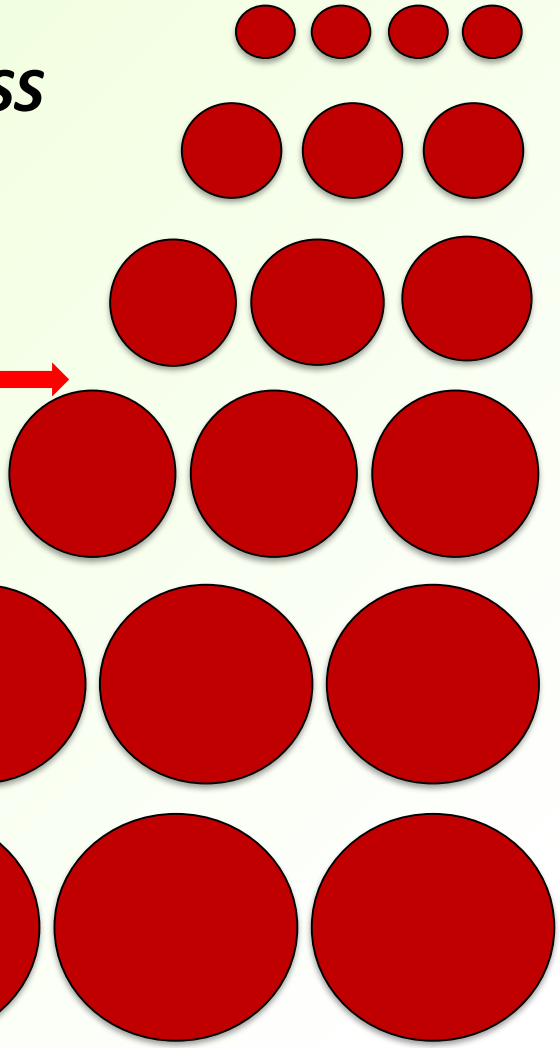
Rarefaction: an aid to estimation

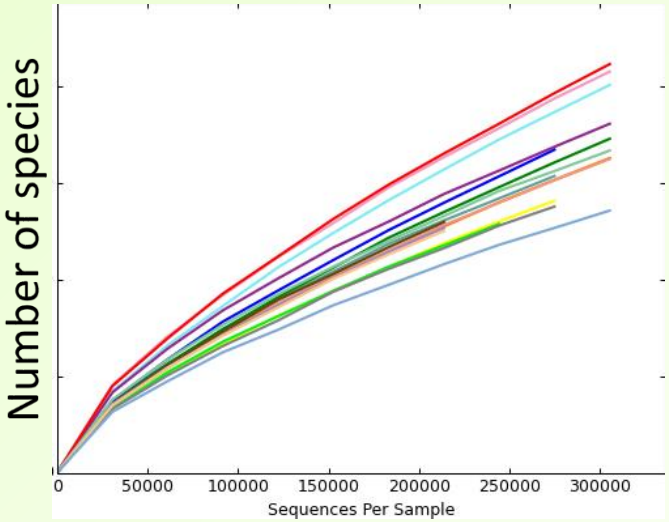


Example metric:

SPECIES RICHNESS

Systematic repeated random sub-sampling using different sub-sample sizes



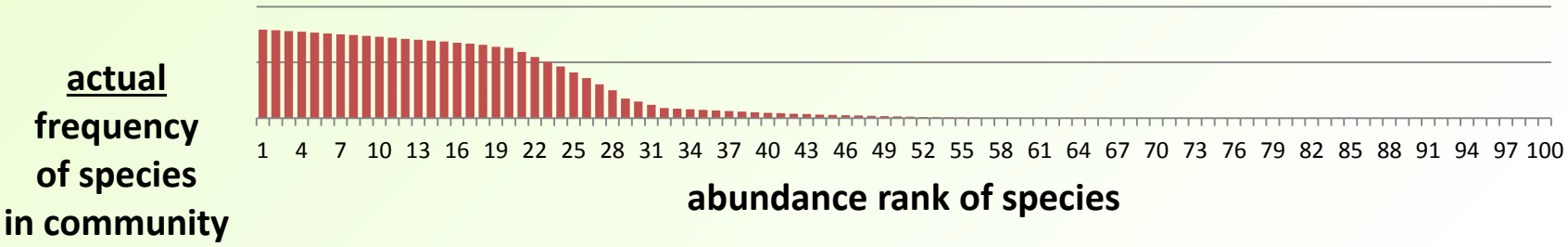


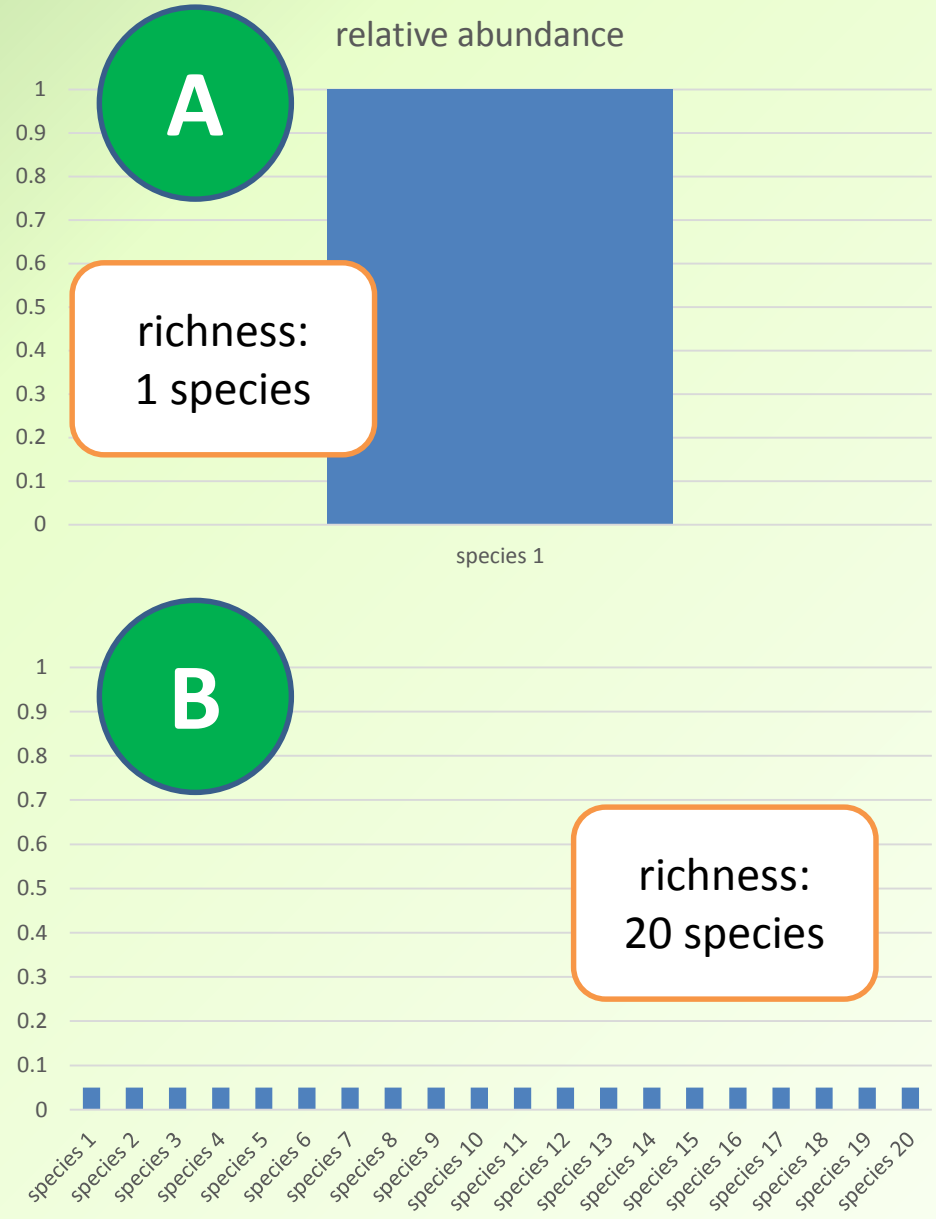
Sample size

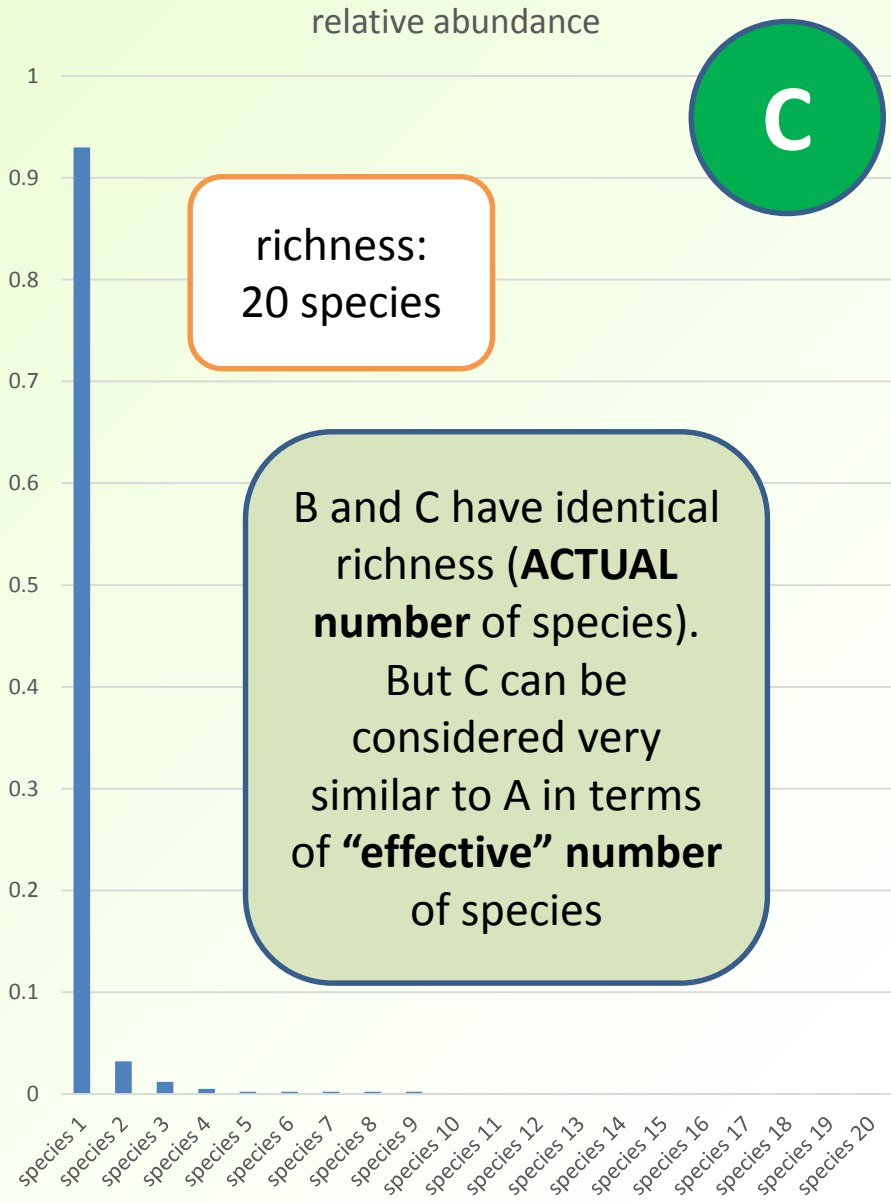
count the number of different species present in each sub-sample

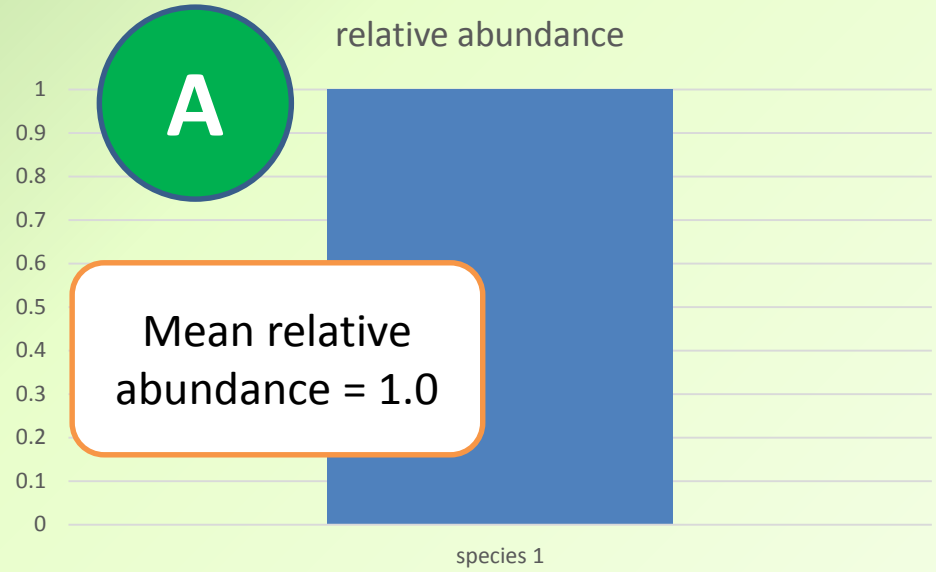
Problems with Richness

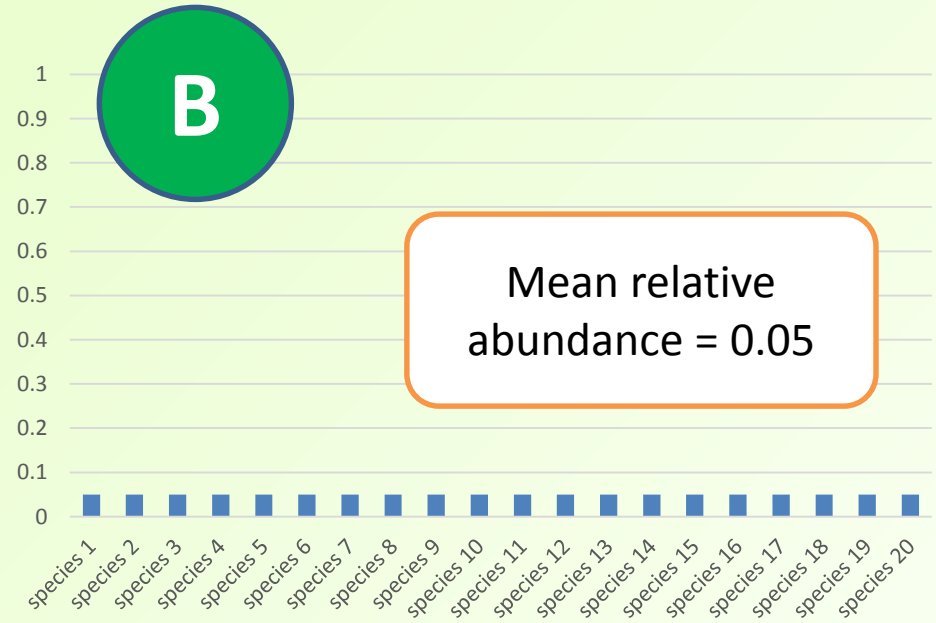
- (21st April) Richness is difficult to estimate
  - Very thin tails (in distribution of abundance versus species)
  - Length of tail makes a big difference
  - Richness is very difficult to estimate reliably in the microbiome - e.g. Haegeman *et al.* (2013)
  - Rarefaction does not help with this
- How interested are we in the extremely low-abundant species?

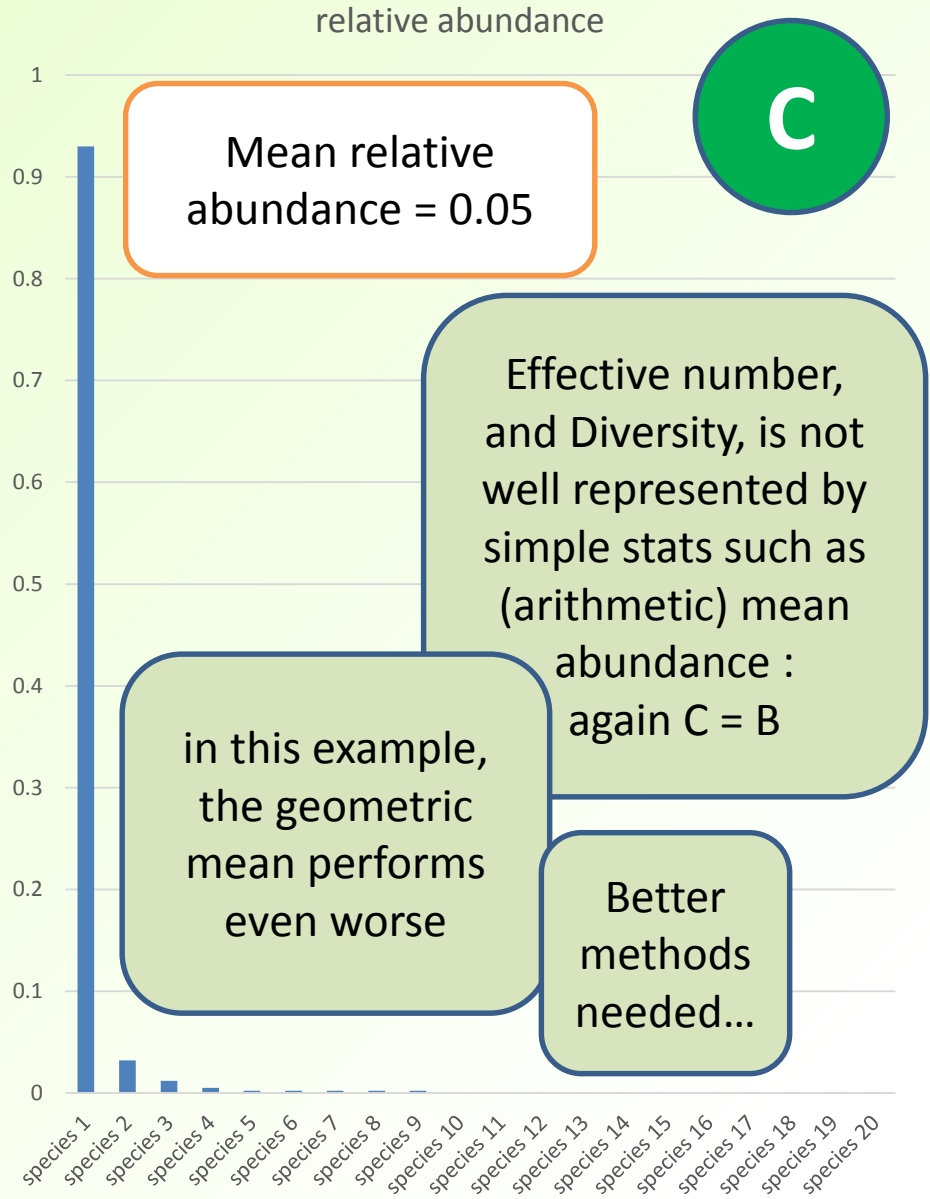


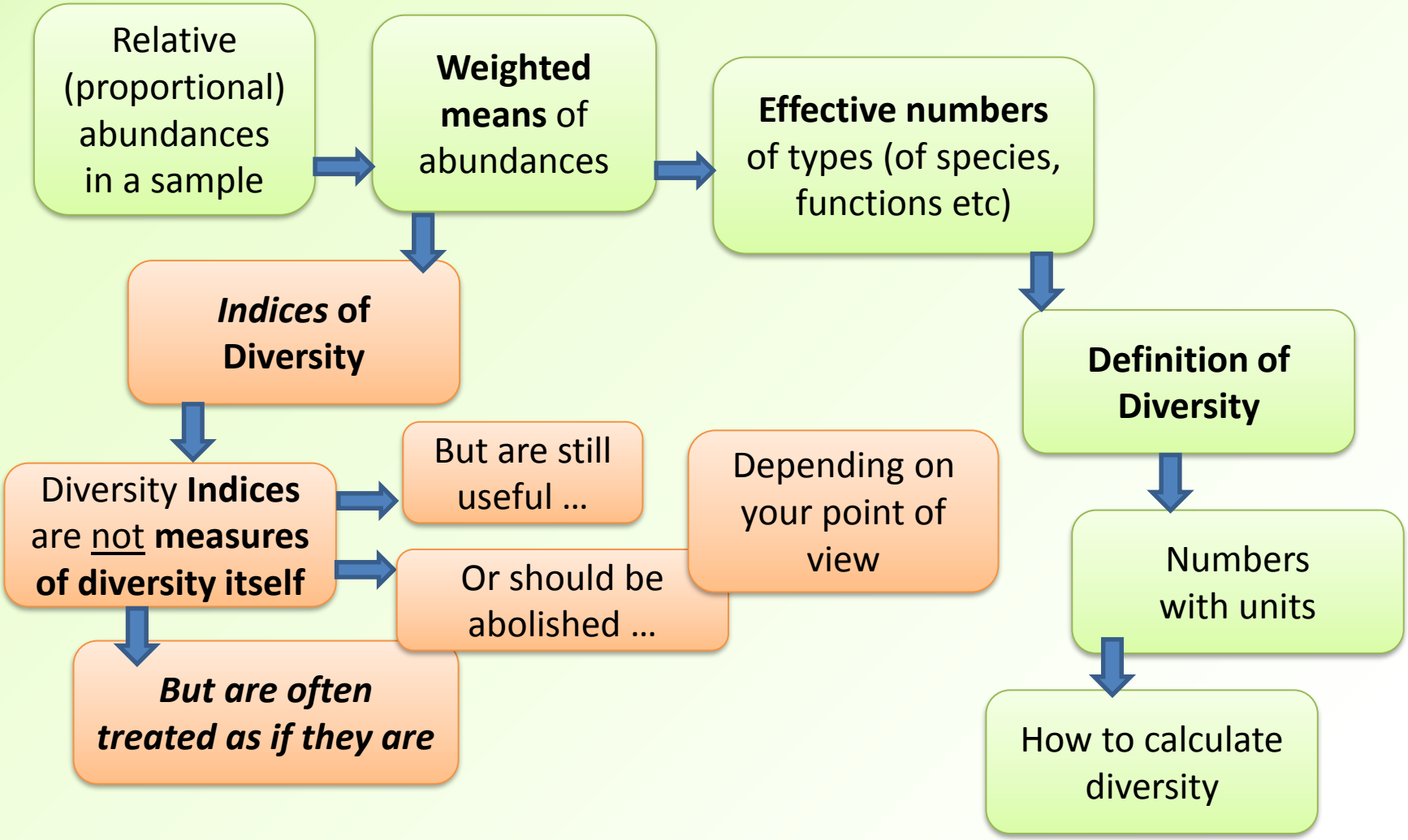

A
relative abundance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
species 1
richness:
1 species
B
richness:
20 species
species 1
species 2
species 3
species 4
species 5
species 6
species 7
species 8
species 9
species 10
species 11
species 12
species 13
species 14
species 15
species 16
species 17
species 18
species 19
species 20

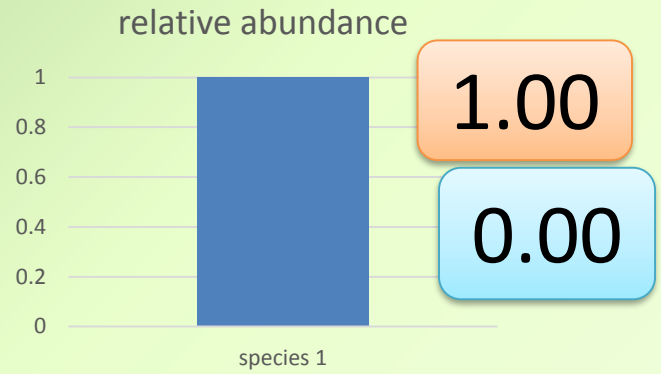

relative abundance
C
richness:
20 species
B and C have identical richness (**ACTUAL number** of species). But C can be considered very similar to A in terms of **"effective" number** of species
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
species 1
species 2
species 3
species 4
species 5
species 6
species 7
species 8
species 9
species 10
species 11
species 12
species 13
species 14
species 15
species 16
species 17
species 18
species 19
species 20

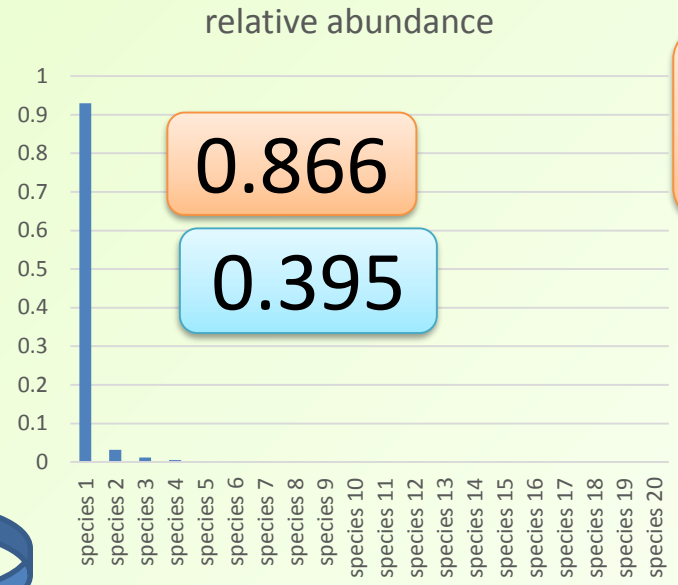

A
relative abundance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
species 1
Mean relative abundance = 1.0


B
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
species 1
species 2
species 3
species 4
species 5
species 6
species 7
species 8
species 9
species 10
species 11
species 12
species 13
species 14
species 15
species 16
species 17
species 18
species 19
species 20
Mean relative abundance = 0.05


relative abundance
C
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
species 1
species 2
species 3
species 4
species 5
species 6
species 7
species 8
species 9
species 10
species 11
species 12
species 13
species 14
species 15
species 16
species 17
species 18
species 19
species 20
Mean relative abundance = 0.05
Effective number, and Diversity, is not well represented by simple stats such as (arithmetic) mean abundance : again C = B
in this example, the geometric mean performs even worse
Better methods needed…


Relative (proportional) abundances in a sample
Weighted means of abundances
Effective numbers of types (of species, functions etc)
***Indices* of Diversity**
Definition of Diversity
Diversity **Indices** are not **measures of diversity itself**
But are still useful …
Depending on your point of view
Or should be abolished …
But are often treated as if they are
Numbers with units
How to calculate diversity

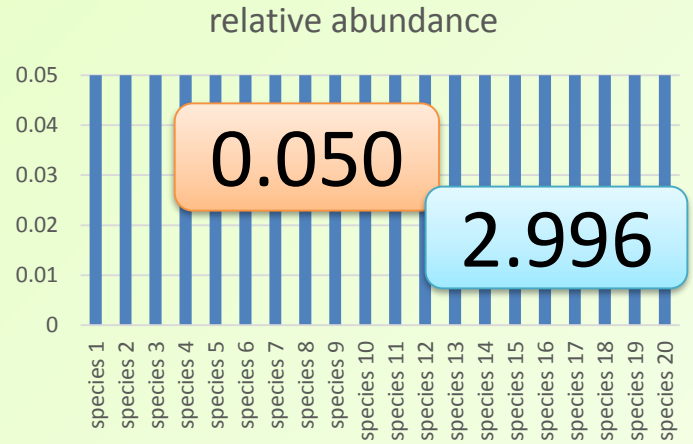


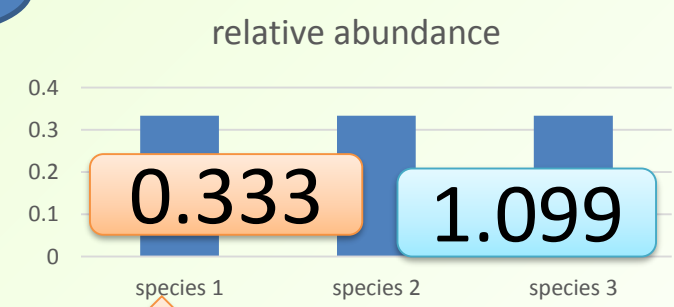


Simpson Index

high

low

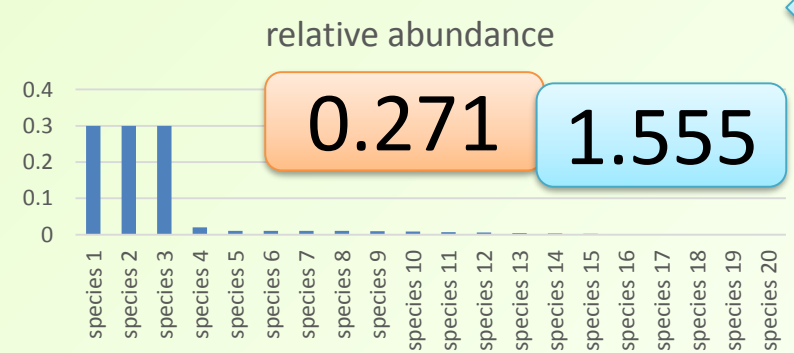


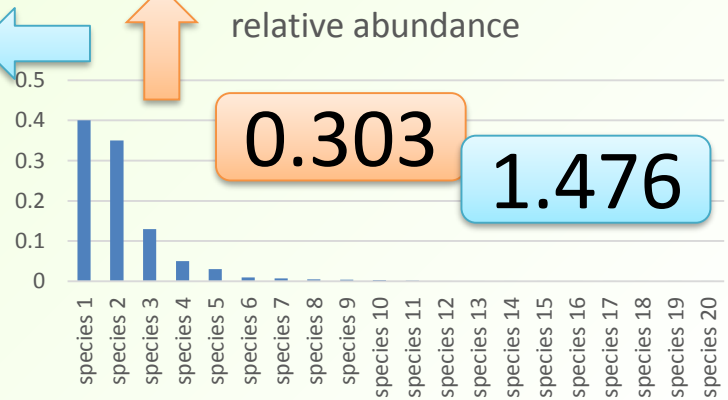


Shannon Index

low

high





Dealing with abundances

- We don't use absolute abundances
  - i.e. counts of each species (or OTU, etc)
- We always deal with the **proportional abundance**
  - often referred to here as "relative abundance"
  - this is also effectively a **probability**
- *N* species: $x_1 , x_2 , x_3 , x_4 , x_5 \ldots , x_N$
  - these will all be $0 < x_i \leq 1$
- $\sum x_i = x_1 + x_2 + x_3 + x_4 + x_5 \ldots + x_N = 1$

Sums, Means and Weights

- Sum (unweighted sum):
  - $\sum = x_1 + x_2 + x_3 + x_4 + x_5 \ldots + x_N = 1$
- Unweighted arithmetic mean = $\sum / N = 1 / N$
- This arithmetic mean is simply a special case of a **weighted** mean
  - where each x_i has a weighting w_i
  - in this special case, all the weights (w_i)are the **same**
  - and all $w_i = 1/N$
  - Mean =
  - $w_1 x_1 + w_2 x_2 + w_3 x_3 + w_4 x_4 + w_5 x_5 \ldots + w_N x_N$
  - **= 1/N**

Non-uniform weighting

- $w_1 x_1 + w_2 x_2 + w_3 x_3 + w_4 x_4 + w_5 x_5 \ldots + w_N x_N$
- Simpson index: use $w_i = x_i$
  - (weight each abundance by itself)
- $= x_1^2 + x_2^2 + x_3^2 + x_4^2 + x_5^2 \ldots + x_N^2 = \sum x_i^2$
- Shannon index: use $w_i = -\ln(x_i)$
- $= -x_1\ln(x_1) - x_2\ln(x_2) - x_3\ln(x_3) - \ldots - x_N \ln(x_N)$
- $= -\sum x_i \ln(x_i)$ i.e. the Shannon entropy
- (often denoted ***H*** or ***H'***)

An actual measurement of something
is not the same as
an ‘index of something’

units are different

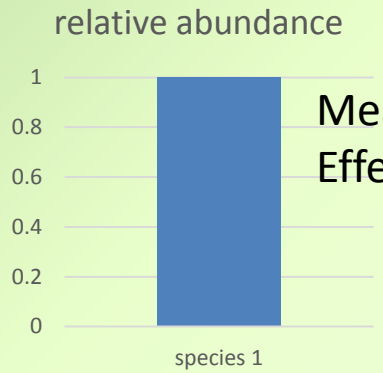
Example: '**the size of a room**'

- Room sizes:
- Ormesby A 9
- Ormesby B 9
- Ranworth 16
- Barton 24
- Rollesby 24
- Lecture Theatre 126

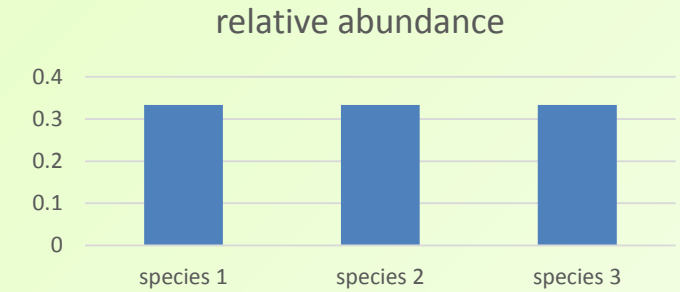
Effective number of species

(or effective numbers of OTUs …
or of genera… function… etc)

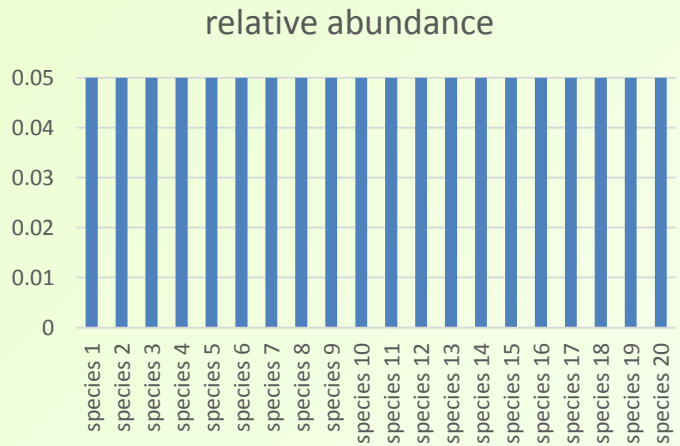
More on effective number of species – the easy cases



Mean relative abundance = 1
Effective number = 1 / mean = **1**



N = 3 species only
All equally abundant
Therefore treated identically
Mean relative abundance = 0.333
Effective number = 1 / mean = **3**



N = 20 species: Mean relative abundance = 0.05
Effective number = 1 / mean = **20**

N = 1000 species:
Effective number = 1 / mean = **1000**

All obvious; but for any number *N*, the effective number will always = N if the distribution is flat irrespective of the weighting scheme

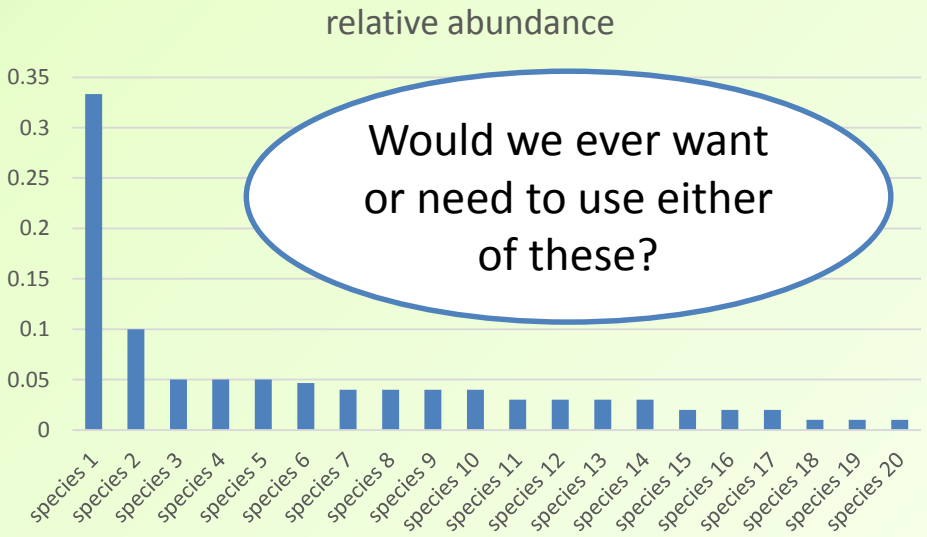
Effective number of species

- Effective number of species is **always:**
- **the reciprocal of the mean of the relative abundances**
  - ***however that mean may be weighted***
- How do we weight that mean?
  - We have a choice
  - We have seen some approaches to this
  - **We can use more than one scheme at a time**
  - But it makes most sense to use a systematic, **generalised weighting scheme**
- For completely flat distributions, the weighted mean will always equal the unweighted mean, irrespective of the weighting applied

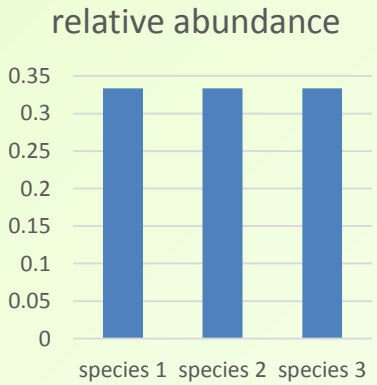
Effective number of species

- We can choose to attach more or less importance to:
  - The more abundant species
  - The rarer species
- At the extremes, we can base the calculation entirely on the abundance of:
  - the single most abundant species
  - the single least abundant species

Extreme weighting



Would we ever want or need to use either of these?



Either way
→ effective number of species
= 1 / 0.33333 = **3.00**

One extreme: we ignore all but the **most** abundant species
So, weighted mean = mean of most-abundant species = 0.33333
→ effective number of species
= 1 / 0.33333 = **3.00**

Other extreme: we ignore all but the **least** abundant species
So, weighted mean = mean of most-abundant species = 0.01
→ effective number of species
= 1 / 0.01 = **100.00**

Effective number of species = Diversity

- The effective number of species
- i.e. the reciprocal of the weighted mean
  - **IS THE DEFINITION OF DIVERSITY**
- It has units: **species**
  - (or OTUs, or functions, or languages spoken by employees, or whatever it is you are assessing the diversity of)
- I.e. its units are identical to **richness**
- If we are using **more than one way** of calculating the weighted mean, in **systematic** approach:
- **Then we have a series of diversity values**

The Hill Numbers

Mark Oliver Hill used a simple system of **weighted generalised means** of the relative abundances

Hill (1973)

(also known as “Hill Diversity” , “true diversity”)

The Hill Numbers

- Consider a value k
- Sum each relative abundance x_i raised to the power k and weighted by w_i
  - $\boldsymbol{w_1 x_1^k + w_2 x_2^k + w_3 x_3^k + w_4 x_4^k + w_5 x_5^k \ldots + w_N x_N^k}$
- i.e. $\sum (w_i x_i^k)$
- The weighted mean is the kth root of this sum
  - $\left(\sum (w_i x_i^k) \right)^{1/k}$
- So the diversity is the reciprocal of that weighted mean:
  - $1 / \left(\sum (w_i x_i^k) \right)^{1/k} \quad = \quad \left(\sum (w_i x_i^k) \right)^{1/-k}$

The Hill Numbers

- This value $\left(\sum (w_i x_i^k) \right)^{1/-k}$
- is the **Hill Diversity of order *k*+1** (thus, ${}^{k+1}D$)
- - and is rarely (ever?) written like the above – but it makes more sense of the *k*th root (IMO)
- The Hill system in fact **always uses $w_i = x_i$**
- and is normally written
  - ${}^{q}D = \left(\sum x_i^q \right)^{1/(1-q)}$
- i.e. **Hill Diversity of order *q*** (i.e., $q = k+1$)
- i.e. the weighted mean abundance is $\left(\sum x_i^q \right)^{1/(q-1)}$

Some interesting properties of ${}^{q}D$

- q can be a non-integer
- q can be negative
- ${}^{q}D$ appears undefined for $q = 1$
- (so too does the reciprocal, i.e. the weighted mean abundance)

$$ {}^{q}D = \left(\sum x_i^q \right)^{1/(1-q)} $$

- Weighted mean abundance
- = 1 / diversity

$$ = 1 / {}^{q}D = \left(\sum x_i^q \right)^{1/(q-1)} $$

$q = 0$

- $q = 0$
- ${}^{0}D =$
- $(x_1^0 + x_2^0 + x_3^0 \ldots x_N^0)^1$
- $= N$
- i.e. the number of different species
- i.e. **RICHNESS**
- (and the weighted mean abundance is the simple arithmetic mean)

$$ {}^{q}D = \left(\sum x_i^q \right)^{1/(1-q)} $$

$$ 1 / {}^{q}D = \left(\sum x_i^q \right)^{1/(q-1)} $$

$q = 2$

- $q = 2$
- ${}^2D =$
- $(x_1^2 + x_2^2 + x_3^2 \ldots x_N^2)^{-1}$
- = the reciprocal of the Simpson index
- (and the weighted mean abundance *is* the Simpson index)

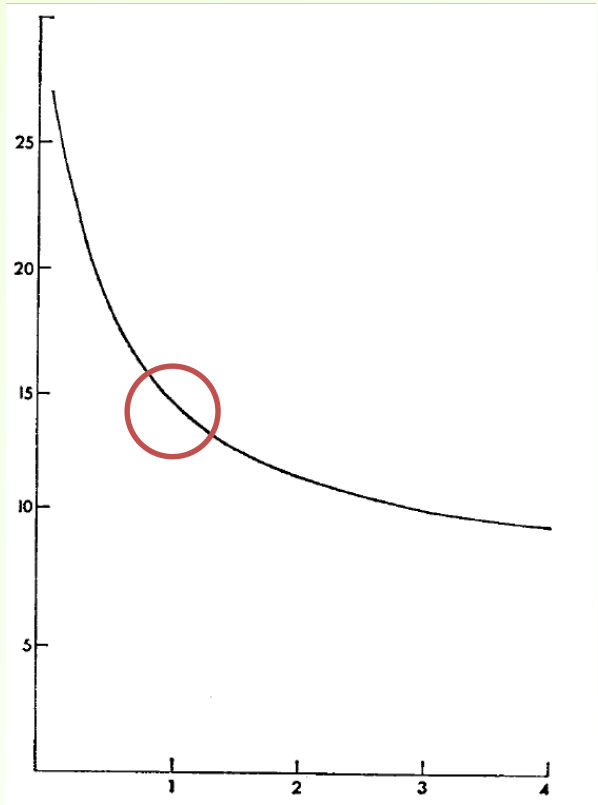
$$ {}^qD = \left(\sum x_i^q \right)^{1/(1-q)} $$

- $1 / {}^qD = \left(\sum x_i^q \right)^{1/(q-1)}$

What about $q = 1$?

- $q = 1$
- ${}^1D$ appears to be
  - $(\sum x_i^q)^{\infty}$ i.e. 1^{∞}
- But do not assume ${}^qD = 1$
- By considering:
- what ${}^qD$ **tends to** as $q \rightarrow 1$
- It can be shown that ${}^qD$ is **continuous** at q = 1
- Hill included a proof of this
- Example data:

$$ {}^qD = (\sum x_i^q)^{1/(1-q)} $$



What about $q = 1$?

- $q = 1$
- ${}^1D = \lim ({}^qD)$

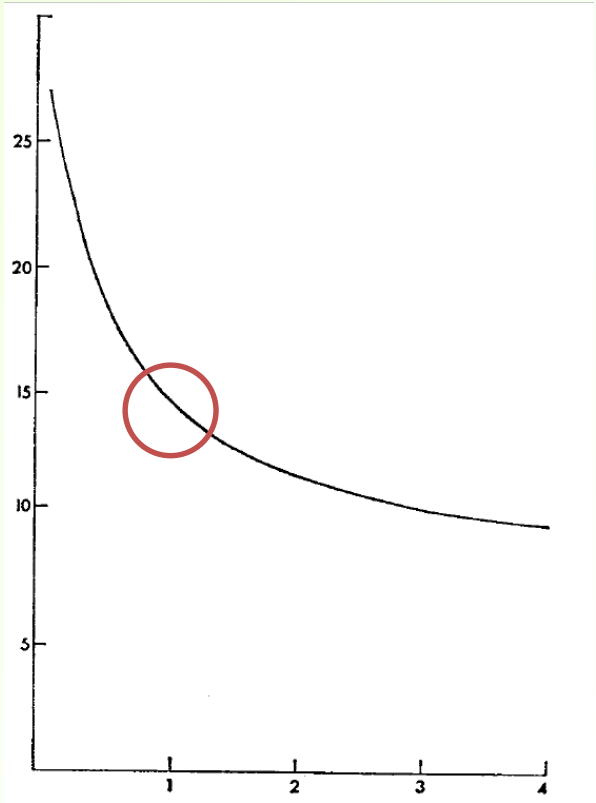
  $q \rightarrow 1$
- It can be shown that:

$${}^1D = e^{(-\Sigma\, x_i \ln(x_i))}$$

$$= e^H$$

- That is, ***e* to the power of the Shannon index**

$${}^qD = \left(\sum x_i^q \right)^{1/(1-q)}$$



$q = \infty$

- $q = \infty$
- Can be shown that:
- ${}^{\infty}D =$
- the reciprocal of the proportional abundance of the commonest species
- and the weighted mean abundance

  = abundance of commonest species

$$ {}^{q}D = \left(\sum x_i^q \right)^{1/(1-q)} $$

$$ 1 / {}^{q}D = \left(\sum x_i^q \right)^{1/(q-1)} $$

$q = -\infty$

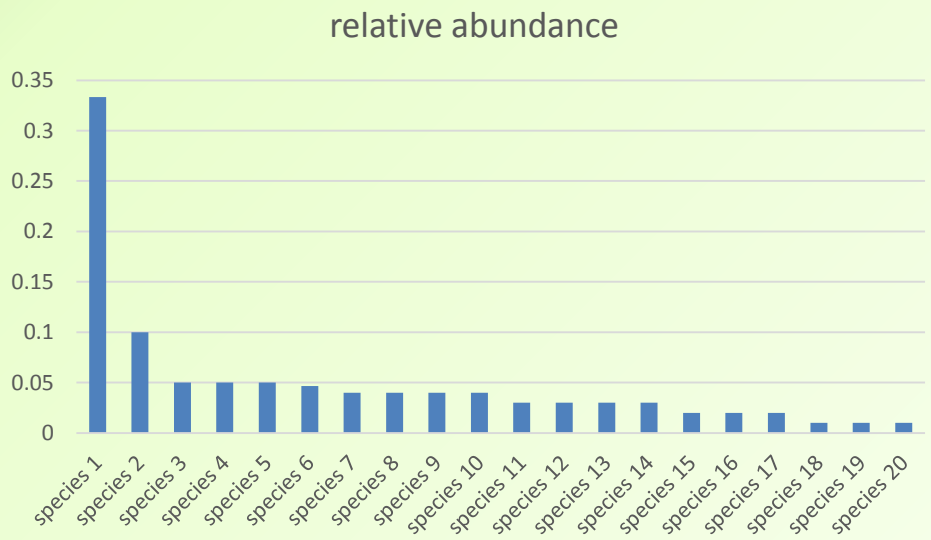
- $q = -\infty$
- Can be shown that:
- ${}^{-\infty}D =$
- the reciprocal of the proportional abundance of the rarest species
- and the weighted mean abundance

  = abundance of rarest species

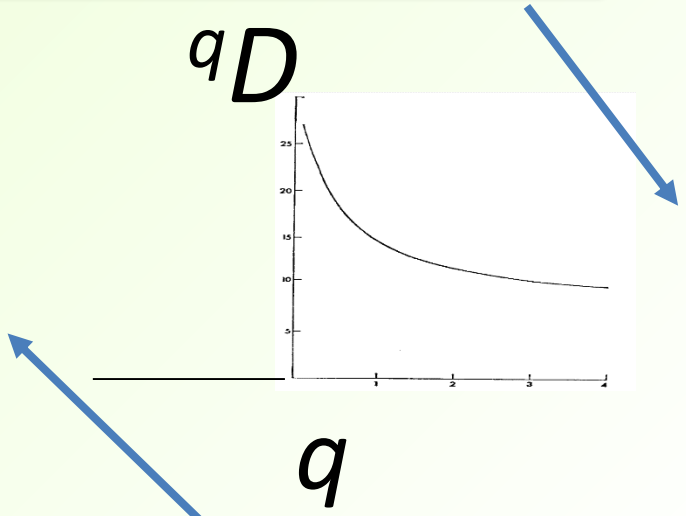
$$ {}^{q}D = \left(\sum x_i^{q} \right)^{1/(1-q)} $$

$$ 1 / {}^{q}D = \left(\sum x_i^{q} \right)^{1/(q-1)} $$

${}^{q}D$: a sliding scale of weighting



One extreme: we ignore all but the **most** abundant species



Other extreme: we ignore all but the **least** abundant species

- As we vary *q* we attach more or less importance to:
  - The more abundant species
  - The rarer species
  - Some balance inbetween

What about units?

- ${}^{q}D = (\sum x_i^q)^{1/(1-q)}$ What are the units?
- Strictly speaking:
  - a proportional abundance (x_i) is unitless
- A mean proportional abundance has units **species^{-1}**
  - Because *N* has units of: **species**
  - mean = 1 / *N*
- In the simple mean case, $\boldsymbol{w_1x_1 + w_2x_2 + w_3x_3 \ldots}$
  - Weightings also have units species^{-1}
  - Because $w_i = 1 / N$

What about units?

- But the longer form of

$$ {}^{q}D = \left(\sum x_i^{q} \right)^{1/(1-q)} $$

- is:

$$ {}^{q}D = \left(\sum \left(w_i \, x_i^{q-1} \right) \right)^{1/(1-q)} $$

- and the units always work (i.e., ${}^{q}D$ has units: **species**) …
- …**as long as** w_i is unitless, and x_i has units: **species^{-1}**
- which is sort of the other way round to what might be expected
- So would it be better written like this? Discuss….

$$ {}^{q}D = \left(\sum \left(x_i \, w_i^{q-1} \right) \right)^{1/(1-q)} $$

So how useful are these numbers?

- For any sample, the series of Hill Diversities ${}^{q}D$ can be easily calculated
- Do you really need to calculate them for 'all'

$$-\infty < q < +\infty \quad ?$$

- Are even just a few integer values of q useful?
- When you **calculate** these Diversity numbers from the **measured** abundances in your sample –
- How well do they **estimate** the Diversity in the original environment?

Using rarefaction with ${}^{q}D$

- As with any rarefaction, the curve can be extrapolated beyond the actual sample size
- The range of uncertainty in the extrapolated region can be calculated
- Haegeman *et al.* (2013)

  Robust estimation of microbial diversity in theory and in practice
- Contains equations
  - for lower and upper estimates

[Figure 3 from Haegeman *et al.* (2013)

http://www.nature.com/ismej/journal/v7/n6/fig_tab/ismej201310f3.html#figure-title]

In silico communities: we know the right answer

- A computer-generated community – i.e. true abundances of all organisms are known
- Here, *N* is the **total number of organisms** in the community
- Obtain a **sample of size *M***
- Perform rarefaction
- Can be done with any metric
  - such as a ${}^{q}D$: for a range of *q*

[Middle panel of top row ($N = 10^{10}$, $M = 10^{4}$), in Figure 4 from Haegeman *et al.* (2013)

http://www.nature.com/ismej/journal/v7/n6/fig_tab/ismej201310f4.html#figure-title]

[Top row *(N* = 10^{10}*),* in
Figure 4 from Haegeman *et al.* (2013)

http://www.nature.com/ismej/journal/v7/n6/fig_tab/ismej201310f4.html#figure-title]

[Figure 4 from Haegeman *et al.* (2013)

http://www.nature.com/ismej/journal/v7/n6/fig_tab/ismej201310f4.html#figure-title]

Haegeman *et al.* (2013)

Using **real samples from real microbiomes** - We don't know the right answer… but do we get estimates in a narrow uncertainty range?

[Figure 5 from Haegeman *et al.* (2013)

http://www.nature.com/ismej/journal/v7/n6/fig_tab/ismej201310f5.html#figure-title]

Haegeman *et al.* (2013)

α-diversity,
β-diversity,
γ-diversity

These are (or should be) related to each other in a straightforward way

α-diversity

- α-diversity is the Diversity of a single “compositional unit”
- What you use as a measure of “Diversity” is your choice
- (but choose wisely)
- E.g. one (or more) of the Hill Diversities

“compositional unit”:
represents a single “compartment”
Which could be:
a locality within a larger region
And also applies to a **sample**

γ-diversity is the Diversity of the entire region

β-diversity is the ratio of these two diversities

$$\beta = \gamma / \alpha$$

(Whittaker, 1960)

Each compositional unit has a Diversity
This is α-diversity

What if …each 'unit' (local environment) had: an identical number of species with identical abundance distributions?

…or, each unit had completely different species?

For a gentle introduction

- See ‘methods.blog’
  - Official blog of Methods in Ecology and Evolution
- https://methodsblog.wordpress.com/
- - see their most-accessed blog article ever
  - “What is beta diversity” Andrés Baselga
- Note that “constituent compositional unit”
  - (such as a localised ecosystem in a larger region, or a sample from it)
- ..is also equivalent to a constituent sampling unit in general
  - Such as multiple faecal samples from the same host

Diversity of diversities

- "A consistent terminology for quantifying species diversity? Yes, it does exist" Tuomisto (2010) *Oecologia* **164** 853-860

"The term 'diversity' has been used in **at least four conceptually different ways** in the ecological literature,

primarily **because indices of diversity have been equated with diversity itself.**

Furthermore, an alpha component, or 'alpha-diversity', has been separated from total or 'gamma diversity' in **at least three different ways**.

The situation is even worse with 'beta diversity', which has been defined in **more than 30 different ways**;

Some of these are **not mathematically derived from alpha and gamma diversity in any way**, and the **values of many are uncorrelated** with each other"

- For more details of some of these, see Tuomisto H. (2010) "A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity", *Ecography* **33:** 2-22

Just two β-diversities for now

- Regional:local diversity ratio β_{Mt}
  - Used with one or more Hill diversities (*q* values)
  - Thus ${}^{q}\beta_{Mt}$
- "Quantifies how many times as rich in effective species an entire dataset is than its constituent sampling units are on average"
  - It is unitless
  - α-diversity involved (α_t) is "mean species diversity within sampling units"
- True beta diversity β_{Md}
  - Put all your constituent units together –how many distinct units does it really look like, in a mathematical sense?
  - If all of your samples are replicates, you hope the answer is 1
  - Thus quantifies the number of composition units
  - β_{Md} has units of: **species/compositional unit**

Summary

- Use true diversities (Hill Diversities)
  - Even if just a small number of q values
- Straightforward to calculate
- Values of the Hill curve for $q \geq 1$ should be comparable
  - So should be similar for e.g. replicate samples
- Also they enable calculation of β-diversity
  - Which is a measure of **how many "distinct units" you <u>really have</u>** amongst your collection of samples

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