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Introduction to Bioindication

- part 2 -

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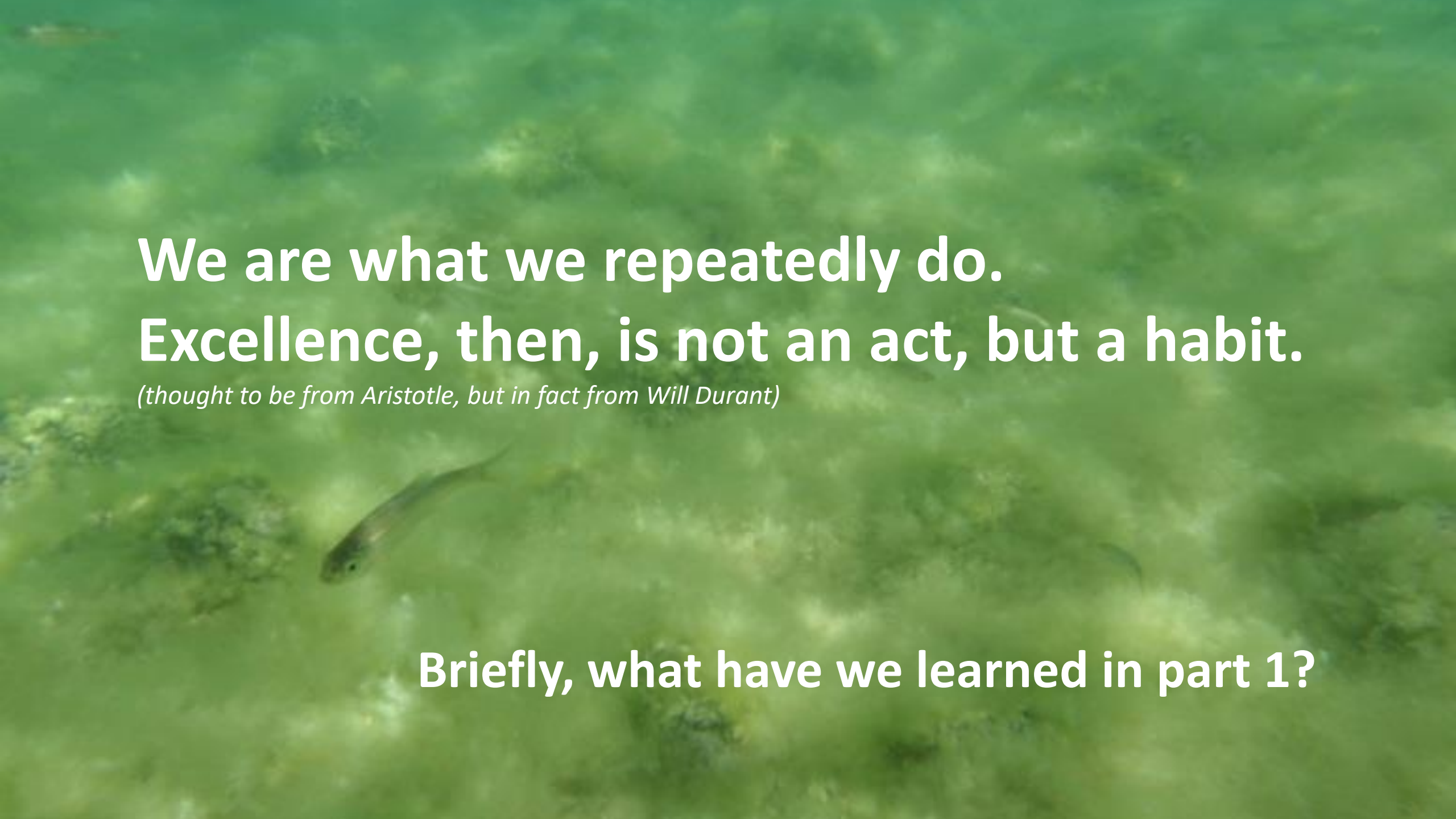
Note:

This presentation is part of a course that has been given in April/May 2023 in Belgrade. To avoid any potential issues with respect to copyright, however, the version which is publicly available has been modified. Specifically, some illustrations were removed.

For this reason, layout and design may appear somewhat “empty”.
The course content has not been changed, however.



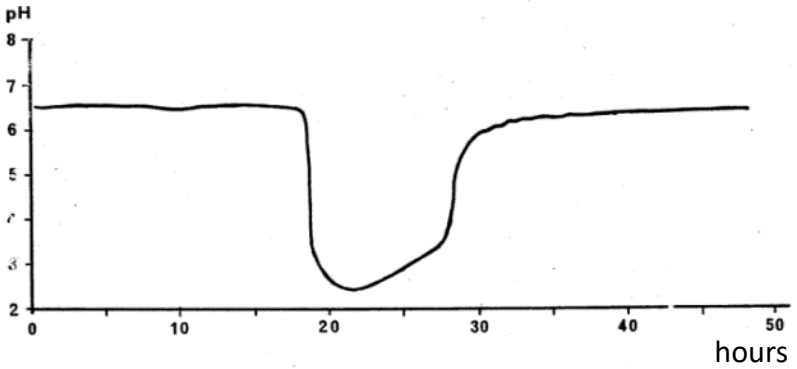
We are what we repeatedly do.
Excellence, then, is not an act, but a habit.

(thought to be from Aristotle, but in fact from Will Durant)

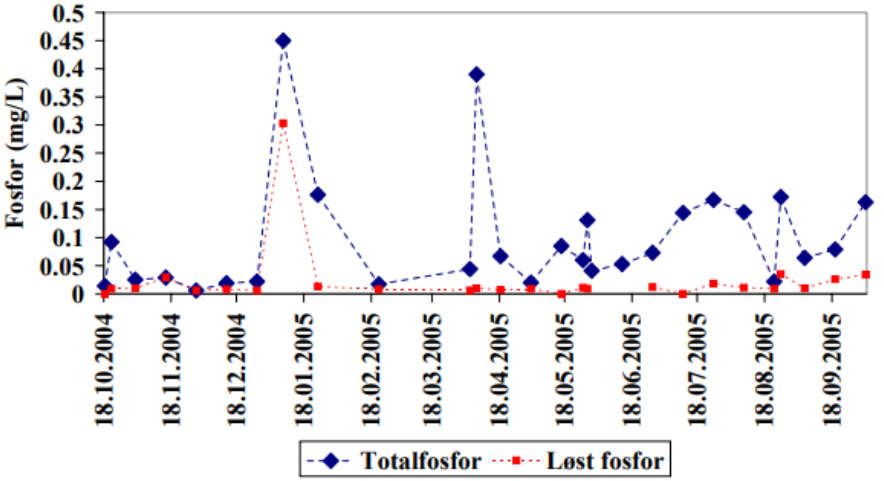
Briefly, what have we learned in part 1?

Why do we need bioindication?

- Detect short-term releases and earlier events
- Show less “random” variation;
  integrate over time
- Water quality has **biological aspects** (ecosystem)
- Register a wide variety of pollutants
- multiple stressors: synergistic and antagonistic effects

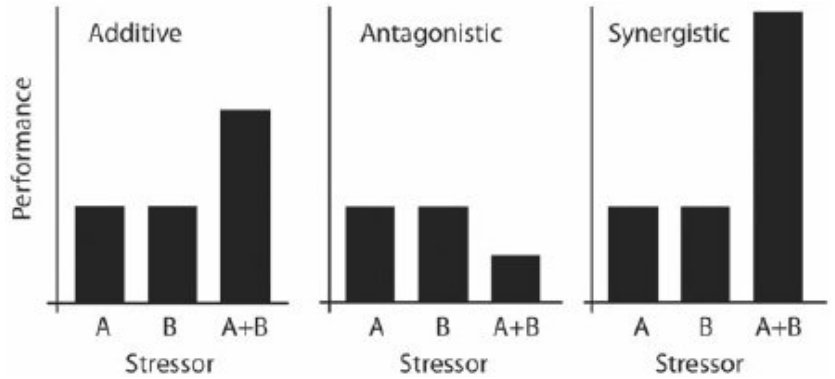


pH in a stream in Oslo, before, during and after a spill of sulfuric acid



TotP and DRP in the Årvoldbekken stream (Norway)

Picture of a fish kill



What is a «good bioindicator»?

Good indicator ability	Provide measurable response (sensitive to the stressor)
	Response reflects the whole population/community/ecosystem
	Responds in proportion to the degree of contamination/degradation
Abundant and common	Adequate local population density (rare species are not optimal)
	Common, including distribution within area of question
	Relatively stable despite moderate climatic and environmental variability
Well-studied	Ecology and life-history well understood
	Taxonomically well documented and stable
	Easy and cheap to survey

Water Framework Directive:

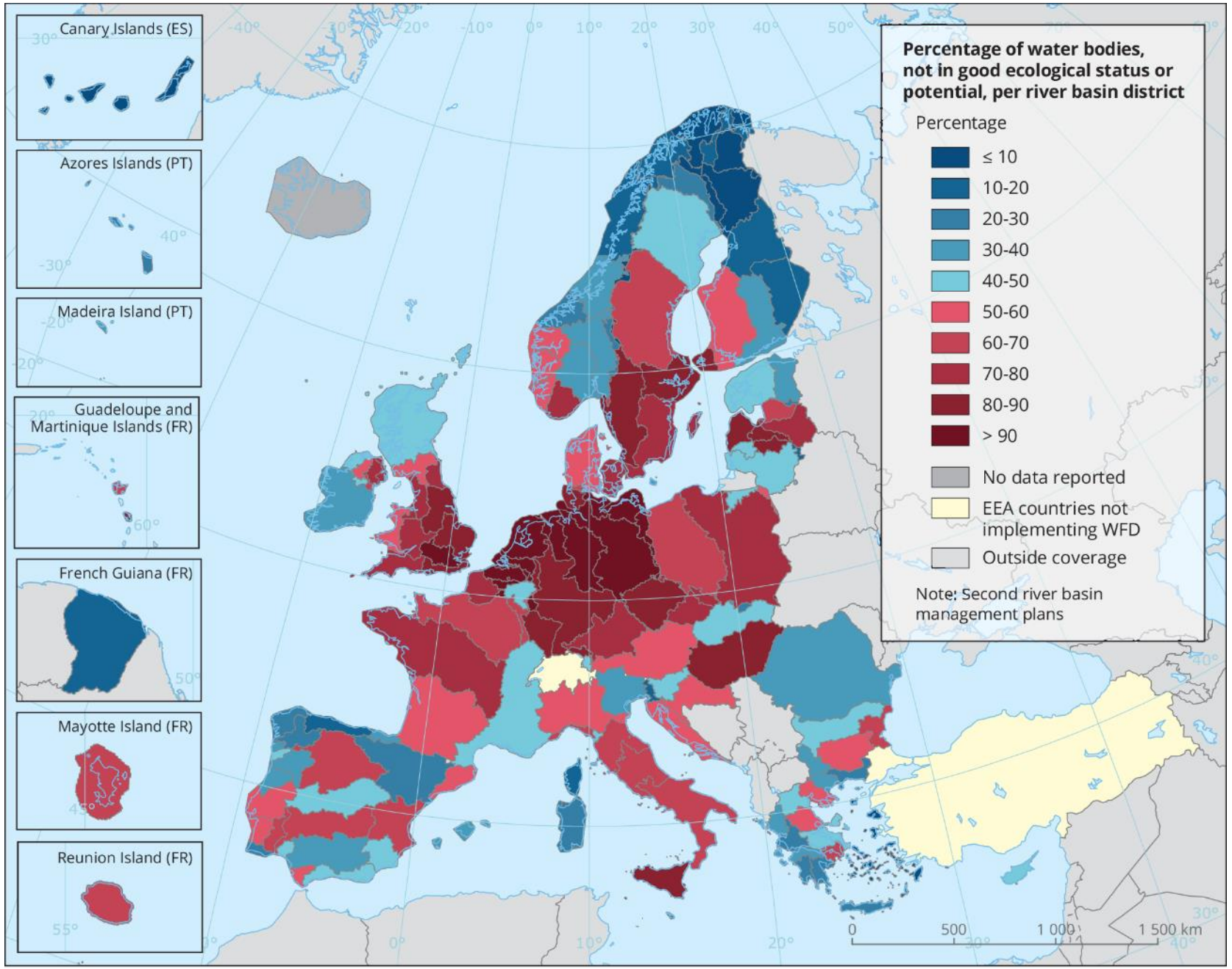
The Water Framework Directive (WFD)

- surface waters are key for supporting society and the economy
- clean, unpolluted waters are essential for healthy ecosystems
- but surface waters have traditionally been used as disposal routes for wastes, they have been altered, e.g. by building dams, to facilitate agriculture and urbanization, to produce energy and protect against flooding
- all of this changed and degraded habitats

The Water Framework Directive (WFD)

=> The WFD stipulates that EU Member States should aim to achieve **good status for all surface water and groundwater bodies**.

However, across Europe, good or better ecological status has been achieved for only around **40% of surface waters** (data from 2015).



Reference data: ©ESRI | ©EuroGeographics

Data from second river basin management plan, i.e. by 2015

Sooner or later you are likely to be responsible for filling some of the grey areas in this map.

So: How are you going to do this?

Before we start: What is ecological status?

- Definitions of ecological status for lakes and rivers
- From Annex V of the WFD

General definition of ecological status classes according to Annex V of the WFD

high	There are no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions. The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion. These are the type-specific conditions and communities.
good	The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions.
moderate	The values of the biological quality elements for the surface water body type deviate moderately from those normally associated with the surface water body type under undisturbed conditions. The values show moderate signs of distortion resulting from human activity and are significantly more disturbed than under conditions of good status.
poor	Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions, shall be classified as poor.
bad	Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large proportions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent, shall be classified as bad.

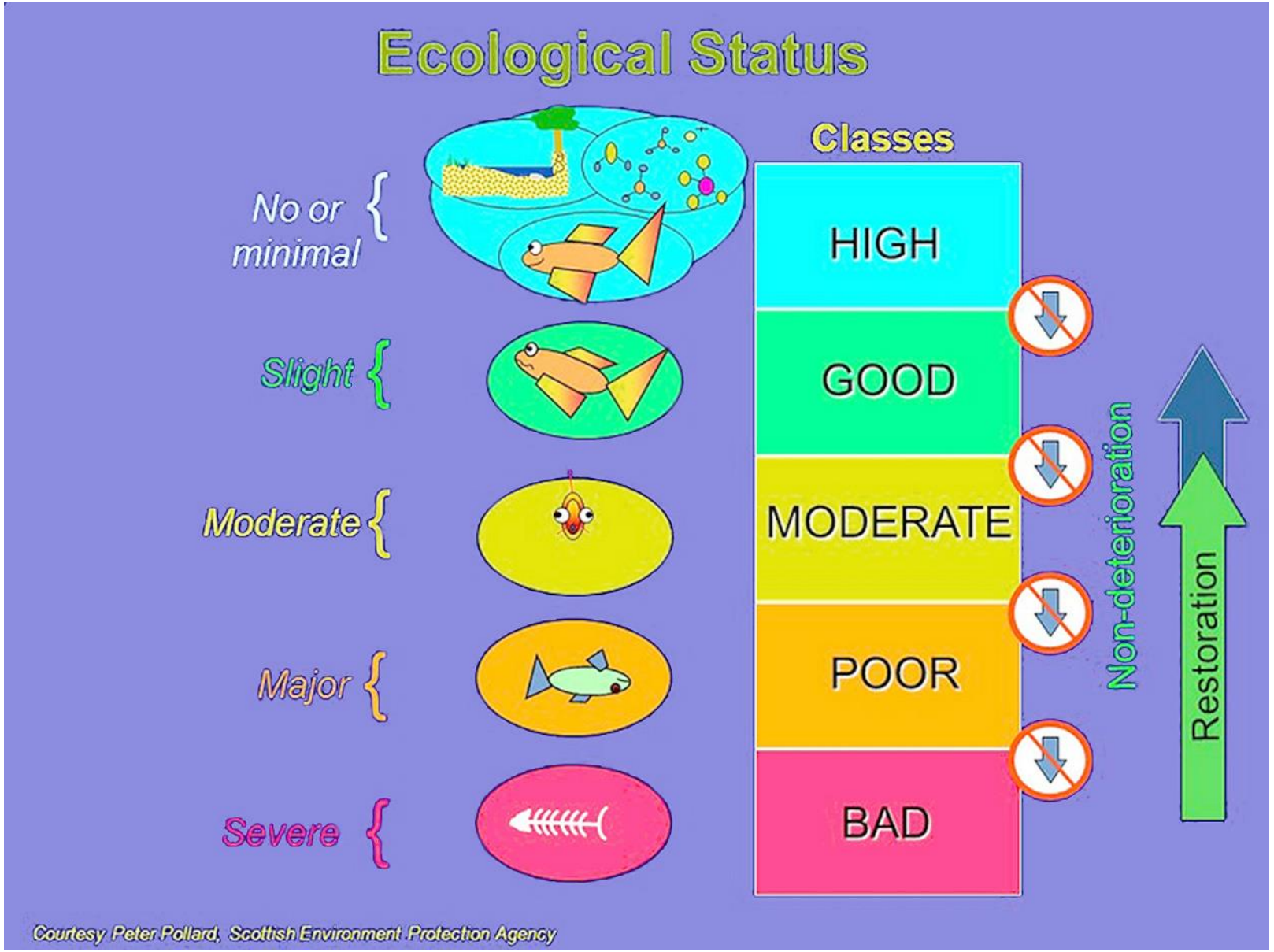
What do you think? Spend 5 minutes to read and discuss these definitions

In addition, there are definitions for high, good and moderate for **each biological quality element**.

Example below: macrophytes and phytobenthos in rivers and lakes

high	The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. There are no detectable changes in the average macrophytic and the average phytobenthic abundance.
good	There are slight changes in the composition and abundance of macrophytic and phytobenthic taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of phytobenthos or higher forms of plant life resulting in undesirable disturbances to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment. The phytobenthic community is not adversely affected by bacterial tufts and coats present due to anthropogenic activity.
moderate	The composition of macrophytic and phytobenthic taxa differs moderately from the type-specific community and is significantly more distorted than at good status. Moderate changes in the average macrophytic and the average phytobenthic abundance are evident. The phytobenthic community may be interfered with and, in some areas, displaced by bacterial tufts and coats present as a result of anthropogenic activities.

Ecological status in brief



Before we start: What is a stressor?

- Driver-Pressure-State-Impact-Response (DPSIR) scheme is much used
- But DPSIR does not mention “stressors”

Driver-Pressure-State-Impact-Response (DPSIR) scheme was developed to communicate the **relationship of socio-economic and environmental policies** in Europe.

driver → pressure → state → impact → response

Picture of agriculture

Picture of nutrient loading

Illustration of somebody angling «dead fish»

icon symbolizing laws and regulations

Socio-economic and environmental forces

- climate change
- agriculture

Direct effect of driver that impacts the environment

- increased nutrient loading
- warmer temperatures

State/condition of the environment; attribute reflecting ecosystem integrity

- extent of suitable habitats for salmon spawning
- abundance of sensitive macroinvertebrate species

Effect on human welfare as a consequence of change of state

- increased costs for water treatment
- loss of fish for food

Societal response to impacts through policy measures

- demand that water bodies must have at least good ecological status
- reduced maximum carbon emissions from cars

Contrastingly, the term '**stressor**' is much more specific and addresses a **measurable environmental variable** that, as a result of an anthropogenic pressure, changes and adversely **affects biological or ecological integrity**. The terms pressure and stressor are not interchangeable: a single pressure (e.g. diffuse pollution) may comprise several stressors (e.g. enhanced concentrations of nitrate, ammonia, phosphorus, pesticides, etc.) that are very likely to act in concert, if the pressure is operating.

pressure → stressor → state

Picture of nutrient loading

Picture of water quality sensor

Direct effect of driver that impacts the environment

- increased nutrient loading

Measurable environmental variable that adversely affects ecosystem integrity

- increased TP concentration
- increased nitrate concentration

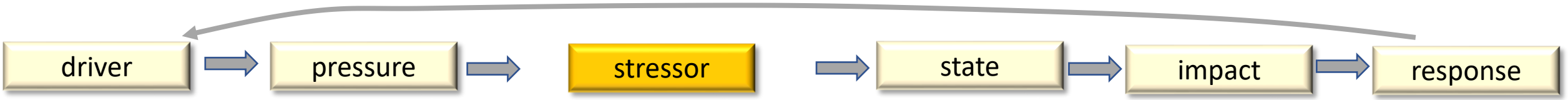
State/condition of the environment; attribute reflecting ecosystem integrity

- cyanobacterial blooms
- mass development of macrophytes

Develop an index, step 1: select a stressor

- The selected stressor must be **relevant**
- **So: what is relevant?**

What is a **relevant** stressor?



Picture of agriculture

Picture of nutrient loading

Picture of water quality sensor

Illustration of somebody angling «dead fish»

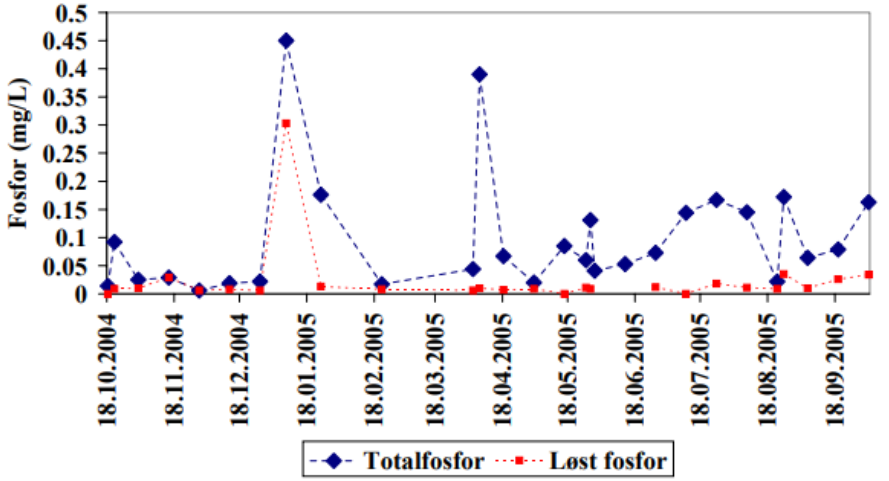
icon symbolizing laws and regulations

- Should be related to both **pressure** and **state**
- The resulting change in state should **impact the society** (otherwise we would not care)
- Societal response should be able to affect the driver, which reduces the pressure and in turn the stressor

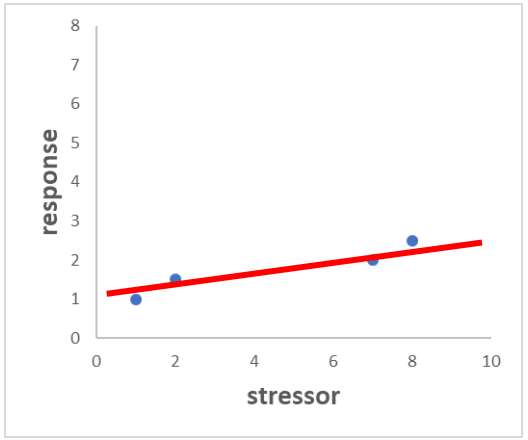
Be aware of these relationships! Make sure to explain them to policy makers, other stakeholders, the general society. Make sure to answer the question “why is this important?”.

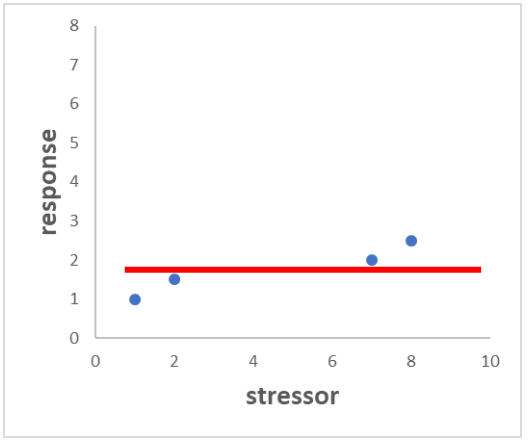
Stressor: High quality data needed!

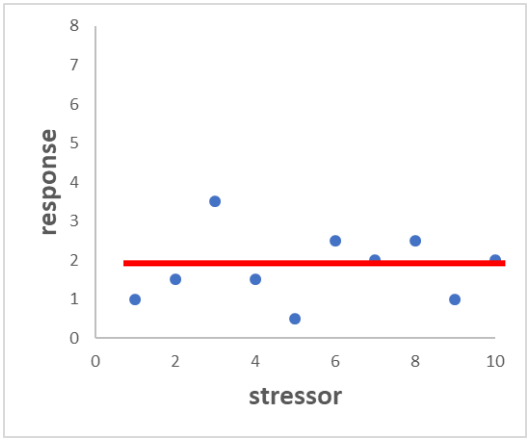
TotP and DRP in the Årvoldbekken stream (Norway)

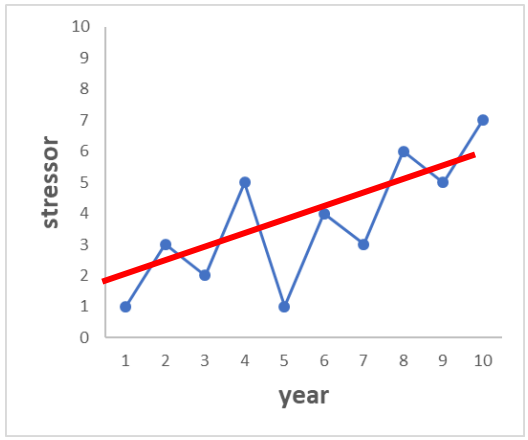


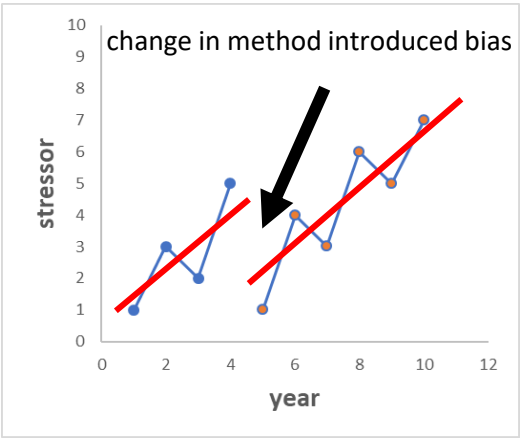
- How often measured?
- How many sites?
- Has the same method been used consistently?











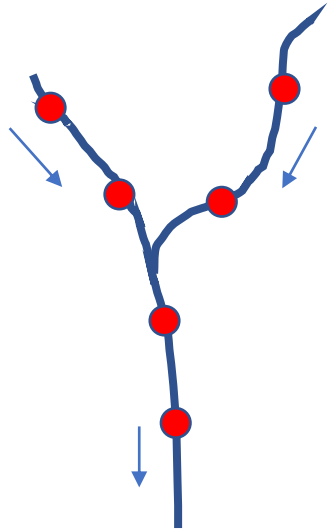
Stressor: make sure sites are («sufficiently») independent!

Be cautious if the data includes dependent observations. This can be **repeated measures at the same site** (temporal dependence) or **observations from different but closely located sites** (spatial dependence, also known as spatial autocorrelation).

temporal dependence

Nr	site	year	TP (µg/l)
1	river A1	2002	23
2	river A1	2003	34
3	river A1	2004	26
4	river A1	2005	19
5	river A1	2006	22
6	river A1	2007	26
7	river A1	2008	26
8	river A1	2009	19
9	river A1	2010	27
10	river B1	2010	89

spatial autocorrelation



Stressor: make sure sites are («sufficiently») independent!

What can be done if data are not independent?

Answer depends on what you want to do with the data:

- use average values of repeated or clustered measures instead of the original measures
- include temporal and/or spatial descriptors as covariates into your models
- split data into a training dataset used for index development (in which the data are independent), and a validation dataset which is used to test the index. This is not exactly according to the rules either (cause the validation is not completely independent), but you might get away with it

Nr	site	year	TP (µg/l)
1	river A1	2002	23
2	river A1	2003	34
3	river A1	2004	26
4	river A1	2005	19
5	river A1	2006	22
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8	river A1	2009	19
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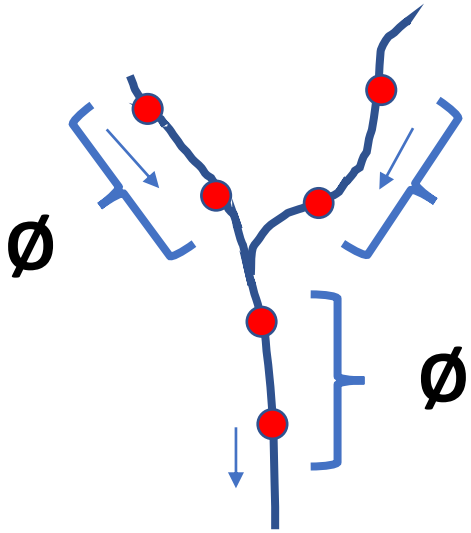
Ø=25

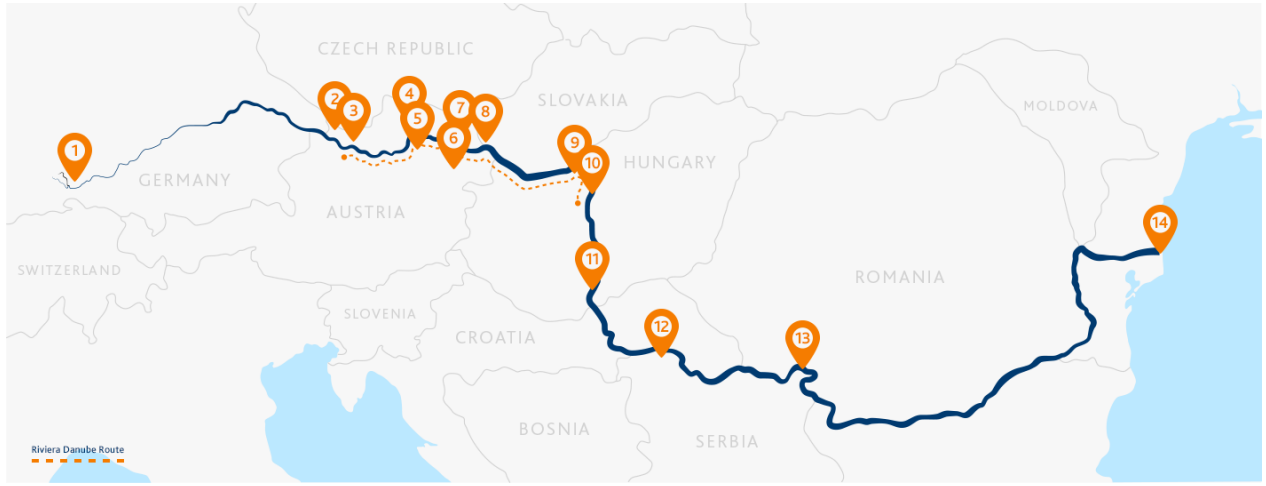


note that this site still is NOT independent from the others

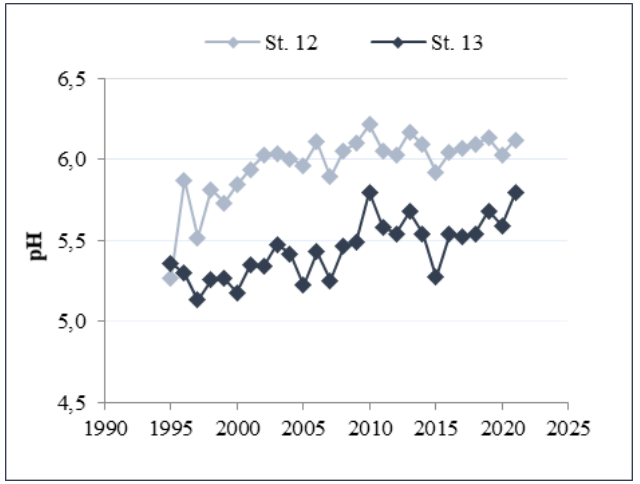
Stressor: make sure sites are («sufficiently») independent!

Note: this is really important, but there is no one-size-fits-all solution, and in my experience, complete independence is rarely achieved in practice (at least not in rivers and streams).

Danube: which of these sites are independent from each other?

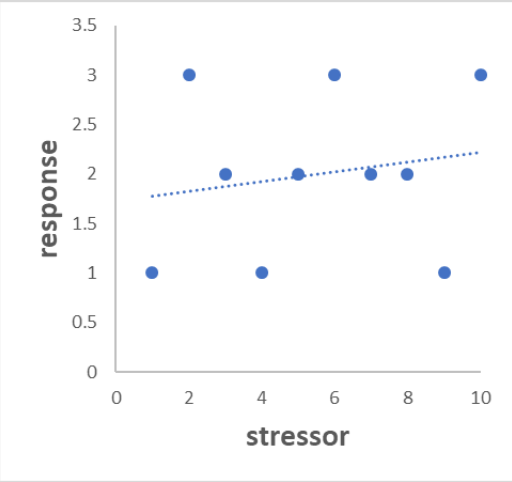


Average annual pH at two stream sites in Norway. Increased pH due to reduced acid deposition. Would averaging be correct in this case?

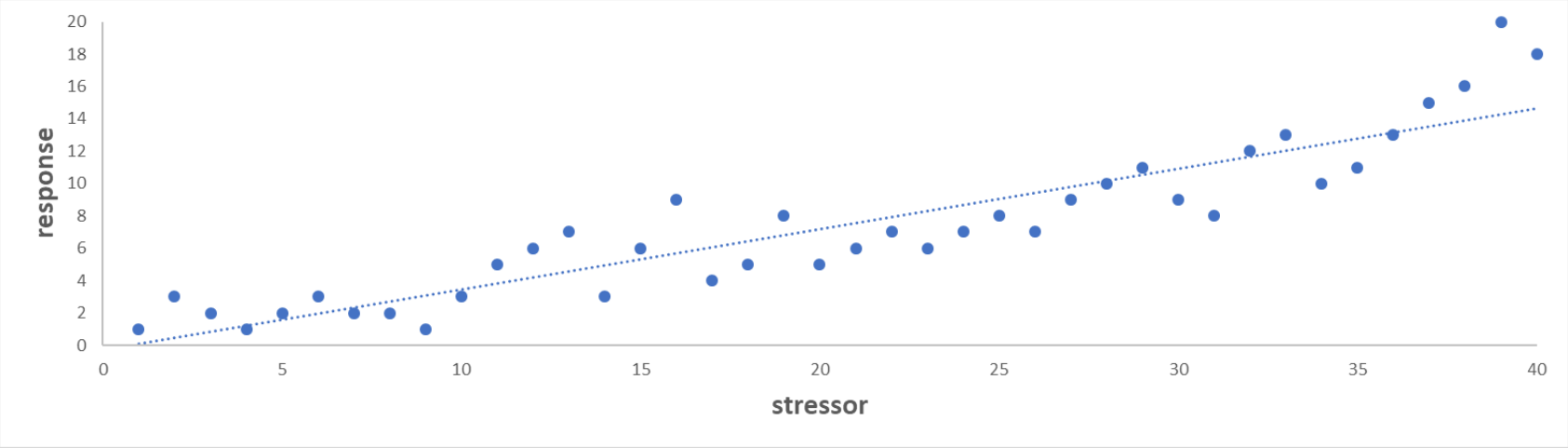


Stressor: make sure you have a sufficient gradient length!

The designated stressor variables need to encompass an environmentally relevant gradient length, i.e. values that include the gradient's end points occurring in the targeted ecosystem.
Compiling information about the stressor's gradient lengths can help estimate as to whether this criterion is met.



No significant *relationship between stressor and response*



Significant *relationship between stressor and response*

Stressor: take care of multiple stressors!

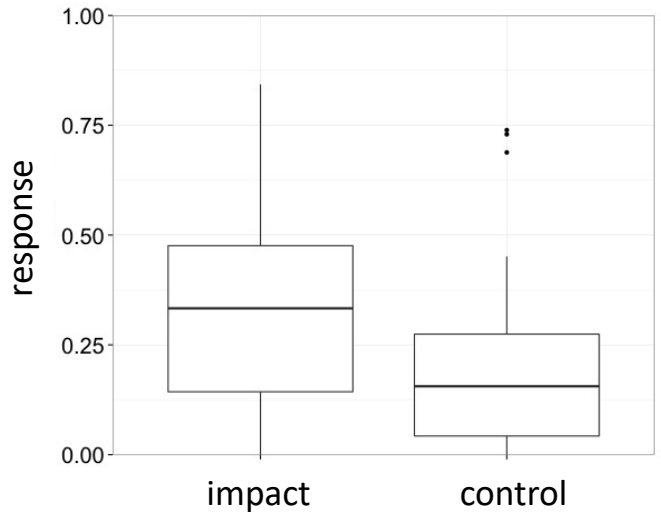
- This is a very complicated point
- It is practically almost impossible to avoid the occurrence of multiple stressors (today, almost all sites are exposed to multiple stressors)
- At the same time, you must try to avoid combining stressors that cancel each other out (sites that are acidified are unlikey to have «eutrophic» algae, even if phosphorus concentrations were high; so including «acidic-eutrophic» sites might «destroy» a correlation between algal assemblages and phosphorus concentrations.

Picture of a polluted river obviously affected by multiple stressors

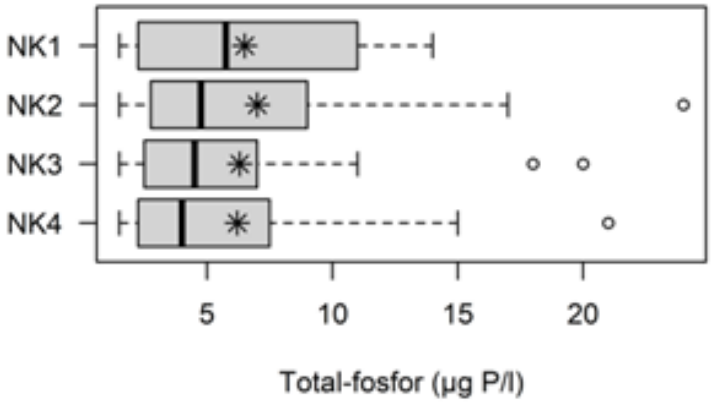
Stressor: take care of outliers!

Outliers and extreme observations need to be detected and handled appropriately early in data analysis.

How would you interpret these outliers?



Does Tot-P concentration decrease from NK1 to NK4?



Stressor: take care of outliers!

Do NOT remove outliers just because they are outliers!
Instead, try to find the reason why a data point is an outlier, and then handle the outlier accordingly!

Outliers may
- be a typo (0.75 instead of 0.57)
- reflect an error in data handling (wrong decimal separator; 0.75 instead of 0.075; 10.75 instead of 1.075)
- be a mistake (something went wrong in the lab)
- reflect unusual but correct conditions

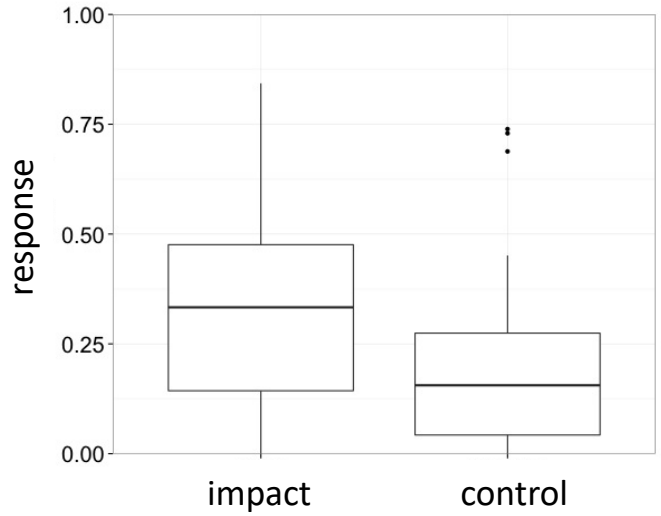


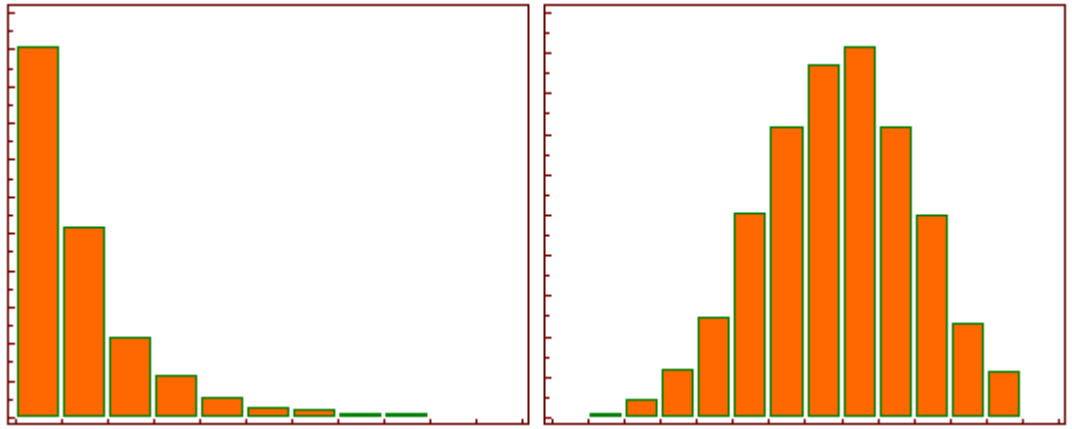
Illustration of an odd number for X1

Illustration of a multivariate outlier

Illustration of an outlier in a low density region of data

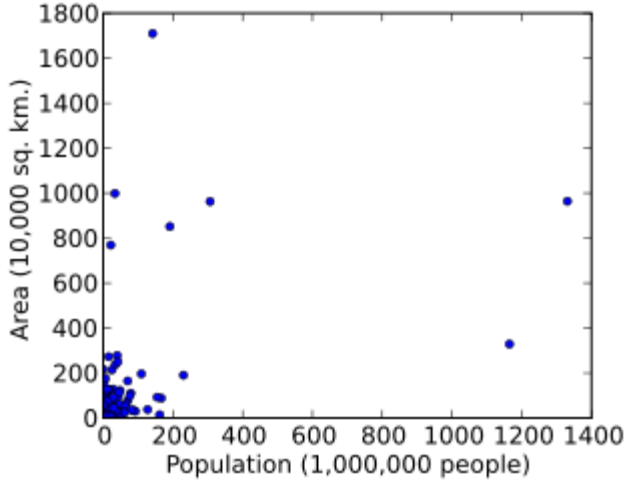
Stressor: data transformation

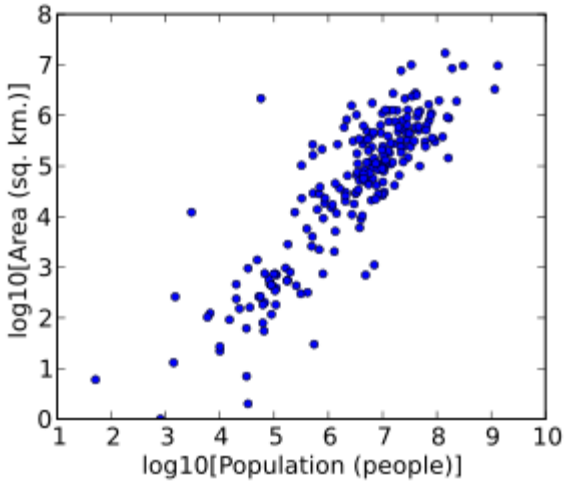
Data transformation aims to approach normal distribution of continuous data in that the influence of high values of a given variable is downweighed. Usual transformations are to calculate the square-root or logarithm. Logit transformation is recommended for proportional (%) values.



https://www.medcalc.org/manual/log-transformation.php

This is not just a statistical exercise. In untransformed data you might not be able to see relationships!

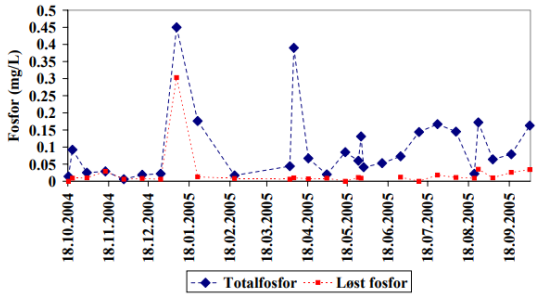


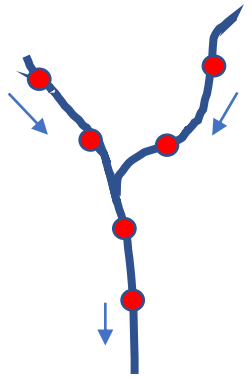


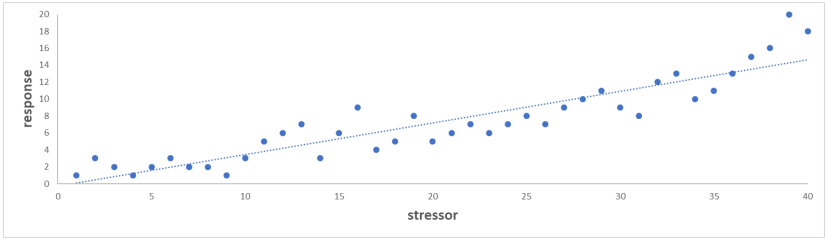
https://en.wikipedia.org/wiki/Data_transformation_%28statistics%29

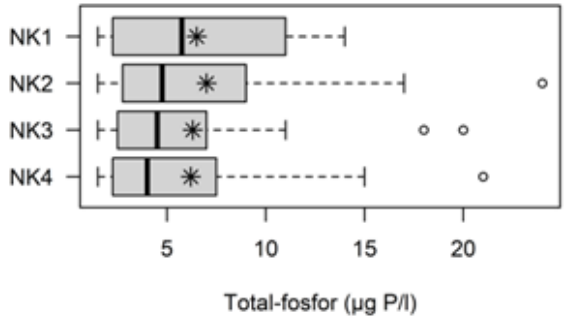
Summary stressor:

- must be relevant
- high quality data needed
- make sure sites are («sufficiently») independent
- make sure you have a sufficient gradient length
- take care of multiple stressors
- take care of outliers
- transform data where necessary



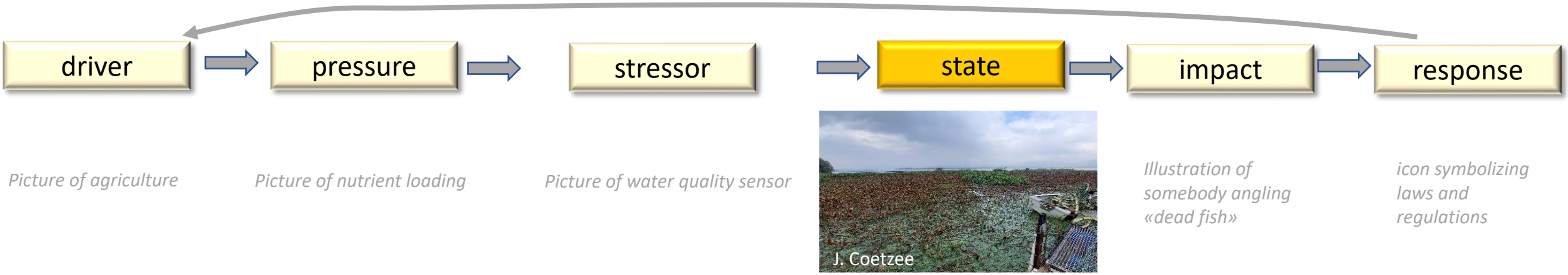






Develop an index, step 2: select an indicator

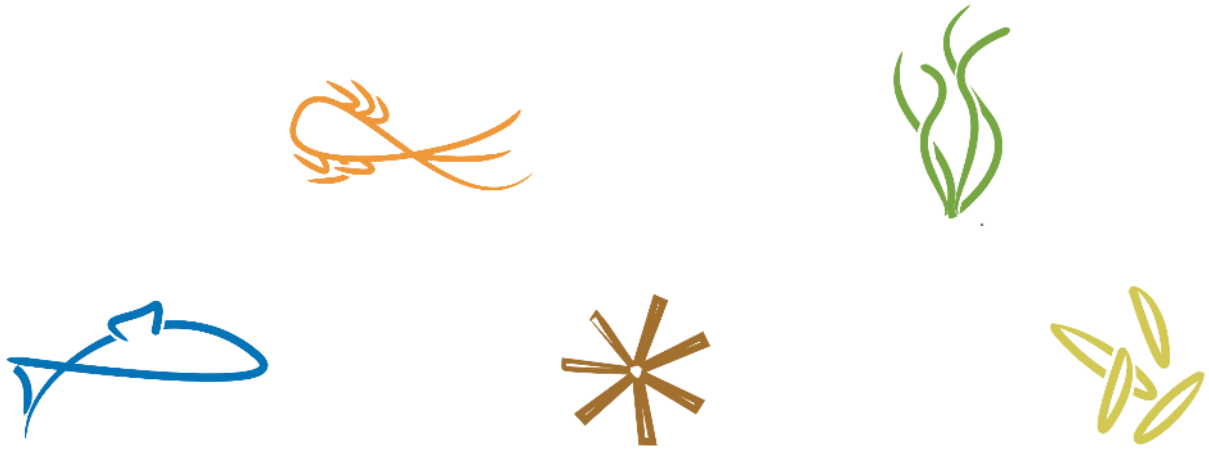
Indicator should be **relevant**



A **relevant** indicator is **affected by the selected stressor** and **affects society.**

Indicator: which organism group?

Water Framework Directive:



But the principles for index development hold true for any organism group.

Indicator: have a hypothesis!

When selecting a species group: **have a hypothesis why and how the indicator is expected to react to the stressor.**

In an ideal setting, the biological response variables used should be **mechanistically relatable** to the stressor variables in the analysis.

Why is this important?

The problem of multiple testing

green jelly beans comic from www.xkcd.com

Indicator: monotonic relationship to the stressor

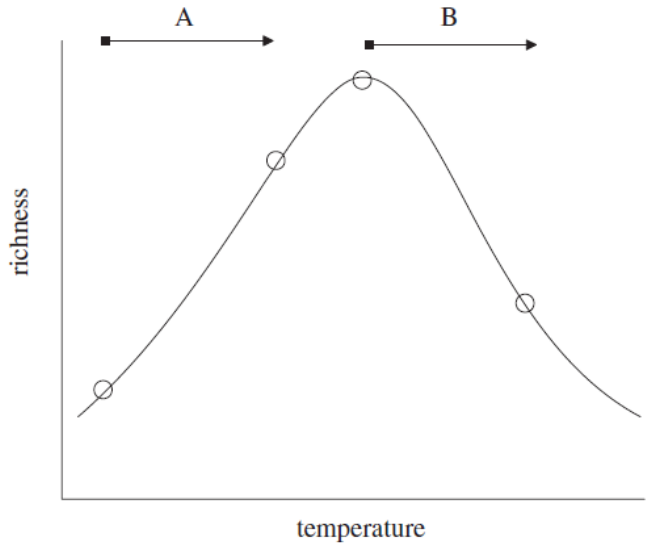
Make sure the expected response of the indicator to the stressor is linear or at least monotonic

Why is this important?

Diversity metrics may fail to detect stressor effects, because species turnover along a stressor gradient may render biodiversity metrics unaffected along the gradient.

Climate change: increase or decrease in biodiversity?

Illustrations of monotonic, monotonic and linear, and non-monotonic relationships

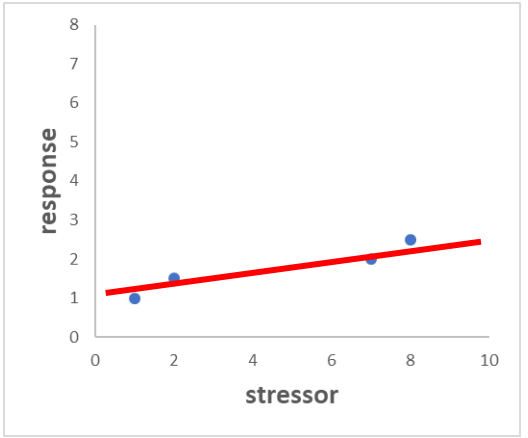


Woodward et al. (2010)

In addition: everything we said for the stressor is also relevant for the indicator!

Indicator: High quality data needed

- Sufficient number of observations
- Field and laboratory analysis must be comparable among observations, **and of sufficient quality** (if the field work was performed poorly then the results will always be poor, no matter how fancy methods you used for data analysis)



Examples:

- taxonomic depth of species determination (genus versus species)
- changing taxonomy (big issue for diatoms)
- scale used for abundance estimates of macrophytes
- mesh size of net used to sample phytoplankton
- stress, long days in the field, insufficient time for field work increase the risk that species are overlooked

Note: it may be possible to develop a good index based on presence-absence data. But if you exclusively have presence-absence data, then you are unable to test if the index could perform better using abundances. If you have good abundances, it is easy to test if the explained variation changes when using abundances compared to presence-absence.

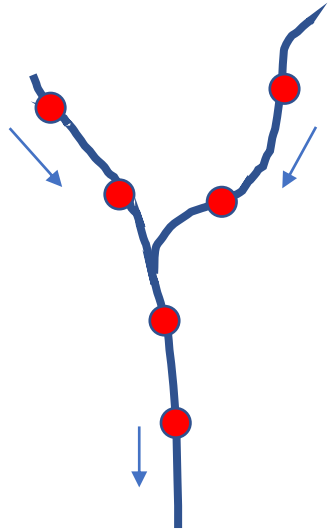
Indicator: make sure sites are («sufficiently») independent!

Exactly the same issue as for the stressor

temporal dependence

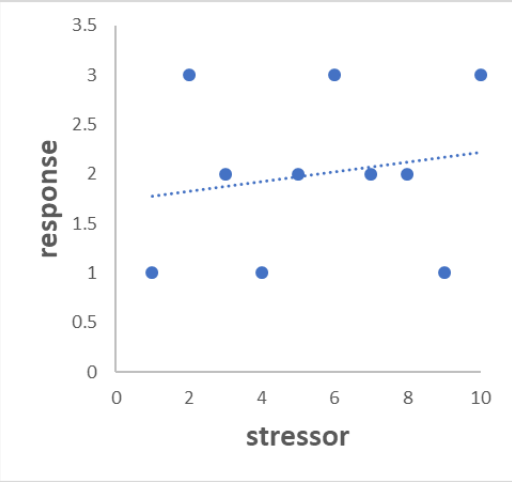
Nr	site	year	TP (µg/l)
1	river A1	2002	23
2	river A1	2003	34
3	river A1	2004	26
4	river A1	2005	19
5	river A1	2006	22
6	river A1	2007	26
7	river A1	2008	26
8	river A1	2009	19
9	river A1	2010	27
10	river B1	2010	89

spatial autocorrelation

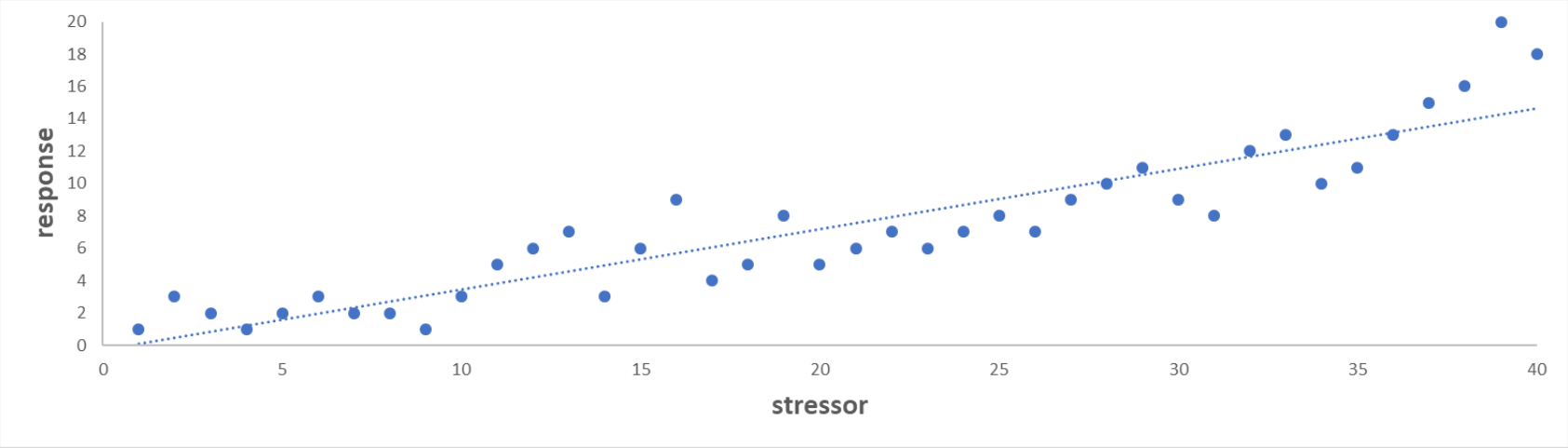


Indicator: make sure you have a sufficient gradient length!

Exactly the same issue as for the stressor



No significant *relationship between stressor and response*



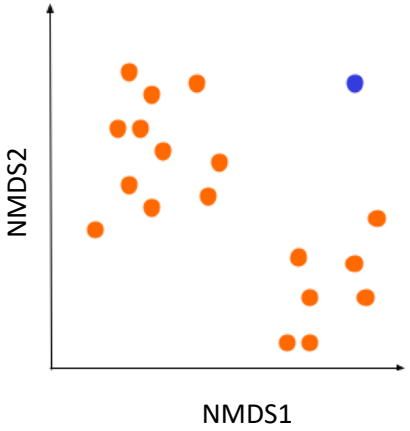
Significant *relationship between stressor and response*

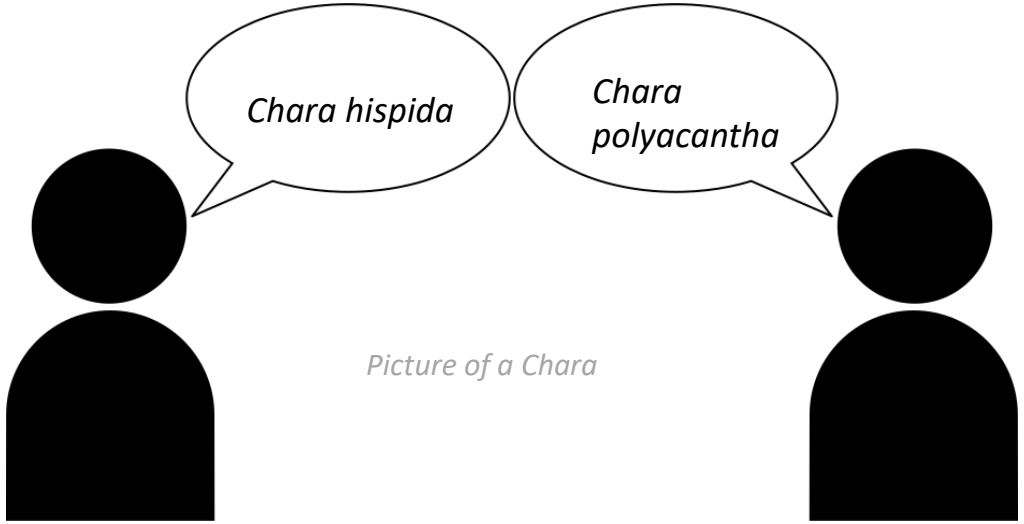
Indicator: take care of outliers!

Do NOT remove outliers just because they are outliers!
Always try to find the reason why a data point is an outlier, and then handle the outlier accordingly!

Outliers may

- be a typo (*Cara hispida* instead of *Chara hispida*)
- reflect poor sampling conditions (field work during high water level)
- be a mistake (wrong species determination)
- change in species name (*Chara polyacantha* => *Chara aculeolata*)
- reflect unusual but correct conditions

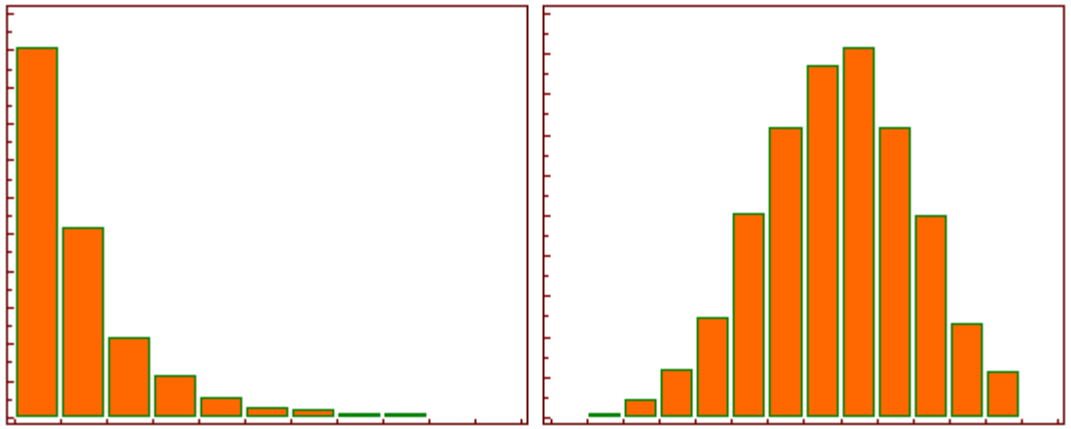




Picture of a Chara

Indicator: data transformation?

Check your data ! Make sure to have a “reasonable” structure in your data.



https://www.medcalc.org/manual/log-transformation.php

Summary indicator:

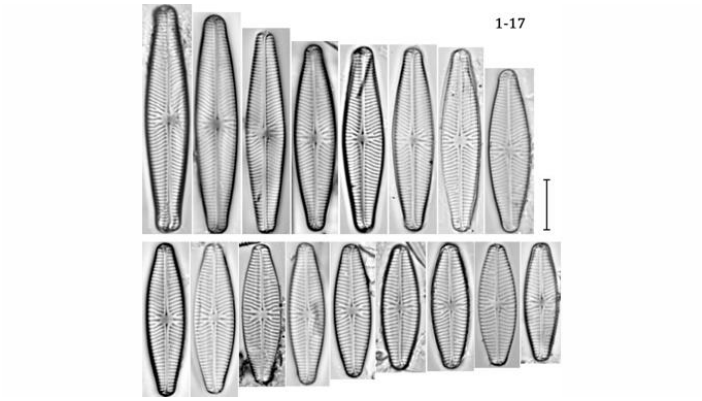
- must be relevant
- have a hypothesis
- monotonic relationship to the stressor
- high quality data needed
- make sure sites are («sufficiently») independent (time and space)
- make sure you have a sufficient gradient length
- take care of multiple stressors
- take care of outliers
- transform data where necessary

Develop an index, step 3: develop index

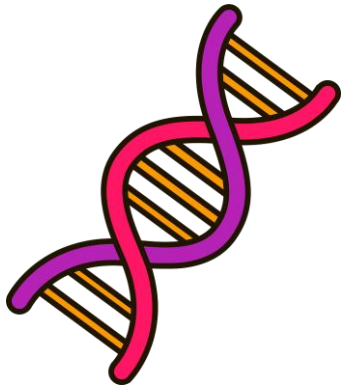
Which type of index?

- Taxonomic, genes or functional?
- Species, genus, family level?
- Entire communities or selected indicator species?
- Diversity indices?

=> No general answer possible. We will discuss advantages and disadvantages with different indices in the seminar.



Vidacovic et al. 2020



Picture illustrating diatom growth forms

Diatoms

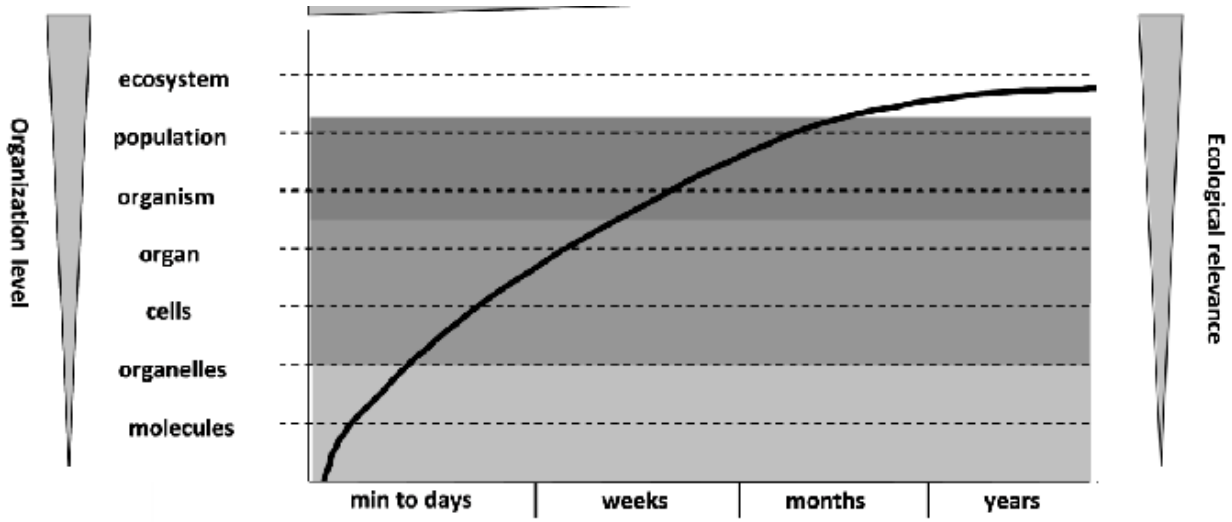
- low profile
- high profile
- motile
- …

Develop index

Make sure data for stressor and response are from the same sites, and from the same time!!

What is «the same time»?

Nr	site	time	TP (µg/l)	species A	species B	species C	species D
1	A1	1	23	1	0	3	5
2	A2	1	34	0	0	3	1
3	A3	1	26	4	3	0	0
4	B1	2	19	3	2	0	0
5	B2	2	22	5	3	0	5
6	B3	2	26	1	2	1	3
7	B4	3	26	2	1	4	3
8	C1	3	19	0	1	3	2
9	D1	3	27	0	0	3	5
10	D2	3	89	2	0	3	5
11	R1	1	12	3	0	0	0
12	R2	1	14	5	0	1	0
13	R3	2	9	3	0	0	0
14	R4	3	11	4	0	2	3



EU, 2014

Ideally: average stressor concentrations from the same year in which biota were taken, and biota data for primary producers from the **main vegetation period** in that year

But: diatoms and phytoplankton generally respond fast, and there are differences in water chemistry throughout a year …

Develop index: calculate indicator values for species/groups

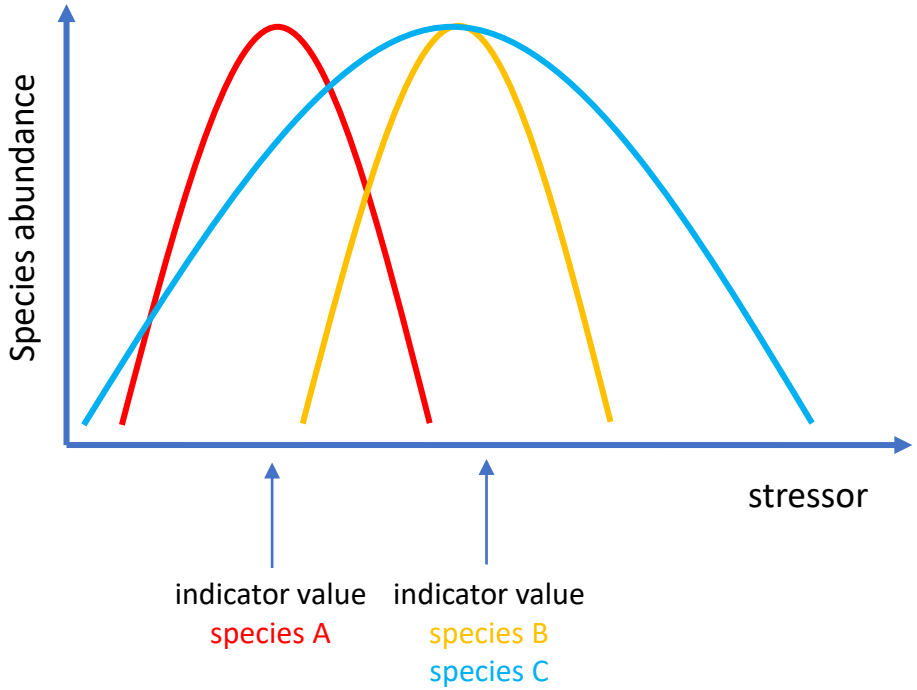
Nr	site	time	TP (µg/l)	species A	species B	species C	species D
1	A1	1	23	1	0	3	5
2	A2	1	34	0	0	3	1
3	A3	1	26	4	3	0	0
4	B1	2	19	3	2	0	0
5	B2	2	22	5	3	0	5
6	B3	2	26	1	2	1	3
7	B4	3	26	2	1	4	3
8	C1	3	19	0	1	3	2
9	D1	3	27	0	0	3	5
10	D2	3	89	2	0	3	5
11	R1	1	12	3	0	0	0
12	R2	1	14	5	0	1	0
13	R3	2	9	3	0	0	0
14	R4	3	11	4	0	2	3

- Often done by averaging stressor values at all sites where a particular species occurs
- Can be weighted by species occurrence
- There are different ways to calculate indicator values … (e.g. median, maximum occurrence, ...)

For example:

	Species A	Species B	Species C	Species D
sumproduct TP*abundances	727	279	742	1066
sum abundances	33	12	23	32
indicator value	**22.0**	**23.3**	**32.3**	**33.3**

Develop index: species indicator values

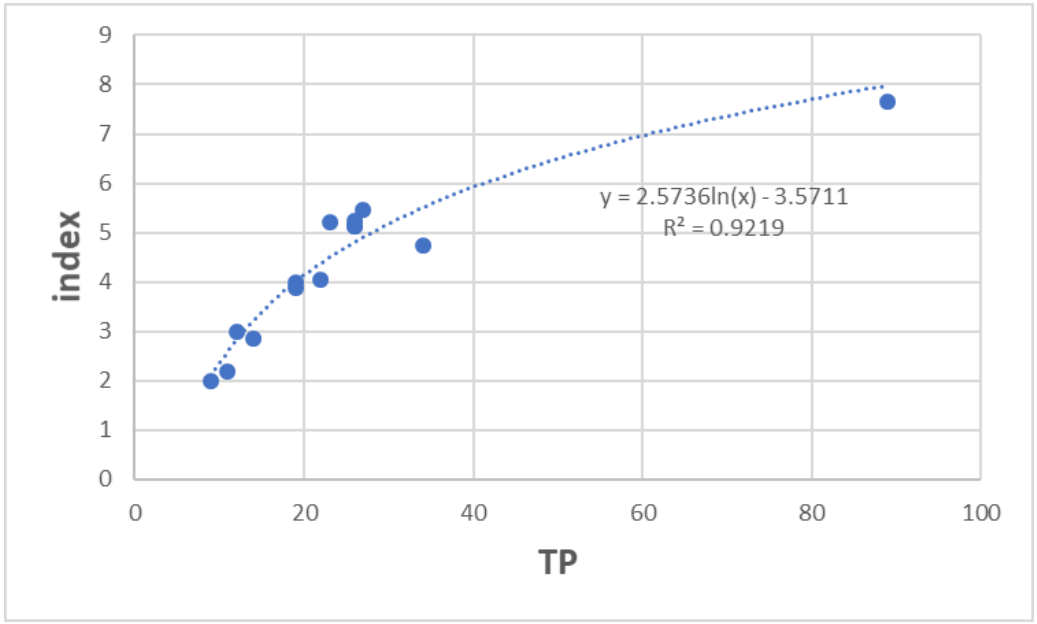


- Do not only calculate the indicator value, but also check the ecological amplitude of each species with respect to the stressor
- Here: species C has a wide ecological amplitude, so species A and B are «better» indicators
- This can be taken into account by introducing weighting factors
- Species with a «too wide» ecological amplitude may just be kicked out (get no indicator value)

Develop index: calculate index for each site from indicator values

- Often just done by averaging the indicator values of the indicator species present at a site, maybe weighted by the weighting factor
- Important: if you calculate the indices for each site from the same dataset as you calculated the species indicator values from, then the data are **not independent and cannot be used for index validation**.
- Ideally you have enough data to split the dataset into an «index development» and «test» dataset
- In reality: set at least some sites aside for independent index validation

Nr	site	time	TP (µg/l)	species A	species B	species C	species D	index
1	A1	1	23	1	0	3	5	5.21
2	A2	1	34	0	0	3	1	4.75
3	A3	1	26	4	3	0	0	5.12
4	B1	2	19	3	2	0	0	3.99
5	B2	2	22	5	3	0	5	4.05
6	B3	2	26	1	2	1	3	5.23
7	B4	3	26	2	1	4	3	5.14
8	C1	3	19	0	1	3	2	3.88
9	D1	3	27	0	0	3	5	5.45
10	D2	3	89	2	0	3	5	7.65
11	R1	1	12	3	0	0	0	2.98
12	R2	1	14	5	0	1	0	2.84
13	R3	2	9	3	0	0	0	1.99
14	R4	3	11	4	0	2	3	2.18



	Species A	Species B	Species C	Species D
sumproduct TP*abundances	727	279	742	1066
sum abundances	33	12	23	32
indicator value	**22.0**	**23.3**	**32.3**	**33.3**

Develop index for WFD: find reference sites

Nr	site	time	TP (µg/l)	species A	species B	species C	species D	index
1	A1	1	23	1	0	3	5	5.21
2	A2	1	34	0	0	3	1	4.75
3	A3	1	26	4	3	0	0	5.12
4	B1	2	19	3	2	0	0	3.99
5	B2	2	22	5	3	0	5	4.05
6	B3	2	26	1	2	1	3	5.23
7	B4	3	26	2	1	4	3	5.14
8	C1	3	19	0	1	3	2	3.88
9	D1	3	27	0	0	3	5	5.45
10	D2	3	89	2	0	3	5	7.65
11	R1	1	12	3	0	0	0	2.98
12	R2	1	14	5	0	1	0	2.84
13	R3	2	9	3	0	0	0	1.99
14	R4	3	11	4	0	2	3	2.18

- **Unimpacted** reference sites
- With respect to which stressor?
- Many countries have just used all sites with average TP < x µg/l
- Other countries have used criteria such as «agriculture in the catchment < x %»

What do you think of this?

The more time you invest into finding good reference sites, the easier the index development will be.

Develop index for WFD: find reference sites

There is no complete agreement within the countries of the EU how «reference state» should be defined.

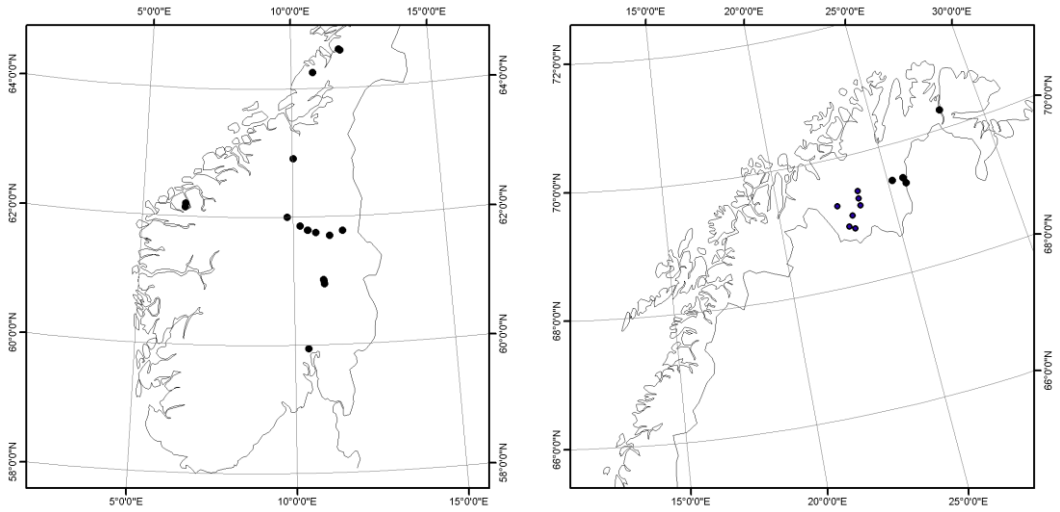
term	explanation
minimally disturbed condition	absence of significant human disturbance
historical condition	a point in the past when this state was achieved (e.g. paleolimnology in lakes)
least disturbed condition	contemporary sites that do not conform to “minimally disturbed condition” but where human disturbance is deemed to fall below thresholds likely to impact ecological condition
best available condition	situation where none of the other criteria are met but where the impact on biota of inevitable land use is minimized

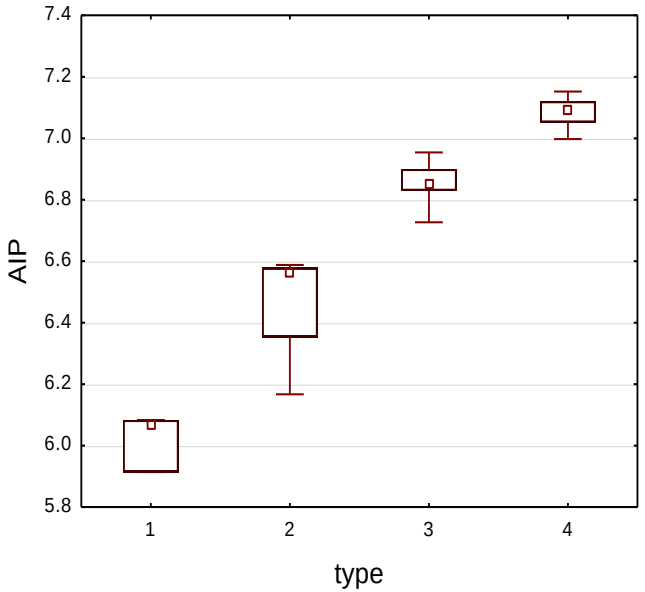
From Stoddard et al. (2006).
See also Kelly et al. (2020).

In most countries, «**reference state**» was defined in a way that roughly alignes with «**least disturbed conditions**».

Develop index: how many reference sites do you need?

Example from Norway:





When AIP was developed in 2009: 28 reference sites in Norway

=> from these data we could differentiate 4 river types

1: very Ca-poor, humic (Ca < 1 mg/l, TOC > 5 mg/l)

2: very Ca-poor, clear (Ca < 1 mg/l, TOC < 5 mg/l)

3: Ca-poor (Ca 1-4 mg/l)

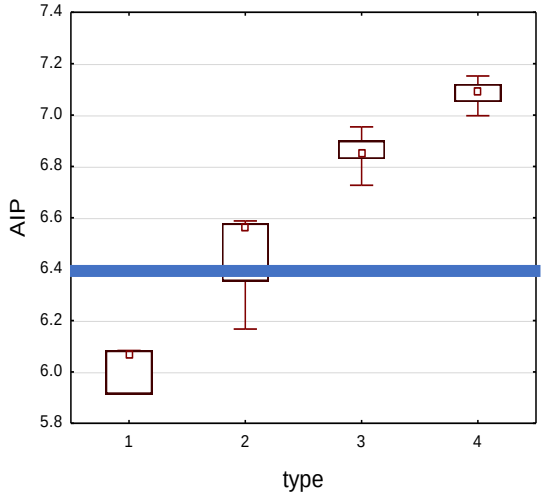
4: moderately Ca-rich (Ca > 4 mg/l)

Develop index: define water body types

- How many reference sites you need depends on how many **water body types** you expect (water body types describe the **natural variation in biota**, i.e. the variation that is **not related to stressors**)
- Generally: the more reference sites you have in your dataset the easier it is to find the correct number of water body types
- Have a hypothesis which parameters affect **natural** variation in biota

  => AIP: river types affected by Ca and TOC concentrations

  => but this likely is different for different indices
  (e.g. altitude, climate zone, alkalinity, …)



Why do you need water body types?

- Describe water body types based on parameters that are **not affected by degradation**
- make **as few water body types as possible** (only those that are **necessary to explain the observed variation in the response** at the reference sites)

1: very Ca-poor, humic (Ca < 1 mg/l, TOC > 5 mg/l)

2: very Ca-poor, clear (Ca < 1 mg/l, TOC < 5 mg/l)

3: Ca-poor (Ca 1-4 mg/l)

4: moderately Ca-rich (Ca > 4 mg/l)

Develop index: assign river type to all sites

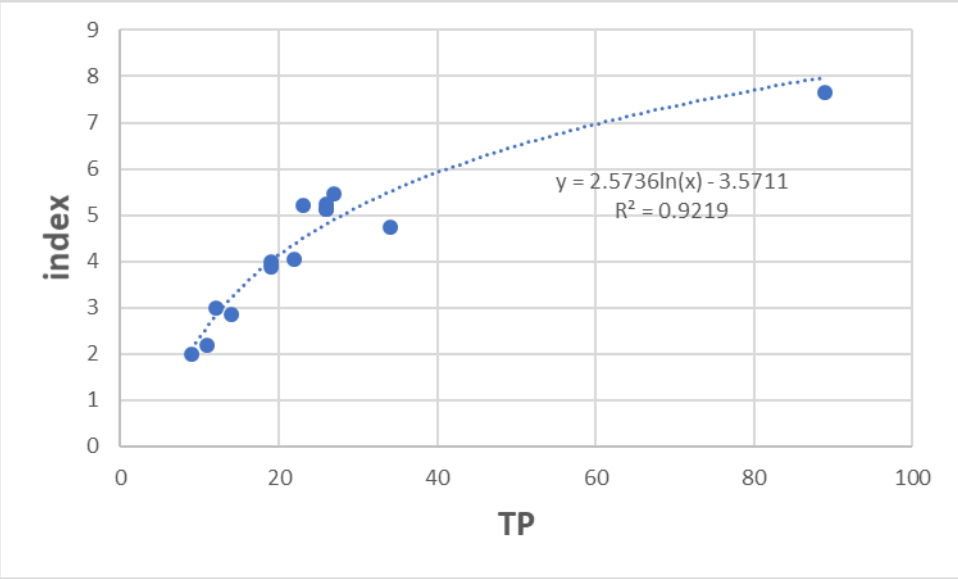
Nr	site	time	TP (µg/l)	species A	species B	species C	species D	index	type
1	A1	1	23	1	0	3	5	5.21	alpha
2	A2	1	34	0	0	3	1	4.75	beta
3	A3	1	26	4	3	0	0	5.12	alpha
4	B1	2	19	3	2	0	0	3.99	alpha
5	B2	2	22	5	3	0	5	4.05	alpha
6	B3	2	26	1	2	1	3	5.23	beta
7	B4	3	26	2	1	4	3	5.14	gamma
8	C1	3	19	0	1	3	2	3.88	alpha
9	D1	3	27	0	0	3	5	5.45	beta
10	D2	3	89	2	0	3	5	7.65	gamma
11	R1	1	12	3	0	0	0	2.98	alpha
12	R2	1	14	5	0	1	0	2.84	alpha
13	R3	2	9	3	0	0	0	1.99	gamma
14	R4	3	11	4	0	2	3	2.18	beta

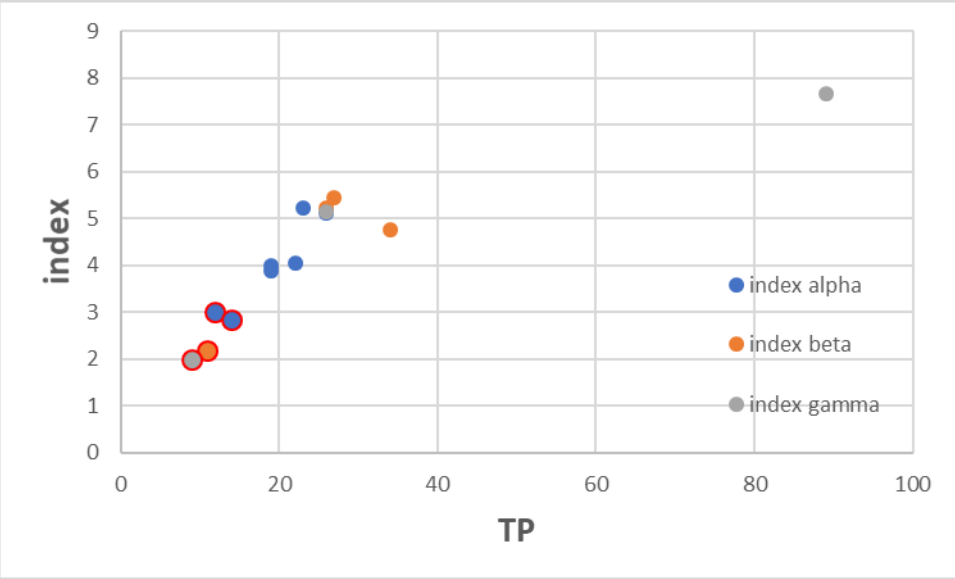
Develop index: make dose-response relationship

- Try both, pooling all data and separate for each water body type. Are there important differences between the regressions?
- Make sure you have data along the entire (at least most of) length of the stressor gradient
- If you lack data: do **not** try to publish the index anyway, but rather explain to authorities why you need more data, and present a plant how these data can be collected
- At the same time, you need to be **pragmatic** (because you will «never» be able to collect a perfect dataset)

Develop index: make dose-response relationship

What do you think of this?

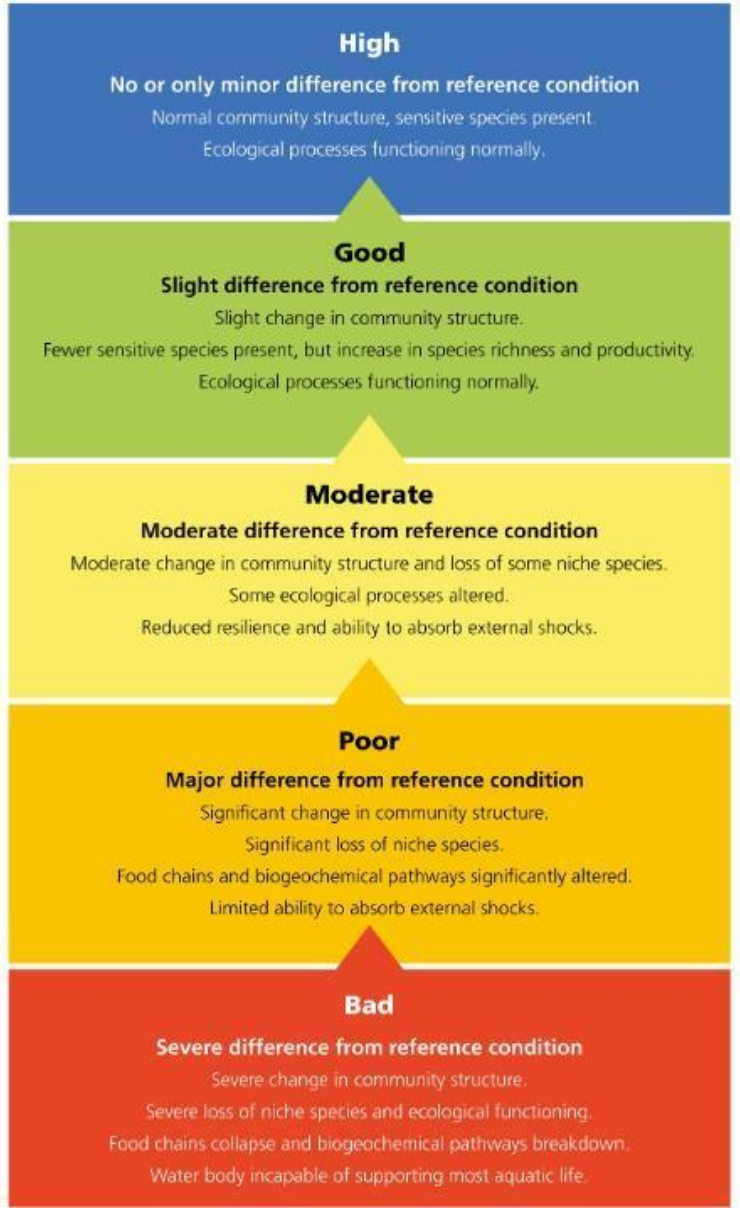




- Too few reference sites
- Same regression for all types, but references may be different
- Can types beta and gamma be combined (reference seems the same)?
- Gradient ok but more data between 40 and 89 µg TP needed, particularly for type alpha

Develop index: set boundaries between status classes

- High/good boundary is determined by the reference conditions.
- If you are really sure you have only true references, then use the maximum/minimum index value
- If some of your references might be «fishy», use e.g. the 75 or 90 percentile



- There are **very many different ways** how to set class boundaries
- Make sure you have a **scientific argument**
- Make sure to re-read the definitions given in the WFD
- Re-calculate your index into **EQR** (scale from 0-1)
- In addition, you need to take care of the **intercalibration results**

Develop an index, step 4: intercalibration of status class boundaries

Intercalibration of status class boundaries

- There are **different ways how to achieve intercalibration of class boundaries**
- Detailed method for intercalibration **depends mainly on your index** (methods used in the field and for index calculation)
- **The general principle is:**

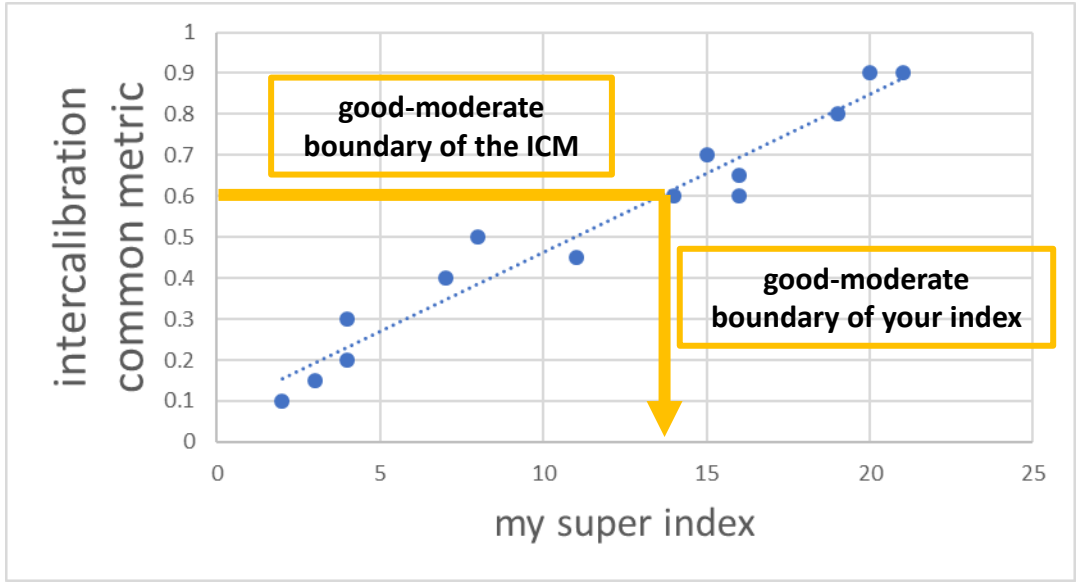
- For (almost) each organism group and each geographical intercalibration group, an **intercalibration common metric (ICM)** was defined
- The ICM is an index that was used for intercalibration, for example «average of IPS and TI» for «diatoms in rivers» in the «Central Baltic» intercalibration group
- Ideally, you can **calculate the ICM for your sites**

Nr	site	time	TP (µg/l)	species A	species B	species C	species D	my super index	intercalibration metric
1	A1	1	23	1	0	3	5	2	0.1
2	A2	1	34	0	0	3	1	4	0.2
3	A3	1	26	4	3	0	0	3	0.15
4	B1	2	19	3	2	0	0	7	0.4
5	B2	2	22	5	3	0	5	4	0.3
6	B3	2	26	1	2	1	3	8	0.5
7	B4	3	26	2	1	4	3	11	0.45
8	C1	3	19	0	1	3	2	14	0.6
9	D1	3	27	0	0	3	5	16	0.6
10	D2	3	89	2	0	3	5	15	0.7
11	R1	1	12	3	0	0	0	16	0.65
12	R2	1	14	5	0	1	0	19	0.8
13	R3	2	9	3	0	0	0	20	0.9
14	R4	3	11	4	0	2	3	21	0.9

Intercalibration of status class boundaries

- And ideally, there is a reasonable linear relationship between «your index» and the ICM

Nr	site	time	TP (µg/l)	species A	species B	species C	species D	my super index	intercalibration metric
1	A1	1	23	1	0	3	5	2	0.1
2	A2	1	34	0	0	3	1	4	0.2
3	A3	1	26	4	3	0	0	3	0.15
4	B1	2	19	3	2	0	0	7	0.4
5	B2	2	22	5	3	0	5	4	0.3
6	B3	2	26	1	2	1	3	8	0.5
7	B4	3	26	2	1	4	3	11	0.45
8	C1	3	19	0	1	3	2	14	0.6
9	D1	3	27	0	0	3	5	16	0.6
10	D2	3	89	2	0	3	5	15	0.7
11	R1	1	12	3	0	0	0	16	0.65
12	R2	1	14	5	0	1	0	19	0.8
13	R3	2	9	3	0	0	0	20	0.9
14	R4	3	11	4	0	2	3	21	0.9

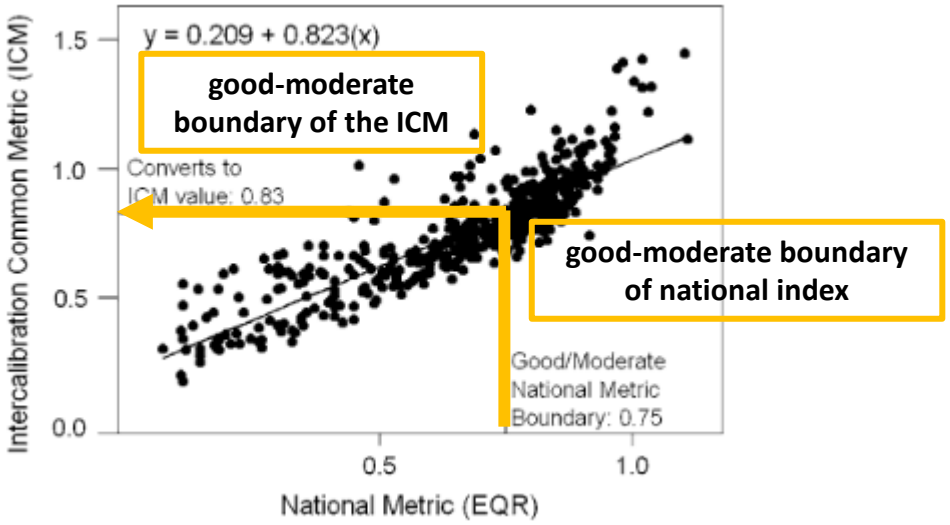


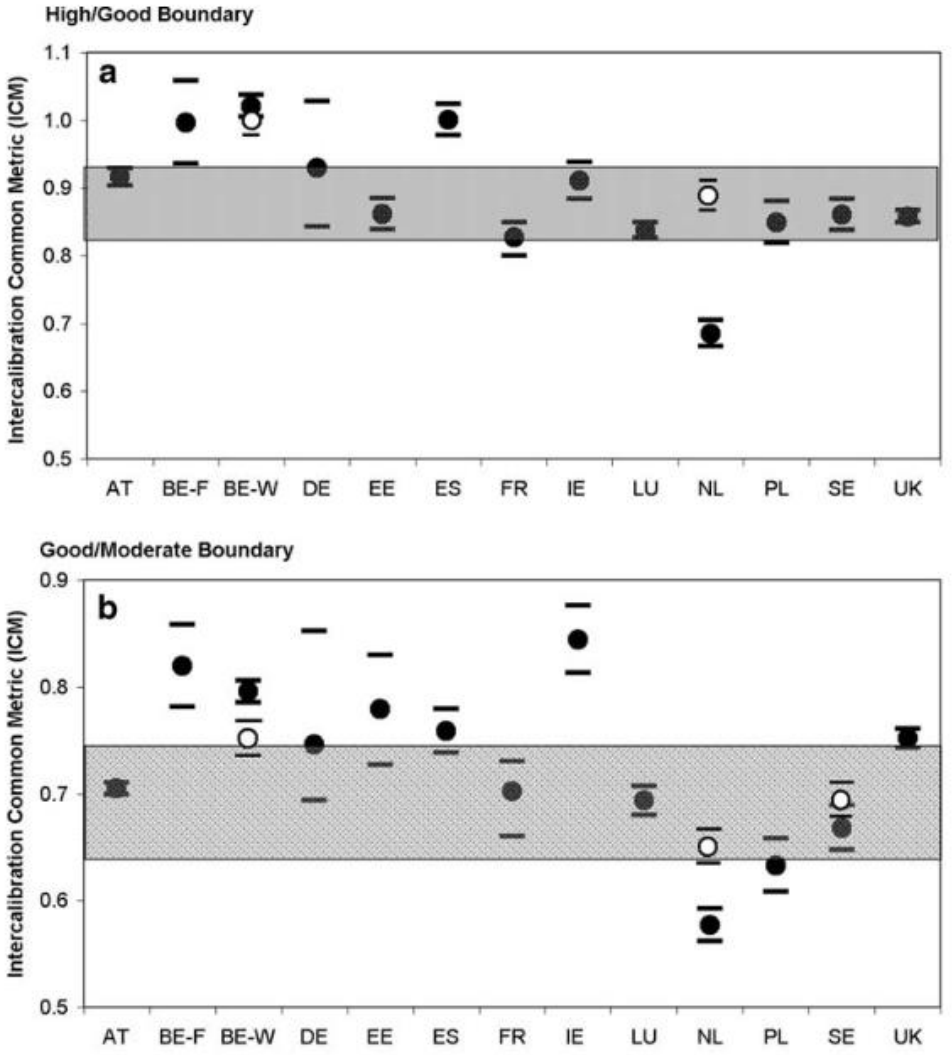
Intercalibration of status class boundaries

Example from **Kelly et al. (2009)**: A comparison of national approaches to setting ecological status boundaries in phytobenthos assessmentfor the European Water Framework Directive: results of an intercalibration exercise.

In the “real” intercalibration exercise, it had to be done the other way round:

- First they proposed the G/M boundary of the national metric
- Then they converted this into the ICM
- Lastly they checked if they agreed
- In case the national boundary was too low, it had to be adjusted





Open circles represent «adjusted» boundaries (during the intercalibration exercise)

But what if I cannot calculate the ICM for my sites?

This may happen when the methods are too different (ICM is on species level, while your index is on genus level; ICM uses abundance data, while your index only uses presence-absence; …)

Possibility 1:

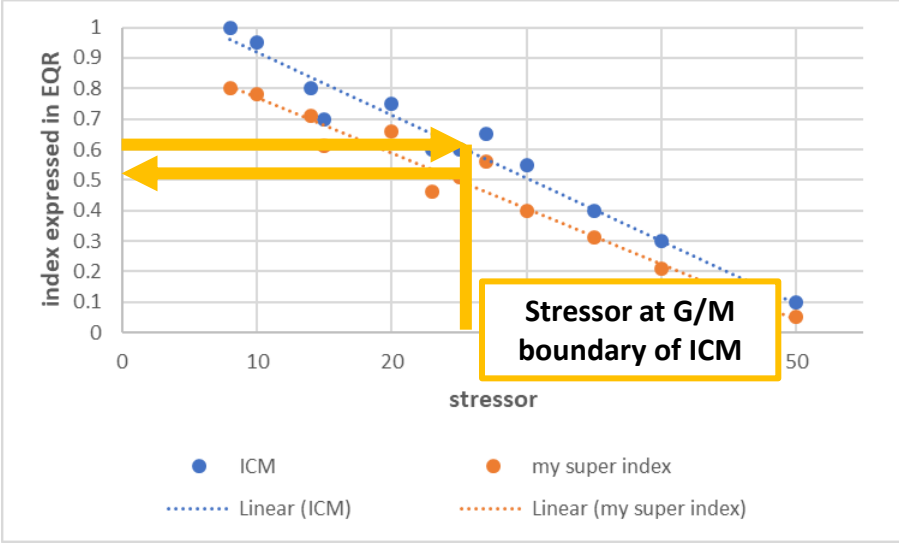
- find a subset of your sites (e.g. 50 sites) **covering all types and the entire gradient length**
- analyse these 50 sites in sufficient detail to calculate **both your index and the ICM**
- determine the **intercalibrated class boundaries for your index from these 50 sites**

Possibility 2:

- plot both your index and the ICM against the stressor
- use the stressor value at the G/M boundary to find the G/M boundary for your index

G/M boundary of ICM

G/M boundary of your index

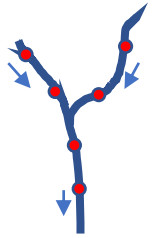


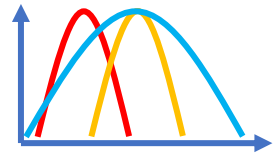
Intercalibration of status class boundaries

There are many more possibilities

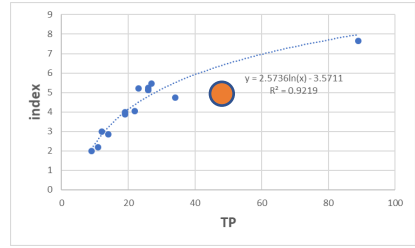
If you have good scientific arguments you are likely going to be fine!

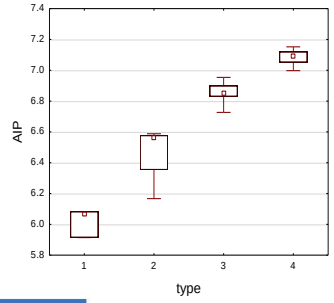
Summary «develop index»:

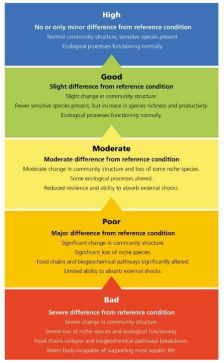




- Make sure data for stressor and response are from the same sites, and from the same time!
- Check ecological amplitudes of potential indicator species, consider weighing the indicator values
- Try to have a least some **independent sites for index validation** (i.e. sites that have not been used for calculating species indicator values)
- Invest some time into finding good reference sites
- Use reference sites to describe water body types
- Do not use more water body types than necessary to describe the natural variation
- Do not publish preliminary indices, but rather show current data to authorities and ask for money for collecting the necessary data. At the same time, be pragmatic where necessary.
- Use reference sites to define high/good boundary
- Make sure to have scientific arguments for the other status class boundaries
- Make sure the class coundaries are consistent with the results of the intercalibration exercise







Develop index: some last words

Multiple stressors

- For example, pH values commonly range from **4–10** in freshwater, nitrate concentrations may range from **0–300** mg/L, while proportional land use ranges **0–100%**.
- The variables would reveal different effect sizes just because of their different numerical scaling.
- Therefore, **standardisation** is a prerequisite for the comparison of effects sizes, i.e. to obtain standardised effect sizes
- A common approach is z transformation, which converts a variable to values with mean = 0 and SD = 1
- But if you only have a short part of the stressor gradient for one of the stressors, then you need to pay **really good attention** …

Develop index: some last words

Multimetric index or single metric index?

What do you think?

- There are good arguments for one and the other
- In the end, availability and quality of the data you may use for index development and intercalibration likeley will determine which approach you are going to use
- Important last words: **Do not make the index more complicated than necessary!**

Develop an index: good luck!

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