Lecture 4
Writing the Methods Section of a Paper

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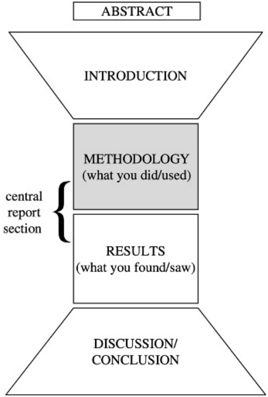
What is the methods section for?

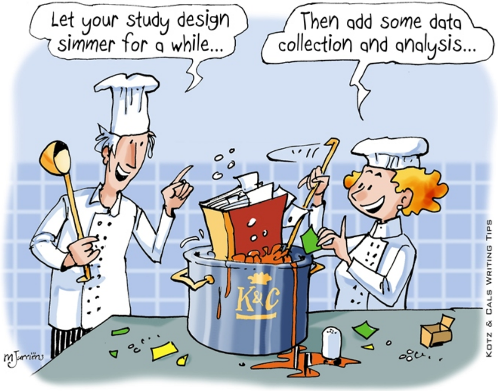
The methods section, as its name suggests, describes the methods and materials used in the paper.



(Glasman-Deal 2010, Science Research Writing for Non-Native Speakers of English)

What is the methods section for?

Description of the methods and materials should be objective with sufficient details so that readers can understand what the authors did and repeat the experiment if desired.



Tip 4 – Methods: provide a cookbook with the study's ingredients!

What is in the the methods section?

The methods section should contain all relevant information on how the research was done. This typically include

- location and time of the work;
- description of the study sites or existing data set utilized;
- type of data collected and the methods of data collection;
- materials used in the work;
- methods for data analysis and software used;

Need for details

The methods section should be detailed and specific, providing information such as GPS coordinates of the study sites or Latin names of species when they were mentioned.

> This study was performed at the Haibei Alpine Grassland Ecosystem Research Station (101°12'E, 37°30'N, 3200 m a.s.l), located in the north-eastern part of the Tibetan Plateau, China. This area has a continental monsoon climate, with a short growing season. From 2008 to 2013, the mean annual air temperature was –1.08 °C (ranging from –1.82 to –0.81 °C). The mean annual precipitation was 416.8 mm (ranging from 350.6 to 501.3 mm) (Table 1), and about 90% of the precipitation was concentrated in the growing season from May to September.

(Wang et al 2018, Soil Biology and Biochemistry)

Need for details

Provide citation information for statistical software and packages. For example, in R, you may use function `citation()` to obtain citation information for the software or packages.

> We fit all the linear mixed effects models using the function lmer in the R package lme4 (Bates et al 2015). The F–test with the Kenward-Roger approximation of degrees of freedom was implemented using R package pbkrtest (Halekoh and Højsgaard 2014). All statistical analyses were performed in R 3.4.1 (R Core Team 2017).

(Song et al 2018, Nature Geoscience)

Need for details

When details of the methods are available in previous publication and are not of critical importance to the current paper, you may simply refer the readers to the published work.

> We conducted this study in six watersheds representing distinct biomes, including tropical forest, tropical savanna, tallgrass prairie, temperate rainfroest, boreal forest, and arctic tundra. Within each watershed, we selected 6–12 streams across a range of stream sizes to capture the physcial gradients within the watershed. A detailed description of the study sites can be found in previous work (Rüegg et al 2016)

(Song et al 2018, Nature Geoscience)

Need for details

Many journals requires original data and software code to be made available upon acceptance of the paper to facilitate **reproducible research**.

Ecological Society of America associated journal requires:

- Raw data and metadata used to generate tables, figures, plots, videos/animations;
- Novel code or computer software utilized to generate results or analyse;
- All methods and protocols utilized to generate the data, both existing and new methods/protocols;
- Derived data products.

Structure of the methods section

The methods section often describes distinct aspects of the experiment. Thus, it can often be divided into individual subsections.

Each subsection should describe one set of experiments or measurements or analyses; Use as many subsections as you need;

If a goal of the Methods section is to help readers evaluate the findings presented in the Results section, then the author needs to make it clear how the two sections relate to each other. Using identical or corresponding subheadings in the methods and results section is a useful strategy.

Study sites and statistical analyses are subsections of the methods commonly found in many ecology papers. Study sites section is often the first one while the statistical analyses section is usually the last one.

Structure of the methods section

forests (with low mean annual temperatures) were characterised by 3–6 fold greater rates of ecosystem respiration, when compared with low latitude forests (high mean annual temperature), after standardising for temperature. Indeed, Enquist *et al.* (2003, 2007) argue that this apparent 'paradox' might be driven by physiological adaptation to climate and/or temperature at the organism level, such arguments being broadly consistent with the 'metabolic cold adaptation' hypothesis i.e. organisms originating from cold environments tend to exhibit elevated rates of metabolism (Krogh, 1916; Clarke, 1991; Addo-Bediako *et al.*, 2002). Similarly, in an experimental study, Luo *et al.* (2001) documented a marked decline in the temperature sensitivity of soil respiration under sustained warming, and attributed this response to acclimatisation driven by changes in microbial communities, reduced respiratory capacity and/or shifts in the underlying physiological response. However, this result may just as easily arise as a result of differential temperature sensitivities of various pools of soil organic matter (Kirschbaum, 2004; Knorr *et al.*, 2005).

Disentangling the relative influence of temperature on the intrinsic biochemical kinetics of respiration from other confounding factors controlling its temperature dependence – e.g. seasonal covariance of substrate availability, multiple limiting carbon pools, nutrients, drought, light etc. – remains elusive because of the difficulty of separating the effects of these variables in natural systems. Here, we attempt to overcome some of these difficulties, to determine the effects of thermal history on the temperature dependence of respiration, by making use of a rare model system: a catchment of Icelandic geothermal streams that vary in temperature (between 5 °C and 25 °C) yet which have comparable physico-chemical properties and an identical regional species pool (Friberg *et al.*, 2009; Woodward *et al.*, 2010; Demars *et al.*, 2011). This system represents a 'natural experiment' with individual streams (each draining a small sub-catchment) acting as replicates. This offered us the opportunity to isolate the effects of temperature on the respiratory capacity of natural stream communities with distinct thermal histories. We combined existing empirical surveys (Demars *et al.*, 2011), *in-situ* measurements, and laboratory experiments to address the following questions:

1 Is the temperature dependence of respiration scale-invariant and constrained by the average activation energy of the respiratory complex (0.6–0.7 eV) for all measurement scales/methods, e.g. between respiration measured in laboratory incubations, under *in-situ* conditions in the benthos, and at the whole-stream scale?
2 Does thermal history and species composition affect the temperature dependence of ecosystem respiration, characterised by the activation energy, *E*, Q_{10} or instantaneous rates of respiration (i.e. the normalisation constant in the Arrhenius model)?
3 Is the Q_{10} of respiration intrinsically related to measurement temperature?

Materials and methods

Field site

The geothermally active Hengill region of Iceland, 30 km east of Reykjavik (64°03′ N: 021 °18′ W, 350–420 m.a.s.l.) contains a large number of streams that are primarily spring-fed, and as such geothermal warming is the principal driver of water temperature differences within the catchment (Friberg *et al.*, 2009). Within the study catchment, temperature differences among streams are consistent across seasons and years (Friberg *et al.*, 2009; Woodward *et al.*, 2010; Demars *et al.*, 2011). Since all streams are tributaries of the same main stream and lie 2 m–2 km apart (Fig. 1) there are few (if any) dispersal constraints on the biota (Woodward *et al.*, 2010). Importantly, the streams have a very similar physicochemistry (Friberg *et al.*, 2009; Woodward *et al.*, 2010; Demars *et al.*, 2011) and temperature accounts for most of the variance in macroinvertebrate community composition (Woodward *et al.*, 2010).

Whole-stream respiration

Whole-stream respiration was measured in 13 tributaries (17–51 m in length) over 2 days per stream within an 11 day period in August 2008 (Demars *et al.*, 2011; Table 1). Measurements were based on a modified open-system oxygen (O_2) change method using two stations (Odum, 1956) corrected for lateral inflows (McCutchan *et al.*, 2003; Hall & Tank, 2005). Essentially, this is an in-stream mass balance of O_2 requiring measurements of inflows and outflows along a river reach with the average of the two records (stations) used to take into account spatial heterogeneity in dissolved O_2 (Demars *et al.*, 2011). Daily (24 h) estimates of whole-stream respiration were calculated by extrapolating the mean night-time value across the hours of daylight, because it is not possible to measure day-time respiration directly (see e.g. Marzolf *et al.*, 1994). The uncertainties of whole-stream respiration rates were calculated based on one standard deviation and propagated for each time step (1-min interval) during the night-time hours (Demars *et al.*, 2011). The necessary measurements and methods on which the calculations are based used state-of-the-art methods (e.g. NaCl and propane tracer studies), equipment (optic oxygen sensors) and calibration care, as detailed in Demars *et al.* (2011). We converted whole-stream respiration rates in units of O_2 as reported in Demars *et al.* (2011) to carbon (C) equivalents assuming a molar respiratory quotient of 0.85 (Hauer & Lamberti, 1996).

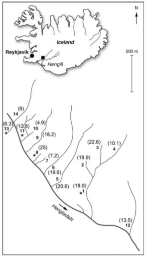


Fig. 1 Map of the streams studied in the Hengill catchment in Iceland. Annotated stream numbers correspond with averaged temperatures over the 2 days of whole-stream metabolism measurements in August 2008 as given in Table 1 which are given here in parentheses. *Indicates the four streams used for *in-situ* benthic and laboratory incubations.

In-situ *benthic respiration*

We selected four streams that spanned a broad temperature range (mean temperatures during study period ~ 6 °C, 13 °C, 21 °C and 25 °C respectively; Table 1, Fig. 1) to measure *in-situ* benthic respiration within the same study period as the whole-stream measurements were made (Demars *et al.*, 2011).

For each stream, *in-situ* benthic respiration was measured using three opaque bottomless benthic chambers (1 L, 8 cm in diameter) per stream. The chambers were screwed into the stream bed (to a depth of approximately 5 cm) secured with baffles facing upstream which deflected flow (after Trimmer *et al.*, 2009). The water inside each chamber was mixed by a small rotating (300 rpm) magnetic flea within the lid, driven by an external magnetic stirrer unit (Rank Brothers Ltd., Cambridge, UK). The chambers were left for 1 h after placement and then water was sampled at the beginning and the end of each 3 h incubation via a port in the lid using a gas-tight syringe (25 ml, SGE, Alltech Assoc. App. Sci., Ltd., Carnforth, UK) and a bladder inside the lid compensated for sample removal (about 4% of the total volume). The duration of the incubations was sufficient to measure changes in O_2 concentrations accurately, whilst ensuring that O_2 uptake during this period was linear. This latter criterion was tested prior to the main incubations in a pilot study where samples were repeatedly removed every 2 h for a total of 8 h from benthic chambers fixed in the two warmest streams (streams 8 and 1; Table 1). Since the uptake of O_2 during incubation was linear (see Supporting Information S1), subsequently only T-zero and T-final samples were taken to determine *in-situ* benthic respiration and to limit sample extraction from the chambers. The samples (25 ml) for dissolved O_2 were gently discharged into gas-tight vials (12 ml exetainers; Labco Ltd., High Wycombe, UK) and allowed to overflow. O_2 was fixed immediately and analysed using Winkler titration (see Hauer & Lamberti, 1996). *In-situ* benthic respiration rate (*R*) was calculated as:

$$R = \Delta O_2(V/S) \quad (1)$$

and expressed as mg ΔO_2 m^{-2} $hour^{-1}$, where ΔO_2 is the change in oxygen concentration between two consecutive O_2 measurements (mg O L^{-1} $hour^{-1}$), *V* is the water volume in the chamber (L) and *S* is the active surface (m^2). Respiration in units of O_2 was converted to C equivalents as above.

Laboratory biofilm incubations

Stones with attached biofilm from the four study streams were collected and transported back to the laboratory in <8 h (in darkened cool boxes with stream water). The principal aim of this experiment was to assess the direct effect of thermal history on the potential for physiological adaptation of respiration at the community-level mediated via changes in the activation energy, *E*, and the normalisation constant, r_0, of the Arrhenius model and/or the Q_{10}. Therefore, respiration rates were estimated over the short-term (e.g. over 30 min incubations see below), to avoid the potential for autotroph and community-level respiratory acclimation to elevated temperature, mediated via possible substrate limitation (Dewar *et al.*, 1999; Atkin & Tjoelker, 2003; Allen *et al.*, 2005).

On arrival at the laboratory, biofilms were maintained at ambient stream temperatures, in temperature-controlled water baths under saturating O_2 conditions. The biofilms were exposed to high power daylight spectrum halogen bulbs (~ 220 μmol photons m^{-2} s^{-1}) with a photoperiod of 20 h: 4 h light to dark (to resemble field conditions, see Demars *et al.*, 2011) to stimulate photosynthesis and prevent carbon limitation of respiration.

In laboratory incubations, (<24 h after initial collection) biofilms (two stones with attached biofilms) from each of the four streams were placed in four 1 L opaque chambers (8 cm in diameter) and submerged in a single temperature-controlled water bath containing freshwater culture medium [Culture Collection of Algae and Protozoa (CCAP); http://www.ccap.ac.uk/media/documents/DM.pdf]. Biofilms were then incubated at six temperatures (~ 5, 10, 15, 20, 25 and 30 °C) in an increasing sequence starting at the lowest (~ 5 °C) through to

(Perkins et al 2012, Global Change Biology)

Writing strategy: the LD structure

The **lead/development (LD)** structure is a useful way to describe methods. It provides an initial overview for all and then details for those who need them.

The LD structure structure intensifies the front-loading of the story. It is effective when readers want to know the general theme but does not need to know all the details.

Writing strategy: the LD structure

Compare the following three ways of describing the methods:

Enzyme inactivation associated with 3-HPAA metabolism was measured by the method of Turman et al. (2008).

PGHS–1 or PGHS–2 was incubated with 25 μM 3–HPAA. When oxygen uptake was complete, arachidonic acid (15 μM) was added, and the maximal rate was determined as described above and normalized to the DMSO control. The concentration dependence of PGHS–2 inactivation was analyzed in a similar manner with varying concentrations of 3–HPAA (from 10nM to 25 μM).

To characterize the extent of enzyme inactivation associated with 3–HPAA metabolism, PGHS–1 or PGHS–2 was incubated with 25 μM 3–HPAA. When oxygen uptake was complete, arachidonic acid (15 μM) was added, and the maximal rate was determined as described above and normalized to the DMSO control. The concentration dependence of PGHS–2 inactivation was analyzed in a similar manner with varying concentrations of 3–HPAA (from 10nM to 25 μM).

Writing strategy: the LD structure

The LD structure can be used in writing each paragraph, i.e., lead the paragraph with a summary sentence before delving into the details.

> We collected data to estimate whole stream metabolism in lower Kings Creek (39.10004 °N, 96.60956 °W) located within the Konza Prairie Biological Station near Manhattan, Kansas, USA. Specifically, we recorded DO concentration, water temperature, and barometric pressure using a YSI ProODO handheld optical DO meter (YSI Instruments, Yellow Springs, Ohio, USA) and photosynthetically active radiation (PAR) using an Odyssey Irradiance logger (DataFlowSystems, Christchurch, New Zealand) at a single location in the stream every 10 minutes for 8 consecutive days (May 28–June 5, 2013). The DO meter was calibrated with water saturated air prior to deployment and readings from the irradiance logger was converted to PAR using a conversion coefficient derived from comparison to a calibrated PAR sensor.

(Song et al 2016, Limnology and Oceanography: Methods)

Writing strategy: the LD structure

An overview of methods at the beginning of the section is useful particularly when the methods are long and complex.

> We simulated meta-analyses consisting of data from 20 papers, each containing a number of studies. For each study, we simulated replicated control and treatment groups, with data from each source paper simulated to obtain various patterns of non-independence among observed effect sizes within the paper. For each study, we calculated a log response ratio and its estimated variance. The log response ratio is the most commonly used effect size metric in ecology, but our qualitative results should apply to other metrics as well. We estimated the overall mean effect size using alternative meta-analysis methods that differ in how they account for non-independence and compared the their performance. We conducted two sets of simulation experiments. In the first experiment, observed effect sizes from the same source paper were correlated with the same correlation coefficients for all pairs. In the second experiment, we varied the correlation between pairs of observed effect sizes.

(Song et al 2021, Ecology)

Grammar and style

Tense: most of the methods section should be written in **past tense** because the methods section describes past action the authors took.

> We recorded DO concentration, water temperature, and barometric pressure using a YSI ProDOD handheld optical DO meter (YSI Instruments, Yellow Springs, Ohio, USA), and photosynthetically active radiation using an Odyssey Irradiance logger (DataFlowSystems, Christchurch, New Zealand) at a single location in each stream. The DO meter was calibrated with water saturated air immediately before deployment. The readings from the irradiance logger were converted to photosynthetically active radiation based on comparison with a calibrated sensor.

(Song et al 2018, Nature Geoscience)

Active and passive voices

Most editors advise authors to **minimize the use of passive voice**. However, passive voice can be validly used in the methods section.

- The readers do not need to know who or what carried out the action. Passive voice is appropriate for this purpose.
- Passive voice can be used to facilitate the flow of the writing, i.e., connecting old and new information.
- It sometimes sounds overly repetitive or immodest to use personal pronoun subjects in active voice.

> We used the results of these analyses to inform the construction of mechanistic candidate functions for the relationship between propagule input, space availability and recruitment. These candidate functions were compared using differences in the Akaike information criteria. We then used model averaging...

(Britton-Simmons and Abbott 2008, Journal of Ecology)

Active and passive voices

When using passive voice, do not write sentences with very long subjects and a short passive verb right at the end.

× Wheat and barley, collected from the Virginia field site, as well as sorghum and millet, collected at Loxton, were used.

✓ Four cereals were used: wheat and barley, collected from the Virginia field site; and sorghum and millet, collected at Loxton.

Abbreviate passive voice sentences to avoid sounding repetitive.

× The data were collected and they were analyzed using...

✓ The data were collected and analyzed using...

When do you write the methods section?

Methods section is straightforward to write. Many suggest starting writing from the methods section to help you get into the mood of writing.

You can also start writing the methods section while experimental work is ongoing so that all the details are still fresh and clear to you.

Tip 1 - How to get started: choose the optimal environment!