

Lecture 4

Writing the methods section of a paper

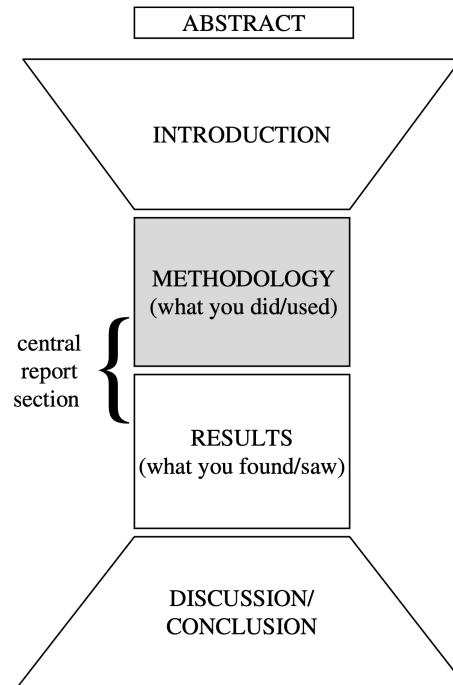
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September 29, 2022

Purpose of the methods section

- This section is often called “Methods” or “Materials and methods”;
- The main purpose of the methods section is to describe the experimental design and provide enough detail so that a competent worker can repeat the experiments;



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

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;
- Study sites (often first) and statistical analyses (often last) are subsections of the methods commonly found in many ecology papers.

Example of methods section structure

1302 D. M. PERKINS *et al.*

THE TEMPERATURE DEPENDENCE OF RESPIRATION 1303

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 physico-chemistry (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 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).

Whole-stream respiration

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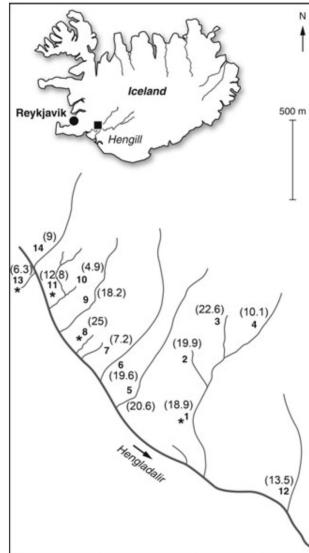


Fig. 1 Map of the streams studied in the Hengill catchment in Iceland. Annotated stream numbers correspond with averaged temperatures over the 2 days of whole-stream metabolism measurements in August 2008 as given in Table 1 which are given here in parentheses. *Indicates the four streams used for *in-situ* 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 extainers; 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 $\Delta O_2 \text{ m}^{-2} \text{ hour}^{-1}$, where ΔO_2 is the change in oxygen concentration between two consecutive O_2 measurements (mg $O \text{ L}^{-1} \text{ hour}^{-1}$), 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, Q_{10} , 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 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) 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

Writing strategy: the LD structure

- An effective way to describe a method is to use a **lead/development (LD)** structure, providing an initial overview for all and then details for those who need them;
- The LD 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 following 3-HPAA metabolism

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

Enzyme inactivation following 3-HPAA metabolism

PGHS-1 or PGHS-2 was incubated with 25 μ M 3-HPAA. When oxygen uptake was complete, arachidonic acid (25 μ M) 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 10 nM to 25 μ M).

Enzyme inactivation following 3-HPAA metabolism

To characterize the extent of enzyme inactivation associated with 3-HPAA metabolism, PGHS-1 or PGHS-2 was incubated with 25 μ M 3-HPAA. When oxygen uptake was complete, arachidonic acid (25 μ M) 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 10 nM to 25 μ M)

Writing strategy: the LD structure

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

We simulated meta-analyses consisting of data from 20 papers, each containing a number of studies (Fig. 1). 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 (Nakagawa et al. 2012), 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 (Fig. A1). 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)

Writing strategy: the LD structure

- The LD structure can also be used in writing each paragraph;

We collected data to estimate whole stream metabolism in lower Kings Creek (39.10004 °N, 96.60959 °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 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)

Need for details

- The methods section should provide sufficient details, such as GPS coordinates of the study sites or the Latin names when listing species etc.

This study was performed at the Haibei Alpine Grassland Ecosystem Research Station (Haibei Station, 101°12'E, 37°30'N, 3200 m a.s.l.), located in the northeastern part of the Tibetan Plateau, China. This area has a continental monsoon climate, with a short growing season (Wang et al. 2014). 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. 2014).

(Wang et al. 2018, Soil Biology and Biochemistry)

Need for details

- Statistical software or packages should be cited.
- In R, 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 the R package pbkrtest (Halekoh and Højsgaard 2014). All statistical analyses were performed in R 3.4.1 (R Core Team 2017).

References

- Bates, D., Mächler, B. Bolker, and S. Walker (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67: 1–48.
- Halekoh, U. and S. Højsgaard (2014). A Kenward–Roger approximation and parametric bootstrap methods for tests in linear mixed models—the R package pbkrtest. *Journal of Statistical Software*, 59: 1–30.
- R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

(Song et al. 2018, *Nature Geoscience*)

Need for details

- When details are available somewhere else and are not of critical importance to the current paper, you may simply refer the readers to previous work.

We conducted this study in six watersheds representing distinct biomes, including tropical forest (LUQ), tropical savanna (AUS), tallgrass prairie (KNZ), temperate rainforest (AND), boreal forest (CPC) and arctic tundra (ARC). Within each watershed, we selected 6–12 streams across a range of stream sizes to capture the physical 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

- **Reproducible:** Research is considered to be reproducible when the exact results can be reproduced if given access to the original data, software, or code;
- Many journals these days requires original data and statistical code to be made available when submitting papers.
- 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 analyses
 - All methods and protocols utilized to generate the data, both existing and new methods/protocols
 - Derived data products

Grammar and style

- Tense: most of the methods section should be written in **past tense** as it describes past action the authors took.
- Authors often are advised to minimize use of passive voice. However, **passive voice often can** validly be used in the methods section, for although what was done must be specified, who did it is often irrelevant or obvious.

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 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)

When to write methods?

- Because the methods section is easier to write, you can draft the methods section first when writing the paper. This helps get the momentum and get into the mood of writing;
- You can start writing the methods section while experimental work is still ongoing so that all the details are still clear.