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# Consistent temperature dependence of respiration across ecosystems contrasting in thermal history

DANIEL M. PERKINS*, GABRIEL YVON-DUROCHER*, BENOÎT O. L. DEMARS†, JULIA REISS*‡, DORIS E. PICHLER*, NIKOLAI FRIBERG†§, MARK TRIMMER* and GUY WOODWARD*

*School of Biological and Chemical Sciences, Queen Mary University of London, London, UK, †Ecological Sciences Group, The James Hutton Research Institute, Craigiebuckler, Aberdeen AB15 8QH, UK, ‡Department of Life Sciences, Whitelands College, University of Roehampton, London, SW15 4JD, UK, §Department of Freshwater Ecology, Aarhus University, National Environmental Research Institute, Vejlsøvej 25, DK-8600, Silkeborg, Denmark

## Abstract

Ecosystem respiration is a primary component of the carbon cycle and understanding the mechanisms that determine its temperature dependence will be important for predicting how rates of carbon efflux might respond to global warming. We used a rare model system, comprising a network of geothermally heated streams ranging in temperature from 5 °C to 25 °C, to explore the nature of the relationship between respiration and temperature. Using this 'natural experiment', we tested whether the natal thermal regime of stream communities influenced the temperature dependence of respiration in the absence of other potentially confounding variables. An empirical survey of 13 streams across the thermal gradient revealed that the temperature dependence of whole-stream respiration was equivalent to the average activation energy of the respiratory complex (0.6–0.7 eV). This observation was also consistent for *in-situ* benthic respiration. Laboratory experiments, incubating biofilms from four streams across the thermal gradient at a range of temperatures, revealed that the activation energy and $Q_{10}$ of respiration were remarkably consistent across streams, despite marked differences in their thermal history and significant turnover in species composition. Furthermore, absolute rates of respiration at standardised temperature were also unrelated to ambient stream temperature, but strongly reflected differences in biofilm biomass. Together, our results suggest that the core biochemistry, which drives the kinetics of oxidative respiratory metabolism, may be well conserved among diverse taxa and environments, and that the intrinsic sensitivity of respiration to temperature is not influenced by ambient environmental temperature.

*Keywords:* carbon cycle, community composition, ecosystem functioning, geothermal streams, global warming, metabolic theory of ecology, respiration, temperature

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## Introduction

Ecosystem respiration is the dominant sink for organic carbon in the biosphere (Valentini *et al.*, 2000; del Giorgio & Williams, 2005). Consequently, it is a critical component of the carbon cycle and an ecosystem service (Schröter *et al.*, 2005) that is likely to be important in regulating the biosphere's response to global warming (IPCC, 2007).

Streams and rivers are 'hotspots' of $CO_2$ emission (Cole *et al.*, 2007; Battin *et al.*, 2008, 2009) because they act as conduits for organic carbon derived from the terrestrial landscape, the majority of which is processed and respired as $CO_2$ to the atmosphere (Cole *et al.*, 2007; Battin *et al.*, 2009). Thus, the role of freshwater ecosystems as major components of the global carbon cycle is being increasingly appreciated (Algesten *et al.*, 2004; Cole *et al.*, 2007; Battin *et al.*, 2008, 2009; Bastviken *et al.*, 2011). The sensitivity of respiration to temperature will therefore be a key factor – in addition to that of primary production (e.g. Yvon-Durocher *et al.*, 2010a; Demars *et al.*, 2011) and sedimentation (e.g. Gudasz *et al.*, 2010) – governing the potential of freshwater ecosystems to modulate climate change as the planet warms (IPCC, 2007).

Numerous models have been proposed to describe the temperature dependence of respiration (Lloyd & Taylor, 1994; Gillooly *et al.*, 2001; Davidson & Janssens, 2006; Davidson *et al.*, 2006). Perhaps the two most common models are the $Q_{10}$, which describes the proportional increase in rates of respiration per 10 °C

Daniel M. Perkins and Gabriel Yvon-Durocher contributed equally to this work.

Correspondence: Gabriel Yvon-Durocher, tel. + 44 207 882 3640, fax + 44 0 20 7882 7732, e-mail: g.yvon-durocher@qmul.ac.uk

rise in temperature, and the Arrhenius equation, which describes how the proportion of molecules with sufficient energy to react changes with temperature. In the Arrhenius equation: $r_i = r_0 e^{-E/kT}$, where $r_i$ describes the rate of respiration of an individual organism, $i$, $r_0$ is an individual-level normalisation constant, $T$ is temperature (in Kelvin) and $k$ is the Boltzmann's constant ($8.62 \times 10^{-5}$ eV $K^{-1}$); $E$, the average activation energy (eV) is the fundamental parameter that determines the temperature sensitivity of respiration (Arrhenius, 1915). Empirical estimates of $E$ tend to fall between 0.6 and 0.7 eV for a wide range of taxa across diverse habitats (Gillooly *et al.*, 2001; Enquist *et al.*, 2003; Brown *et al.*, 2004; Allen *et al.*, 2005). This suggests that $E$ might represent a relatively fixed property across species and environments, reflecting the conservation of a core biochemistry of oxygenic metabolism among the main domains of life (Lane & Martin, 2010). A growing body of work also demonstrates that $E$ tends to be well conserved across multiple levels of biological organisation, from the respiratory complex in a single mitochondrion through to whole ecosystems (Enquist *et al.*, 2003; Allen *et al.*, 2005; López-Urrutia *et al.*, 2006; Allen & Gillooly, 2009; Yvon-Durocher *et al.*, 2010a,b).

The 'universality' of the temperature dependence of respiration has, however, been a topic of considerable debate (Atkin & Tjoelker, 2003; Clarke, 2004, 2006; Clarke & Fraser, 2004; Davidson *et al.*, 2006; Gillooly *et al.*, 2006), in part, because a substantial body of work has demonstrated significant variability in the $Q_{10}$ of ecosystem respiration both among (Kirschbaum, 1995; Fierer *et al.*, 2006) and within sites (Acuña *et al.*, 2008). It is important to note that variation in the more commonly used $Q_{10}$ can arise from shifts in either $E$ (the slope of the temperature response) or $r_0$ (the intercept of the temperature response), because the $Q_{10}$ is intrinsically dependent on the temperature response curve (Atkin & Tjoelker, 2003; Davidson & Janssens, 2006). Variability in the temperature dependence of respiration (either in the form of $E$ or $Q_{10}$) can be driven by a range of mechanisms that can be contingent on the temporal and spatial scales of analysis and on the specific type of ecosystem being studied. Disentangling the relative influence of extrinsic scale dependent factors from the intrinsic physiological sensitivity of respiration to temperature is a fundamental stepping stone towards developing a mechanistic understanding of this process, but has thus far proved challenging (Davidson & Janssens, 2006). Herein, we attempt to provide clarity to one piece of the jigsaw; the frequently observed phenomenon of decline in the temperature dependence with increasing temperature.

Diminishing temperature sensitivity with increasing temperature is itself a complex process, and its mechanistic basis is also dependent on the temporal scale of its analysis. Perhaps the most commonly observed example is that of respiratory acclimation to elevated environmental temperature (e.g. Atkin & Tjoelker, 2003) for which at least two types of responses have been observed. First, instantaneous declines in the $Q_{10}$ with increasing measurement temperature have been consistently observed in plants (Atkin & Tjoelker, 2003) and soils (Kirschbaum, 1995; Fierer *et al.*, 2006). This phenomenon is broadly consistent with principles of physical chemistry, and at least in part reflects the fact that the $Q_{10}$ is intrinsically temperature dependent, when respiration follows Arrhenius kinetics. Thus, as temperatures rise the rate of increase in the proportion of molecules with sufficient energy to react slows down (Gillooly *et al.*, 2001; Davidson & Janssens, 2006). In this example, a declining $Q_{10}$ can occur whilst $E$ remains constant i.e. the underlying biochemistry is unaffected by temperature.

The second type of response results from long-term exposure to elevated temperatures predominantly explored in experimental warming studies (Luo *et al.*, 2001; Atkin & Tjoelker, 2003; Kirschbaum, 2004; Hartley *et al.*, 2006; Zhou *et al.*, 2007; Bradford *et al.*, 2010) but also from a bigeographical context (Giardina & Ryan, 2000; Addo-Bediako *et al.*, 2002; Enquist *et al.*, 2003, 2007; Stæhr & Wernberg, 2009). Herein, studies have revealed that sustained temperature increases of weeks to months in duration elicit a decline in the temperature sensitivity characterised by either the $Q_{10}$ (Luo *et al.*, 2001; Addo-Bediako *et al.*, 2002; Atkin & Tjoelker, 2003; Zhou *et al.*, 2007; Stæhr & Wernberg, 2009) or $r_0$ – i.e. the normalisation constant of the Arrhenius function (Giardina & Ryan, 2000; Enquist *et al.*, 2003) – for plants (Atkin & Tjoelker, 2003; Zhou *et al.*, 2007; Stæhr & Wernberg, 2009), soils (Giardina & Ryan, 2000; Luo *et al.*, 2001), insects (Addo-Bediako *et al.*, 2002) and whole forest ecosystems (Enquist *et al.*, 2003, 2007). The mechanisms offered to explain these patterns are diverse, but perhaps the two most common are acclimatisation or adaptation responses (Giardina & Ryan, 2000; Luo *et al.*, 2001; Addo-Bediako *et al.*, 2002; Enquist *et al.*, 2003, 2007) and substrate limitation, driven by either differential temperature dependencies of mineralisation of labile and recalcitrant soil carbon pools (Kirschbaum, 2004; Knorr *et al.*, 2005), or limitation of plant respiration by the supply rate of reduced substrates from photosynthesis (Dewar *et al.*, 1999; Atkin & Tjoelker, 2003; Allen *et al.*, 2005).

Across a broad biogeographical and climatic gradient, Enquist *et al.* (2003) found that high latitude

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*

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).

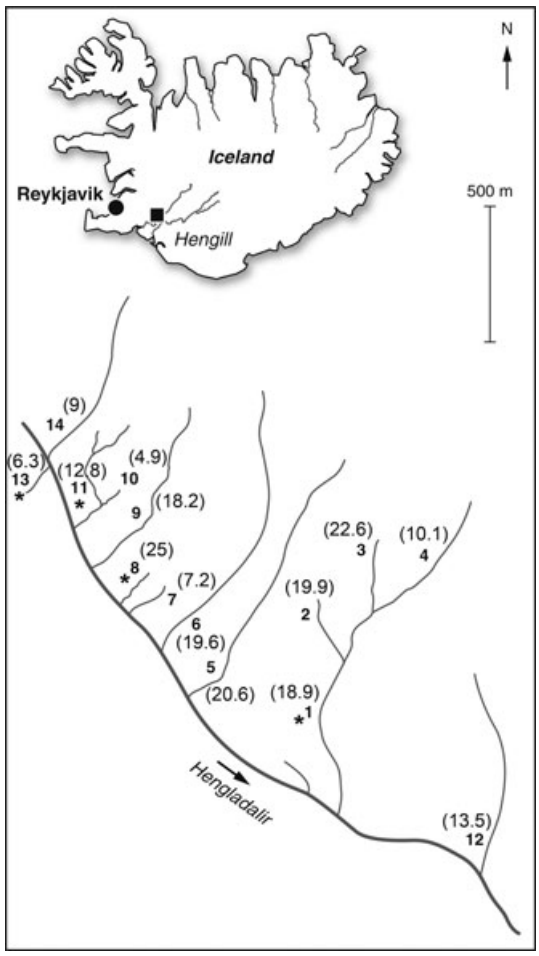


**Fig. 1** Map of the streams studied in the Hengill catchment in Iceland. Annotated stream numbers correspond with averaged temperatures over the 2 days of whole-stream metabolism measurements in August 2008 as given in Table 1 which are given here in parentheses. *Indicates the four streams used for *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 $\Delta O_2$ $m^{-2}$ $hour^{-1}$, where $\Delta O_2$ 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 $\mu$mol 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

**Table 1** Primary physicochemical variables in the study streams during the 2008 measurement period for whole-stream respiration (Demars *et al.*, 2011) with the four study streams used for *in-situ* benthic and laboratory incubations highlighted in bold

| Stream n.o | Mean temperature during measurements (ºC) | 1 SD (°C) | pH | Cond ($\mu$S/cm) | DOC ($\mu$g/ml) | Total N ($\mu$g/ml) | Total P ($\mu$g/ml) |
|---|---|---|---|---|---|---|---|
| 10 | 4.9 | 0.4 | 7.7 | 129 | 0.314 | 0.054 | 0.018 |
| **13** | **6.3$^{(5.9)}$** | **0.1** | **7.6** | **201** | **0.294** | **0.114** | **0.006** |
| 7 | 7.2 | 0.5 | 7.6 | 110 | 0.208 | 0.012 | 0.025 |
| 14 | 9.0 | 0.8 | 8.1 | 254 | 0.403 | 0.123 | 0.010 |
| 4 | 10.1 | 2.2 | 7.7 | 153 | 0.465 | 0.041 | 0.008 |
| **11** | **12.8$^{(12.4)}$** | **1.6** | **8.0** | **624** | **0.581** | **0.085** | **0.015** |
| 12 | 13.5 | 0.9 | 7.9 | 223 | 0.618 | 0.098 | 0.011 |
| 9 | 18.2 | 0.9 | 8.1 | 262 | 0.263 | 0.036 | 0.036 |
| **1** | **18.9$^{(19.9)}$** | **1.6** | **7.8** | **294** | **0.767** | **0.062** | **0.015** |
| 6 | 19.6 | 0.4 | 8.1 | 283 | 0.317 | 0.016 | 0.028 |
| 2 | 19.9 | 0.6 | 8.0 | 281 | 0.424 | 0.036 | 0.024 |
| 5 | 20.6 | 0.5 | 8.0 | 282 | 0.427 | 0.032 | 0.019 |
| 3 | 22.6 | 0.7 | 7.9 | 275 | 0.226 | 0.019 | 0.028 |
| **8** | **25.0$^{(24.7)}$** | **0.1** | **8.1** | **300** | **0.330** | **0.014** | **0.031** |

Temperatures given in parentheses correspond to mean temperatures during measurements of *in-situ* benthic respiration.
Note site 13 was excluded from whole-stream metabolic estimates because lateral inflows were too large (79% of discharge at the bottom station, see Demars *et al.*, 2011) to accurately estimate whole stream metabolism.

the highest (~ 30 °C) temperature. Each incubation lasted for 30 min, during which time dissolved $O_2$ measurements were recorded every 20 s using micro-oxygen electrodes (50 $\mu$m tip) connected to in-line amplifiers and a four-channel data logger (Unisense, Aarhus, Denmark) inserted into a custom-built gas-tight port in the lid of each chamber. After each 30 min incubation, the chamber lids were removed to allow re-equilibration of $O_2$ for a further 30 min before starting the subsequent incubation at the next temperature. Oxygen electrodes were calibrated in 100% and 0% air-saturated water, at the start of each incubation. After the end of the final incubation (i.e. at ~ 30 °C), biofilms were removed from stones, frozen in darkened bottles and digital photographs of the stones were taken to determine active surface calculated using image analysis software (IMAGE PRO PLUS 6.3; Media Cybernetics, Inc®). Each suite of incubations was repeated four times, each with new biofilms from each of the four donor streams. This gave a total of 96 experimental units; i.e. 4 streams × 6 incubation temperatures × 4 biofilm replicates.

Biofilm respiration rate ($R$) was calculated (equation 1) and $\Delta O_2$ was described by the slope of a regression between $O_2$ vs. time for the first 15 min of each incubation (to avoid the potential effects of $O_2$ limitation that might have resulted in non-linear $O_2$ consumption at the higher temperatures). Relationships where the $r^2$ values did not exceed 90% were excluded from further analysis ($n$ = 5 out of 96 experimental units).

## *Biofilm biomass*

Total biofilm biomass was measured for each of the stones used in the laboratory experiments. Ash free dry mass (AFDM) was measured from biofilm samples from the stone scrapes (after Hauer & Lamberti, 1996) via combustion of samples at 550 °C for 20 min in a muffle furnace. AFDM was converted to C units by applying an empirical ratio of 0.53 (Wetzel, 2001).

## *Biofilm characterisation*

We determined the species composition of the local microbial and meiofaunal assemblages of each of the four streams used for the *in-situ* benthic and laboratory incubations. To carry out this process we collected eight stones with attached biofilms from each of the four study streams during the 2 weeks, period for taxonomic characterisation of diatoms, ciliates and micro-metazoans (species < 2 mm in length). Species richness and composition within these groups was characterised for each of the four study streams. Three samples were preserved in Lugol's solution for diatom identification. Diatoms were identified to species in >95% of total cases, but when this was not possible, they were categorised as morpho-types within Genus, Family or Order level (see Supporting Information S2). An Olympus BX50 microscope with 400–1000 × magnification and the taxonomic keys by Krammer & Lange-Bertalo (1986a,b, 1991a,b) were used. For identification of heterotrophic groups, biofilms from the five remaining stones were scraped off using a toothbrush and transferred into sampling tubes containing 50 ml stream water at the study stream. All samples were examined the same day they were collected: ciliates and micro-metazoans (the 'meiofauna') were identified alive and counted (in subsamples until at least 50 individuals had been recorded) at 1000 × magnification as previously described.

### Data analyses

The activation energy of stream respiration in all analyses was estimated from a linearised Arrhenius function of the form:

$$\ln R(T) = -E\left(\frac{1}{kT} - \frac{1}{kT_c}\right) + \ln[\mathrm{R}(\mathrm{T}_c)], \quad (2)$$

where $R(T)$ is the rate of respiration at temperature, $T$ and, $E$ and $k$ are the activation energy and the Boltzmann constant as previously described. Here, we express the natural logarithm (ln) of respiration as a function of standardised temperature ($1/kT$-$1/kT_c$) which centres the inverse temperature data around zero, to make the intercept of the model ($\ln[R(T_c)]$) equal to the rate of respiration at standardised temperature, $T_c$ (here $T_c$ = 15 °C = 288.15K). This greatly reduces the correlation between the slope and the intercept and makes the intercept biologically more meaningful (i.e. gives information on metabolic rates at a standardised temperature, as opposed to infinite temperature-e.g. Yvon-Durocher *et al*., 2010b). In equation 2, which is similar in form to those derived from the metabolic theory of ecology (Enquist *et al*., 2003; Allen *et al*., 2005; Yvon-Durocher *et al*., 2010a,b; López-Urrutia *et al*., 2006), $\ln[R(T_C)]$ is hypothesised to be determined by the total mass-corrected biomass of organisms in the ecosystem (Allen *et al*., 2005).

The temperature dependence of whole-stream respiration measured using *in-situ* methods across the 13 streams was analysed by ordinary least squares regression analysis because the data satisfied the assumption of independence. However, this assumption was not met for the *in-situ* measurements of benthic respiration because multiple measurements were made in each stream, which constitute spatial pseudoreplicates. We therefore carried out a linear mixed effects analysis using the *lmer* function in the lme4 package in R version 2.11.0 (R Development Core Team, 2010) to determine the activation energy ($E$) and the intercept ($\ln[R(T_c)]$) of these data. Linear mixed effects models provide a powerful statistical method to describe relationships between a response variable and covariates, which can have a nested covariance structure and may be unbalanced (Pinheiro & Bates, 2000). To account for the spatial pseudoreplication and autocorrelation in the data intercepts were treated as random variables that may vary among streams.

The activation energy of respiration of the biofilms determined in laboratory incubations was also analysed using the *lmer* function, this time allowing slopes and intercepts to vary among streams. Using these models, it was possible to investigate the relationship between biofilm respiration and standardised temperature, whilst accounting for the grouped structure of our data i.e. respiration-temperature relationships for each subject (biofilm replicate) are nested within the relationship at the stream-level. This method also accounted for our unbalanced design, as 5 of the 96 experimental units were excluded from the analysis (as described above).

We also estimated the $Q_{10}$ of respiration for the biofilms in the laboratory incubations. The $Q_{10}$ was estimated from:

$$log_{10}R(T) = b(T - T_c) + log_{10}R(T_c) \quad (3)$$

$$\mathrm{Q}_{10} = 10^{b\times 10} \quad (4)$$

where $T$ and $T_c$ are the measurement temperature and standardised temperature ($T_c$ = 15 °C), respectively, in degrees Celsius. We first tested whether the $Q_{10}$ declined systematically with measurement temperature for each experimental subject by fitting a *lmer* model to the relationship in Eq (3), allowing the slope and intercept to vary randomly among streams. In our model, we also additionally included cubic and quadratic orthogonal polynomial coefficients of $T-T_c$. If the $Q_{10}$ changed systematically with temperature, we would expect a model including either cubic and/or quadratic terms to be a better fit to the data than a model with only the linear coefficient. We then used the most appropriate mixed effects model (either linear, quadratic or cubic) to test for differences in the $Q_{10}$ between streams.

In all mixed effects analyses, we used a top-down approach, starting with the most complex model, to determine the significance of the fixed and random effects in a two-stage analysis. In stage-one, we determined the correct random effects structure of the data which accounted for the grouped structure of these data, where the most complex random effects structure included random variation in slopes and intercepts by streams and subjects (biofilm replicates). To assess the significance of each of these random effects terms, we compared a series of models from the most complex to the simplest – i.e. random effect on intercepts between streams only – using their AIC scores (Pinheiro & Bates, 2000). Using this method, parameters are deleted sequentially from the most complex model to determine whether their removal significantly decreased the fit to the data. In the second stage of the analysis, we applied the random effects structure determined in stage one to a range of fixed effects – e.g. stream interactions – to test for differences in slopes and intercepts among streams. Models were fit using maximum likelihood and were compared using the likelihood ratio test. Finally, we carried out a multiple comparison test to assess which between-stream differences in the model parameters were significantly different from zero.

We carried out a binary (i.e. presence/absence) community-level analysis of similarity of the biofilm assemblages (i.e. species from the major groups: diatoms, ciliates and meiofauna) as a function of stream temperature. To do this, we calculated the Sørensen similarity index (Sørensen, 1948) as a measure of the between-stream ß-diversity in the biofilm assemblages, where index values can range from 0 (no overlap) to 1 (exactly the same taxa are present in both assemblages). Differences among the four streams were analysed against their respective pairwise temperature differences by means of a linear regression.

## Results

### Whole-stream and in-situ benthic respiration

Rates of whole-stream respiration, and *in-situ* benthic respiration were strongly correlated with stream temperature (Fig. 2). The activation energies ($E$) derived for whole-stream (0.67 eV, 95% confidence interval: 0.17–1.17 eV; Fig. 2a) and benthic respiration (0.66 eV, 95%

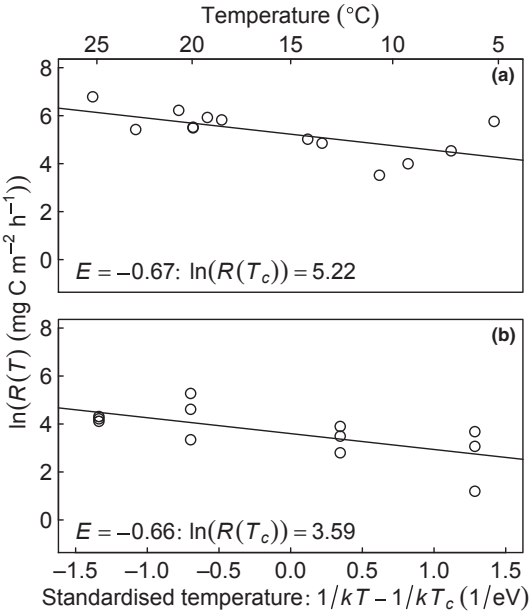


**Fig. 2** Arrhenius plots across streams for (a) whole-stream respiration ($r^2$ values = 0.44) and (b) *in-situ* benthic respiration. Data reveal that across the catchment the temperature dependences of both whole-stream and benthic respiration – given by the slope of the relationship between the natural logarithm of respiration rate as a function of standardised stream temperature ($1/kT$ -1 $/kT_c$) – were indistinguishable from the average activation energy of the respiratory complex (0.6–0.7 eV). Data in (a) are redrawn from Demars *et al.* (2011).

confidence interval: 0.21–1.11 eV; Fig. 2b) were indistinguishable from one another and from the activation energy of the respiratory complex (~0.65 eV).

### *Laboratory incubations*

Biofilm respiration was strongly and positively related with the temperature of incubation (Fig. 3). Measured values of $E$ were statistically indistinguishable across streams (likelihood ratio test for *lmer* models see Table 2). Holding slopes constant across streams yielded a common $E$ of 0.47 (95% confidence intervals: 0.31–0.63 eV), which, although somewhat lower than predicted, included the lower end of the expected range (0.6–0.7eV) within the 95% confidence limits. Conversely, the intercepts (i.e. $\ln[R(T_C)]$) of the Arrhenius relationships showed significant variation among streams (likelihood ratio test for *lmer* models see Table 2). Specifically, differences between streams 1 and 13, 8 and 11, and 11 and 13 were significant in *Tukey post-hoc* tests (all $P < 0.001$, Table 2). A Pearson's correlation analysis of the variation in $\ln[R(T_C)]$ between streams revealed that it was not correlated with mean stream temperature during the study period (Table 1; $r = 0.23$, $n = 4$, $P = 0.761$) but was strongly and positively correlated with mean total biofilm biomass ($r = 0.98$, $n = 4$, $P = 0.012$).

Likelihood ratio tests with *lmer* models containing cubic, quadratic and linear coefficients of standardised temperature for the estimation of $Q_{10}$, revealed that models with cubic ($X_2^2 = 2.76$, $P = 0.25$), and quadratic coefficients ($X_2^2 = 3.41$, $P = 0.18$) were not significantly better fits to the data than the linear coefficient model. Thus, in our experiment $Q_{10}$ did not change systematically with measurement temperature. Applying the linear coefficient model, we found that the $Q_{10}$ did not vary significantly among streams ($\chi_1^2 = 0.65$, $P = 0.88$), and a model with a common $Q_{10}$ among streams converged on an average $Q_{10}$ of 1.9 (95% confidence intervals: 1.7–2.0), in strong agreement with the activation energy analysis.

### *Biofilm characterisation*

A total of 146 species and morpho-types of microscopic biofilm-dwelling taxa were identified from across the four streams (Supporting Information S2). Within-stream richness of diatoms ranged between 25 and 37 taxa, similar to the 26–36 ciliate and meiofaunal taxa (Supporting Information S2). Within the meiofauna, rotifers, aquatic mites and nematodes were the dominant groups in terms of both species richness and abundance. Values of the between-stream ß-diversity in the biofilm assemblages, as indicated by the Sørensen index, ranged between 0.37 and 0.46 (Fig. 4), revealing low similarity in species composition across the four streams. There was also a significant progressive decline in similarity as pairwise temperature differences increased ($r^2 = 0.92$, $n = 6$, $P = 0.003$). Although, the coldest and the warmest stream (streams 8 and 13, Table 1) showed the greatest dissimilarity in biofilm species composition, these two streams were statistically indistinguishable in terms of values of $E$, $Q_{10}$ and $\ln[R(T_C)]$.

## Discussion

The geothermally heated catchment in Iceland allowed us to test the effects of long-term differences in environmental temperature on the respiratory capacity of stream communities, in the absence of otherwise potentially confounding variables (e.g. biogeography, seasonality and physico-chemical conditions). Our results highlighted a surprising consistency in the activation energy, $E$ and the $Q_{10}$ of respiration across communities comprising different species composition, diversity and thermal history, as well as some consistency across spatial scales of measurement (e.g. from biofilms within

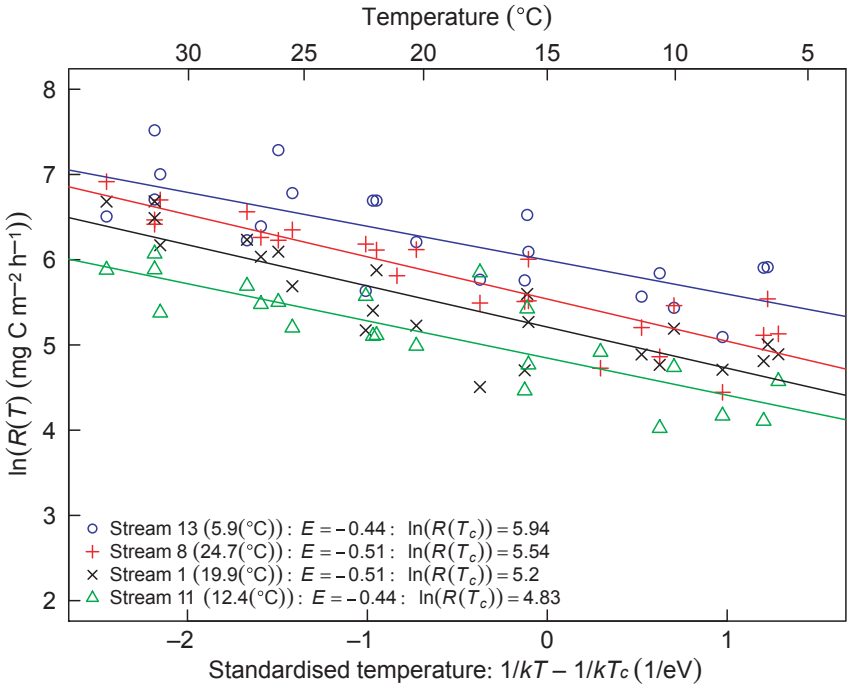


**Fig. 3** Arrhenius plot for laboratory incubations showing the relationship between the natural logarithm of biofilm respiration, as a function of standardised experimental temperature ($1/kT$-$1/kT_c$) for biofilm assemblages originating from streams contrasting in ambient temperature (Table 1). Analysis using linear mixed effects models (Table 2) found no significant differences in slope of the Arrhenius relationship (activation energy, $E$) between streams (likelihood test ratios Table 2). In contrast, the elevation of the Arrhenius relationship i.e. intercepts $\ln R(T_c)$ varied significantly between streams (likelihood test ratios Table 2), with *Tukey post-hoc* tests highlighting significant contrasts between streams 1 and 13, 8 and 11, and 11 and 13 (Table 2). Biofilm respiration rates (mg C $m^{-2}$ $h^{-1}$) at standardised temperature (15 °C) for each stream were as follows; stream 13 = 372, stream 8 = 252, stream 11 = 126 and stream 1 = 179.

microhabitat patches to whole-stream reaches within the catchment). These findings offer important insights into the possible mechanisms that control the temperature dependence of respiration, and have implications for predicting how this ecosystem process (Valentini *et al.*, 2000; del Giorgio & Williams, 2005) might respond to global warming (IPCC, 2007).

Both whole-stream and *in-situ* benthic respiration were positively related to stream temperature, and both displayed remarkably similar values of $E$ (0.67 and 0.66 eV respectively), consistent with the average activation energy of the respiratory complex (0.6–0.7 eV) (Gillooly *et al.*, 2001; Brown *et al.*, 2004; Allen *et al.*, 2005; Allen & Gillooly, 2009). At the community and ecosystem level, respiration is largely determined by the distribution of abundance with body mass, and the rate of metabolism of that biomass (Allen *et al.*, 2005; Yvon-Durocher *et al.*, 2010b). Therefore, the apparent activation energy of respiration across streams should reflect both between-stream differences in the biomass structure, and temperature dependent differences in the metabolism of that biomass. The similar temperature dependence of whole-stream and *in-situ* benthic respiration across streams suggests that the distribution of abundance with body mass was largely independent of stream temperature and, therefore, unlikely to account for the observed temperature dependence. Previous surveys of the Hengill catchment have revealed strong temperature-driven shifts in macrofaunal community composition and food web structure (Friberg *et al.*, 2009; Woodward *et al.*, 2010), whilst maintaining consistent patterns in the slope of the relationship between abundance and body mass (Pichler *et al.*, unpublished data), across the same set of streams studied here. Our results, therefore, provide further evidence to suggest that 'process sensitivities' at the ecosystem-level in freshwater systems might be largely independent of changes in community composition (i.e. species identity), but more strongly related to the biomass and size structure of the community (e.g. Perkins *et al.*, 2010; Reiss *et al.*, 2010).

The laboratory experiments revealed remarkable consistency in the activation energy (0.44–0.51 eV), and $Q_{10}$ (1.6–2.1) of respiration among biofilm assemblages from streams with different natal thermal regimes (between 6 °C and 25 °C) that differed markedly in spe-

**Table 2** Summary results from linear mixed effect models used in analysis of data from laboratory incubations. **a**) Models were fit using restricted maximum likelihood and the random effects structure that best described the data was assessed using the AIC scores (lower scores equals better fit). **b**) The significance of the fixed effects were then determined by comparing models (fit with maximum likelihood, and the random effects determined in stage a) of reduced complexity using the likelihood ratio test to assess the significance of each parameter (Pinheiro & Bates, 2000). **c**) *Tukey post-hoc* multiple comparison test used to assess which between-stream differences in intercepts were significantly different from zero

| Model | No. parms | AIC | logLik | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| ***a)*** *Random effects structure* | | | | | |
| R1. $E$*subject + $\ln R(T_C)$*stream | 11 | 68.67 | | | |
| R2. $E$*stream + $\ln R(T_C)$*stream | 11 | 86.15 | | | |
| R3. $E$*subject + $\ln R(T_C)$*stream*subject | 12 | 72.78 | | | |
| R4. $E$*stream*subject + $\ln R(T_C)$*subject | 12 | 73.43 | | | |
| R5. $E$*stream+ $\ln R(T_C)$*stream*subject | 12 | 79.36 | | | |
| R6. $E$*stream*subject+ $\ln R(T_C)$*stream | 12 | 84.57 | | | |
| R7. $E$*stream*subject+ $\ln R(T_C)$*stream*subject | 13 | 77.50 | | | |
| ***b)*** *Fixed effects structure* | | | | | |
| 1. lnR ~ inv.temp \| random=R1 | 5 | 84.08 | −37.04 | | |
| 2. lnR ~ inv.temp+stream \| random=R1 | 8 | 63.50 | −23.75 | 26.58 | **<0.0005** |
| 3. lnR ~ inv.temp*stream \| random=R1 | 11 | 68.67 | −23.34 | 0.823 | 0.843 |
| ***c)*** *Tukey Post-hoc* | | Est | SE | *Z* | *P* |
| S11 – S1 | | −0.37 | 0.16 | −2.32 | 0.09 |
| S13 – S1 | | 0.74 | 0.16 | 4.66 | **<0.001** |
| S8 – S11 | | 0.34 | 0.16 | 2.18 | 0.13 |
| S13 – S11 | | 1.11 | 0.16 | 6.91 | **<0.001** |
| S8 – S11 | | 0.71 | 0.16 | 4.49 | **<0.001** |
| S8 – S13 | | −0.38 | 0.16 | −2.52 | 0.06 |

cies composition of both autotrophic and heterotrophic groups. This temperature dependence was characterised by a common activation energy of 0.47 eV and a $Q_{10}$ of 1.9, which did not differ significantly from that observed for whole-system and *in-situ* benthic respiration or mean values of $E$ observed for stream biofilms in a study by Acuña *et al.* (2008). Our experiment demonstrates that the thermal history of the assemblages had little or no effect on their respective activation energies or $Q_{10}$ values, in contrast to previous observations in stream biofilms (Acuña *et al.*, 2008) and soil microorganisms (Fierer *et al.*, 2006). Surprisingly, our analyses also revealed that the $Q_{10}$ was a constant function of measurement temperature, in contrast to observations in terrestrial plants (Atkin *et al.*, 2000; Atkin & Tjoelker, 2003) and soils (Davidson & Janssens, 2006; Davidson *et al.*, 2006), and contrary to expectations from the Arrhenius equation. However, this difference is likely to reflect the fact that the effect of temperature on the $Q_{10}$ under Arrhenius kinetics is very subtle (e.g. $Q_{10}$ declines from 2.65 to 2.25 over 5 to 30 °C with an $E$ = 0.65 eV), and may have been masked by measurement error in our analyses.

Studies on plants (Dewar *et al.*, 1999; Atkin *et al.*, 2000; Atkin & Tjoelker, 2003; Gifford, 2003; Zhou *et al.*, 2007; Stæhr & Wernberg, 2009) and in soils (Luo *et al.*, 2001; Kirschbaum, 2004) have revealed that over extended time scales – e.g. weeks to months – the temperature dependence of respiration characterised by the $Q_{10}$ declines with long-term increases in temperature, a phenomenon referred to as respiratory acclimation. The proposed mechanisms underlying this pattern fall into two broad categories, those based on adaptation or acclimatisation (Luo *et al.*, 2001; Enquist *et al.*, 2003, 2007) – i.e. shifts in the underlying physiology

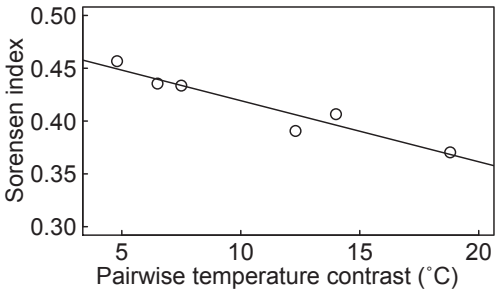


**Fig. 4** Sørensen similarity index for pairwise comparisons of taxonomic biofilm assemblages (diatoms, ciliates and micrometazoans) from benthic biofilm samples from the four streams used in *in-situ* benthic and laboratory incubations, as a function of pairwise temperature contrasts ($r^2$ = 0.92, $n$ = 6, $P$ = 0.003).

and/or altered community structure – and those based on greater substrate limitation of heterotrophs (Kirschbaum, 2004; Knorr *et al.*, 2005; Davidson & Janssens, 2006;) and autotrophs (Dewar *et al.*, 1999; Gifford, 2003), where respiration is ultimately constrained by the weaker temperature dependence of substrate supply by photosynthesis (Allen *et al.*, 2005). Our finding of a consistent activation energy and $Q_{10}$ among stream assemblages contrasting in thermal history does not conflict with substrate driven hypotheses of respiratory acclimation. Crucially, our experimental design was focused on assessing whether the long-term environmental thermal conditions organisms are subjected to (i.e. each stream had its own distinct thermal history) influence the intrinsic physiological response reflected in the instantaneous temperature dependence of respiration. In our experiment, biofilm communities were kept under high light regimes to stimulate photosynthesis, and experimental incubations were kept as short as possible, to rule out the effects of substrate limitation and ensure that potential acclimatory effects did not confound our results. Therefore, our finding that the activation energy and $Q_{10}$ were independent of species composition and thermal history suggest that the core biochemistry, which drives the kinetics of oxidative respiratory metabolism is well conserved among diverse taxa and environments and the intrinsic sensitivity of respiration to temperature is unaffected by environmental temperature.

Rates of biofilm respiration at standardised temperature, $\ln[R(T_c)]$, exhibited distinct variation among streams. This was uncorrelated with mean stream temperature, but was strongly and positively correlated with biofilm biomass. These results, therefore, provide further evidence against physiological thermal adaptation and the 'metabolic cold adaptation' hypothesis (Clarke, 1991; Addo-Bediako *et al.*, 2002) under which we might have expected instantaneous rates of respiration at standardised temperature to be the greatest for biofilms from colder streams. Our results are broadly consistent with the work of Enquist *et al.* (2003, 2007) who also observed significant (3–6 fold) variation in rates of respiration at standardised temperature across forests spanning a broad biogeographical gradient in temperature. Enquist *et al.* (2003) hypothesised that these patterns were driven by individual-level physiological adaptation to local climatic regimes, but our study neither supports nor refutes this because it was conducted at a vastly different scale. However, because we were able to remove the influence of possible confounding biogeographical variables, our study reveals that variation in $\ln[R(T_C)]$ cannot be explained by temperature *per se*. Variation in $\ln[R(T_c)]$ observed by Enquist *et al.* (2003) may well be driven by physiological adaptation to factors that are confounded with temperature, e.g. the extent of seasonality and the length of the growing season. By contrast, they may also be driven by systematic declines in heterotrophic biomass with increasing mean annual temperature in terrestrial ecosystems, as hypothesised by Allen *et al.* (2005); a result that seems to be supported by our data – i.e. $\ln[R(T_c)]$ was strongly correlated with biofilm biomass.

The 'natural experiment' provided by the geothermal field setting enabled us to assess whether the long-term environmental temperature organisms are subjected to influences the acute temperature dependence of respiration at the ecosystem-level. Our findings revealed that the temperature dependence of ecosystem respiration, whether characterised by the activation energy in the Arrhenius equation, or by the $Q_{10}$, is well conserved across diverse taxa and environments. This may be modulated in the longer term by other factors – e.g. substrate limitation – which could have important consequences for the potential feedbacks between the biota and the climate as the planet warms in the coming decades. Nevertheless, our work has revealed a new insight, and a missing piece of the puzzle surrounding the mechanisms which control the temperature dependence of respiration i.e. its underlying physiological temperature sensitivity is unaffected by environmental temperature and thermal history. This suggests a common parameter may be used to model the short-term temperature dependence of respiration from diverse ecosystems.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Linearity of oxygen uptake in stream benthos.
**Appendix S2.** Table of species and morpho-types identified in biofilms across the four streams used for *in-situ* benthic and laboratory incubations.

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**Appendix S1: Linearity of oxygen uptake in stream benthos.**

Incubations were performed in the stream benthos for the two warmest streams (stream 8 and 1, respectively) used in measurements of *in-situ* benthic respiration, to check for the linearity of oxygen ($O_2$) uptake over time. A fixed area of the stream benthos was isolated for an incubation period of eight hours with water samples (25 ml) repeatedly removed every two hours for determination of dissolved $O_2$ concentrations as described in main methods. In stream 8, the warmest of the streams, $O_2$ uptake displayed a significant linear relationship whereas only a marginal significant relationship was observed for stream 1 (Figure S1). Crucially, these results suggest that estimates of benthic respiration measured *in-situ* over 3 hours reflected a linear uptake of $O_2$ over this time period.

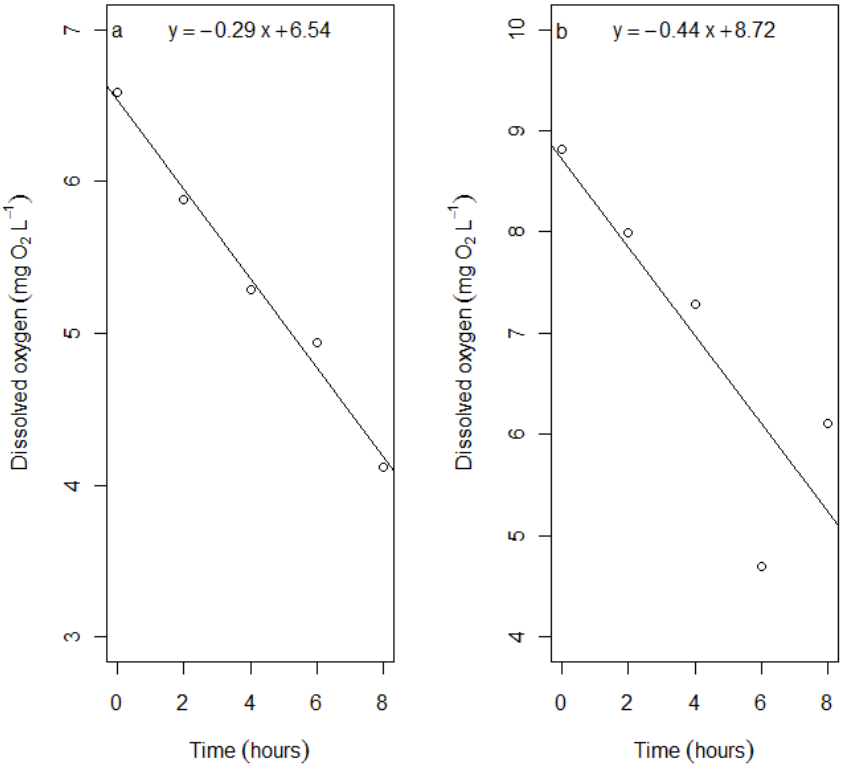


**Figure S1.** Benthic respiration measured over 8 hours for the warmest streams used in *in-situ* benthic incubations. Panel a) stream 8 (24.7ºC), $r^2 = 0.99$, $n = 5$, $P < 0.001$; and b) stream 1 (19.9 ºC), $r^2 = 0.73$, $n = 5$, $P = 0.067$.

**Appendix S2:** Table of species and morpho-types identified in biofilms across the four streams used for *in-situ* benthic and laboratory incubations.

| Group | Taxanomic identity | Stream n.o. present | Group | Taxanomic identity | Stream n.o. present |
|---|---|---|---|---|---|
| Diatom | *Achnanthes exigua* | 1,8 | Diatom | *Melosira varians* | 1,8,11 |
| Diatom | *Achnanthes grana* | 11 | Diatom | *Meridion circulare* | 1,8,11,13 |
| Diatom | *Achnanthes lanceolata* | 1,8,11,13 | Diatom | *Navicula atomus* | 1,11,13 |
| Diatom | *Achnanthes minutissima var. minutissima* | 1,13 | Diatom | *Navicula atomus var. atomus* | 11 |
| Diatom | *Achnanthes stolida* | 13 | Diatom | *Navicula gallica* | 1,8,11,13 |
| Diatom | *Amphora inariensis* | 8,11,13 | Diatom | *Navicula minima* | 1,8,11,13 |
| Diatom | *Amphora ovalis* | 1,11,13 | Diatom | *Navicula obsidialis conf.* | 13 |
| Diatom | *Amphora pediculus* | 1,8,11,13 | Diatom | *Navicula placentula* | 1,8,11,13 |
| Diatom | *Cocconeis pediculus* | 1,8,11,13 | Diatom | *Navicula subatomoides* | 13 |
| Diatom | *Cocconeis placentula* | 1,8,11 | Diatom | *Navicula viridula* | 1,11 |
| Diatom | *Cyclotella spp* | 8 | Diatom | *Nitzschi heufleriana conf.* | 11 |
| Diatom | *Cymbella minuta* | 11,13 | Diatom | *Nitzschia clausii conf.* | 11 |
| Diatom | *Cymbella sinuata* | 1,8 | Diatom | *Nitzschia dissipata* | 1,11,13 |
| Diatom | *Diatoma mesodon* | 8,11,13 | Diatom | *Nitzschia inconspicua* | 8,11 |
| Diatom | *Diploneis ovalis* | 11 | Diatom | *Nitzschia lanceolata* | 1 |
| Diatom | *Epithemia sorex* | 1,11 | Diatom | *Nitzschia obtusa* | 1 |
| Diatom | *Epithemia turgida* | 1,11 | Diatom | *Nitzschia palea* | 1,11,13 |
| Diatom | *Eunotia bilunaris* | 1,11,13 | Diatom | *Nitzschia paleacea* | 1,8,11,13 |
| Diatom | *Fragilaria capucina var. capucina* | 1,8,11,13 | Diatom | *Pinnularia borealis* | 1 |
| Diatom | *Fragilaria capucina var. rumpens* | 1,8,11,13 | Diatom | *Pinnularia intermedia* | 11,13 |
| Diatom | *Fragilaria pinnata* | 1,8,11,13 | Diatom | *Pinnularia simils* | 1 |
| Diatom | *Frustilia vulgaris* | 1,13 | Diatom | *Rhoicosphenia abbreviata* | 1,8,11 |
| Diatom | *Gomphonema angustatum* | 8 | Diatom | *Rhopalodia gibba* | 1,8,11 |
| Diatom | *Gomphonema clavatum* | 1,8 | Diatom | *Synedra ulna* | 1,8,11,13 |
| Diatom | *Gomphonema clevei* | 11 | Diatom | *Tetracyclus glans* | 1 |
| Diatom | *Gomphonema parvulum* | 1,8 | Diatom | *Navicula lucinensis conf.* | 1 |
| Diatom | *Gomphonema type D* | 1,11 | | | |

| Group | Taxanomic identity | Stream n.o. present | Group | Taxanomic identity | Stream n.o. present |
|---|---|---|---|---|---|
| Acari | Acari genus sp. 1 | 1 | Ciliate | Hypotrich genus sp. 3 | 11 |
| Acari | Acari genus sp. 2 | 11 | Ciliate | Hypotrich genus sp. 4 | 11 |
| Acari | Acari genus sp. 3 | 1 | Ciliate | Hypotrich genus sp. 5 | 8 |
| Acari | Acari genus sp. 4 | 1 | Ciliate | Hypotrich genus sp. 6 | 1 |
| Acari | Acari genus sp. 5 | 1 | Ciliate | Hypotrich genus sp. 7 | 1 |
| Acari | Acari genus sp. 6 | 1 | Ciliate | *Lacrymaria olor* | 11 |
| Acari | Acari genus sp. 7 | 1 | Ciliate | *Stentor sp.* | 8 |
| Acari | Acari genus sp. 8 | 1 | Gastrotrich | Gastrotrich genus sp. | 1,11 |
| Acari | Acari genus sp. 9 | 8 | Harpacticoid copepod | Harpacticoid genus sp. | 1,13 |
| Acari | Acari genus sp. 10 | 11 | Monogonont rotifer | *Cephalodella apocoela* | 8,11 |
| Bdelloid rotifer | *Adineta sp.* | 8 | Monogonont rotifer | *Cephalodella* sp. 1 | 11 |
| Bdelloid rotifer | Bdelloid genus sp. 1 | 1,13 | Monogonont rotifer | *Cephalodella* sp. 2 | 1,11 |
| Bdelloid rotifer | Bdelloid genus sp. 2 | 8 | Monogonont rotifer | *Cephalodella* sp. 3 | 1 |
| Bdelloid rotifer | Bdelloid genus sp. 3 | 1,11 | Monogonont rotifer | *Colurella sp.* | 1,8,11,13 |
| Bdelloid rotifer | Bdelloid genus sp. 4 | 11 | Monogonont rotifer | *Dicranophorus* sp. 1 | 1 |
| Bdelloid rotifer | Bdelloid genus sp. 5 | 11 | Monogonont rotifer | *Dicranophorus* sp. 2 | 11 |
| Bdelloid rotifer | Bdelloid genus sp. 6 | 11 | Monogonont rotifer | *Dicranophorus* sp. 3 | 8 |
| Bdelloid rotifer | *Philodina sp.* | 11 | Monogonont rotifer | *Dicranophorus* sp. 4 | 13 |
| Chironomid | Chironomid genus sp. 1 | 13 | Monogonont rotifer | *Encentrum* sp. 1 | 13 |
| Chironomid | Chironomid genus sp. 2 | 8,11,13 | Monogonont rotifer | *Encentrum* sp. 2 | 11 |
| Chironomid | Chironomid genus sp. 3 | 11 | Monogonont rotifer | *Lecane sp.* | 1,8,11,13 |
| Chironomid | Chironomid genus sp. 4 | 11 | Monogonont rotifer | *Monostyla sp.* | 1 |
| Ciliate | *Aspidisca sp.* | 1 | Monogonont rotifer | *Paraencentrum sp.* | 1 |
| Ciliate | *Bursaria sp.* | 8,13 | Monogonont rotifer | *Proales sp. conf.* | 13 |
| Ciliate | Ciliate genus sp. 1 | 8 | Monogonont rotifer | Rotifer genus sp. 1 | 11 |
| Ciliate | Ciliate genus sp. 2 | 8 | Monogonont rotifer | Rotifer genus sp. 2 | 1 |
| Ciliate | Ciliate genus sp. 3 | 8 | Monogonont rotifer | Rotifer genus sp. 3 | 13 |
| Ciliate | Ciliate genus sp. 4 | 8 | Monogonont rotifer | Rotifer genus sp. 4 | 8 |
| Ciliate | Ciliate genus sp. 5 | 8 | Monogonont rotifer | Rotifer genus sp. 5 | 11 |
| Ciliate | Ciliate genus sp. 6 | 8 | Monogonont rotifer | Rotifer genus sp. 6 | 13 |
| Ciliate | Ciliate genus sp. 7 | 11 | Monogonont rotifer | Rotifer genus sp. 7 | 1 |
| Ciliate | Ciliate genus sp. 8 | 11 | Nematodes | Nematode genus sp. 1 | 8,11,13 |
| Ciliate | Ciliate genus sp. 9 | 1 | Nematodes | Nematode genus sp. 2 | 13 |
| Ciliate | Ciliate genus sp. 10 | 8 | Nematodes | Nematode genus sp. 3 | 1,8,13 |
| Ciliate | Ciliate genus sp. 11 | 1,8,11,13 | Nematodes | Nematode genus sp. 4 | 1,8,13 |
| Ciliate | Ciliate genus sp. 12 | 8 | Nematodes | Nematode genus sp. 5 | 13 |
| Ciliate | Ciliate genus sp. 13 | 1,8 | Nematodes | Nematode genus sp. 6 | 11 |
| Ciliate | Ciliate genus sp. 14 | 13 | Nematodes | Nematode genus sp. 7 | 1,11,13 |
| Ciliate | Ciliate genus sp. 15 | 8 | Nematodes | Nematode genus sp. 8 | 11 |
| Ciliate | Ciliate genus sp. 16 | 8 | Nematodes | Nematode genus sp. 9 | 13 |
| Ciliate | Ciliate genus sp. 17 | 11 | Oligochaet | *Aelosoma sp.* | 8 |
| Ciliate | Ciliate genus sp. 18 | 13 | Oligochaet | *Chaetogaster diastrophus* | 1,8 |
| Ciliate | *Dileptus sp.* | 1 | Ostracods | Ostracode genus sp. 1 | 1,8 |
| Ciliate | *Euplotes sp.* | 1,11 | Ostracods | Ostracode genus sp. 2 | 11,13 |
| Ciliate | Hymenostomatida genus sp. 1 | 1 | Turbellaria | *Stenostomum sp.* | 13 |
| Ciliate | Hypotrich genus sp. 1 | 8 | Turbellaria | Turbellar genus sp. 1 | 13 |
| Ciliate | Hypotrich genus sp. 2 | 11 | | | |