

## RESEARCH ARTICLE

# Heat transfer in fish: are short excursions between habitats a thermoregulatory behaviour to exploit resources in an unfavourable thermal environment?

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## ABSTRACT

Temperature is the primary environmental factor affecting physiological processes in ectotherms. Heat-transfer models describe how the fish's internal temperature responds to a fluctuating thermal environment. Specifically, the rate coefficient ( $k$ ), defined as the instantaneous rate of change in body temperature in relation to the difference between ambient and body temperature, summarizes the combined effects of direct thermal conduction through body mass, passive convection (intracellular and intercellular fluids) and forced convective heat transfer (cardiovascular system). The  $k$ -coefficient is widely used in fish ecology to understand how body temperature responds to changes in water temperature. The main objective of this study was to estimate the  $k$ -coefficient of brook charr equipped with internal temperature-sensitive transmitters in controlled laboratory experiments. Fish were first transferred from acclimation tanks (10°C) to tanks at 14, 19 or 23°C (warming experiments) and were then returned to the acclimation tanks (10°C; cooling experiments), thus producing six step changes in ambient temperature. We used non-linear mixed models to estimate the  $k$ -coefficient. Model comparisons indicated that the model incorporating the  $k$ -coefficient as a function of absolute temperature difference ( $\Delta T$ : 4, 9 and 13°C) best described body temperature change. By simulating body temperature in a heterogeneous thermal environment, we provide theoretical predictions of maximum excursion duration between feeding and resting areas. Our simulations suggest that short (i.e. <60 min) excursions could be a common thermoregulatory behaviour adopted by cold freshwater fish species to sustain body temperature below a critical temperature threshold, enabling them to exploit resources in an unfavourable thermal environment.

**KEY WORDS:** Ectotherm, Internal fish tags, Non-linear mixed modelling, Radiotelemetry, *Salvelinus fontinalis*, Temperature, Thermoregulation

## INTRODUCTION

In ectotherms, the primary environmental factor affecting physiological processes is temperature (Crawshaw, 1977; Clarke and Johnston, 1999; Gillooly et al., 2002). Not surprisingly, a thermal niche theory has emerged, and the potential consequences in terms of individual fitness and thermal adaptation have been investigated (Magnuson et al., 1979; Angilletta et al., 2006; Martin

and Huey, 2008). As mobile animals, fish have developed behavioural thermoregulatory tactics to cope with the thermal heterogeneity in the environment (Tanaka et al., 2000; Sims et al., 2006; Goyer et al., 2014). For example, diel vertical migration was hypothesized to be an energy conservation strategy in a benthic marine-fish predator, the dogfish shark (*Scyliorhinus canicula*), which forages in warm water during the night and rests and digests in cool water during the day (Sims et al., 2006).

Heat-transfer models, primarily derived from Newton's law, best describe how internal fish temperature responds to a fluctuating thermal environment (Stevens and Fry, 1974; Fechhelm and Neill, 1982; Azumaya and Ishida, 2005). The rate coefficient  $k$ , expressed as the instantaneous rate of change in body temperature in relation to a difference between ambient and body temperature, represents the combined effects of direct thermal conduction through body mass, passive convection (intracellular and intercellular fluids) and forced convective heat transfer (cardiovascular system) (Stevens and Fry, 1974; Fechhelm and Neill, 1982). Thus, accurate estimates of this coefficient under different environmental conditions are needed to accurately model internal fish temperature in a fluctuating thermal environment. These properties have been extensively investigated in different species of tuna, which have unique vascular counter-current heat exchangers to maintain stable body temperature, and used to explain physiological and behavioural thermoregulatory mechanisms (e.g. Holland et al., 1992; Brill et al., 1994; Malte et al., 2007; Lawson et al., 2010). Short excursions between warm and cold waters (typically of minutes to hours) are also observed in other fish species, but their ecological significance is not yet completely resolved (e.g. Tanaka et al., 2000; Azumaya and Ishida, 2005; Sims et al., 2006). Recently, Nakamura et al. (2015) found that ocean sunfish, *Mola mola*, exhibit vertical migration of hundreds of metres to rewarm at the surface and forage for siphonophores in deeper water, below their thermal preference.

Current telemetry systems enable researchers to simultaneously track a large number of individuals with frequent data recordings (e.g. 5–15 s intervals; Cooke et al., 2005; Brewitt and Danner, 2014). Furthermore, advances in technology continue to reduce the size and mass of temperature-sensitive tags, which allows study of the thermal ecology of a wide range of fish species and sizes (applying the 2% rule of thumb for tag/fish mass ratio; Mellas and Haynes, 1985; Bridger and Booth, 2003; Cooke et al., 2004). The final choice of external or internal tags mainly depends on the goal of the study, the fish species and the habitat type (Bridger and Booth, 2003). For example, the results of Bertolo et al. (2011) versus those of Goyer et al. (2014) in the same lake system suggest that survival is higher for brook charr (*Salvelinus fontinalis*) equipped with internal temperature-sensitive transmitters. While external tags are generally well suited to record ambient water temperature, internal tags are preferable if the risk of entanglement is high (e.g. presence of

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**List of symbols and abbreviations**

$dT$	absolute temperature difference between the transfer tanks (°C)
$k$	rate coefficient for thermal equilibration ( $\text{min}^{-1}$ )
$M$	fish mass (kg)
$M_c$	centred fish mass (kg)
$M_{\text{mean}}$	mean fish mass (=0.419 kg)
$t$	time (min)
$T_0$	initial temperature at $t=0$ min (°C)
$T_a$	ambient temperature (°C)
$T_b$	body temperature (°C)
$T_m$	rate of temperature change due to internal heat production ( $^{\circ}\text{C min}^{-1}$ )
$T_{\text{op}}$	optimal temperature (°C)
$T_{\text{tol}}$	tolerance temperature (°C)

vegetation, wood debris, high flow velocity). However, internal tags may bias the interpretation of habitat temperature because of the delay in equilibrating body cavity temperature relative to ambient water temperature when fish are rapidly moving among different temperatures (e.g. depth in stratified lakes).

The main objective of this study was to estimate the  $k$ -coefficient of adult brook charr equipped with internal temperature-sensitive transmitters based on the heat-transfer models. We used experimental design and a non-linear mixed modelling approach to estimate this coefficient over different thermal conditions. In a second step, we simulated thermoregulatory behaviours of fish and provided theoretical prediction of maximum excursion duration between feeding and resting areas that could be tested in future field studies using acoustic telemetry systems. The insights revealed by this study should lead to a better understanding of the thermal biology of fish species and better predictions of thermoregulatory behaviours in a heterogeneous thermal environment.

**MATERIALS AND METHODS****Fish tagging**

Fifteen brook charr, *Salvelinus fontinalis* (Mitchill 1814) (mass: 0.204–0.625 kg, total length: 261–390 mm), from the Alléghany hatchery (St-Alexis-des-Monts, QC, Canada) were equipped with 3.6 g thermo-sensitive radio transmitters [model ATS-F1540, Advanced Telemetry System (ATS), Isanti, MN, USA]. After fish were anaesthetized (50  $\text{mg l}^{-1}$  clove oil), transmitters were implanted in the peritoneal cavity through a small incision on the ventral side in front of the pelvic fins. To prevent infection, oxytetracycline (50  $\text{mg kg}^{-1}$  of fish mass) was injected before closing with three stitches of non-absorbable synthetic monofilament (Ethilon noir 4/0+FS2; CDMV, St-Hyacinthe, QC, Canada). Fish were then placed in recuperation tanks and held at a constant acclimation temperature (10°C) for 5–7 days before the heat-transfer experiments.

**Heat-transfer experiments**

Fish were subjected to six step changes of ambient water temperature (absolute temperature difference,  $dT$ , of 4, 9 or 13°C). These temperature changes were chosen after considering 3 years of empirical data based on observations of wild brook charr in an oligotrophic stratified lake (Bertolo et al., 2011; Goyer et al., 2014). Fish were first transferred from acclimation tanks (10°C) to tanks held at a constant temperature (14, 19 or 23°C; warming experiments). After 2–3 h, fish were returned to the acclimation tanks (10°C; cooling experiments). All fish performed the six heat-transfer experiments and each warming/cooling transfer was separated by at least 24 h. Body temperature was recorded by a radio receiver (ATS model R4500) connected to a data logger (ATS-Data Collection computer model R4500) and to two loop antennas installed near the tanks. Body temperature of each fish was recorded every 2–5 min, depending on wave interference with other electrical equipment in the laboratory (data are available upon

request). The fish-tagging and heat-transfer experiments were approved by the Animal Care Committee of the University of Québec at Trois-Rivières (Comité de Bons Soins aux Animaux de l'UQTR – CBSA; certificate no. 2010-P.M.26).

**Data analyses**

We used a non-steady-state heat-transfer differential equation (e.g. Holland et al., 1992; Brill et al., 1994; Azumaya and Ishida, 2005; Nakamura et al., 2015) for modelling the body cavity temperature ( $T_b$ ) relative to the ambient water temperature ( $T_a$ ):

$$\frac{dT_b}{dt} = k(T_a - T_b) + T_m, \quad (1)$$

where  $t$  is time (min),  $k$  is the rate coefficient for thermal equilibration ( $\text{min}^{-1}$ ) and  $T_m$  is the rate of temperature change due to internal heat production ( $^{\circ}\text{C min}^{-1}$ ). By assuming that  $T_a$ ,  $k$  and  $T_m$  are constant through time, integrating Eqn 1 gives:

$$T_b(t) = \left( T_0 - T_a - \frac{T_m}{k} \right) \exp(-kt) + T_a + \frac{T_m}{k}, \quad (2)$$

where  $T_0$  (°C) is the initial value of  $T_b$ . The values of  $T_b$ ,  $T_0$  and  $T_a$  are given by the experiments whereas  $T_m$  and  $k$  are parameters to be estimated. The  $k$ -coefficient is of primary interest as it is proportional to the exponential decay of body temperature changes (Fechhelm and Neill, 1982). The  $k$ -coefficient, sometimes defined as the whole-body heat-transfer coefficient (e.g. Holland et al., 1992; Azumaya and Ishida, 2005; Nakamura et al., 2015), is widely used in fish ecology to understand how body temperature responds to water temperature change. Fish mass is an important variable explaining the range of the  $k$ -coefficient (Stevens and Fry, 1974; Fechhelm and Neill, 1982). The relationship between fish mass and the  $k$ -coefficient was empirically described by a power law, by analogy to an allometric relationship (Stevens and Fry, 1974; Fechhelm and Neill, 1982; Weller et al., 1984). The thickness of the body wall, a proxy for the fish thermal conductivity property of a specific body shape, increases with fish mass, which results in a lower  $k$ -coefficient and a longer time to equilibrate to ambient temperature.

We used a non-linear mixed modelling approach to account for the hierarchical structure of the data, with experiments nested within individuals (Pinheiro and Bates, 2000). Random terms for the  $k$ -coefficient and for the  $T_m$ -coefficient were included in all models as well as a power variance function for modelling within-fish heteroscedasticity, as suggested by preliminary analyses. For the null model (model 0), the fixed terms included (i) a linear relationship between fish mass and the  $k$ -coefficient (Stevens and Fry, 1974; Fechhelm and Neill, 1982), and (ii) the  $T_m$ -coefficient specific to each of the six experiments, as suggested by preliminary analyses. Competing models differed in how  $k$  was modelled: model 1 assumes that the  $k$ -coefficient is different for cooling ( $k_{\text{low}}$ ) and warming ( $k_{\text{high}}$ ) experiments (Weller et al., 1984; Hight and Lowe, 2007; Malte et al., 2007); model 2 assumes that the  $k$ -coefficient varies depending on the temperature difference, but the direction of temperature change is not considered (i.e. absolute temperature difference,  $dT$ ); and model 3 assumes that the  $k$ -coefficient varies in all experiments (i.e. full model). Fish mass was centred ( $M_c$ ) before analyses for easier interpretation of the  $k$ -coefficients (i.e. deviance of the  $k$ -coefficients from mean fish mass;  $M_{\text{mean}}=0.419$  kg). Model comparisons were based on Akaike information criteria, AIC (Burnham and Anderson, 2002). All analyses were carried out with the R software (R Development Core Team, 2014; nlme package, v3.1-113). R code is available upon request.

**Theoretical predictions of maximum excursion duration**

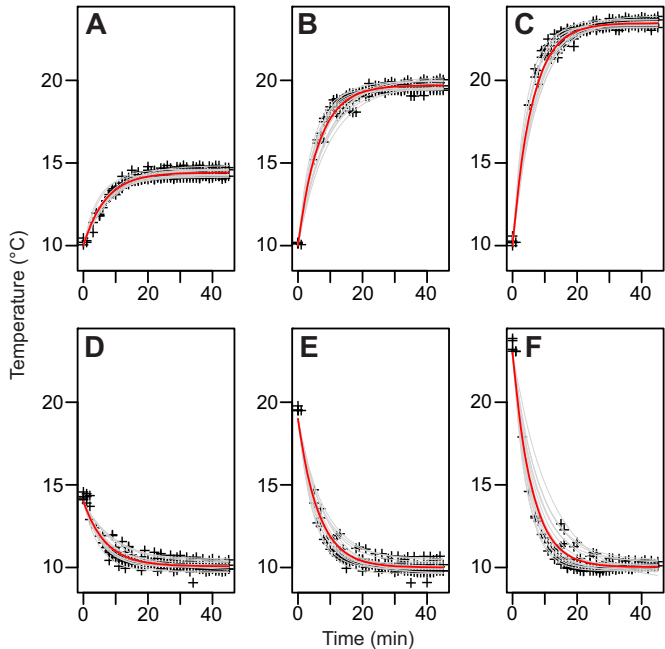
Estimates of the  $k$ -coefficient obtained from our laboratory experiments were used to simulate the body temperature of fish inhabiting a heterogeneous thermal environment. The objective of the simulations was to explore two types of thermoregulatory behaviour: (i) cyclic oscillations between warm and cool water and (ii) sporadic excursions into warm water (e.g. epilimnion) from cool water (e.g. metalimnion). The former behaviour simulated attack rate or feeding bouts in time budget experiments (Marchand et al., 2003) or

when fish swam rapidly to eat at the surface and then returned to deep water. Previous telemetry studies suggested that the latter behaviour, equivalent to our step change experiments, could be adopted by lacustrine brook trout as a response to summer lake warming (Bertolo et al., 2011; Goyer et al., 2014). Although cyclic oscillations are mainly presented as illustrative examples, we provide quantitative theoretical predictions of the maximum duration of sporadic excursions. These simulations were mainly based on thermoregulatory behaviours of brook charr inhabiting stratified lakes (e.g. Goyer et al., 2014), but the results are easily extendable to other heterogeneous thermal environments such as tributary/stream systems.

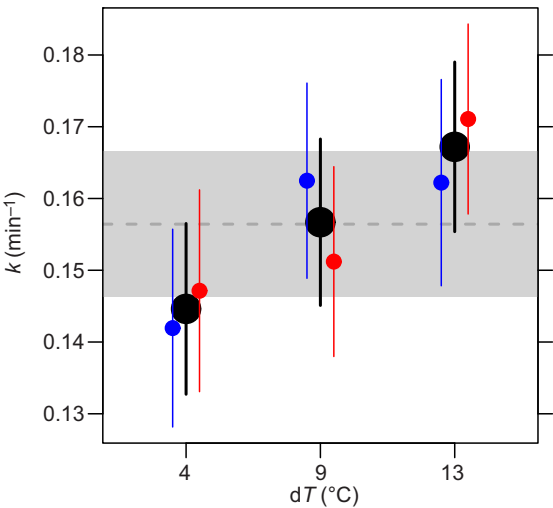
Numerical solutions of cyclic oscillations were obtained by solving the differential Eqn 1 using the deSolve package (v. 1.10-10) of the R software (R Development Core Team, 2014). Maximum excursion duration was obtained from Eqn 2 by calculating the time when body temperature exceeded the critical temperature threshold. For brook trout, the critical temperature threshold (hereafter tolerance temperature,  $T_{tol}$ ) was set to 22.4°C and optimal temperature ( $T_{op}$ ) was set to 12°C (Goyer et al., 2014). Optimal temperature was equivalent to initial temperature ( $T_0$ ) of the step temperature change experiments. Ambient temperature ( $T_a$ ) referred to the temperature of the epilimnion and ranged from 22.5 to 30°C. In all simulations, the  $k$ -coefficient was interpolated by linear regression with absolute temperature difference (model 2; see Results) and  $T_m$  was set to 0°C min<sup>-1</sup> for simplicity. Theoretical predictions could be adjusted to other systems or fish species by modifying the above parameter set. R code is available upon request to reproduce the simulations.

RESULTS

Our statistical approach modelled the exponential decay of heat transfer in a unified framework by incorporating the source of variation at different levels (Fig. 1). Model comparisons (Table 1) indicated that the model incorporating  $k$ -coefficients as a function of absolute temperature difference (model 2) clearly outperformed the null model (model 0) and the model differentiating the cooling and warming experiments (model 1). Assuming the  $k$ -coefficients to be



**Fig. 1. Heat-transfer experiments of 15 radio-tagged brook charr.** Warming experiments consisted of transferring individuals from 10°C (acclimation temperature) to 14°C (A), 19°C (B) or 23°C (C). Cooling experiments consisted of transferring individuals from 14°C (D), 19°C (E) or 23°C (F) to 10°C (acclimation temperature). Crosses are individual data points. The grey and red lines refer to individual and global (mean fish mass: 0.419 kg) predicted values of the best overall model (model 2; Tables 1, 2), respectively.



**Fig. 2. Rate coefficient ( $k$ ) estimates from the best overall model (model 2).** The black symbols represent  $k$  for model 2 (Tables 1 and 2), plotted against the absolute temperature difference ( $dT$ ). Coefficient estimates of the null model (model 0; Tables 1, 2; dashed grey line) and the full model [model 3; Tables 1, 2; blue symbols (cooling experiments) and red symbols (warming experiments); see Fig. 1 for details] are shown for comparison only. Vertical lines through symbols and the grey area represent the 95% confidence intervals.

specific to each of the six experiments (model 3) did not further improve the model fit (Table 1). The best improvement of model 2 over the null model was the linear increase of  $k$ -estimates with increasing absolute temperature differences (Fig. 2, Table 2). The null model (model 0) overestimated the  $k$ -coefficient for the low step temperature change ( $dT=4^\circ\text{C}$ ) and underestimated the  $k$ -coefficient for the high step temperature change ( $dT=13^\circ\text{C}$ ). The large overlap of estimates between the full model (model 3) and the best overall model (model 2; Fig. 2) supported our conclusion based on AIC comparison (no substantial fit improvement of the full model; Table 1). As previously suggested (Stevens and Fry, 1974; Fechhelm and Neill, 1982), the  $k$ -coefficient was negatively related to fish mass (Fig. 3). Our statistical approach directly

**Table 1. AIC-based comparison of four candidate models differing in their fixed component**

Model	Terms in fixed component of model*	$K$	AIC	$\Delta\text{AIC}$
0	$k$	16	-1453	11
1	$k_{low}$ , $k_{high}$	17	-1451	13
2	$k_{dT4}$ , $k_{dT9}$ , $k_{dT13}$	18	-1464	0
3	$k_{10-14}$ , $k_{10-19}$ , $k_{10-23}$ , $k_{14-10}$ , $k_{19-10}$ , $k_{23-10}$	21	-1463	1

The number of model parameters ( $K$ ), Akaike information criterion (AIC) and difference in AIC relative to the best-fitting model ( $\Delta\text{AIC}$ ) are presented. Model 0: the  $k$ -coefficient is constant in all experiments; model 1: the  $k$ -coefficient is different for cooling ( $k_{low}$ ) and warming ( $k_{high}$ ) experiments; model 2: the  $k$ -coefficient varies depending on the absolute temperature difference ( $dT$ ) but does not account for the direction of temperature change; model 3: the  $k$ -coefficient varies in all experiments. All models are derived from Eqn 2 (see Materials and methods).

\*All models include a linear relationship between fish mass and the  $k$ -coefficient. The rate of temperature change due to internal heat production ( $T_m$ ) varies in all experiments. All random terms for the  $k$ -coefficient and  $T_m$ -coefficient are included to take into account the nested design (experiments repeated within individuals). For each model, all parameters were fitted in a single global analysis (see details in Table 2).

Table 2. Parameter estimates for the best overall model (model 2 in Table 1)

Parameter	Estimate	95% confidence interval	
		Lower	Upper
Fixed			
$k_{Mc}$	−0.1650	−0.2329	−0.0972
$k_{dT_4}$	0.1446	0.1327	0.1565
$k_{dT_9}$	0.1567	0.1451	0.1683
$k_{dT_{13}}$	0.1672	0.1554	0.1790
$T_{m,10-14}$	0.0614	0.0454	0.0774
$T_{m,10-19}$	0.1108	0.0948	0.1269
$T_{m,10-23}$	0.0777	0.0616	0.0938
$T_{m,14-10}$	0.0097	−0.0063	0.0258
$T_{m,19-10}$	−0.0003	−0.0164	0.0158
$T_{m,23-10}$	0.0023	−0.0146	0.0191
Random			
$\sigma_{f,k}$	0.0181	0.0115	0.0287
$\sigma_{f,T_m}$	0.0257	0.0172	0.0382
$\sigma_{f,k-T_m}$	0.0901	−0.6264	0.7241
$\sigma_{e,k}$	0.0174	0.0136	0.0222
$\sigma_{e,T_m}$	0.0189	0.01523	0.0236
$\sigma_{e,k-T_m}$	0.5716	0.2971	0.7588
$\sigma_e$	0.0924	0.0696	0.1226
$\delta$	0.2005	0.0950	0.3061

Note: in the fixed component, the  $k$ -coefficient is a function of centred fish mass ( $M_c$ ) and absolute temperature difference ( $dT$ ). The heat-transfer production ( $T_m$ ) was adjusted for each of the six experiments. For the random component, the standard deviation of the  $k$ -coefficient ( $\sigma_{f,k}$ ) and the heat-transfer production ( $\sigma_{f,T_m}$ ) is provided at the fish ( $i=f$ ) and the experiment ( $i=e$ ) levels. The within-fish standard deviation ( $\sigma_e$ ) and the correlation between random effects ( $\sigma_{f,k-T_m}$ ) at each level are also provided. We used a power ( $\delta$ ) variance function for modelling within-fish heteroscedasticity.

incorporated this relationship in a global analysis, which should provide better parameter estimation and generalization (e.g. Table 2). The rate of temperature change due to internal heat production,  $T_m$ , was always lower than 0.13°C and was not significantly different from 0°C for cooling experiments (Table 2; see 95% confidence intervals).

Simulations showed that fish could maintain body temperature under the critical temperature threshold by (i) reducing the periodicity of cyclic oscillations between warm and cool water (Fig. 4) or (ii) reducing the excursion duration with increasing water temperature when sporadically entering into the epilimnion of stratified lakes (Fig. 5).

DISCUSSION

Our study proposed an original experimental design to quantify the  $k$ -coefficients of fish equipped with internal temperature-sensitive transmitters by using a non-linear mixed modelling approach. Previous studies reported an effect of fish mass (Stevens and Fry, 1974; Fechhelm and Neill, 1982) and cooling/warming experiments (Weller et al., 1984; Hight and Lowe, 2007). Surprisingly, our information-theoretic approach indicated that the  $k$ -coefficient was related to fish mass and absolute differences in ambient temperature between the transfer tanks, but not to cooling/warming experiments (Fig. 2, Tables 1, 2). To the best of our knowledge, our study is the first to show an effect of absolute temperature differences on the  $k$ -coefficient. We found no other studies that evaluated the influence of the step temperature change used in their experimental setup on the estimation of the  $k$ -coefficient. The  $k$ -coefficient was traditionally presented as an empirically derived lumped parameter that includes conditions both within and outside the animal (Stevens and Fry, 1974; Fechhelm and Neill, 1982; Brill et al., 1994) and is

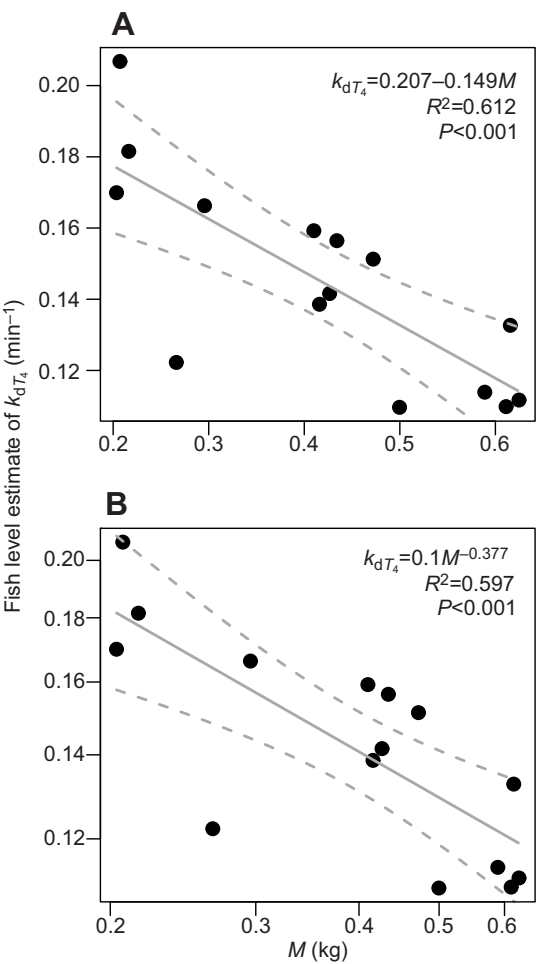
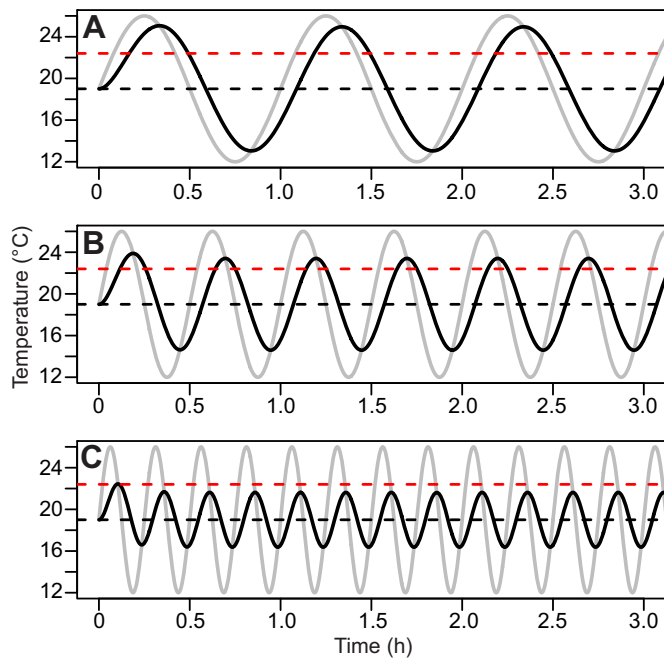


Fig. 3. Linear and power relationship between fish mass and  $k$ -coefficient estimates for experiments with a  $dT$  of 4°C ( $dT_4$ ). (A) Linear relationship. (B) Power relationship (log–log scale). The regression lines (solid lines) and 95% confidence intervals (dashed lines) are shown. Estimates were obtained from the fish-level random residuals of the best overall model (model 2; Tables 1, 2), where fish mass ( $M$ ) is not included in the fixed effects. Relationships are shown for comparison only with previous articles by adopting similar methodology (i.e. individual fitting in a first step and  $M$ – $k$  relationship in a second step). In the present study, we propose including this relationship directly in a global analysis (see details in Tables 1 and 2).

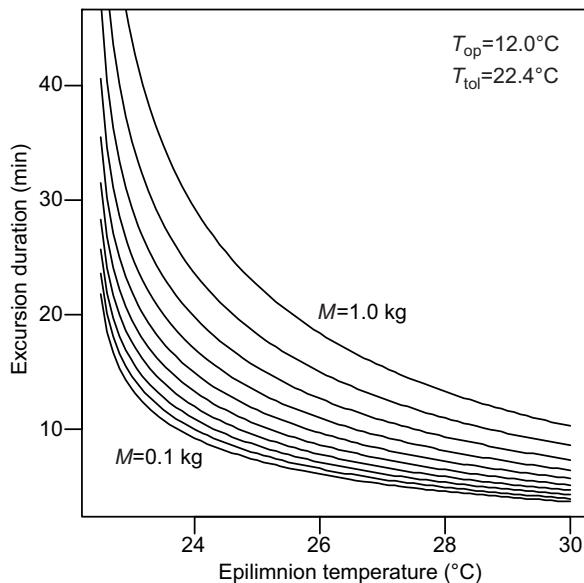
now broadly applied in fish ecology (e.g. Azumaya and Ishida, 2005; Nakamura et al., 2015). Although this model generally fits accurately with recorded body temperature data, the precise factors explaining our results (e.g. behavioural or physiological processes, heat-transfer model) will need to be further investigated.

Because all environmental variables were controlled across all the experiments (e.g. oxygen, water flow, temperature, etc.), thermal conduction through body mass or active convection due to the cardiovascular system (i.e. blood flow) are the two potential mechanisms explaining the observed variation of the fish thermal properties (i.e. estimates of the  $k$ -coefficient). Previous studies comparing thermal properties of live and dead fish reported that the contribution of thermal conduction relative to active convection ranged from 20% to 80% (Stevens and Sutterlin, 1976; Kubb et al., 1980; Weller et al., 1984). This high variation in the relative contribution of conduction and convection could be related to fish morphology (surface/volume ratio) and muscle content. It is suggested that the relative contribution of conduction should decrease as fish mass increases (Kubb et al., 1980). In our



**Fig. 4. Simulations of cyclic oscillations between preferred (12°C) and maximum (26°C) epilimnion temperatures of a hypothetical stratified lake.** Solid lines represent ambient (grey) or body (black) temperature. Dashed lines represent upper tolerance (red) or mean body (black) temperature. The oscillation periods are 60 min (A), 30 min (B) or 15 min (C). Simulation parameters were derived from Table 2 by omitting the  $T_m$ -coefficient ( $M=0.419$  kg,  $k=0.1695$  min<sup>-1</sup>,  $T_m=0^\circ\text{C min}^{-1}$ ).

experiments, we observed ~16% variation in the estimation of the  $k$ -coefficient. Although the thermal conductivity of the flesh generally increases with ambient temperature (Farak et al., 2008; Abbas et al., 2009), our model comparison suggested that active



**Fig. 5. Theoretical graph illustrating the maximum duration of excursions to the epilimnion as a function of temperature constraints.** The excursions are from the optimal temperature in the metalimnion ( $T_{op}$ ) to the temperature of the epilimnion (x-axis). Excursion duration is the period needed to reach the upper tolerance temperature ( $T_{tol}$ ). For simplicity, the  $T_m$ -coefficient was omitted ( $T_m=0^\circ\text{C min}^{-1}$ ) and the  $k$ -coefficient was interpolated from Table 2 by considering the difference between the optimal temperature and the epilimnion temperature. Solid lines refer to predictions for different fish mass ( $M$ ; Table 2).

convection was the most plausible process involved in the variation of the  $k$ -coefficient because the estimates were a function of the absolute temperature difference ( $dT$ ), whatever the direction of heat flow (i.e. cooling or warming; Fig. 2). It is possible that, in response to a thermal environmental stress, the cardiovascular system of the fish increases as the absolute temperature difference increases, resulting in a higher  $k$ -coefficient and a faster temperature equilibration. Potential improvements of our modelling framework include: (i) empirical relationship between  $k$ -coefficient and absolute temperature difference or (ii) changing heat-transfer models to explicitly differentiate thermal conduction from active convection (e.g. Boye et al., 2009). Whatever the mechanisms involved, previous studies could have reported biased estimates of the  $k$ -coefficient due to experimental design (see Fig. 2). Extrapolation outside the experimental temperature range should thus be made with caution.

Modelling body temperature dynamics in varying thermal environments has both applied and fundamental implications (Figs 4, 5). We demonstrated that using internal temperature-sensitive transmitters to infer ambient water temperature can produce biased results if fish behaviour includes periodic oscillations between warm and cool water (Fig. 4). Although mean body temperature was equal irrespective of the periodicity of the oscillations, bias in discrete temperature recorded by telemetry systems increased as the periodicity of the oscillations decreased, ranging from less than 1.0°C for a periodicity of 60 min to more than 3.5°C for a periodicity of 15 min (Fig. 4). Therefore, internal temperature alone cannot unambiguously determine thermal habitat use by the fish at short temporal scales (i.e. <60 min). For example, a body temperature recording of 24°C from a fish inhabiting a stratified lake could result from one of two distinct behaviours: (i) the fish selects this temperature (equilibrium state) or (ii) the fish exhibits a short excursion into warmer water (non-steady state). This limitation could be avoided in at least two ways: (i) combining heat-transfer models with an external transmitter recording temperature over short temporal scales, or (ii) using an internal transmitter that records both pressure and temperature data so that ambient water temperature can be inferred from the lake's thermal profile (not a valid option in river environments; e.g. Brewitt and Danner, 2014).

From a theoretical point of view, fish could also take advantage of heat-transfer properties. For example, reducing the periodicity of cyclic oscillations is a way for fish to avoid excessive body temperatures that are detrimental to physiological processes while exploiting food resources that are present in an unfavourable thermal environment (see Fig. 4). In oligotrophic lakes of the Canadian Shield, littoral habitats provide rich food resources for brook charr (Lacasse and Magnan, 1992; Bourke et al., 1999), but elevated summer temperatures could act as a thermal barrier that prevents brook charr from foraging in this area (Goyer et al., 2014). Brook charr followed with radiotelemetry spent most of their time in the metalimnion, which corresponds to their preferred temperature (i.e. depth <8 m; Bertolo et al., 2011; Goyer et al., 2014), and rarely entered the hypolimnion. Considering a cruising speed of two body lengths per second (Bjornn and Reiser, 1991; Rouleau et al., 2010; Castro-Santos et al., 2013), a brook charr of 300 mm total length could reach the surface within 15 s. Extrapolating our experimental results to natural populations, we can predict excursion times into the epilimnion according to the thermal constraints of the species under study (see Fig. 5). From this, we can make two predictions. (i) Individuals should select heterogeneous thermal environments when the foraging habitat temperature exceeds the temperature tolerance of the species so that swimming costs between foraging and thermal refuge habitats are minimized. These habitats include

tributary/mainstream junctions and abrupt changes in depth profile in stratified lakes. (ii) Individuals should adjust their excursion times to avoid non-optimal physiological temperatures (Fig. 5). New tag technologies provide an exciting avenue to test these predictions in natural fish populations, especially small fish species in freshwater systems (Roy et al., 2013; Brewitt and Danner, 2014).

Thermal refuges were described as critical habitats in a warming environment to explain summer movements and the spatial distribution of salmonids (Snucins and Gunn, 1995; Curry et al., 1997; Breau et al., 2011) or their diel movement patterns (Brewitt and Danner, 2014). Most studies on fish behavioural thermoregulation concern diel vertical or horizontal migration between warm and cool habitats, and these were interpreted as behaviours to maximize growth efficiency (Neverman and Wurtsbaugh, 1994; Armstrong et al., 2013) or as energy-saving strategies (Sims et al., 2006). We suggest that short excursions (i.e. <60 min) could also be a strategy adopted by fish species to exploit food resources during long periods of unfavourable thermal conditions in the feeding environment. Data acquisition of foraging times is an old and difficult issue in field behavioural studies on wild populations because of the problem of correctly classifying individual activity and obtaining movement data at high spatio-temporal resolution (Hixon, 1982). Such thermoregulatory behaviours have already been described in large marine organisms that exhibit vertical migration of hundreds of metres in typically minutes to hours (Holland et al., 1992; Tanaka et al., 2000; Azumaya and Ishida, 2005; Nakamura et al., 2015). Our simulations based on heat-transfer models showed that scaling these thermoregulatory behaviours to small freshwater fish species inhabiting stratified lakes could provide similar benefits. By quantifying the  $k$ -coefficient for brook charr and providing examples of plausible thermoregulatory behaviours in stratified lakes, we feel that our modelling framework and theoretical predictions are easily extendable to most freshwater fish species inhabiting heterogeneous thermal environments. These theoretical predictions could be easily tested in future field studies using acoustic telemetry systems.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

M.P., K.G. and P.M. conceived the study. K.G. tagged the fish and collected the data. M.P. analysed the data. M.P., K.G. and P.M. wrote the manuscript.

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