Imperial College London

Effect of thermal acclimation on the respiration and feeding rate of freshwater snails

Eléa Giraud

August 2017

A thesis submitted for the partial fulfillment of the requirements for the degree of Master of Science at Imperial College London

Formatted in the journal style of Global Change Biology.

Submitted for the MSc in Ecological Applications

Project declaration

All the experimental design, feeding rate experiments, statistical analyses, writing up and production of images was done by the author. Guidance, advices and support was provided by Dr Eoin O'Gorman, who also helped collecting snails from the streams and provided data on stream temperatures.
Data on the respiration rate of *Radix balthica* were produced and given by Dr Rebecca Kordas, who also guided me through the respiration protocol and gave me some advises.
None of the statistical methods used were developed by either the author or the supervisor.

Eléa Giraud, 31st of August 2017

Supervisor: Dr Eoin J. O'Gorman

Abstract

Climate change is expected to have strong impacts on freshwater ecosystems, with warming likely to alter physiological rates and thus consumer-resource relationships. While many studies have assessed the thermal thresholds of species and their life history, still little is known about the connection between thermal ranges, physiological rates and consumer-resource interactions. The purpose of this study was to investigate the effect of thermal acclimation on the temperature dependence of respiration and feeding rate of freshwater snails. The influence of laboratory-grown or field-grown biofilm was also investigated. Freshwater snails from three different thermal acclimation streams but with similar chemical properties, were brought back into the lab in order to test the effect of temperature increase on the respiration, feeding rate and energetic efficiency of the wandering snail *Radix balthica*. Analysis of Covariance highlighted an increase in respiration and feeding rates with increasing experimental temperature, as well as a dampening effect of thermal acclimation on the temperature dependence of these two biological rates. Energetic efficiency was found to decrease with increasing experimental temperature, however, thermal acclimation had no effect on the temperature dependence of energetic efficiency. The overall reduction in energetic efficiency with warming could be explained by a greater increase in metabolic demand with warming relative to feeding rate, potentially resulting in predator starvation. The effect of thermal acclimation on the thermal response of biological rates deserves further attention and research, in order to better understand the dependence between physiological rates and environmental temperature.

Keywords: Respiration, feeding rate, energetic efficiency, thermal acclimation, *Radix balthica*, temperature, freshwater, diatoms.

Introduction

Climatic fluctuations have been frequent during the earth's history, but current warming rates are the fastest on record (IPCC 2013). Global land and ocean surface temperature increased by 0.80°C between 1880 and 2012, representing the warmest period over the last millennia, and will increase by at least 1.5°C over the next century (Hansen et al. 2006; IPCC 2013). Polar regions are expected to undergo even greater rates of warming than the global average, with a predicted increase in air temperature of more than 2.4°C by 2100 (Sala et al. 2000). The arctic is thus expected to experience the largest ecological impacts, with even small temperature changes leading to very large alterations to the constituent biota (Broecker 2017).

The major ecological impacts of global warming that have been documented include biodiversity loss and altered ecosystem services (Chapin et al. 2000). For example, diurnal temperature ranges have shortened, reducing the freezing period and amount of precipitation, with a resultant 10% loss in snow cover since the middle of the 20th century (Walther et al. 2002). These alterations in physical characteristics and hydrological properties in turn impact freshwater systems that highly depend on melting glacial ice, precipitation, water supply and temperature (Barnett et al. 2005). Indeed, a change in the flow regime of a river or stream changes the sediment and habitat characteristics, influencing a species' ability to disperse, and the resource availability of the stream (Hart & Finelli 1999). On the other hand, thermal characteristics of a river or stream play a key role in freshwater ecosystems, impacting primary productivity and nutrient availability and cycling (Caissie 2006; Hannesdóttir et al. 2013).

Freshwater ecosystems represent a very small fragment of the biosphere, covering as little as 0.01% of the globe, yet they account for about 6% of identified species (Dudgeon et al. 2006). They also provide many important ecosystem services to humans, such as food, drinking water, and recreation (Postel & Carpenter 1997). Freshwater ecosystems found at higher latitude can be considered as 'sentinel systems' because they are early indicators of predicted climatic impacts, due to the rapid rate of warming there (Woodward, Dybkjaer, et al. 2010). Freshwater ecosystem are highly vulnerable to environmental perturbation for three main reasons; (1) freshwater species have a very limited capability to spread or change their expansion range; (2) the physical properties of water, such as temperature and volume, are climate sensitive; and (3) they are experiencing strong anthropogenic pressure (Woodward, Perkins, et al. 2010).

Scientific studies on freshwater systems highlight that climate change and warming impacts have already been observed over all levels of biological organisation, from changes in individual-level rates (such as growth and metabolism), to altered structure of communities and ecosystem functioning (Walther 2010; Parmesan & Yohe 2003). At higher levels of biological organisation, warmer

temperature can shift communities toward smaller species, exclude species close to their thermal maxima, but can also support longer food chains (O'Gorman et al. 2012). At the individual level, warming can affect physiology, body size and velocity, with the rate of change in the individual response being dependant on the species and its foraging strategy (i.e. herbivorous, carnivorous), leading to changes in consumer resource interactions (Dell et al. 2014). This is because the metabolic rate of an organism determines its resource usage and energy allocation and is intrinsically linked to the body size of the animal and its environmental temperature (Brown et al. 2004). The Metabolic Theory of Ecology states that most biological reaction rates increase exponentially as temperature increases following the Van't Hoff-Arrhenius relationship $e^{-E/kT}$, where k is the Boltzmann constant, E is the activation energy and T is the temperature in Kelvin (Brown et al. 2004).

Following the principle from the Metabolic Theory of Ecology, at warmer temperatures organisms are expected to show higher energetic demand a result of the metabolic rate increase (Gillooly et al. 2001). To meet this increase in energetic demand, consumer should require more resources. Previous research found that warmer temperature increased attack rates of predators onto preys and lowered the handling time, resulting in higher feeding rates (Rall et al. 2010; Rall et al. 2012). The relative temperature dependence of feeding rate and metabolism are then very important in determining the energetic efficiency and population persistence of an organism (Van M Savage et al. 2004). If respiration rate increases more rapidly than feeding rate, as the environmental temperature increases, then consumer should be less efficient in energy intake and may risk starvation it is not able to meet its metabolic demands.

Temperature being one of the factor shaping biological activity, it could be argue that organism evolving in the environment adapt, or thermally acclimate to environment to perform better. Thermal acclimatization is thought to be a physiological process that influences the thermal sensitivity of an organism, by physiologically compensating temperature fluctuations (Grigaltchik et al. 2012). Following this principle, organisms spending time in warmer environment should be able to lower their metabolic cost under increasing temperature. This may have a knock-on effect on the metabolic and feeding rates, as organism would need less energy to survive in the warmer environment than would be predicted if thermal acclimation was not taken into account. Thus, it is important to investigate the effect of thermal acclimation on the temperature dependence of metabolism and feeding.

Here, the effects of thermal acclimation on the temperature dependence of feeding and respiration rates were studied using snails collected from geothermally heated stream in Iceland. By examining the ratio of metabolism to feeding, it was also possible to investigate the energetic efficiency of the snails. The following hypotheses will thus be investigated:

(i) There will be an increase in the respiration rate of snails with increasing experimental temperature.

(ii) There will be an increase in the feeding rate of snails on diatoms with increasing experimental temperature.

(iii) There will be a reduction in the energetic efficiency of snails with increasing experimental temperature.

(iv) Increasing acclimation to warmer temperature will dampen the temperature effect on respiration, feeding rate, and energetic efficiency.

Methods

Study site

All organism collections were carried on in the Hengill geothermal valley (64°.03N – 021°.18W) in southwest Iceland. The Hengill valley is located in a so called "high temperature area" as a result of volcanic activity and thermal heat transfers (Arnason et al. 1969). Differential heating of the bedrock in this area results in a series of spring-fed streams that span a temperature gradient of 5-25 °C (Friberg et al. 2009). These streams have been intensively studied over the past decade, with temperature effects described from the individual to the ecosystem level (Gudmundsdottir et al. 2011; Hannesdóttir et al. 2013; Woodward, Dybkjaer, et al. 2010). Three streams were chosen for use in this study because they allowed the target organism for all experimental work to be collected from a cold (IS9 = 10 °C), tepid (IS5 = 14 °C), and warm (IS8 = 18 °C) environment.

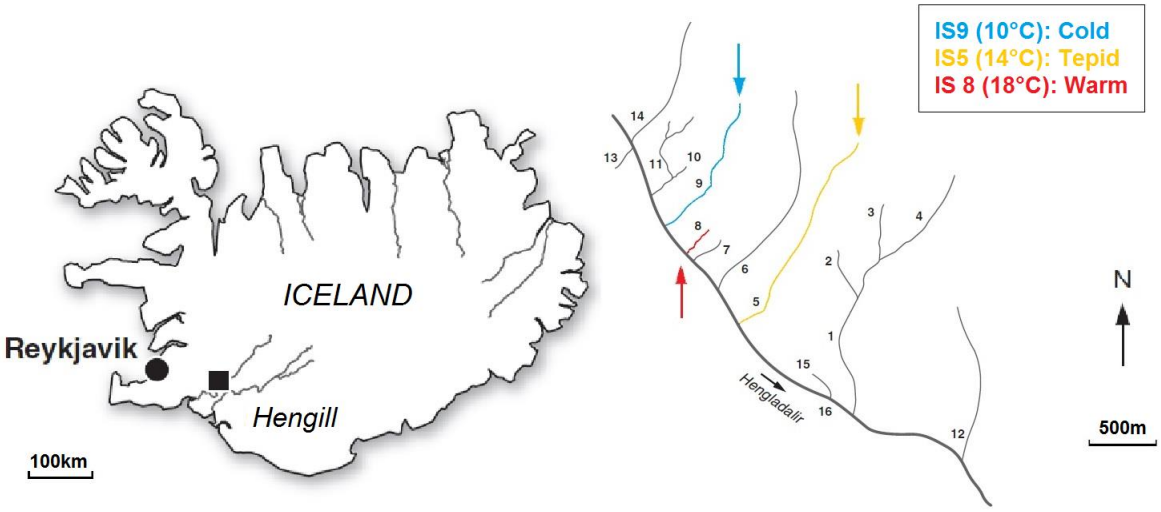

Figure 1: Hengill Geothermal Valley, Iceland (adapted from Woodward, Dybkjaer, et al. (2010)).

Study organism

The freshwater snail *Radix balthica* (Linnaeus 1758) was chosen for this experiment because it is one of the dominant macroinvertebrates in the Hengill geothermal system and throughout European freshwaters (Johansson 2015). This species is the main grazer in the Hengill stream system, found over a wide range of stream temperature and in higher abundances in warmer channels (O'Gorman et al.

2012; Hannesdóttir et al. 2013). These snails usually scrape biofilm off the surface of rocks, but they can also feed on detritus or bacteria (Brönmark 1989). When feeding on aquatic plants, *R. balthica* prefers to graze on leaves which are covered by periphyton (Li et al. 2009).

Grazers are an important conduit of energy flow from autotrophs to heterotrophs (Schaum et al. 2017). Gastropods and particularly snails, play an important role in the productivity of freshwater ecosystems, by controlling the population of primary producers and periphyton (Brönmark 1989; Zębek & Szymańska 2014) and forming an important link to higher trophic level consumers (O'Gorman et al. 2012). They also play a role in the community through mutualistic interaction with macrophytes (Jones et al. 1999). For example, the freshwater snail, *R. balthica*, has been found to stimulate growth of macrophytes by feeding on the periphyton covering leaves, and suppressing the negative effect it has on the plant (Li et al. 2009). Given the strength of grazing exhibited by freshwater snails (Brönmark 1989), it is important to understand how their respiration and feeding rate may respond to warming, with the potential for cascading effects throughout the food web (Brönmark 1994).

In this experiment, snails of a variety of sizes were randomly collected from streams IS9 (10°C), IS5 (14°C), and IS8 (18°C) in the Hengill system, representing acclimation to a cold, tepid, and warm stream, respectively. Snails were brought back to the lab, stored in small aquaria that were aerated with air pumps and maintained in climate chambers at temperatures that closely matched the temperature of the stream they were collected from.

Respiration measurements

In order to quantify the metabolic activity of the snails, the respiration rate of *R. balthica* collected from stream IS9, IS5 and IS8 was measured at 5, 10, 15, 20, 25, and 30 °C. The snail respiration measurement followed the protocol from Brodersen et al. (2008). Snails were acclimatised to the experimental temperature for 15 minutes prior to the start of measurements to avoid a shock response. Snails were individually placed into a glass chamber filled with stream water, a magnet and a protective mesh in order to homogenize the oxygen within the chamber. The water had previously been filtered and oxygenated until the water reached 100% oxygen saturation. In each trial, one individual snail was placed in seven of the chambers and the eighth chamber was used as an organism-free control to account for sensor drift. Oxygen depletion was then measured with the help of a micro electrode fitted through a capillary in the gas-tight stopper of each chamber. Three periods of measurements were recorded for each chamber (10-15 seconds each, where oxygen concentration was measured every second) until the oxygen concentration declined to 70-80% saturation. Metabolic rate was measured for 5-10 individuals of each species at each experimental temperature, with a new

individual used in every experiment. Measurements were repeated three times for each individual chamber. The length of the snails was also measured and converted into dry weight following a length-weight relationship from Hannesdóttir et al. (2013a)

I performed respiration measurements on snails at 2 experimental temperatures to learn all the methodologies for this work. However, due to the length of time that would have been needed to measure all the respiration rates required for this study, I acquired data from Dr Rebecca Kordas at Imperial College London, who performed all the respiration experiments from May to July 2015 and June to July 2016.

The following equation from the Metabolic Theory of Ecology (Gillooly et al. 2001; Van M Savage et al. 2004) was used to estimate the temperature dependence of respiration rate, *I*:

$$I = I_0 e^{E_I \frac{T_I - T_0}{k T_I T_0}}, \quad (1)$$

where I_0 is a constant, E_I is the activation energy of respiration rate, T_I is the experimental temperature, and T_o is a sets the intercept of the temperature relationship at 283.15 K (10 °C), and *k* is the Boltzmann constant (8.62×10^{-5} eV K^{-1}).

Biofilm procedure

Diatoms are found in most water bodies, accounting for a quarter of the biosphere's primary production, with more than 200 000 species recorded (Snoeijs et al. 2002; Mann & Droop 1996). They are single celled, identifiable by their silicon dioxide (SiO_2), and produce high energy lipids that make them a good resource for grazers (Smol & Stoermer 2010). They play an important part in the ecosystem by allowing the transformation of energy used up at higher trophic levels (Komulaynen 2009; Adams et al. 2013), while they also play an important role in the carbon biochemical cycle, allowing its transfer through the foodweb (Ragueneau et al. 2006).

Diatoms are the main dominant group at colder temperatures or in arctic and sub-arctic regions, whereas cyanobacteria or green algae are dominant in warmer environment (Gudmundsdottir et al. 2011; Komulaynen 2009). The three genera *Cyclotella*, *Nitzschia*, and *Navicula* were chosen because they are commonly found in Hengill streams (Gudmundsdottir et al. 2012; Gudmundsdottir et al. 2013).

Biofilms for use in the experiments were grown on 500 glass coverslips (15 mm diameter) and 75 microscope slides (76 × 26 mm), which were placed at the bottom of three large aquaria. Each aquarium was then filled with 8mL of stock medium solution (see Table S1), 2L of distilled water and 250mL of solution containing diatoms from the genera *Cyclotella*, *Nitzschia*, and *Navicula* (ordered

from Sciento Algae, UK). The aquaria and coverslips were maintained at 20.8 ± 0.79 °C (mean ± SD) near a north-facing window in a laboratory at the University of Iceland. Coverslips with attached biofilms were removed from the aquaria after 25, 30, 37, and 44 days and immediately used in laboratory experiments performed on each day (see below). Microscope slides (76 × 26 mm) were also placed in a large plastic container in IS13 (5 °C) in Hengill for 44 days, to allow biofilm to naturally colonise them. IS13 was chosen because it does not contain any snails and previous experiments in the system have shown that it has the fastest accumulation of algal biofilms (O'Gorman unpublished).

Feeding experiments

A total of four feeding experiments were carried out. The first two trials were used to fine-tune the methodology and determine the optimum experimental duration for snails to graze the biofilm on the coverslips. The third experiment was used to collect data to test the four main hypotheses. The fourth experiment was used to determine whether snail grazing differed between the cultured diatom biofilms and natural biofilms grown in a stream at Hengill.

Snails were starved for 48 hours prior to each feeding trial to standardise hunger levels while preventing metabolic down regulation due to extended starvation (Vucic-Pestic et al. 2011). For each acclimation temperature, 35 snails of 5 different size classes were randomly selected from the holding tanks: small, medium-small, medium, medium–large, and large. One individual from each size class was then randomly allocated to each of seven different experimental temperatures (see Table 1).

For each experimental temperature, the 5 snails of each stream acclimation were placed onto the lid of a labelled petri dish and a picture was taken for the snail to be measured with ImageJ software (Rasband 2011). One coverslip containing cultured biofilm (or microscope slides with cultured or natural biofilm in the fourth experiment) was added to each petri dish and then filled with 25mL of stream water from the main river in Hengill, warmed to the experimental temperature where the petri dish would go. Once all pictures were taken, the snails were moved from the lid into the water and the petri dishes were closed to prevent the snails from escaping. Petri dishes were placed into the allocated chambers with LED lighting (note that the fourth experiment lasted 24 hours and was exposed to a 20:4 hour light:dark cycle to match outdoor light conditions in Iceland for that time of year). Experimental temperature chambers were set up in a different order for each of the four feeding trials (Table 1). Petri dish were left in the chamber at the experimental temperature for several hours to allow snails to graze the biofilm. Three petri dishes containing 1 coverslip and 25ml of water but no snail were also added to each chamber to act as controls in the absence of grazing.

Once the feeding trial was done, petri dishes were removed in the same order as they were placed into chambers, in order to make sure they were all exposed to experimental conditions for the same amount of time. Coverslips were removed from each petri dish and placed into a falcon tube with the corresponding label, and containing 5ml of ethanol for chlorophyll extraction.

Table 1: Experimental design of feeding trials; Biofilm growth indicating the amount of time culture of diatoms were let to grow; Feeding trial duration indicating the duration snails were let to graze on biofilm (including the two pilot feeding trials 1 and 2); Type of material being the material on which cultures were grown; Chamber set up order indicating the order at which feeding trials were set up in the chamber.

	Trial 1	**Trial 2**	**Trial 3**	**Trial4**
Biofilm growth	25 days	30 days	37 days	44 days
Feeding trial duration	4 hours	2 hours	3 hours	24 hours
Type of material	Lab grown coverslip	Lab grown coverslip	Lab grown coverslip	Field and Lab grown microscope slides
Chamber set up order	5, 10, 14, 18, 22, 26 and 30°C.	5, 14, 22, 30, 10, 18 and 26°C	10, 18, 26, 5, 14, 22 and 30°C	22, 14, 5, 18 and 10°C

Photospectometry

All falcon tubes containing coverslips for chlorophyll extraction were immediately placed in a dark 5°C fridge for chlorophyll extraction over an 18 hour period (following Steinman et al. 1996). After 18 hours, the ethanol solution was poured into 4-mL photospectrometry cuvettes and absorbance was measured with the photospectrometer at 665nm for chlorophyll pigments and 750 nm wavelength for the background absorption (Tett et al. 1975; Anon n.d.), with the photospectrometer GENESYS. Biomass of chlorophyll was calculated as follows:

Chlorophyll $(mg.m^{-2}) = ((A_{665} - A_{750}) \times E \times 10^{4})/ (83.4 \times A),$ (2)

where A_{665} is the absorbance at 665nm, A_{750} the absorbance at 750nm, E is the volume of ethanol used for extraction, A the coverslip or microscope slide area and the constant 83.4 corresponds to the absorption coefficient for chlorophyll in ethanol ($l.g^{-1}.cm^{-1}$).

Estimation of feeding rate

The feeding rate, *F*, was estimated using the chlorophyll results from the equation above, with the following equation:

$$F = (Chl_C - Chl_E) / D_F, \quad (3)$$

where Chl_C is the chlorophyll measured on the controls at the end of the experiment, Chl_E is the chlorophyll measured on each coverslip or microscope slide exposed to snail grazing at the end of the experiment, and D_F is the duration of the feeding trial in hours.

The temperature dependence of feeding rate was estimated in a similar fashion to respiration rate:

$$F = F_0 e^{E_F \frac{T_C - T_0}{kT_C T_0}}, \quad (4)$$

where F_0 is a constant, E_F is the activation energy of feeding rate, T_C is the experimental temperature of the climate chambers, and all other parameters are the same as Equation 1.

Energetic Efficiency

The energetic efficiency, γ, of the snails was estimated using the following equation (Vucic-Pestic et al. 2011):

$$\gamma = \frac{\omega F}{\lambda I}, \quad (5)$$

where ω is the assimilation efficiency, which is independent of temperature and specific to feeding strategies of organisms (i.e. grazers) and λ is a constant for converting basal metabolic rate to field metabolic rate. Here, ω = 0.45 for herbivores (Yodzis and Innes 1992) and λ = 3 (Savage & Allen 2004; Sciences et al. 2010). Since respiration rates were not measured at exactly the same experimental temperature as the climate chambers, *I* in Equation 5 was estimated by inserting the temperature of the climate chambers during the third feeding experiment into Equation 1 for each acclimation temperature of the snails.

The temperature dependence of energetic efficiency was estimated in a similar fashion to respiration rate:

$$\gamma = \gamma_0 e^{E_\gamma \frac{T_C - T_0}{kT_C T_0}}, \quad (6)$$

where γ_0 is a constant, E_γ is the activation energy of energetic efficiency, and all other parameters are the same as Equations 1 and 4.

Statistical analysis

All statistical analyses were carried out in R version 3.3.1. The effect of thermal acclimation on the temperature dependencies of the response variables respiration rate, feeding rate, and energetic efficiency were explored with an analysis of covariance (ANCOVA). Here, experimental temperature was incorporated into the model as a continuous explanatory variable, while acclimation temperature was included as a categorical variable with three levels: cold, tepid, and warm. The analysis of all three response variables followed the same format:

$$\ln RV = RV_0 + E_{RV}\frac{T_C - T_0}{kT_CT_0} + T_{acc} + E_{RV}\frac{T_C - T_0}{kT_CT_0} : T_{acc}\,, \tag{7}$$

where RV is the response variable (either respiration rate, feeding rate, or energetic efficiency), RV_0 is the intercept of the relationship, E_{RV} is the activation energy, T_{acc} is the acclimation temperature. The interaction term describes the effect of thermal acclimation on the temperature dependence of the response variable.

Results

Respiration rate

There was a significant increase in the respiration rate of *R. balthica* with increasing experimental temperature (ANCOVA: $F_{1,178}$=356.29, p < 0.0001). There was a significant effect of acclimation on respiration rate (ANCOVA: $F_{2,178}$=4.89, p = 0.00855), and acclimation was found to significantly alter the temperature effect on respiration rate (ANCOVA: $F_{2,178}$=8.438, p = 0.0003). The temperature dependence of respiration rate was lowest in the cold acclimated snails (activation energy = 0.68 ± 0.08 eV), followed by the warm acclimated snails (0.97 ± 0.12 eV), while it was highest for the tepid acclimated snails (1.14 ± 0.12 eV; see Figure 2).

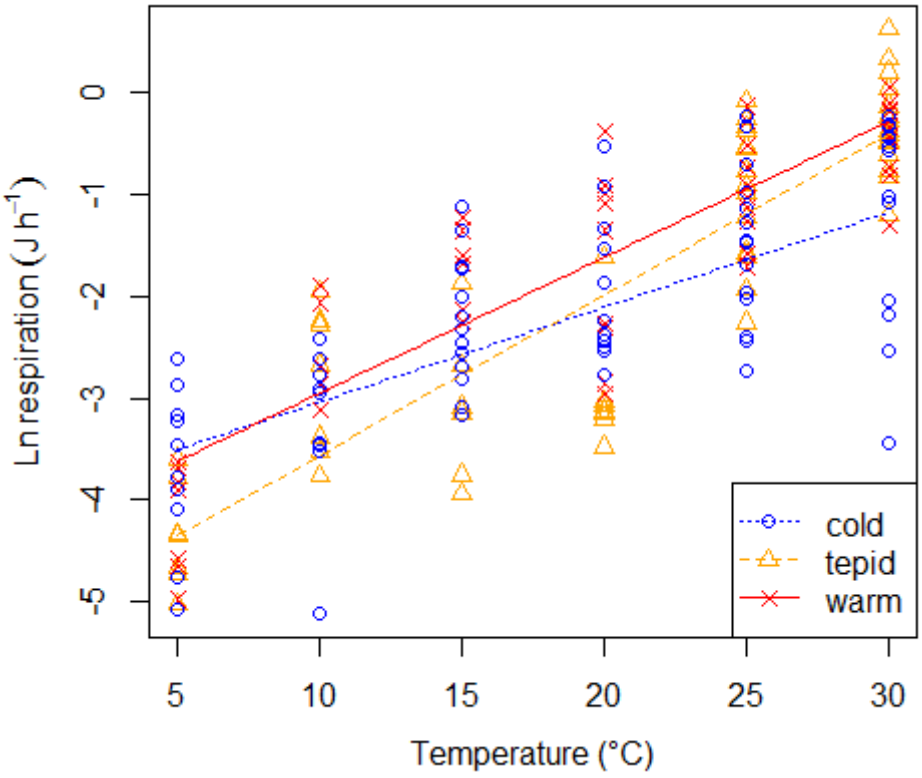

Figure 2 : Respiration rate (J.h^{-1}) of *R. balthica* in response of experimental temperature, for cold (IS 9, 10°C), tepid (IS5, 14°C) and warm (IS8, 18°C) acclimated snails.

Feeding rate

There was a significant increase in the feeding rate of *R. balthica* with increasing experimental temperature (ANCOVA: $F_{1,63}$ = 58.01,p < 0.0001). There was no significant main effect of acclimation

on feeding rate (ANCOVA: $F_{2,63}$ = 0.917, p = 0.4049), but acclimation significantly changed the temperature effect on feeding rate (ANCOVA: $F_{2,63}$ = 3.240, p < 0.0458). Here, the temperature dependence of feeding rate was weakest in the cold acclimated snails, with a lower activation energy (0.14 ± 0.09 eV) than both the tepid (0.40 ± 0.12 eV) and warm (0.43 ± 0.12 eV) acclimated snails (see Figure 3).

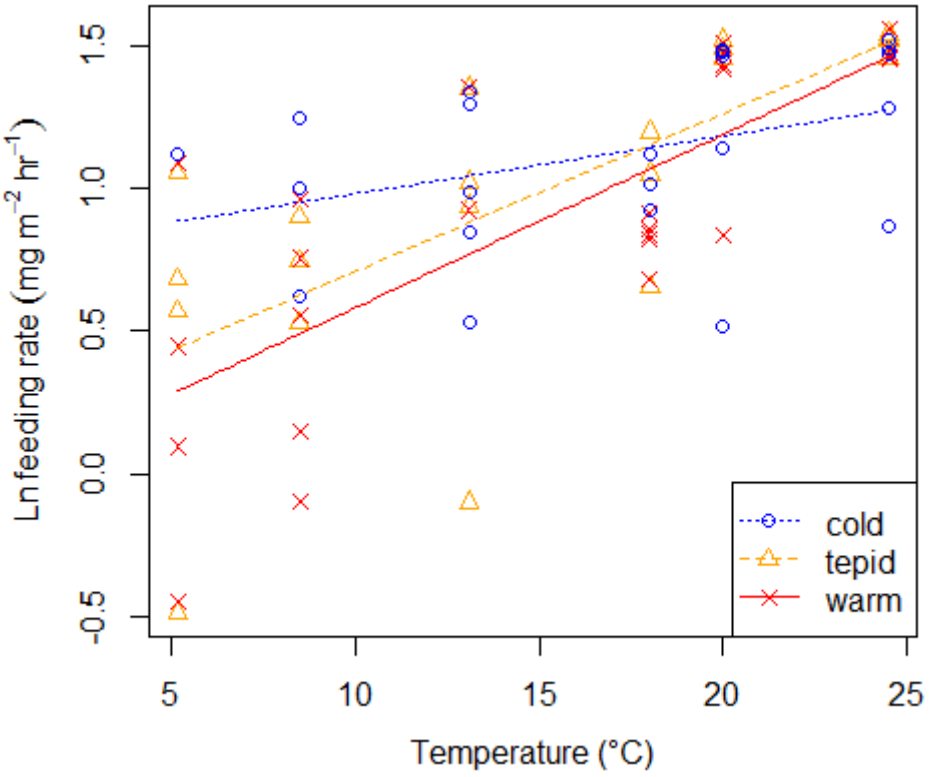

Figure 3 : Feeding rate (mg.m^{-2}.hr^{-1}) of *R. balthica* in response of experimental temperature, for cold (IS 9, 10°C), tepid (IS5, 14°C) and warm (IS8, 18°C) acclimated snails.

Energetic efficiency

There was a significant decrease in energetic efficiency of *R. balthica* with increasing experimental temperature (ANCOVA: $F_{1,68}$ = 41.23, p < 0.0001). There was a significant main effect of acclimation on energetic efficiency (ANCOVA: $F_{2,68}$ = 3.45, p = 0.0375), but acclimation did not significantly alter the temperature effect on energetic efficiency (ANCOVA: $F_{2,68}$ = 1.52, p = 0.23; see Figure 4).

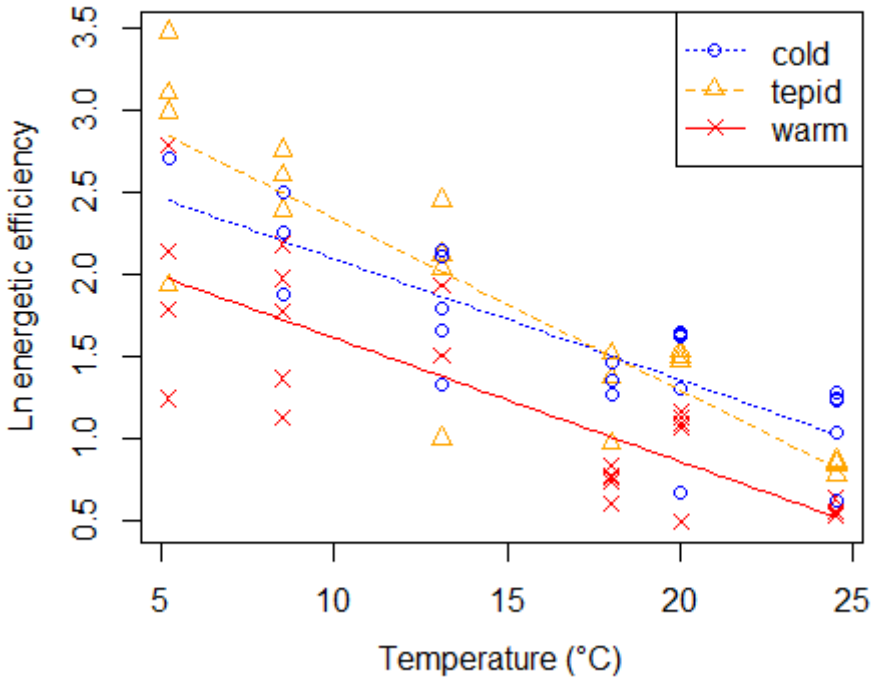

Figure 4 : Energetic efficiency of *R. balthica* in response of experimental temperature, for cold (IS 9, 10°C), tepid (IS5, 14°C) and warm (IS8, 18°C) acclimated snails.

Laboratory to field comparison

There was no significant difference in the feeding rate of *R. balthica* on biofilm grown in the field compared to biofilm grown in the laboratory (ANCOVA: $F_{1,48} = 0.835$, $p = 0.3653$), and location where biofilm was grown did not significantly change the temperature effect on feeding rate (ANCOVA: $F_{1,48} = 0.0009$, $p = 0.976$; see Figure 5).

There was a significant increase in the feeding rate of *R. balthica* with increasing experimental temperature for both field and laboratory-grown biofilm combined (ANCOVA: $F_{1,46} = 6.596$, $p = 0.0135$). There was a significant effect of acclimation on feeding rate (ANCOVA: $F_{2,46} = 3.563$, $p = 0.0364$), but acclimation did not significantly change the temperature effect on feeding rate (ANCOVA: $F_{2,46} = 0.2222$, $p = 0.8016$).

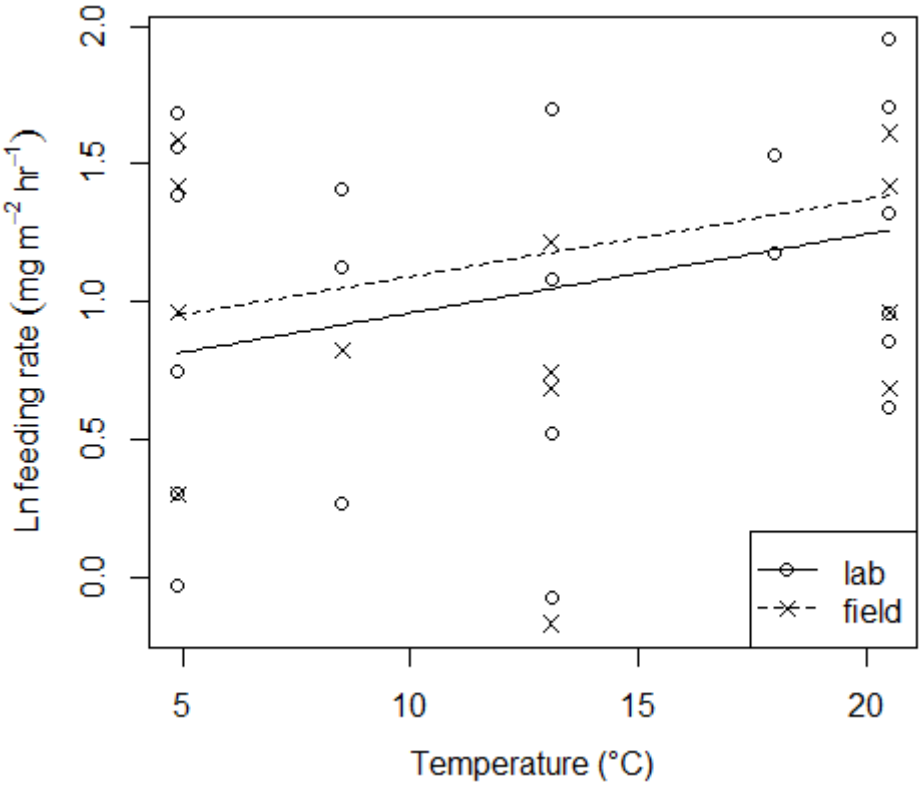

Figure 5 : Feeding rate (mg.m^{-2}.hr^{-1}) of *R. balthica* in response of experimental temperature, with laboratory-grown biofilm and field-grown biofilm.

Discussion

Global warming is expected to have strong impacts on freshwater food webs and especially ectotherms which are highly vulnerable to environmental temperature. Consequently it is important to understand how the respiration and consumption rate of herbivores will respond to an increase in environmental temperature (O'Connor 2009). This study indicates that warming increases the metabolic and feeding rate of organisms. More interestingly, thermal acclimation has an effect on the biological response of consumer species to warming and explains how freshwater snails may cope with environmental temperature increases by down-regulating their metabolism.

Respiration rate

The knowledge of the impact of climate warming on ectotherms is still not fully comprehensive nor the process understood (Pörtner 2010). Present results indicate that warming strongly increased the respiration rate of snails. They are coherent with the expected output and previous studies, which found that metabolic rates and oxygen consumption increased at higher temperature in invertebrates and herbivores (Rall et al. 2010; Beisner et al. 1997).

Present results also support an effect of thermal acclimation on the metabolic activity of snails. Most interestingly, thermal acclimation impacts the metabolic response to thermal change. Acclimation of organisms to environmental temperature is subject to debate (Gillooly et al. 2006; Clarke 2004; Clarke 1993; Clarke & Fraser 2004), but a recent research found it affects metabolic response of molluscs (Schaum et al. 2017; Falfushynska et al. 2016). Comparable to the present results, Schaum et al. (2017) found that activation energy was different according to thermal acclimation, suggesting snail are adapted to the river's thermal conditions. Considering the lowest activation energy was found in cold water snails and the highest in tepid acclimated ones, it suggests the metabolic activity of tepid snails respond faster than warm acclimated snails. This could be explained by the fact that tepid water snails may have a higher thermal phenotypic plasticity, allowing them to quickly adapt to colder or warmer environments, at the cost of their metabolic activity which will require more energy if 'upregulation of metabolism' needs to be maintained (Schaum et al. 2017).

Feeding rate

Thermal stress, which impacts the metabolic activity and respiration, in turn leads to a higher demand for food and increased consumption rates (Sanford 2002). Results obtained in this study were consistent with the hypothesis, and with a previous studies which found that feeding rate increases

with warming in many organisms (Rall et al. 2012; Sentis et al. 2012). Sentis et al. (2012) also argue that this increase is the result of an increase in searching rate and a decrease in handling time, which they consider being the two main determinants of feeding rate.

Interestingly, this result also underlined the dampening effect of thermal acclimation on the temperature dependence of feeding rate, such that activation energy of feeding rate was higher for warm acclimated snails than for cold acclimated ones. This is consistent with previous research realised on fish, which found that warm acclimated organisms had higher attack rates and capture success, and that 'predation pressure' was higher in warm acclimated organisms, independently to experimental temperature (Grigaltchik et al. 2012). This increase in feeding rate of grazers under global warming could have further implications by increasing the top down effect on primary producers and periphytic algae (Zębek & Szymańska 2014) and playing a bottom up effect on higher trophic level predators (O'Gorman et al. 2012).

Laboratory versus field

In the context of global warming, it is important to compare how the effect of thermal acclimation and consumer-resource interactions observed in the laboratory can relate to what is happening in the field. Fortunately, Hengill geothermal streams could be used to answer the question whether the feeding rate of snails would be different if feeding on a natural or laboratory grown substrate. Results suggested the feeding rate was similar on both field and laboratory substrates, consistent with Scrimgeour et al. (1991) who found that mayfly feeding behaviour and depletion rates of the diatom patch obtained in the laboratory was similar to observations from the field. Few studies compare the feeding rates on substrate from laboratory or field grown culture in aquatic environments, however, it appears that predation behaviours, attack and handling time rates observed in the laboratory often scale up to field observations for terrestrial organisms (Xia et al. 2003). These results should not be taken as proof that what happen in the laboratory perfectly reflect the field, but rather as a way to understand the general outcomes of environmental warming.

Energetic efficiency

In this study, energetic efficiency of snails decreased with increasing temperature. These results are in accordance with previous research which found that ingestion efficiency decreases with increasing temperature (Rall et al. 2010). This decrease in energetic efficiency could be explained by the fact that as the consumption demand increases it does not match the physiological demand (Rall et al. 2010;

Vucic-Pestic et al. 2011). It could also result in predator starvation and eventual extinction if temperature increase reaches very high levels (Sentis et al. 2012; Vucic-Pestic et al. 2011).

Thermal acclimation was also found to play a role on the energetic efficiency, with a lower energetic efficiency in warm acclimated snails, than tepid and cold acclimated snails. This is consistent with research from Seebacher et al. (2005) who found that cold acclimated organisms have the ability to cope with increasing temperature. They argue that thermal specialization (i.e. adaptation of an organism to better perform at a certain temperature) would be obtained through a trade-off where the thermal plasticity is lost (Seebacher et al. 2005). In this study, cold acclimated snails seem to be less impacted as the temperature increases (i.e. cold acclimated snails have a lower decrease in energetic efficiency than warm acclimated snails) inferring that cold acclimated snails may not have thermal specialization. On the contrary to the belief that cold environment organisms are thermally specialised (Seebacher et al. 2005), this study highlight that organism living in cold environment have the ability to cope with temperature changes.

Most importantly, whereas thermal acclimation influences the energetic efficiency, results indicate that thermal acclimation does not alter the effect of temperature on energetic efficiency, which was found to decrease in a similar fashion for all thermal acclimations. Altogether, it infers that increase in temperature eventually impacts all thermally acclimated organisms. This could be explained by the fact that, independently from thermal acclimation, as the temperature gets warmer, resource consumption increases to a level exceeding resource production (O'Connor 2009). Indeed, the feeding rate of the predator increases at a faster pace than does the turnover rate of prey, eventually leading to a lack of resources for the predator, resulting in its decrease or death and a likely increase in the biomass of algal populations (Beisner et al. 1997).

By combining these results with previous research, it can be argued that thermal acclimation dampens the temperature dependence of respiration and feeding rate. Indeed, as environmental temperature increases, an organism's first response is stress and phenotypic plasticity of an individual, while the second level is acclimatization; it implies the adjustment of the metabolism through a change in thermal performances and thermal window of the organism, eventually leading to genetic and niche modifications over longer time scale (Pörtner 2010). After a period of time, going from hours to days, organisms are believed to be able to acclimate to the environmental temperature (O'Connor 2009). However, it is also found that warming increases metabolic activity and feeding rates to such levels, that energetic efficiency will eventually be impacted, independent of thermal acclimation.

Implications and limitations

The main limitation of this study was found in the measurement of chlorophyll for feeding rate measurements. Indeed chlorophyll could not be measured before and after the feeding experiment on the same coverslips, due to the process of chlorophyll extraction. Direct measurement of chlorophyll before and after the feeding experiment being the ultimate way to measure feeding rate, further research could potentially use cell count estimations through microscope imagery as a way of quantifying diatoms, which is more a precise but time consuming method.

Additionally, studying the individual level cannot be used to extrapolate on possible impact at higher trophic levels (Woodward, Perkins, et al. 2010). It can only be used to better understand individual biological rates and lower levels of interaction which are as important as higher trophic levels, as studying the big picture is not enough to understand processes underpinning changes.

Further studies could also investigate the effect of culture quality on the feeding rate of grazers. Past studies highlighted that global warming was found to impact phytoplankton by decreasing their size, and impacting the community through a lower diatom biomass and a decrease in biomass quality (Yvon-D et al. 2011). As phytoplankton play a key role in the ecosystem through biomass production and carbon sequestration, changes in their ecology could potentially further impact consumer-resources interactions and feeding rates, while the decrease in biomass quality could accentuate the decrease in energetic efficiency.

Conclusions

Climate change is predicted to impact freshwater ecosystems and ectothermic grazers through modification of environmental temperature. The present study highlights the effect of temperature on metabolic activity, physiological rates and how acclimation set the ability of organisms to cope with thermal variations. Metabolic and feeding rates were both found to increase as a result of experimental warming. Thermal acclimation was also found to dampen the temperature dependence of respiration and feeding rates, but ultimately the energetic efficiency will decrease for all snails, independently of their thermal acclimations. Ideally, further research could investigate the role of thermal acclimation, directly measuring metabolic rates and efficiencies in the field.

Acknowledgements

I would like to considerably thank Dr Eoin O'Gorman for his continuous supervision, help, availability and support throughout the course of this research. Many thanks to Dr Rebecca Kordas for her support during laboratory work at the University of Iceland, for teaching me the respiration protocol and for providing me with respiration data.

References:

Adams, G.L. et al., 2013. Diatoms can be an important exception to temperature – size rules at species and community levels of organization. *Global Change Biology*, 19(11), pp.3540–3552.

Anon, Estimation of algal biomass from stones.

Arnason, B. et al., 1969. Hengill , a High Temperature Thermal Area in Iceland. *Bulletin of Volcanology*, 33(1), pp.245–259.

Barnett, T.P., Adam, J.C. & Lettenmaier, D.P., 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature*, 438(November), pp.303–309.

Beisner, B.E., Mccauley, E. & Wrona, F.J., 1997. The influence of temperature and food chain length on plankton predator-prey dynamics. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(3), pp.586–595.

Brodersen, K.P. et al., 2008. Respiration of midges (Diptera; Chironomidae) in British Columbian lakes: oxy-regulation, temperature and their role as palaeo-indicators. *Freshwater Biology*, 53(3), pp.593–602.

Broecker, W.S.., 1975. Climatic Change : Are We on the Brink of a Pronounced Global Warming? *Science*, 189(4201), pp.460–463. Available at: http://www.jstor.org/stable/1740491.

Brönmark, C., 1994. Effects of Tench and Perch on Interactions in a Freshwater , Benthic Food Chain. *Ecology*, 75(6), pp.1818–1828.

Brönmark, C., 1989. Interactions between Epiphytes , Macrophytes and Freshwater Snails : A Review. *Journal of Molluscan Studies*, 55(2), pp.299–311.

Brown, J.H. et al., 2004. Toward a metabolic theory of ecology. *Ecology*, 85(7), pp.1771–1789.

Caissie, D., 2006. The Thermal Regime of Rivers : A Review The thermal regime of rivers : a review. *Freshwater Biology*, 51(8), pp.1389–1406.

Chapin, I.F.S. et al., 2000. Consequences of changing biodiversity. *Nature*, 405(6783), pp.234–242.

Clarke, A., 2004. Is there a Universal Temperature Dependence of metabolism ? *Functional Ecology*, 18(2), pp.252–256.

Clarke, A., 1993. Seasonal Acclimatization and Latitudinal Compensation in Metabolism : Do They Exist ? *Functional Ecology*, 7(2), pp.139–149. Available at: http://www.jstor.org/stable/2389880.

Clarke, A. & Fraser, K.P.P., 2004. Why does metabolism scale with temperature ? *Functional Ecology*, 18(2), pp.243–251.

Dell, A.I., Pawar, S. & Savage, V.M., 2014. Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. *Journal of Animal Ecology*, 83(1),

pp.70–84.

Dudgeon, D. et al., 2006. Freshwater biodiversity : importance , threats , status and conservation challenges. *Biological reviews*, 81(2), pp.163–182.

Falfushynska, H.I., Phan, T. & Sokolova, I.M., 2016. Long-Term Acclimation to Different Thermal Regimes Affects Molecular Responses to Heat Stress in a Freshwater Clam Corbicula Fluminea. *Nature Publishing Group*, (November), pp.1–17. Available at: http://dx.doi.org/10.1038/srep39476.

Friberg, N. et al., 2009. Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology*, 54(10), pp.2051–2068.

Gillooly, J.F. et al., 2001. Effects of Size and Temperature on Metabolic Rate. *Science*, 293(5538), pp.2248–2252.

Gillooly, J.F. et al., 2006. Response to Clarke and Fraser : effects of temperature. *Functional*, 20(2), pp.400–404.

Grigaltchik, V.S., Ward, A.J.W. & Seebacher, F., 2012. Thermal acclimation of interactions : differential responses to temperature change alter predator – prey relationship. *Proceedings of the Royal Society of London B: Biological Sciences*, 279(1744), pp.4058–4064.

Gudmundsdottir, R. et al., 2013. Diatoms as indicators : The influences of experimental nitrogen enrichment on diatom assemblages in sub-Arctic streams. , 32, pp.74–81.

Gudmundsdottir, R. et al., 2011. Effects of temperature regime on primary producers in Icelandic geothermal streams. *Aquatic Botany*, 95(4), pp.278–286. Available at: http://dx.doi.org/10.1016/j.aquabot.2011.08.003.

Gudmundsdottir, R. et al., 2012. Variation in diatom and bryophyte communities along a temperature gradient in sub-Arctic streams : model surrogates for trends in larger ecosystems ? *Inland Waters*, 2(4), pp.163–176.

Hannesdóttir, E.R. et al., 2013. Increased Stream Productivity with Warming Supports Higher Trophic Levels. *Advances in Ecological Research*, 48, pp.285–342.

Hansen, J. et al., 2006. Global temperature change. *Reviews of Geophysics*, 103(39), pp.14288–14293.

Hart, D.D. & Finelli, C.M., 1999. Physical-Biological Coupling in Streams: The Pervasive Effects of Flow on Benthic Organisms. *Annual Review of Ecology and Systematics*, 30(1), pp.363–395.

IPCC, 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia,* Cambridge, United Kingdom and New York, NY, USA.

Johansson, M., 2015. *Adjusting to the extreme: Thermal adaptation in a freshwater gastropods*.

Uppsala Universitet.

Jones, J.I. et al., 1999. Do Submerged Aquatic Plants Influence Their Periphyton to Enhance the Growth and Reproduction of Invertebrate Mutualists ? *Oecologia*, 120, pp.463–474.

Komulaynen, S., 2009. Diatoms of periphyton assemblages in small rivers in Northwestern Russia. *Studi Trent. Sci. Nat.*, 84, pp.153–160.

Li, K., Liu, Z. & Gu, B., 2009. Density-dependent effects of snail grazing on the growth of a submerged macrophyte, Vallisneria spiralis. *Ecological Complexity*, 6(4), pp.438–442.

Mann, D.G. & Droop, S.J.M., 1996. Biodiversity, biogeography and conservation of diatoms. In *Biogeography of freshwater algae*. Springer, pp. 19–32.

O'Connor, M.I.., 2009. Warming Strengthens an Herbivore : Plant Interaction. *Ecology*, 90(2), pp.388–398. Available at: http://www.jstor.org/stable/27650994.

O'Gorman, E.J. et al., 2012. Impacts of Warming on the Structure and Functioning of Aquatic Communities : Individual- to Ecosystem-Level Responses. *Advances in Ecological research*, 47, pp.81–176.

Parmesan, C. & Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918), pp.37–42.

Pörtner, H., 2010. Oxygen- and capacity-limitation of thermal tolerance : a matrix for integrating climate-related stressor effects in marine ecosystems. *The Journal of Experimental Biology*, 213(6), pp.881–893.

Postel, S. & Carpenter, S., 1997. Freshwater ecosystem services. *Nature's services: Societal dependence on natural ecosystems*, 195.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URLhttps://www.R-project.org/.

Ragueneau, O. et al., 2006. Si and C interactions in the world ocean : Importance of ecological processes and implications for the role of diatoms in the biological pump. , 20, pp.1–15.

Rall, B.C. et al., 2010. Temperature , predator-prey interaction strength and population stability. *Global Change Biology*, 16(8), pp.2145–2157.

Rall, C. et al., 2012. Universal temperature and body-mass scaling of feeding rates. , pp.2923–2934.

Rasband, W., 2011. ImageJ.

Sala, O.E. et al., 2000. Biodiversity : global biodiversity scenarios for the year 2100. *Science*, 287, pp.1770–1774.

Sanford, E., 2002. Water Temperature , Predation , and the Neglected Role of Physiological Rate Effects in Rocky. *Integrative and Camparative Biology*, 42(4), pp.881–891.

Savage, V.M. et al., 2004. Effects of body size and temperature on population growth. *The American Naturalist*, 163(3), pp.429–441.

Savage, V.M. et al., 2004. The predominance of quarter-power scaling in biology. *Functional Ecology*, 18(1932), pp.257–282.

Schaum, E.C. et al., 2017. Adaptation to warming increases the strength of an algal-grazer interaction in naturally heated streams. *BioRxiv*.

Sciences, P., Fields, D. & Road, L., 2010. Temperature , predator – prey interaction strength and population stability.

Scrimgeour, G.J. et al., 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly-diatom systems. *Oecologia*, 85(3), pp.343–348.

Seebacher, F. et al., 2005. A falsification of the thermal specialization paradigm : compensation for elevated temperatures in Antarctic fishes. *Biology Letters*, 1(2), pp.151–154.

Sentis, A., Hemptinne, J. & Brodeur, J., 2012. Using functional response modeling to investigate the effect of temperature on predator feeding rate and energetic efficiency. *Oecologia*, 169, pp.1117–1125.

Smol, J.P. & Stoermer, E.F., 2010. *The diatoms: applications for the environmental and earth sciences*, Cambridge University Press.

Snoeijs, P., Busse, S. & Potapova, M., 2002. The importance of diatom cell size in community analysis. *Journal of Phycology*, 38(2), pp.265–281.

Steinman, A.D., Lamberti, G.A. & Leavitt, P.R., 1996. Biomass and pigments of benthic algae. *Methods in Stream Ecology*, (eds. Hauer FR & Lamberti GA), pp.357–380.

Tett, P., Kelly, G.M. & Hornberger, M. george, 1975. A method for the spectrophotometric measurement of chlorophyll a and Pheophytin a in benthic microalgae. *Limnology and Oceanography*, 20(5), pp.887–896.

Vucic-Pestic, O. et al., 2011. Warming up the system : higher predator feeding rates but lower energetic efficiencies. *Global Change Biology*, 17(3), pp.1301–1310.

Walther, G., 2010. Community and ecosystem responses to recent climate change. , 365(1549), pp.2019–2024.

Walther, G.R. et al., 2002. Ecological responses to recent climate change. *Nature*, 416(6879), pp.389–395.

Woodward, G., Dybkjaer, J.B., et al., 2010. Sentinel systems on the razor ' s edge: effects of warming on Arctic geothermal stream ecosystems. *Global Change Biology*, 16(7), pp.1979–1991.

Woodward, G., Perkins, D.M. & Brown, L.E., 2010. Climate change and freshwater ecosystems : impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365(1549), pp.2093–2106.

Xia, A.J.Y., Rabbinge, R. & Werf, W. Van Der, 2003. Multistage Functional Responses in a Ladybeetle-Aphid System : Scaling up from the Laboratory to the Field. *Environmental Entomology*, 32(1),

pp.151–162.

Yodzis, P. & Innes, S., 1992. Body Size and Consumer-Resources Dynamics. *The American Naturalist*, 139(6), pp.1151–1175. Available at: http://links.jstor.org/sici?sici=0003-0147%28199206%29139%3A6%3C1151%3ABSACD%3E2.0.CO%3B2-V.

Yvon-D et al., 2011. Warming Alters the Size Spectrum and Shifts the Distribution of Biomass in Aquatic Ecosystems. *Global Change Biology*, 17(4), pp.1681–1694.

Zębek, E. & Szymańska, U., 2014. Gastropods and periphytic algae relationships in the vicinity of a small hydroelectric plant on the Paslęka River in northeast Poland. *Archives of Polish Fisheries*, 22(1), pp.69–80. Available at: http://www.degruyter.com/view/j/aopf.2014.22.issue-1/aopf-2014-0007/aopf-2014-0007.xml.

Appendix

Table S1: Stock culture for growing biofilm on laboratory coverslips and microscope slides. This stock culture was added to the aquaria in order to give the nutrients for diatom cultures to grow.

Stock	**Per 200ml**
$Ca(NO_3)_2 . 4H_2O$	4.00g
KH_2PO_4	2.48g
$MgSO_4 . 7H_2O$	5.00g
$NaHCO_3$	3.18g
EDTAFeNa	0.45g
$EDTANa_2$	0.45g
H_3BO_3	0.496g
$MnCl_2.4H_2O$	0.278g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.20g
Cyanocobalamin	0.008g
Thiamine HCl	0.008g
Biotin	0.008g
$Na_2SiO_3.H_2O$ (Sigma S4392)*	11.4g
Medium	**Per litre**
Stock solution 1-8	1.0 ml each

*Sigma Chemical Co