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RESEARCH ARTICLE

# Seasonal changes in the behaviour and respiration physiology of the freshwater duck mussel, *Anodonta anatina*

**Glenn Lurman*, Johanna Walter and Hans H. Hoppeler**

## ABSTRACT

For low-energy organisms such as bivalves, the costs of thermal compensation of biological rates (synonymous with acclimation or acclimatization) may be higher than the benefits. We therefore conducted two experiments to examine the effect of seasonal temperature changes on behaviour and oxygen consumption. In the first experiment, we examined the effects of seasonal temperature changes on the freshwater bivalve *Anodonta anatina*, taking measurements each month for a year at the corresponding temperature for that time of year. There was no evidence for compensation of burrowing valve closure duration or frequency, or locomotory speed. In the second experiment, we compared *A. anatina* at summer and winter temperatures (24 and 4°C, respectively) and found no evidence for compensation of the burrowing rate, valve closure duration or frequency, or oxygen consumption rates during burrowing, immediately after valve closure or at rest. Within the experimental limits of this study, the evidence suggests that thermal compensation of biological rates is not a strategy employed by *A. anatina*. We argue that this is due to either a lack of evolutionary pressure to acclimatize, or evolutionary pressure to not acclimatize. Firstly, there is little incentive to increase metabolic rate to enhance predatory ability given that these are filter feeders. Secondly, maintained low energetic demand, enhanced at winter temperatures, is essential for predator avoidance, i.e. valve closure. Thus, we suggest that the costs of acclimatization outweigh the benefits in *A. anatina*.

**KEY WORDS: Bivalve, Locomotion, Oxygen consumption, Thermal acclimation, Unionid, Valve closure**

## INTRODUCTION

Acclimation, defined here as physiological adjustment to a laboratory-induced temperature change, and acclimatization, defined here as physiological adjustment to a seasonally induced temperature change, are two terms that can be linked by the core idea of thermal compensation of physiological rates following temperature change. The study of thermal compensation mechanisms has resulted in a number of explanatory models and hypotheses (e.g. Prosser, 1955; Precht, 1958; Lagerspetz, 2006; Angilletta, 2009). One of the earliest of these, developed by Precht (Precht, 1958), categorised five different types of thermal compensation based on thermal coefficients, or $Q_{10}$ values, where the $Q_{10}$ is defined as the factorial increase of a (biological) rate for every 10°C increase in temperature, where a $Q_{10}$ of 2 indicates a doubling of a rate over 10°C, indicative of a thermodynamically driven increase. In Precht's model, $Q_{10}$ values below 1 indicate type 1 acclimation, or supra-optimal compensation; values equal to 1 indicate type 2 acclimation or ideal compensation; values of 1–2 indicate type 3 or partial compensation; values of 2–3 indicate type 4 or missing compensation; and values greater than 3 indicate type 5 acclimation or inverse compensation (Fig. 1A). The effect of these different thermal compensation types on the slope of a rate–temperature (R–T, also known as Arrhenius) curve can be seen in Fig. 1B. Thus, on the one hand, animals can maintain performance capacity in the face of changing temperatures, Precht's types 1, 2 and 3 acclimation. For example, fish re-model skeletal muscle on a number of levels to maintain locomotory performance (Rome, 1995; Johnston and Temple, 2002; Catalán et al., 2004), a function necessary to outswim predators and/or catch prey. On the other hand, some animals have adopted the strategy of 'enhancing' thermodynamic effects to save energy, i.e. hibernation (Storey and Storey, 1990; Geiser, 2004) and torpor (Holopainen et al., 1997), or Precht's type 5 acclimation. Yet there is a cost implicit in Precht's model associated with thermal compensation.

Because compensation comes at a cost, and this can be significant (Leroi et al., 1994; Hoffmann, 1995), the question of whether compensation is a relevant strategy in low-energy organisms such as bivalves comes to the fore. On the face of it, bivalves do not appear to compensate oxygen consumption rates at low temperatures, but do partially compensate at intermediate, and in some species, at high temperatures. Evidence for this can be drawn from several marine species, namely *Mytilis edulis* (Newell and Pye, 1970a; Widdows, 1973), *Ostrea edulis* (Newell et al., 1977), *Crassostrea virginica* (Pernet et al., 2007; Pernet et al., 2008), *Perna perna* (Resgalla et al., 2007), *Protothaca thaca* (Riascos et al., 2012) and *Littorea littorea* (Newell and Pye, 1970a). Similar patterns have also been observed in freshwater bivalves, namely *Dreissena polymorpha* (Alexander and McMahon, 2004) and *Amblema plicata* (Baker and Hornbach, 2001). Yet this phenomenon is best exemplified by the fingernail clam *Sphaerium striatinum* following seasonal acclimatization. The $Q_{10}$ values for metabolic rate were highest in the winter months, generally ranging between values of 2 and 5, and lowest in the summer months, between 2 and 0.2 (Hornbach et al., 1983). At intermediate to high temperatures, bivalves may become increasingly limited in their oxygen consumption, quickly reaching their maximal metabolic rate (Pörtner, 2012), preventing significant increases in oxygen consumption. The lack of compensation at lower temperatures, in contrast, may be a thermodynamically driven energy-saving mechanism and a response to reduced food supply, akin to the inverse thermal compensation seen in hibernation and states of torpor.

We elected to use a freshwater bivalve, *Anodonta anatina* (Linnaeus 1758), from a local lake, Lake Murten (Murtensee/Lac de Morat), to explore the effects of seasonal temperature changes on behaviour and oxygen consumption. Lake Murten is a small, shallow lake on the Swiss plateau. It is roughly rectangular, ~5 km

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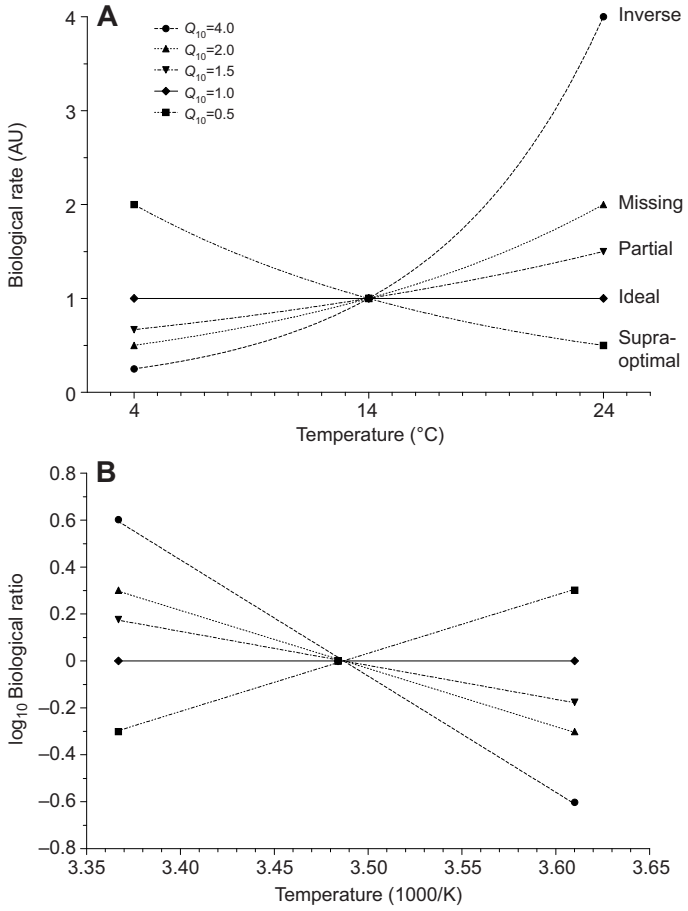


**Fig. 1. Thermal compensation types and their effects on biological rate.** The compensation types given in A are taken from Precht (Precht, 1958) and are based on the $Q_{10}$ values indicated. (B) A rate–temperature (R–T) plot of the same model data accordingly transformed, and with the corresponding slope indicated for each of the different compensation types.

long, 3 km wide and 45 m deep at its deepest point. Given the small size and the shallow bathymetry of Lake Murten, it is subject to pronounced seasonal variation in temperature. In winter, temperatures drop to 4°C, while in summer temperature increases to 25°C (Service de l'environnement Fribourg, 2011). Near shore, at 1 m depth, this variation is presumably even more pronounced given that the shores have a very gentle slope and exhibit minimal turbulence. The shoreline is characterised by reeds (*Phragmites australis*), hornwort (*Ceratophyllum submersum*) and a fine sediment, which provides habitat for burrowing bivalves.

In our study, we conducted two experiments with *A. anatina*. In Experiment 1, we examined the changes in behavioural parameters, namely burrowing rate, valve closure (known elsewhere as gape) duration and frequency, as well as locomotion each month, throughout 1 yr, with measurements taken at the corresponding temperature for that month. In Experiment 2, we then examined the energetic cost of burrowing and valve closure and hypothesised that: (1) the resting oxygen consumption rate would be lower in winter; (2) oxygen consumption rates during burrowing and immediately after valve closure would be lower in winter; (3) the recovery rates would be longer in winter; and (4) all of these decreases would be of a magnitude that corresponded with thermodynamic laws, i.e. $Q_{10}$ values for these rates would range between 2 and 3.

## RESULTS

### Lake temperature

Lake temperature at 1 m changed as a result of season (Fig. 2). A frequency distribution of temperature measurements revealed a bimodal distribution with peaks at 21.4±0.41 and 6.8±0.69°C (supplementary material Fig. S1), representing the summer and winter averages, respectively. Seasonal rates of temperature change were ~0.2°C day$^{-1}$ in spring and autumn. Considerable variation was also seen on shorter scales, particularly in summer and winter, with rates of change as high as 1–2°C day$^{-1}$ for several days running, although daily variation was low.

### Experiment 1: changes in behavioural rates due to seasonal temperature changes

There were significant differences in the whole mass of the mussels used (supplementary material Table S1), with the lowest mean mass being 41.4±3.8 g in March and the highest being 71.1±3.9 g in September (see supplementary material Table S1 for a summary of whole mussel masses and lengths for each month). There was a statistically significant effect of month on all the rates measured (see Table 1 for the complete statistical summary). Summer-acclimatized

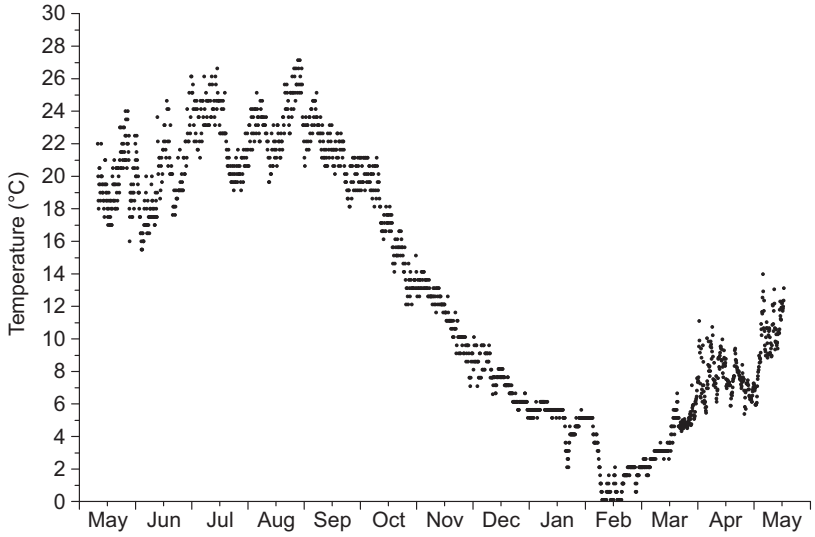


**Fig. 2. Seasonal changes in water temperature in Lake Murten (46°54′24″N, 7°2′59″E) at 1 m depth from 9 May 2011 until 10 May 2012.**

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**Table 1. Summary of the statistical analysis of the seasonal changes in behavioural rates using a non-parametric Kruskal–Wallis one-way ANOVA with Dunn's multiple comparison**

| | Mass | BRI | Closure duration | Closure frequency | Locomotion |
|---|---|---|---|---|---|
| Kruskal–Wallis *P* | <0.001 | <0.001 | <0.001 | <0.001 | 0.027 |
| Dunn's multiple comparison | | | | | |
| Jan vs Feb | ns | ns | ns | ns | ns |
| Jan vs Mar | ns | ns | ns | ns | ns |
| Jan vs Apr | ns | ns | ns | ns | ns |
| Jan vs May | ns | ns | ns | ns | ns |
| Jan vs Jun | ns | ns | ns | * | ns |
| Jan vs Jul | ns | * | ns | *** | ns |
| Jan vs Aug | ns | ns | ns | ** | ns |
| Jan vs Sep | ns | ns | ns | * | ns |
| Jan vs Oct | ns | ns | ns | ns | ns |
| Jan vs Nov | ns | ns | ns | ns | – |
| Jan vs Dec | ns | ns | ns | ns | ns |
| Feb vs Mar | ns | ns | ns | ns | ns |
| Feb vs Apr | ns | ns | * | ns | ns |
| Feb vs May | ns | ns | ns | ns | ns |
| Feb vs Jun | ns | ns | ns | ns | ns |
| Feb vs Jul | ns | *** | *** | ns | ns |
| Feb vs Aug | ns | ns | ns | ns | ns |
| Feb vs Sep | ** | ns | ns | ns | ns |
| Feb vs Oct | * | ns | ns | ns | ns |
| Feb vs Nov | ns | ns | ns | ns | – |
| Feb vs Dec | * | ns | ns | ns | ns |
| Mar vs Apr | ns | ns | ** | ns | ns |
| Mar vs May | ns | ns | ns | ns | ns |
| Mar vs Jun | ns | ns | ns | ** | ns |
| Mar vs Jul | ns | ** | *** | *** | ns |
| Mar vs Aug | ns | ns | * | *** | ns |
| Mar vs Sep | * | ns | ns | ** | ns |
| Mar vs Oct | ns | ns | ns | ns | ns |
| Mar vs Nov | ns | ns | ns | ns | – |
| Mar vs Dec | ns | ns | ns | ns | ns |
| Apr vs May | ns | ns | ns | ns | ns |
| Apr vs Jun | ns | ns | ns | ns | ns |
| Apr vs Jul | ns | ns | ns | ns | ns |
| Apr vs Aug | ns | ns | ns | ns | ns |
| Apr vs Sep | ** | ns | ns | ns | ns |
| Apr vs Oct | * | ns | ns | ns | ns |
| Apr vs Nov | ns | ns | ns | ns | – |
| Apr vs Dec | * | ns | ns | ns | ns |
| May vs Jun | ns | ns | ns | ns | ns |
| May vs Jul | ns | ns | ns | ns | ns |
| May vs Aug | ns | ns | ns | ns | ns |
| May vs Sep | ns | ns | ns | ns | ns |
| May vs Oct | ns | ns | ns | ns | ns |
| May vs Nov | ns | ns | ns | ns | – |
| May vs Dec | ns | ns | ns | ns | ns |
| Jun vs Jul | ns | ns | ns | ns | ns |
| Jun vs Aug | ns | ns | ns | ns | ns |
| Jun vs Sep | ** | ns | ns | ns | ns |
| Jun vs Oct | * | ns | ns | ns | ns |
| Jun vs Nov | ns | ns | ns | ns | – |
| Jun vs Dec | ns | ns | ns | ns | ns |
| Jul vs Aug | ns | ns | ns | ns | ns |
| Jul vs Sep | ** | ns | ns | ns | ns |
| Jul vs Oct | * | ns | ns | ns | ns |
| Jul vs Nov | ns | ns | ** | ** | – |
| Jul vs Dec | ns | * | * | ns | ns |
| Aug vs Sep | * | ns | ns | ns | ns |
| Aug vs Oct | ns | ns | ns | ns | ns |
| Aug vs Nov | ns | ns | ns | * | – |
| Aug vs Dec | ns | ns | ns | ns | ns |
| Sep vs Oct | ns | ns | ns | ns | ns |
| Sep vs Nov | ns | ns | ns | ns | – |
| Sep vs Dec | ns | ns | ns | ns | ns |
| Oct vs Nov | ns | ns | ns | ns | – |
| Oct vs Dec | ns | ns | ns | ns | ns |
| Nov vs Dec | ns | ns | ns | ns | – |

The statistical significance in the pairwise analysis of mussel whole mass, burrowing rate index (BRI), valve closure duration, valve closure frequency and locomotory speed for each month is indicated by: ns, not significant; –, no data; *$P$<0.05; **$P$<0.01; ***$P$<0.001.

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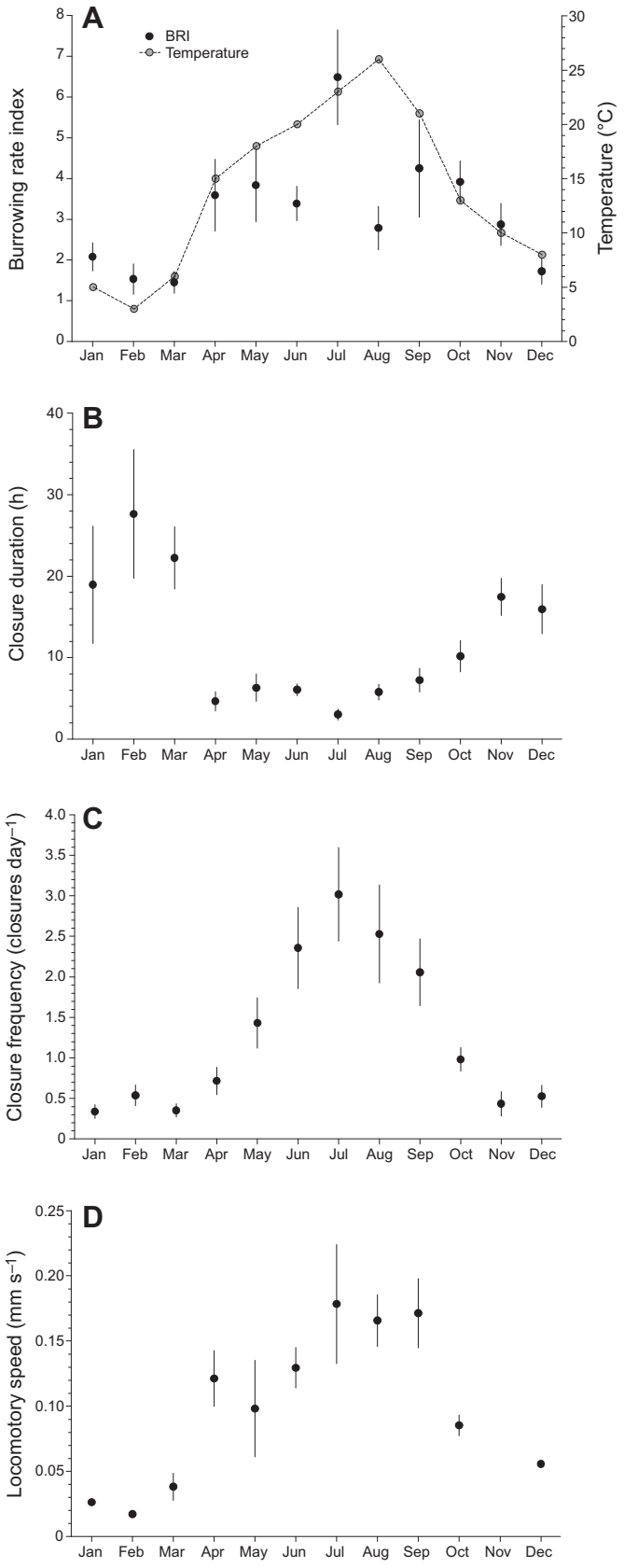


**Fig. 3. Seasonal changes in burrowing (A), valve closure duration (B), valve closure frequency (C) and locomotory speed (D) in *Anodonta anatina*.** The temperature used each month is also given in A. See Table 1 for a summary of the statistical analysis.

mussels burrowed faster, closed for shorter durations and more frequently, and had a greater locomotory speed (Fig. 3). A pairwise comparison of individual months (Table 1) revealed statistically significant differences between summer (primarily July) and the winter months (December, January, February and March) in rates of burrowing and valve closure duration and frequency. Linear models were fit to each of the different data sets on R–T plots. The derived descriptors of each of these fits are given in Table 2.

### Experiment 2: energetic cost of burrowing and valve closure

With the exception of the whole mussel mass and the degree to which mussel buried (i.e. % buried), all parameters were significantly different between summer and winter (Table 3). The burrowing duration was 2.8 times longer in winter mussels, resulting in a 3.4-fold lower burrowing rate index (BRI) (Fig. 4). The closure duration was 3.6-fold longer and the closure frequency was 6.5 times lower in winter mussels (Fig. 5). Oxygen consumption rates were between 5.2- and 6.5-fold lower in winter mussels in comparable states (Fig. 6, Table 4). In the summer mussels, the oxygen consumption rate during burrowing and immediately after valve closure were of a similar magnitude, being 1.8- and 1.6-fold higher than the resting oxygen consumption rate, respectively. While the oxygen consumption rate during burrowing was 2.3-fold higher than resting in the winter mussels, the oxygen consumption rate following valve closure was ~4-fold higher than that during resting and thus 2-fold higher than that during burrowing.

A two-way repeated-measures ANOVA found no significant differences in the recovery rates after burrowing. This was due largely to the fact that the oxygen consumption rate was no longer significantly higher than the resting oxygen consumptions rate a mere 30 min after burrowing had stopped in both summer- and winter-acclimatized mussels. Thus, if there were any differences, they were below our level of detection.

## DISCUSSION

There was a very clear effect of seasonal temperature change on the burrowing rate, valve closure behaviour and locomotory speed in *A. anatina*. In addition, stark differences were seen in the oxygen consumption rates between summer- and winter-acclimatized *A. anatina*. We found only very limited evidence of thermal compensation. We are unaware of any data relating the behaviours we studied to temperature change; however, a comparison of previously published data examining the effects of temperature change on oxygen consumption from a range of bivalves is possible. Evidence from a number of marine (Newell and Pye, 1970a; Newell and Pye, 1970b; Widdows, 1973; Newell et al., 1977; Pernet et al., 2007; Resgalla et al., 2007; Pernet et al., 2008; Riascos et al., 2012) and freshwater (Hornbach et al., 1983; Baker and Hornbach, 2001; Alexander and McMahon, 2004) bivalve species indicates that bivalves compensate oxygen consumption rates at intermediate, and in some species, higher temperatures, while there is no evidence of compensation at lower temperatures. As our primary interest was the difference between summer- and winter-acclimatized mussels, we did not measure oxygen consumption rates in *A. anatina* at intermediate temperatures as well, and cannot conclusively say whether *A. anatina* partially compensates oxygen consumption at intermediate temperatures. Nonetheless, from Experiment 2, it is clear that there is no compensation of oxygen consumption rates evident between the summer and winter temperatures. If we consider parameters other than just the oxygen consumption for which we do have measurements at intermediate temperatures, i.e.

**Table 2. Descriptive parameters (means ± s.e.m.) derived from the linear model fits to R–T transformed rate ($log_{10}$ of rate) versus temperature (1000/K) data**

| Rate | Slope | Intercept | *P* | $r^2$ |
|---|---|---|---|---|
| BRI | −1.53±0.49 | 5.69±1.7 | 0.010 | 0.50 |
| Valve closure duration (h) | 2.41±0.60 | −7.50±2.1 | 0.003 | 0.62 |
| Valve closure frequency (closures $day^{-1}$) | −2.63±0.23 | 9.19±0.97 | <0.001 | 0.90 |
| Locomotory speed (mm $s^{-1}$) | −3.33±0.41 | 10.5±1.42 | <0.001 | 0.88 |

Experiment 1's monthly measurements of BRI, valve closure duration and frequency and locomotory speed, we can see from the R–T plot linear regression parameters that there is only slight evidence of partial compensation for BRI and inverse compensation of locomotion, while valve closure behaviour was not compensated. Consequently, we would not expect to find significant compensation of the oxygen consumption rates at intermediate temperatures either.

The lack of seasonal acclimatization to cold, winter temperatures seen in *A. anatina* and the aforementioned species may be an energy-saving mechanism, allowing for a thermodynamically driven reduction in energetic requirements, which would correlate with seasonal reductions in food supply. This parallels similar conclusions from another study of the marine gastropod *Littorina saxatalis* (Sokolov et al., 2003). Cold-acclimated specimens from the White Sea (where food is extremely scarce in winter) had a significantly lower metabolic rate than cold-acclimated conspecifics from the North Sea, which continue to feed and be active in winter. Yet warm-acclimated specimens from both areas had comparable oxygen consumption rates, and were both significantly higher than cold-acclimated rates.

One theoretical model that integrates dynamic energy budgeting (Kooijman, 2000) and the oxygen and capacity limitation of thermal tolerance (OCLTT) model (Pörtner, 2012) suggests that as acute thermal stress increases, oxygen supply and, as a consequence, oxygen consumption become increasingly limited, which leads to a progressive limitation in biological functioning. Working through a hierarchy of biological functions, an animal will progressively switch off less essential functions as oxygen supply and consumption become more limited. The first to go are lipid and carbohydrate storage, then reproduction and growth, followed by activity and finally homeostatic maintenance (Sokolova et al., 2012; Sokolova, 2013). What is neglected here is the process of feeding, which can come at a considerable energetic cost, provoking a significant increase in metabolic rate in bivalves (Lurman et al., 2013). Thus, we would expect that feeding would be one of the first processes, along with storage, that would be switched off, although in winter this may occur in response to a restricted food supply (see below). Much more work needs to be done to determine whether this model can be equally applied to acclimatization processes in *A. anatina* and other animals, or whether all biological processes are downregulated/upregulated to the same degree as a result of decreasing/increasing temperature, such that the entire system is thermodynamically driven, or whether less essential biological functions are switched off in winter. We predict that it may turn out to be a mix of both.

We are unable to know whether other biological rates such as feeding, growth or cellular processes were compensated as a result of seasonal temperature; however, the fact remains that *A. anatina* did not (within the experimental errors of this study) thermally compensate BRI, valve closure behaviour, locomotory speed or oxygen consumption. Given that this species is in a temperate zone and experiences wide seasonal thermal variation as well as temperature fluctuations across days (rather than hours), current theory would suggest it should be highly plastic between seasons. These are all the conditions where we would expect thermal plasticity to evolve (Gabriel and Lynch, 1992; Angilletta, 2009). So, why do they not acclimatize? In short, because there is no evolutionary pressure to do so as the costs may outweigh the benefits. As exemplified by fishes, the primary factor driving thermal compensation is predation. A fish is either a predator or prey. In either case, it is of obvious advantage to maintain locomotory performance. Consequently, cold acclimation typically leads to compensation of a suite of biological processes that ultimately affect locomotion (e.g. Johnston and Temple, 2002; Woytanowski and Coughlin, 2013). Changes typically include alterations in the energy production machinery either at a molecular level, taking the form of alterations in enzyme isoform expression and membrane lipid compensation, or at the cellular level, with changes in mitochondrial volume density (Sidell, 1983; Johnston et al., 1998; Guderley, 2004b; Abele, 2012). In the muscle itself, there can be changes in ion channel function (McArdle and Johnston, 1982; Godiksen and Jessen, 2002) as well as changes in muscle fibre protein composition (Johnston and Temple, 2002; Tattersall et al., 2012) and recruitment order (Rome, 1990; Catalán et al., 2004). At a physiological level, there are also changes in cardiac function (Keen and Farrell, 1994; Lurman et al., 2012). These factors combined lead to maintained swimming performance despite changes in temperature, and thus maintained ability to out-swim predators or catch prey. Of course we cannot rule out similar molecular or cellular acclimation processes in *A. anatina*, despite our integrative measures generally showing otherwise.

Indeed, similar compensatory mechanisms have been identified in bivalves (Abele, 2012). The only examination of bivalve

**Table 3. Summary of the statistical analysis of behavioural parameters**

| Parameter | Summer | Winter | *P* | Test type |
|---|---|---|---|---|
| Burrowing duration (h) | 1.15±0.14 (7) | 3.17±0.65 (5) | 0.034 | Mann–Whitney |
| BRI | 4.15±0.90 (7) | 1.23±0.27 (5) | 0.010 | Mann–Whitney |
| % Buried | 74.3±10.6 (7) | 80.0±7.07 (5) | 0.934 | Mann–Whitney |
| Closure duration (h) | 6.30±1.11 (8) | 22.6±4.45 (8) | 0.007 | *t*-test, Welch's correction |
| Closure frequency ($day^{-1}$) | 2.07±0.27 (8) | 0.32±0.06 (8) | <0.001 | Mann–Whitney |
| Time at rest (%) | 20.1±2.58 (8) | 48.0±5.72 (8) | <0.001 | Logit, *t*-test |

Values are means ± s.e.m. (*N*).

The Journal of Experimental Biology (2014) doi:10.1242/jeb.093450

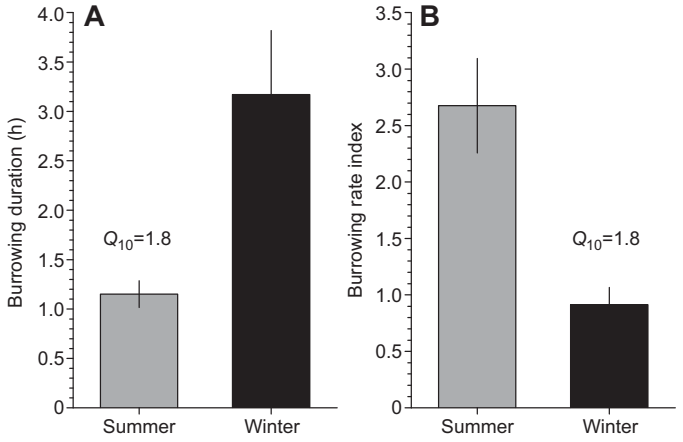


**Fig. 4. Burrowing duration and burrowing rate index (BRI) in summer (24°C)- and winter (4°C)-acclimatized *Anodonta anatina*.** Significant differences were seen between summer and winter mussels for both duration and BRI.

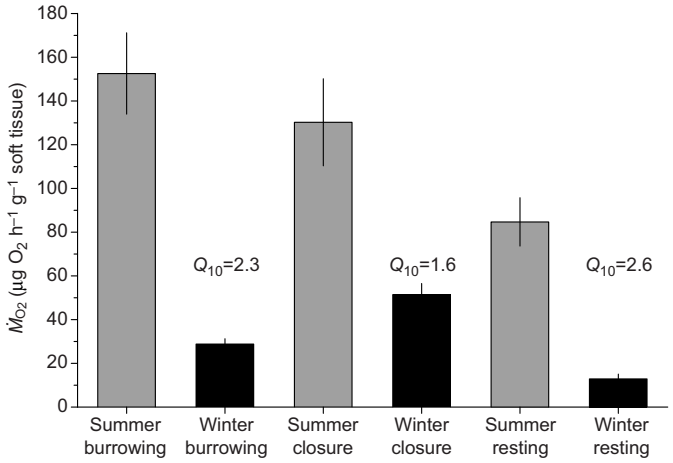


**Fig. 6. Oxygen consumption rate ($\dot{M}_{O_2}$) during burrowing and immediately after valve closure, and resting oxygen consumption rate in summer (24°C)- and winter (4°C)-acclimatized *Anodonta anatina*.** Significant differences were seen between summer and winter mussels for all three oxygen consumption rates.

locomotory performance we can find showed that swimming performance in the scallops *Chlamys islandica* and *Euvola ziczac* was not affected by changes in temperature; however, the recovery duration was prolonged (Guderley, 2004a). This can be explained in part by the fact that swimming is anaerobic, and thus relatively temperature independent. Recovery, by contrast, depends upon aerobic machinery, which is in turn dependent upon metabolic rates set, at least in part, by temperature.

With respect to *A. anatina*, there is no reason to believe that these mussels are predated upon in their natural setting. What is more, even if they were, they would have no chance of out-running a predator given that their maximal 'walking' speed is less than 1 cm $min^{-1}$ between 20 and 26°C. Indeed the best strategy they can, and indeed do, adopt is to simply shut their valves and wait for any predator to leave. In light of this, the longer a mussel can stay closed, the better. The consequence of which is that their metabolic rates are kept as low as possible. Moreover, these mussels are not active predators and compensation of locomotory rates to enable prey capture is equally unnecessary, and with respect to keeping the metabolic rate low, also of potential disadvantage. If they want to increase their feeding rate they must simply increase filtration rates, as seen in other bivalves (Thompson and Bayne, 1972; Riisgård et al., 2003). However, given that algal concentrations in freshwater bodies are generally low in winter months, there is little reason to increase filtration rates. Thus, being neither predator nor prey, there is little for *A. anatina* to gain by increasing locomotory ability, as the increased metabolic rate would reduce their ability to close their valves when under threat, i.e. the costs must outweigh the benefits.

That said, there are other forms of thermal compensation and acclimation that we did not explore. The phenomena of heat and cold hardening have been well studied in plants, bacteria and fruit flies. There is a clear demonstrable advantage where short-term exposure to very high or low, but non-lethal temperatures can result in a favourable shift in the upper or lower critical temperature, respectively, while other physiological and performance characteristics remain unchanged (Hoffmann, 1995). One study recently found that acclimation can have an (albeit minimal) affect on the upper critical thermal maximum in North American freshwater mussels, with an increase of 1–2°C following acclimation seen in two of three species tested (Galbraith et al., 2012). With many species living close to their upper thermal limit, a difference of 1–2°C may be the difference between life and death. It remains to be seen whether the thermal tolerance limits show signs of thermal compensation in *A. anatina*.

To summarise, *A. anatina*, like many bivalves, has a very low-energy lifestyle. It inhabits a relatively stable thermal environment (in the short-term when compared with the marine intertidal), where temperature changes are slow. We suggest that these two factors serve as selective pressures for these mussels to not compensate behaviour or metabolic rate. Because their only predator avoidance mechanism is to close their valves, combined with the fact that they are subject to considerable seasonal fluctuations in food supplies, they instead simply allow their behaviour and metabolic rate to fluctuate at the behest of the seasons. Given the 500 million year fossil record for bivalves, this is most clearly an ecologically and evolutionary successful strategy that needs to be further explored and more explicitly considered in acclimation/acclimatization models.

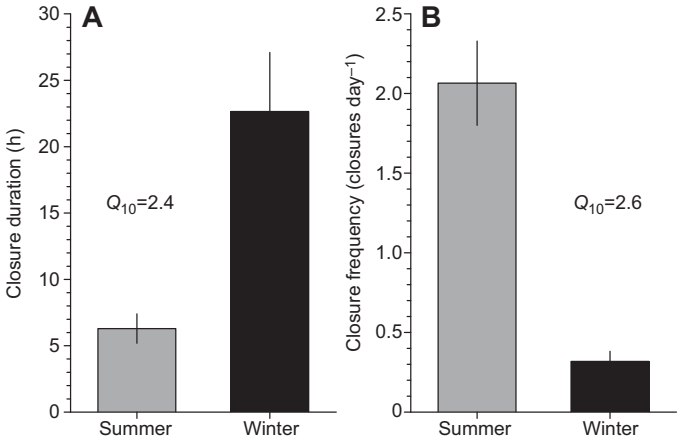


**Fig. 5. Valve closure duration and valve closure frequency in summer (24°C)- and winter (4°C)-acclimatized *Anodonta anatina*.** Significant differences were seen between summer and winter mussels for both duration and frequency.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.093450

**Table 4. Summary of the statistical analysis of oxygen consumption ($\dot{M}_{O_2}$; μg $O_2$ $h^{-1}$ $g^{-1}$ soft tissue)**

| Parameter | Summer | Winter | *P* | Test type |
|---|---|---|---|---|
| Burrowing $\dot{M}_{O_2}$ | 152±18.6 (7) | 28.8±2.44 (5) | 0.025 | Mann–Whitney |
| $\dot{M}_{O_2}$ immediately post closure | 130±19.9 (8) | 51.4±5.12 (8) | 0.007 | *t*-test, Welch's correction |
| Resting $\dot{M}_{O_2}$ | 84.7±11.0 (8) | 12.9±2.22 (9) | <0.001 | *t*-test, Welch's correction |

Values are means ± s.e.m. (*N*).

## MATERIALS AND METHODS

Lake Murtensee water temperature (±0.5°C) was recorded at 4-hourly intervals between 9 May 2011 and 10 May 2012 using an iButton temperature logger (Embedded Data Systems, Lawrenceburg, KY, USA) placed on the lake bottom at a depth of 1 m, ~50 m from the shore (46°54′21.5″N, 7°3′0.6″E).

In the first experiment (hereafter Experiment 1), designed to determine changes in burrowing, valve closure behaviour and locomotion as a result of seasonal temperature changes, mussels (*A. anatina*) were collected by hand in ~1 m of water from the same location as above, each month for a year, from May 2011 until April 2012. Lake sediment was also collected from the same location for the burrowing experiments. Mussels were cleaned of epibiota before being transported to the University of Bern (~40 km) in a 2 litre sealed box filled with lake water, and were then measured and weighed (supplementary material Table S1). Mussels were kept in a 60 litre aquarium filled with 100% air-saturated, charcoal-filtered, aged tap water at the same temperature as the lake for a maximum of 3 weeks. Mussels were not fed during this time. Water was constantly aerated. The aquarium was kept in a cold-room and temperature was maintained within 0.5°C of the target temperature using a timer-controlled 50 W aquarium heater. The water concentrations of nitrate and nitrite were checked regularly and kept below 0.1 and 10 mg $l^{-1}$, respectively, with weekly water changes.

The total length of time that the individual mussels were observed varied between ~1 and 5 days, with longer observation periods required at lower temperatures (see supplementary material Table S1 for exact details). Artificial lighting followed the natural light:dark cycle, and a low wattage red light was constantly on to allow behavioural observation at night (see below).

In the second experiment (hereafter Experiment 2), designed to quantify the energetic cost of burrowing and valve closure using respirometry, naturally acclimatized mussels were collected from the same location in either summer, with experiments run between August and September 2011, or winter, with experiments run between January and March 2012, and held at 24±1°C or 4±1°C, respectively. Mussel collection, transportation and holding were as described above.

### Behavioural observation

For Experiment 1, single mussels were placed in one of three rectangular (210×100 mm) 1.5 litre containers containing autoclaved lake sediment in the aquarium, and were allowed to move and burrow voluntarily. Behaviour was recorded for both experiments using a webcam connected to a computer that took a time-stamped picture every 30 s. Pictures were then compiled into time-lapse film at a rate of 2 frames $s^{-1}$ using Quicktime Player Pro 7.7.3 (Apple Inc., Cupertino, CA, USA). The time at which given activities, namely burrowing, valve closure and opening, as well as locomotory activity, occurred were recorded manually.

Several phases involved with burrowing were identified, firstly probing with the foot, and then erection into a vertical position, before the actual burrowing typically began. The burrowing duration was defined by the start of the burrowing cycle, once the mussel was in a vertical position, and the end, i.e. once the mussel was completely inactive. The burrowing rate index (BRI) was calculated using the following equation, as given by Peck et al. (Peck et al., 2004):

$$\mathrm{BRI} = (M_{\mathrm{mw}} / d_{\mathrm{burrow}})^{1/3} \times 1000, \quad (1)$$

where $M_{\mathrm{mw}}$ is mussel whole mass (g) and $d_{\mathrm{burrow}}$ is burrowing duration (s).

A 20 mm linear black and white scale marked directly on both long edges of the 1.5 litre containers allowed for the determination of voluntary locomotory speed. This was calculated from the distance moved along the scale in a given time period.

### Respirometry

The respirometry chamber, a custom-made plastic respiration chamber made of clear Perspex with a middle section that could be filled with autoclaved lake sediment (1.4 litres, see Fig. 7), was connected to a 600 l $min^{-1}$ water pump with two one-way valves that allowed the chamber to be flushed with aerated, fully oxygenated water for two out of every 30 min using an automatic timer. The air saturation of the chamber water never decreased below 90% in this time. Two minutes was sufficiently long to completely recharge the chamber with 100% air-saturated water. The gas-tightness of the chamber was regularly checked by bubbling the water with nitrogen gas and then monitoring oxygen concentration over a 24 h period. A fluorescence oxygen electrode (Model FDO925, WTW, Weilheim, Germany) recorded the water oxygen concentration (mg $l^{-1}$) at 30 s intervals. All measurements were automatically temperature compensated.

Mussels were held for at least 2 days before being used for the respirometry experiments. Mussels of a similar size (Table 5) were used for the determination of oxygen consumption rates to avoid allometric scaling effects. For Experiment 2, an individual mussel was selected at random and placed in the chamber, atop the sediment on its side. Mussels were observed and behaviours quantified as per Experiment 2. Sediment was autoclaved to

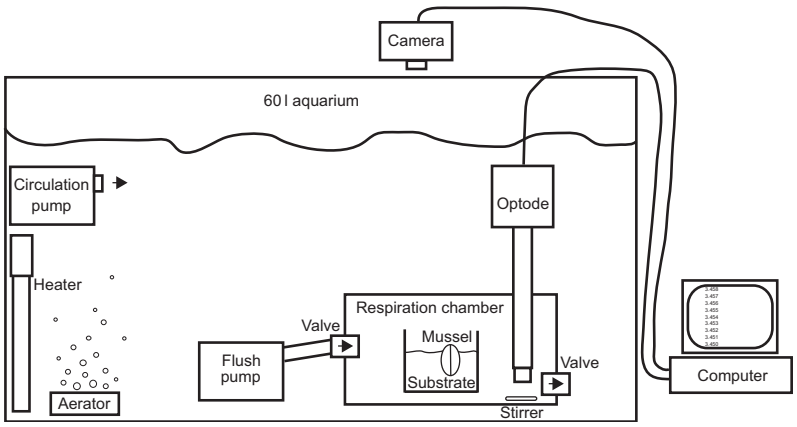


**Fig. 7. A schematic diagram of the custom-made respiration chamber used for determining oxygen consumption in summer and winter acclimatized *Anodonta anatina* during burrowing.** The flush pump was connected to an electronic timer programmed to flush the chamber with fully aerated water via the one-way valves for 2 min every half hour.

**Table 5. Morphometric data from mussels used for the determination of oxygen consumption**

| Parameter | Summer | Winter | *P* | Test type |
|---|---|---|---|---|
| Whole mussel mass (g) | 43.4±4.94 | 40.7±6.58 | 0.752 | *t*-test |
| Length (mm) | 74.7±2.63 | 76.5±4.08 | 0.723 | *t*-test |

Values are means ± s.e.m.

minimise background oxygen consumption and provided a substrate for voluntary burrowing. The mussel was then left in the chamber for 5–7 days and allowed to burrow voluntarily. The drop in the oxygen concentration (mg $l^{-1}$) in the chamber once sealed was equivalent to the mussel's oxygen consumption rate minus the background oxygen consumption. Specifically, the amount of oxygen in the chamber ($A_{O_2}$; mg) was calculated according to:

$$A_{O_2} = [O_2] \times V_c , \quad (2)$$

where $V_c$ is the chamber volume (l).

The background oxygen consumption (i.e. of the closed chamber without a mussel; mg $O_2$ $h^{-1}$) was measured for a minimum of 2.5 h at the beginning ($\dot{M}_{O_2start}$) and end ($\dot{M}_{O_2end}$) of the trial, with each calculated using:

$$\dot{M}_{O_2} = (\Delta O_2 / \Delta T) , \quad (3)$$

where $\Delta T$ is the change in time in hours. The rate of drift ($D$; mg $O_2$ $h^{-1}$ $h^{-1}$), that is, the steady increase in background oxygen consumption throughout each individual trial, was calculated using:

$$D = (\dot{M}_{O_2end} - \dot{M}_{O_2start}) / \Delta T . \quad (4)$$

The rate of drift was then used to calculate the drift factor (DF; mg $O_2$ $h^{-1}$) using:

$$DF = D \times T_{meas} , \quad (5)$$

where $T_{meas}$ is the measurement time (h). The drift factor was subtracted from the $\dot{M}_{O_2}$ at each time point using the following equation:

$$\dot{M}_{O_2} = (\Delta O_2 / \Delta T) - DF . \quad (6)$$

Expression of oxygen consumption rates per whole mussel (including shell and cavity water), per gram of whole mussel or per gram wet soft tissue made little difference to the results. To enable comparison with other data, oxygen consumption rates are expressed here in μg $O_2$ $h^{-1}$ $g^{-1}$ soft tissue, where the oxygen consumption derived using Eqn 6 was multiplied by the wet soft tissue mass of the mussel. Wet soft tissue masses ($M_{sw}$) were calculated from the whole mussel mass using Eqn 7, which was derived from a correlation of mussel whole mass and soft tissue wet mass.

$$M_{sw} = (0.29 \times M_{mw}) + 1.09 . \quad (7)$$

No significant differences were found between correlations for summer and winter mussels ($F_{2,77}$=0.49, $P$=0.61), so samples were pooled. Whole mussel mass spanned a range from 23.3 to 105.6 g.

## Statistics

All analyses were performed using Prism 5.0 (Graphpad Software, La Jolla, CA, USA). All data were first checked for a Gaussian distribution using a Shapiro–Wilk test before analysis. Significant differences in seasonal changes of each rate, namely BRI, valve closure duration and frequency, and locomotory speed, as well as for the mass of the mussels used each month were assessed using a non-parametric one-way Kruskal–Wallis ANOVAs. Dunn's multiple comparison test was used to isolate significant differences between individual months. Linear models were fit to R–T plots of $\log_{10}$ rate versus temperature (1000/K). For the second experiment comparing summer- with winter-acclimatized mussels, the following parameters were checked for significance differences using parametric Student's two-tailed *t*-tests: mussel whole mass, burrowing time, BRI, proportion of mussel buried, resting oxygen consumption, proportion of time spent closed, valve closure duration, frequency of valve closure. The following parameters were non-Gaussian and were thus subjected to non-parametric Mann–Whitney two-tailed *t*-tests: burrowing duration, BRI, % buried, oxygen consumption during burrowing and valve closure frequency. A two-way repeated-measures ANOVA was used to examine differences in the recovery rate after burrowing.

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**Competing interests**
The authors declare no competing financial interests.

**Author contributions**
G.L. made significant and substantial contributions to the conception, design, execution and interpretation of the findings being published, and drafting and revising the article. J.W. made significant and substantial contributions to the design, execution and interpretation of the findings being published. H.H.H. made significant and substantial contributions to the conception, design and interpretation of the findings being published, and drafting and revising the article.

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