

# Cyclic temperatures influence growth efficiency and biochemical body composition of vertically migrating fish

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## SUMMARY

1. Diel vertical migrations (DVM) are frequently observed in pelagic fish and zooplankton populations. In addition to predator avoidance and foraging opportunity, bioenergetics optimisation has been hypothesised to cause the selective advantage of migrating fish. However, experimental confirmation of growth advantages of fish held at naturally observed cyclic temperatures, and food densities are surprisingly rare.

2. We compared growth rates, growth efficiencies and energy budgets of vendace (*Coregonus albula*, Salmoniformes) fed daily rations of 10% body mass and held at low (4.5 °C), high (8 °C) and cyclic (switch between 4.5 and 8 °C) temperatures over a period of 6 weeks. Biochemical body composition was evaluated by bioimpedance analyses and direct determination of water, lipid and protein content in a subset of fish.

3. Growth rate and food conversion efficiency were similar in fish held at high and cyclic temperatures, but higher than those found at low temperatures. Body condition of fish at cyclic temperatures was maximised, but these fish also had the highest water content. The proportion of lipids and proteins was slightly depleted in fish held at cyclic and low temperatures relative to the high temperature treatment. Accordingly, growth and biochemical composition of fish responded specifically to cyclic temperatures and were not simply intermediate between those found at low and high temperatures.

4. We could not confirm a bioenergetics advantage of fish switching between high and low temperatures. However, there were no substantial extra metabolic costs through exposure to cyclic temperatures, and hence, fish performing DVM may benefit from predation avoidance without compromising their metabolic balance and hence growth rates. These results suggest that the evolution of DVM is a multi-faceted process with no single ultimate explanation.

*Keywords:* bioenergetics advantage, bioimpedance analysis, *Coregonus*, diel vertical migration, lipids

## Introduction

Diel vertical migration (DVM) is a cyclic habitat switch frequently observed in marine and freshwater fish populations. Usually, fish ascend from deep into

shallow water layers at dusk and descend back at dawn (Scheuerell & Schindler, 2003). The proximate trigger of DVM is the gradual change of illumination strength in the water during the crepuscular periods (Scheuerell & Schindler, 2003). Several ultimate

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explanations for DVM have been proposed. Primarily, DVM is seen as a behavioural response to avoid visual predators (Scheuerell & Schindler, 2003; Hrabik *et al.*, 2006). In some other cases, feeding opportunity has been suggested explaining DVM because prey seemed to track the spatial movements of their resources (Janssen & Brandt, 1980; Rosland & Giske, 1994).

A third ultimate explanation of DVM is referred to as bioenergetics optimisation. Planktivorous fish gain metabolic benefits when switching between layers which differ in temperature and resource density (Brett, 1971; Biette & Geen, 1980a; Bevelhimer & Adams, 1993). Dogfish [*Scyliorhinus canicula* (L.)] could lower daily energy costs by 4% by adopting a 'hunt warm – rest cool' strategy (Sims *et al.*, 2006). Just the opposite strategy, 'feed cool – digest warm', has been suggested to explain the bioenergetics benefits of DVM in Bear Lake sculpin (*Cottus extensus* Bonce & Baird) (Wurtsbaugh & Neverman, 1988). In contrast, low bioenergetics benefits of DVM have been concluded from observations of fish growth in several field studies (Johnston, 1990; Levy, 1990).

Surprisingly, few experimental studies have addressed the bioenergetics efficiency of DVM. Growth rates and gross growth efficiency were measured in underyearling sockeye salmon (*Oncorhynchus nerka*) held at constant and cyclic temperatures (Biette & Geen, 1980a). At moderate resource densities, salmon grew more rapidly and efficiently at cyclic than at constant temperatures (Biette & Geen, 1980a). Juvenile Bear Lake sculpin held at a temperature and in feeding regimes that mimicked those experienced by migrating fish grew substantially faster than those reared at low temperatures (Neverman & Wurtsbaugh, 1994). Both experiments thus demonstrate that DVM is energetically favourable at low to moderate resource densities. However, fish performing DVM feed most intensively while ascending and descending through layers with high resource densities during crepuscular periods (Scheuerell & Schindler, 2003). Therefore, the certainly most appropriate simulation of the natural situation is to offer high food rations at crepuscular periods in the experiments. Furthermore, temperature gradients along the migration amplitude may differ between lakes, and therefore, bioenergetics advantages of DVM for a specific system can be demonstrated only by experimentally applying the *in situ* observed temperature range between daytime and nighttime habitats.

To elucidate the potential bioenergetics advantages of DVM at high resource densities, we compared conversion and production efficiencies of vendace (*Coregonus albula* L.) held at cyclic, low or high temperatures over 6 weeks. Vendace are pelagic, zooplanktivorous fish (order Salmoniformes) commonly inhabiting deep lakes of the Baltic and Scandinavian region of Europe (Mehner *et al.*, 2007a). The fish used in our experiments originated from Lake Stechlin (Germany) where vendace perform DVM (Mehner, Kasprzak & Hölker, 2007b). We confined the temperature difference between cold and warm water to 3.5 °C, thus matching the temperature gradient vendace experienced during their daily migrations (Mehner *et al.*, 2007b). We run the experiments at high resource densities simulating summer and autumn conditions in Lake Stechlin when zooplankton density is highest and the vertical temperature gradient is established because of stable thermal stratification of the lake (Mehner *et al.*, 2010). The applied feeding and temperature conditions mimicked the intense feeding periods of vendace at dusk and dawn and the presumed quick digestion in the warmer water at night.

We predicted that (i) fish held at the highest temperatures (8 °C) would grow fastest, similar to earlier results obtained from temperature-dependent growth experiments in salmonids (Koskela, Pirhonen & Jobling, 1997b; Elliott & Hurley, 1999; Lyytikäinen & Jobling, 1999; Gunther, Moccia & Bureau, 2007). We calculated energy budgets to compare the metabolic effects of maintenance at cyclic, high or low temperatures. We predicted that (ii) fish held at cyclic temperatures (switch between 4.5 and 8 °C) should have a higher growth rate than fish held at low temperatures (4.5 °C), caused by two processes. First, fish can digest the food offered at the switch to high temperatures quickly, thus enhancing the capacity to feed during the switch to low temperatures, and second have lower rates of defecation and excretion in comparison to fish held at constant low temperature (Biette & Geen, 1980b; Wurtsbaugh & Neverman, 1988). Finally, we measured the biochemical body composition of fish from the different temperature regimes. The proximate body composition of fish, in particular the lipid and water content, is a sensitive indicator for resistance of fish against malnutrition and environmental stress (Adams, 1999; Pangle & Sutton, 2005). We predicted that (iii) the lipid and

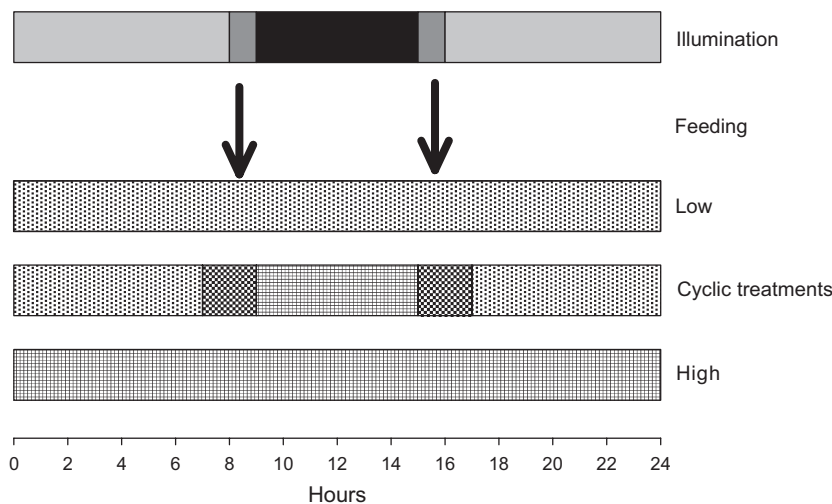
protein proportions of the fish may be higher at the high than at low temperatures (Gunther *et al.*, 2007), but cyclic temperatures may induce even higher lipid concentrations relative to fish held at constant high temperatures (Biette & Geen, 1980b).

## Methods

### Experimental protocol

We used *c.* 1½-year-old European vendace raised from artificial reproduction of parents originating from Lake Stechlin (53°10'N, 13°02'E, Germany). Larvae were hatched at 4 °C and subsequently raised in circular basins (80 L). They were fed with rotifers, brine shrimp and commercially available dry food. The juveniles were maintained in a 500-L basin at about 6–7 °C and fed twice per day *ad libitum* with commercial salmon dry food (*Trouvit Pro Aqua Brut*, Milkivit, Burgheim, Germany). Experiments were conducted at three temperature regimes, namely high (constant 8 °C), low (constant 4.5 °C) and cyclic (two daily switches between 4.5 °C for about 16 h and 8 °C for about 8 h). These temperatures represent the amplitude covered by vendace migrating between the upper and lower hypolimnion in Lake Stechlin during thermal stratification (Helland *et al.*, 2007; Mehner *et al.*, 2010). To obtain temperatures of the cyclic regime, fish were held in 140-L glass

aquaria with a computer-controlled temperature-regulation system with heating and cooling units (adapted from Staaks, Kirschbaum & Williot, 1999). The cooling and heating phases lasted two hours each (Fig. 1), similar to the duration of DVM of vendace in Lake Stechlin (Mehner, 2006; Busch & Mehner, 2009). For the constant high and low temperature treatments, fish were kept in cylindrical fibre-glass tanks with a volume of about 120 L, because further glass tanks similar in dimension to those used for cyclic temperatures were not available. We do not assume that the differing construction and dimension of the tanks caused systematic differences between the experimental treatments. All tanks were surrounded by light-impermeable curtains to avoid disturbances and were illuminated with dimmable fluorescent lamps (30 W) fixed above. The lamps were covered with blue plastic foil to reduce the light intensity and to simulate the light colour spectrum of deeper water layers. All tanks were exposed to a 16:08 hours light:twilight photoperiod. Light intensity was measured in 15 cm water depth using a research radiometer IL 1700 and SUD033/Y/W sensor (International Light Technologies, Peabody, MA, U.S.A.). The daytime light intensity was 25 lx and twilight represented 22% of daylight illumination (5.5 lx). Illumination strength during twilight mimicked the natural illumination during daytime in hypolimnetic layers and the



**Fig. 1** Schematic view of the 24 h cycle during the experiments. Illumination was changed from bright illumination (light grey) over a dimming period (dark grey) to twilight (black) and back. The cyclic temperature treatment consisted of low (4.5 °C, sparse dots) and high (8.0 °C, dense dots) phases with intermittent phases (intermittent dots) during which temperatures were adjusted to the next phase. Low and high treatments had unchanged temperatures. The two feeding phases are indicated by arrows.

illumination experienced during migrations at crepuscular periods in Lake Stechlin, thus facilitating efficient feeding of vendace (Ohlberger *et al.*, 2008a; Gjelland *et al.*, 2009). The light regime was computer-controlled with a transition period of one hour between minimum and maximum light (Fig. 1). Accordingly, the constant low or high temperature regimes mimicked the environmental conditions of potentially stationary fish in either lower or upper hypolimnetic layers of a stably stratified lake. The cyclic regime mimicked the temperature and light variations fish experience while migrating during crepuscular periods, but did not mimic the complete darkness that may occur naturally in moonless nights.

Each treatment tank was stocked with 13 fish of approximately the same size (mean total length  $\pm$  SE:  $12.4 \pm 0.09$  cm, range 10.6–15.0 cm), randomly selected from the main holding tank and assigned to the experimental temperature regimes. There were two replicate groups for each treatment ( $n = 6$  replicates in total,  $n = 78$  fish in total). Although a different design with 26 replicates (tanks) per treatment with a single fish each would have been the statistically superior approach, this was not achievable because vendace are shoaling fish, sensitive to stress, and stop feeding when held in isolation or too small groups. Total experimental duration was limited to 6 weeks (42 days) to prevent strong tank effects. Both oxygen content ( $\text{mg L}^{-1}$ ) and temperature ( $^{\circ}\text{C}$ ) were checked in all six tanks daily. Vendace were fed constant rations of commercial dry pellet feed (*Trouwit Pro Aqua Brut*, Milkivit; 57% protein, 15% lipid, digestible energy  $19.1 \text{ kJ g}^{-1}$ ), the ration approximating the *a priori* estimated maximum daily ration [ $0.1 \text{ g food (g fish wet mass)}^{-1} \text{ d}^{-1}$ ]. The daily ration was split into equal amounts of food given during both crepuscular periods. Food was supplied manually in one batch spread slowly into the tank. Fish usually caught the sinking food within the water column, but occasionally took also particles from the bottom.

### Calculations

To obtain individual growth rates, individual recognition of vendace was necessary. An individual-specific photo-ID was created at the start of the experiment using the particular coloured patterns of the head region and visible bone structures of the

skullcap and the operculum and circumorbital bones from both dorsal and lateral views. These patterns did not change during the six experimental weeks and allowed unequivocal recognition of individuals at the end of the experiments (Data S1).

Fish were weighed (g fish wet mass,  $w_m$ ) at the beginning ( $w_{m1}$ ) and the end ( $w_{m2}$ ) of the experiment. Because vendace mature after the second summer, final gonad mass (g) was estimated after dissection of fish at the end of the experiment in 77 of 78 individuals (in one fish gonads could not be quantitatively removed from the body cavity). In altogether 67 individuals, sex could be determined visually, but there were no effects of sex on any of the measured variables (results not shown). Individual instantaneous growth rates  $g$  ( $\text{day}^{-1}$ ) were calculated from the individual mass increment over the 42 experimental days, by

$$g = (\log_{10} w_{m2} - \log_{10} w_{m1})/42$$

A measure to indicate condition of fish was obtained by calculating a linear regression between  $\log_{10}$ -transformed final length and  $\log_{10} w_{m2}$  of fish, and by calculating the residuals from this regression separately for each fish (Kaufman *et al.*, 2007).

### Energy budget

We used Fish Bioenergetics 3.0 (Hanson *et al.*, 1997) to calculate respiration ( $R$ ), egestion ( $F$ ), excretion ( $U$ ) and specific dynamic action (SDA) rates of vendace (all in  $\text{J g}^{-1} \text{ day}^{-1}$ ). We applied the parameter set originally developed for bloater (*Coregonus hoyi*) characterised by fixed proportions of consumption for  $F$ ,  $U$  and SDA (see Hanson *et al.*, 1997), but slightly modified the parameters for respiration according to own measurements of oxygen consumption of vendace (Ohlberger, Staaks & Hölker, 2007) (according to the common parameter notation of Fish bioenergetics:  $RA = 0.022$ ,  $RB = -0.21$ ,  $RQ = 0.071$ ,  $RTO = 0.021$ ). We calculated the relative daily consumption rate ( $C$ ,  $\text{J g}^{-1} \text{ day}^{-1}$ ) from the observed average mass increase (somatic production  $P$ ,  $\text{J g}^{-1} \text{ day}^{-1}$ ) per replicate, by taking into account the differing temperatures per treatment (4.5, 8 and  $5.67^{\circ}\text{C}$  as daily average from the cyclic temperature treatment). The energetic density of fish wet mass ( $E$ ,  $\text{J g}^{-1}$ ) was calculated by

$$E = 5083 + 32.4 \text{ wm} \quad (\text{Gjelland, 2008}).$$

The main variations between treatments in bioenergetics budgets were the temperature-dependent respiration rates, the differing growth and hence production rates, and the re-calculated consumption rates. To compare the treatments, we calculated the gross food conversion efficiency ( $K_1 = P/C$ ) and the net production efficiency [ $K_2 = P/(P + R)$ ] (Winberg, 1956), separately for all replicates. In replicate 2 of the high temperature treatment, fish fed the food almost entirely every feeding event, equivalent to a rate of about  $191 \text{ J g}^{-1} \text{ day}^{-1}$ . This value was used to validate the bioenergetics calculations. In all other treatments, some food was left unfed and mixed with faeces. These remains were removed about two hours after feeding, dried and weighed. However, it was impossible to differentiate between unused food and faeces, and therefore, these data could not be used to estimate real consumption rates of fish.

#### Bioimpedance analysis

At the end of the experiment, all fish were stunned by a blow to the head and immediately killed by spinal transection. Subsequently, bioelectrical impedance analyses (BIA) were conducted. Tissues with low water content (e.g. adipose tissues) have a different conductivity than tissues with high water content (e.g. protein matrix) (Lukaski *et al.*, 1985). Water offers less resistance to electrical current than lipids, and therefore, higher resistance equates to higher amounts of lipids or non-conductive material such as bone (Pothoven *et al.*, 2008). BIA is commonly used in human medical diagnostics for assessing nutritional condition, but increasing numbers of studies on fish become available (Cox & Hartman, 2005; Pothoven *et al.*, 2008; Willis & Hobday, 2008).

BIA was conducted with a bioelectrical impedance analyzer (BIA-Quantum II Bioelectrical Body Composition Analyzer, RJL Systems, Clinton Township, MI, U.S.A.; current  $800 \mu\text{A}$  AC and frequency  $50 \text{ kHz}$ ). The system was calibrated by the provided test resistor. The BIA system consists of two sets of needle electrodes, one signal and one detecting electrode, placed  $1 \text{ cm}$  apart (tetrapolar measurement). The two sets of electrodes were placed in a consistent morphological position at the left side of

fish. One set of electrodes was placed near the operculum, whereas the other set was placed near the adipose fin. The four electrodes were softly penetrated  $2 \text{ mm}$  into the fish. The distance between signal and detector electrodes was kept constant, while the distance between the two inner detecting electrodes differed from fish to fish.

Series resistance ( $R_m$ ,  $\Omega$ ), series reactance ( $X_m$ ,  $\Omega$ ) and the distance between inner electrodes ( $D$ ,  $\text{mm}$ ) were recorded. Parallel resistance  $R_p$  was calculated by

$$R_p = R_m + (X_m^2 R_m^{-1})$$

(Pothoven *et al.*, 2008). The composition index (CI,  $\text{mm}^2 \Omega^{-1}$ ) was determined as

$$\text{CI} = D^2 \cdot R_p^{-1}$$

(Cox & Hartman, 2005; Willis & Hobday, 2008). The composition index increases linearly with the absolute amount (in g) of lipids, protein or water in the fish, but the slopes of these regressions differ (Pothoven *et al.*, 2008).

#### Biochemical body composition of a subsample of fish

Three fish randomly selected from each of the six experimental replicates ( $n = 18$  in total) were analysed for their biochemical body composition (body protein,  $\text{g g}^{-1} \text{ wm}$ ; lipid concentration,  $\text{g g}^{-1} \text{ dry mass (dm)}$ ; water content,  $\text{g g}^{-1} \text{ wm}$ ). More fish could not be analysed because of logistic reasons. For all measurements, small pieces of muscle from the left side of the fish (about  $1 \text{ g}$ ) were excised. The tissue was shortly exposed to liquid nitrogen, wrapped in aluminium foil, bagged and stored at  $-80 \text{ }^\circ\text{C}$  until chemical analysis. Protein determination followed standard protocols (Lowry *et al.*, 1951; Schacterle & Pollock, 1973). Lipid was determined gravimetrically from dry mass of tissue, following the standard extraction protocol (Bligh & Dyer, 1959; Manirakiza, Covaci & Schepens, 2001). Water content was determined by comparing  $\text{wm}$  and  $\text{dm}$  (drying at  $90 \text{ }^\circ\text{C}$  over  $24 \text{ h}$ ).

Subsequently, we correlated the BIA composition index measured at the 18 undissected fish with the proximate body composition (g water, g lipid, g protein in the whole fish), as extrapolated from the proportions of the body constituents in the white



muscle from the same 18 fish (Cox & Hartman, 2005). These linear correlations were significant for all three body constituents (Pearson's  $r > 0.92$ ,  $n = 18$ ,  $P < 0.0001$ ). However, if measured and predicted (from bioimpedance composition index) relative proportions (%) of water, lipid and protein in the whole fish were compared, these correlations were not significant (Pearson's  $r < 0.46$ ,  $n = 18$ ,  $P > 0.054$ ), indicating that the between-individual variance in BIA measurements reflects predominantly the size variability between fish, and less the relatively minor changes of proximate body composition in response to the treatments. Therefore, in contrast to our original intention, we could not extrapolate the proximate body composition from the bioimpedance data for all fish in which biochemical composition was not directly measured.

#### Statistical analysis

Most of the variables ( $n = 78$  individual observations) were normally distributed (Lilliefors test) and showed homogeneity of variances (Levene's test). Minor deviations from normality, but homogeneous variances, were determined for growth rate and bioelectrical composition index from one replicate. Accordingly, we used analysis of variance (ANOVA) and analysis of covariance (ANCOVA) because these tests are quite robust to small deviations from normal distributions (Sokal & Rohlf, 1995).

Unfortunately, the randomised collection of fish from the holding tank created significant differences of initial fish mass between the six replicates (ANOVA,  $F_{5,72} = 7.43$ ,  $P < 0.0001$ , minimum mean mass 10.3 g in replicate 2 of the cyclic temperature treatment, maximum mean mass 15.9 g in replicate 2 of the high temperature treatment). However, the overall differences between the treatments were less substantial because of the inclusion of two replicates each (mean initial mass  $\pm$  SE: high:  $13.7 \pm 0.84$  g; cyclic:  $11.6 \pm 0.52$  g; low:  $12.4 \pm 0.73$  g). To account for these differences, we used nested ANCOVAs with initial fish mass as covariate and replicate nested in treatment to compare the effect of treatment (high, low, cyclic) on instantaneous growth rates, final gonad mass, residuals from the length-mass regression (fish condition) and bioimpedance composition index. The nested analysis of variances is recommended if each value of one nominal variable (here the replicates) is

found in combination with only one value of the higher-level nominal variable (here the treatments) (McDonald, 2009). The nested approach allows testing for overall differences between treatments while controlling for the effect of replicates on the individual measurements (tank effects) and thus prevents pseudoreplication from measurements of individual fish in the same tank (Hurlbert, 1984). If overall tests were significant, the estimated marginal means (group means corrected for effects of covariates) were pairwise compared by Tukey's tests with Bonferroni correction for multiple comparisons.

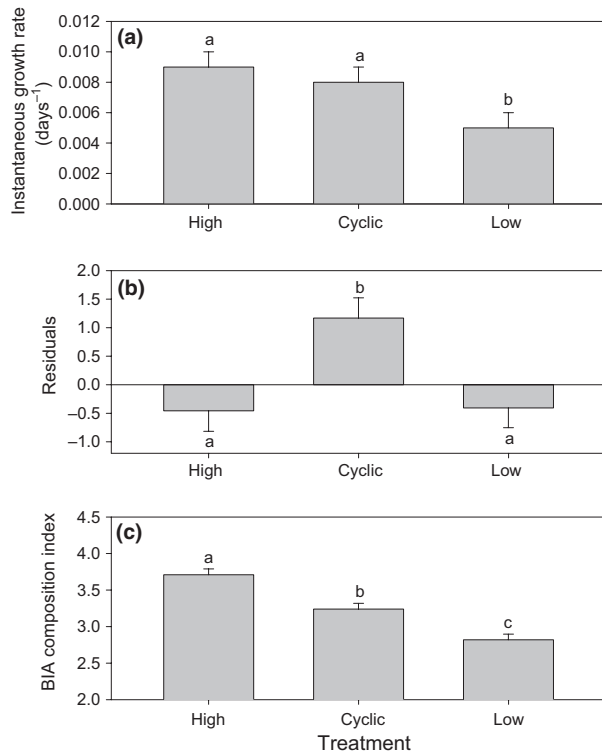
The proximate body composition (water, lipid and protein content) of the  $n = 6$  fish from each treatment was compared by ANCOVA with Sidak-corrected Tukey *post hoc* tests, by using final wm of fish as covariate to account for potential mass-dependency of body composition.

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, U.S.A.) and SIGMAPLOT 11.0 (Systat Software Inc., San Jose, CA, U.S.A.).

#### Results

Instantaneous growth rate was about 50% lower in vendace kept at low temperatures when compared with the high temperature treatment (Fig. 2a, Table 1, Tukey tests of estimated marginal means with Bonferroni correction: low-high:  $P < 0.001$ , low-cyclic:  $P < 0.001$ ), but did not differ between high and cyclic temperatures ( $P = 0.99$ ). The final gonad mass did not differ between the treatments or replicates (Table 1).

The linear regression between  $\log_{10}$ -transformed final fish length and mass was highly significant ( $F_{1,76} = 358.2$ ,  $R^2 = 0.82$ ,  $P < 0.0001$ ). The residuals from the regression indicating fish condition significantly differed between the treatments (Fig. 2b, Table 1). They were significantly higher in the cyclic than in both high and low temperature treatments (Tukey tests of estimated marginal means: high-cyclic:  $P = 0.007$ , cyclic-low:  $P = 0.006$ , high-low:  $P = 0.99$ ). The bioimpedance composition index differed significantly between the treatments ( $P = 0.049$ , Table 1) and the replicates ( $P = 0.033$ ). However, the effect of the covariate (initial fish mass) was highly significant ( $P < 0.0001$ , Table 1) in this comparison, suggesting that the average treatment-specific BIA composition index was strongly influenced by the differences in



**Fig. 2** Estimated marginal means  $\pm$  SEM of instantaneous growth rate ( $\text{day}^{-1}$ , a), residuals from the length-mass linear regression (fish condition, b) and bioimpedance composition index of parallel resistance (c) from three treatments with 26 vendace each, held at high ( $8.0\text{ }^{\circ}\text{C}$ ), cyclic (switch between  $4.5$  and  $8\text{ }^{\circ}\text{C}$ ) or low ( $4.5\text{ }^{\circ}\text{C}$ ) temperatures. Differing letters above the bars indicate significant differences between treatments, as tested by nested ANCOVA (see Table 1) with Bonferroni-corrected Tukey *post hoc* tests on estimated marginal means.

fish mass between the treatments. The treatment-specific estimated marginal means of the bioimpedance composition index (i.e., means corrected for the effect of fish mass) significantly declined from high over cyclic to low treatments (Fig. 2c, Table 1;

**Table 1** Results of nested ANCOVAs to compare the effect of treatment, replicate (nested in treatment) and initial fish mass (covariate) on instantaneous growth rate ( $\text{day}^{-1}$ ), final gonad mass (g), residuals from the length-mass regression (condition factor) and bioimpedance composition index ( $\text{mm}^2\ \Omega^{-1}$ ). Treatments consisted of 13 vendace each held at high ( $8.0\text{ }^{\circ}\text{C}$ ), cyclic (switch between  $4.5$  and  $8\text{ }^{\circ}\text{C}$ ) or low ( $4.5\text{ }^{\circ}\text{C}$ ) temperatures with two replicates per treatment

Variable	Effect	F	d.f.	P
Growth rate ( $\text{day}^{-1}$ )	Treatment	14.47	2,2.81	0.033
	Replicate (nested in treatment)	1.38	3,71	0.255
	Initial fish mass	0.09	1,71	0.766
Final gonad mass (g)	Treatment	0.43	2,2.87	0.684
	Replicate (nested in treatment)	1.94	3,70	0.131
	Initial fish mass	3.13	1,70	0.073
Length-mass residuals	Treatment	19.67	2,2.31	0.035
	Replicate (nested in treatment)	0.37	3,71	0.777
	Initial fish mass	2.55	1,71	0.115
BIA composition index ( $\text{mm}^2\ \Omega^{-1}$ )	Treatment	10.05	2,2.91	0.049
	Replicate (nested in treatment)	3.08	3,71	0.033
	Initial fish mass	212.3	1,71	<0.0001

BIA, bioelectrical impedance analyses.

Tukey tests of estimated marginal means: high-cyclic:  $P < 0.001$ , high-low:  $P < 0.001$ , cyclic-low:  $P = 0.001$ .

The bioenergetics model calculated a maximum consumption rate of  $189.8\ \text{J g}^{-1}\ \text{day}^{-1}$  from the average somatic growth observed for the 13 vendace in replicate 2 of the high temperature treatment (Table 2). This replicate was the only tank in which all food offered was completely eaten during each feeding event. Therefore, fish in this replicate have actually consumed the daily ration of  $191\ \text{J g}^{-1}\ \text{day}^{-1}$ . The good correspondence between bioenergetics estimate and observed consumption rate indicates that the bioenergetics model reliably reproduced the

**Table 2** Energy budgets of vendace held at high ( $8.0\text{ }^{\circ}\text{C}$ ), cyclic (switch between  $4.5$  and  $8\text{ }^{\circ}\text{C}$ ) and low ( $4.5\text{ }^{\circ}\text{C}$ ) temperatures with two replicates per treatment, as calculated by a bioenergetics model according to the average observed somatic growth of 13 fish in each replicate. Consumption (C), egestion (F), excretion (E), somatic production (P), respiration (R) and specific dynamic action (SDA) rates are given in  $\text{J g}\ \text{wm}^{-1}\ \text{day}^{-1}$ ,  $K_1 = P/C$  = gross food conversion efficiency,  $K_2 = P/(P + R)$  = net production efficiency

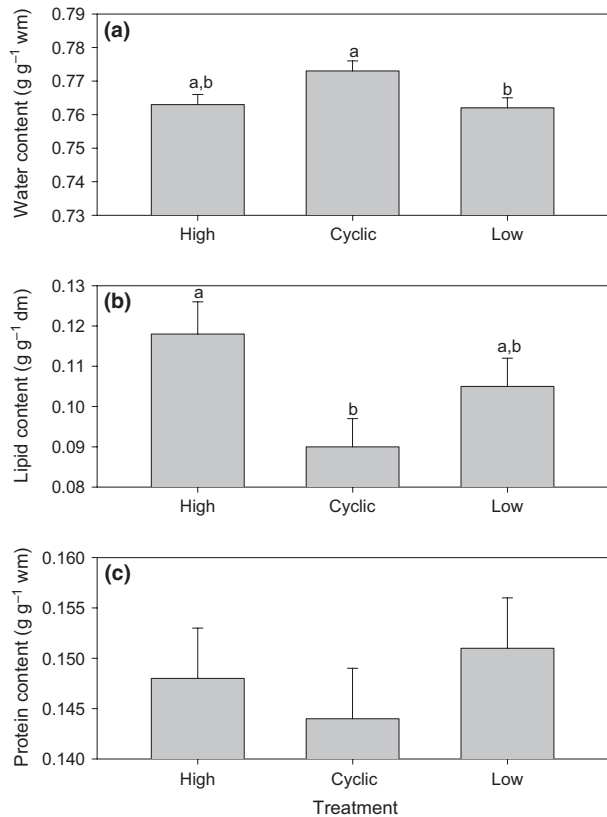
Treatment	Replicate	C	F	E	P	R	SDA	$K_1$	$K_2$
High	1	179.4	44.8	13.5	58.3	39.9	22.9	0.33	0.59
High	2	189.8	47.4	14.2	62.3	41.7	24.2	0.33	0.60
Cyclic	1	155.7	38.9	11.7	50.5	34.7	19.9	0.32	0.59
Cyclic	2	172.1	43.0	12.9	58.3	35.9	21.9	0.34	0.62
Low	1	119.6	29.9	9.0	33.7	31.8	15.2	0.28	0.51
Low	2	102.6	25.6	7.7	23.6	32.6	13.1	0.23	0.42

metabolic balance of vendace in the experiment. The calculated energy budgets indicated that daily respiration rates ( $R$ ) were about 13% lower in the cyclic when compared with the high temperature treatments, and even 21% lower in the low temperature treatment (Table 2). Daily consumption rates were likewise 13% lower in the cyclic than in the high temperature treatments, but were reduced by 40% from high to the low temperature treatments (Table 2). The gross food conversion efficiency ( $K_1$ ) was comparable in fish held at high and cyclic temperatures (average of two replicates 33% for both treatments), but substantially lower in fish held at low (26%) temperatures (Table 2). Similarly, the net production efficiency ( $K_2$ ) was comparable in high and cyclic temperature treatments, but more than 20% lower in the low temperature treatment (Table 2).

The average proximate body composition differed between the subsets of six fish from each treatment. Fish from the cyclic temperature treatment had higher water content (estimated marginal means:  $0.773 \text{ g g}^{-1} \text{ wm}$ ) than fish from the low temperature treatment ( $0.762 \text{ g g}^{-1} \text{ wm}$ ) (Fig. 3a; ANCOVA of treatment effect:  $F_{2,14} = 4.70$ ,  $P = 0.027$ ; *post hoc* tests of estimated marginal means with Sidak correction: high-cyclic:  $P = 0.10$ ; high-low:  $P = 0.99$ ; low-cyclic:  $P = 0.050$ ). Conversely, fish from the cyclic temperature treatment had lower lipid content (estimated marginal means  $0.090 \text{ g g}^{-1} \text{ dm}$ ) than fish from the high temperature treatment ( $0.118 \text{ g g}^{-1} \text{ dm}$ ) (Fig. 3b; ANCOVA,  $F_{2,14} = 3.70$ ,  $P = 0.050$ ; Holm-Sidak *post hoc* tests: high-cyclic:  $P = 0.055$ ; high-low:  $P = 0.63$ ; low-cyclic:  $P = 0.41$ ). Protein content of fish did not differ between the treatments (Fig. 3c; ANCOVA,  $F_{2,14} = 0.58$ ,  $P = 0.58$ ).

## Discussion

We demonstrate that the exposure to cyclic temperatures as experienced during DVM did not result in metabolic benefits for vendace, when compared with fish held at high temperatures. However, as predicted, fish grew more efficiently at cyclic than at low temperatures. Evaluation of biochemical body composition indicated that fish exposed to cyclic temperatures had a lower lipid and higher water content than fish held at high temperatures and thus resembled fish from the low temperature treatments. These data indicate that the exposure to cyclic temperatures modified growth and biochemical body composition



**Fig. 3** Estimated marginal means  $\pm$  SEM of water content ( $\text{g g}^{-1}$  wet mass, a), lipid content ( $\text{g g}^{-1}$  dry mass, b) and protein content ( $\text{g g}^{-1}$  wet mass, c) of a subset of  $n = 6$  vendace each from high ( $8.0 \text{ }^\circ\text{C}$ ), cyclic (switch between  $4.5$  and  $8 \text{ }^\circ\text{C}$ ) or low ( $4.5 \text{ }^\circ\text{C}$ ) temperature treatments. Differing letters above the bars indicate significant differences between treatments, as tested by ANCOVA with Sidak-corrected Tukey *post hoc* tests.

of fish in a specific way, thus differing from results to be expected for fish held at constant temperatures intermediate between the high and low temperature treatments. Our results challenge the assumption that vendace may grow faster while migrating over vertical temperature gradients than stationary fish occurring permanently in warm water layers (Biette & Geen, 1980a; Wurtsbaugh & Neverman, 1988; Bevelhimer & Adams, 1993).

The low growth performance of vendace at  $4 \text{ }^\circ\text{C}$  confirms earlier experiments on temperature-dependent growth in fishes. Growth rate and food conversion efficiency of salmonids usually show an unimodal response to temperature, with peaks at  $12$ – $15 \text{ }^\circ\text{C}$  (Koskela *et al.*, 1997b; Elliott & Hurley, 1999; Lyytikäinen & Jobling, 1999; Gunther *et al.*, 2007), and impaired growth performance below  $6 \text{ }^\circ\text{C}$  (Koskela, Pirhonen & Jobling, 1997a). We found lowest absolute



respiration rates for fish held at 4.5 °C, but in the low temperature treatment, these rates were higher relative to consumption rates (i.e.,  $R/C = 28\%$ ) than in the high and cyclic temperature treatments ( $R/C = 22\%$ ). Consequently, our data show the poorest absolute growth of fish and the lowest conversion and production efficiencies in the low temperature treatments. According to our consumption estimates by the bioenergetics model, growth of vendace at low temperatures was constrained by the amount of food eaten daily that was even 40% lower than in the fish held at cyclic or constant high temperatures. This may have been caused by the slow digestion speed imposed by low temperatures that limits overall food uptake (Giske & Salvanes, 1995 and references therein). These results suggest that a constant occurrence of fish in hypolimnetic waters without ascent into shallower and warmer waters results in the lowest growth rates and is bioenergetically a sub-optimum strategy, even when food density is high.

We could not confirm a generally higher growth rate of fish held at cyclic temperatures over fish exposed to constant high temperatures, as suggested from earlier experiments (Biette & Geen, 1980a; Spigarelli, Thommes & Prepejchal, 1982; Neverman & Wurtsbaugh, 1994). Two experimental details may have contributed to this difference. First, the difference between high and low temperatures in our experiment (3.5 °C) was substantially smaller than the difference applied in some of the previous approaches (10–13 °C) (Biette & Geen, 1980a; Neverman & Wurtsbaugh, 1994). However, our data from Lake Stechlin unequivocally show that the mean population depth of vendace is continuously located in water layers of about 8–9 °C at night, whereas fish reside in hypolimnetic waters at 4–5 °C during daytime (Mehner *et al.*, 2007b, 2010). Therefore, our experiments closely mimicked the natural temperature gradients. Second, the relatively low temperature at high conditions in our experiments may be the reason that fish from the high temperature treatments grew fastest. At 8 °C (our high temperature treatment), vendace show the lowest net energetic costs of swimming (per unit distance), whereas swimming costs are higher at 4 and 15 °C (Ohlberger *et al.*, 2008b). Therefore, if vendace would have been exposed to high temperatures of about 15 °C as applied in the earlier experiments with salmonids (Biette & Geen, 1980a), their growth rates at the high temperature presumably would have been lower than growth rates at cyclic

temperatures because of the enhanced swimming costs beyond the optimum temperature.

The food ration offered also influences the relative growth performance of fish held at cyclic or constant temperatures. If fish were fed submaximal rations, cyclic temperatures resulted in the highest growth rates (Biette & Geen, 1980a). However, if fish were fed *ad libitum* with daily rates >8% on dry mass basis, fish held at high temperatures grew faster than those held at cyclic temperatures (Biette & Geen, 1980a; Neverman & Wurtsbaugh, 1994). Similarly, larval burbot (*Lota lota*) fed *ad libitum* grew fastest in high temperature relative to cyclic and low temperatures, but the product of growth and mortality rates indicated higher ecological performance of migrating burbot relative to stationary behaviour (Donner & Eckmann, 2011). The daily ration in our experiment was 10% on wet mass basis, and therefore, our result confirms that fish held at high temperatures can achieve comparable growth rates as fish held at cyclic temperatures if the feeding rate is sufficiently high.

Finally, we could not demonstrate that fish exposed to cyclic temperatures could profit from fast digestion at high temperatures, subsequent higher feeding, and low respiration costs at low temperatures. Both consumption and respiration rates were about 12% lower at cyclic than high temperatures, resulting in almost identical conversion efficiencies in these two treatments. This may suggest that cyclic and high temperatures are energetically comparable at high food densities. However, we may have underestimated the respiration costs at cyclic temperatures by modelling intermediate constant temperatures. When respiration was directly measured in fish held at cyclic temperatures, rates occasionally exceeded those measured in fish held at constant high temperatures (Vondracek, Cech & Longanecker, 1982; Lyttikainen & Jobling, 1999). Higher rates at cyclic temperatures may be caused by the energy needed for the frequent temperature acclimation (Guderley, 2004). In this case, enhanced metabolic costs at cyclic temperatures would reduce the growth efficiency (e.g.,  $K_2$ ) relative to the high temperature treatment.

An unexpected result of our study was the treatment-specific effect of temperature on biochemical body composition of fish. Body condition of fish held at cyclic temperature was highest, but the high mass at unit length was obviously caused by a high water content of fish muscles. In contrast, lipid content of

fish at cyclic temperatures was lower and comparable with body composition of fish held at constant low temperatures. Accordingly, water content (Pangle & Sutton, 2005) is a more reliable predictor of biochemical body composition than the often applied body condition factor (Salam & Davies, 1994; Sutton, Bult & Haedrich, 2000). Water content is usually inversely correlated with lipid and protein content in fish (Gunther *et al.*, 2007). Lipids are the secondary energy source of fish after the easily metabolisable carbohydrates, whereas proteins are catabolised only at severe and enduring starvation (Pierce *et al.*, 1980; Post & Evans, 1989; Mehner & Wieser, 1994). The slightly reduced lipid content of fish held at low and cyclic temperatures suggests temperature-dependent differences in metabolism of body constituents. A higher lipid and protein content of fish held at higher temperature was likewise found in other studies (Gunther *et al.*, 2007), whereas cyclic temperatures induced higher lipid content in brown trout (*Salmo trutta*) in comparison to fish held at constant intermediate temperature (Spigarelli *et al.*, 1982). The proximate body composition of vendace in our experiments may have been influenced also by the artificial feed used, which had higher lipid content and energy density than zooplankton, the natural prey of vendace (Helland *et al.*, 2008). Thus, we have to be cautious to extrapolate these specific results from the laboratory to natural situations. However, the influence of cyclic temperatures on proximate body composition of migrating fish warrants further investigations.

The changes in the biochemical body composition may have consequences for the population dynamics and ecology of vendace. Lipid storage and dynamics within the fish are particularly important attributes of fish health and future population success. Processes affected by reduced lipid storage encompass lowered overwinter survival, lower reproductive performance and impaired survival of descendants, and a compromised response to environmental stress (reviewed by Adams, 1999). In addition, lipids play a substantial role in buoyancy regulation of fish. Lipid-dense fishes can occupy greater depths than lean ones because they can passively compensate for acute changes in their buoyancy by lift *via* high lipid content (Fleischer & TeWinkel, 1998; Clemens & Crawford, 2009). It may be questioned whether a reduction of lipids by about 22% (from 11.6 to 9.0 g g<sup>-1</sup> dm) as found in our cyclic relative to the high temperature treatments may

decisively influence population dynamics or buoyancy regulation of vendace. However, a higher water content and lower lipid concentration of migrating fish may be an energetic advantage because the active buoyancy regulation required during daily ascent and descent may be impeded by the passive and static buoyancy from lipids.

We have applied bioimpedance analyses (BIA) to facilitate a fast and cheap determination of biochemical body compositions of fish. The close correspondence found between BIA composition index and the total amount of water, lipid or protein mass confirms other studies in which BIA has been applied (Cox & Hartman, 2005; Pothoven *et al.*, 2008; Willis & Hobday, 2008). However, biochemical analyses have to be conducted in addition, because a direct estimation of the relative water, protein or lipid content (% wm) *via* BIA is usually not successful (Pothoven *et al.*, 2008). The poor predictive power of regressions between BIA measurements and the percentage of body constituents in fish was confirmed by our results. Unfortunately, this negative result prevented the direct extrapolation of proximate body composition from BIA composition index for all fish for which we could not determine body composition directly. Therefore, the effect of cyclic temperatures on lipid and water content of vendace could only be demonstrated indirectly by the similar trends found in both the subset of fishes with direct biochemical analyses and the significant difference of BIA composition index between the treatments. BIA measurements seem to reflect primarily the length- and mass-dependent absolute amount of body constituents in fish, whereas the subtle differences in relative body compositions as induced by our experimental conditions cannot be derived from BIA measurements.

In conclusion, bioenergetics benefits are hardly the sole ultimate cause for DVM of vendace in Lake Stechlin. Under the conditions of our experiment which closely mimicked the naturally occurring temperature gradients and feeding periods, non-migrating fish with a permanent occurrence in the warm water performed as well or even better physiologically and biochemically than fish occurring either in permanently cold waters or those following the cyclic migrating strategy. A similar rejection of the bioenergetic efficiency hypothesis for the adaptive significance of DVM was found in juvenile kokanee salmon where growth of migrating fish was not higher than

that of non-migrating salmon (Johnston, 1990). Alternative ultimate explanations for DVM in fish are mainly related to predation avoidance. Owing to the low predator density in Lake Stechlin (Mehner *et al.*, 2010), a genetic fixation of migration behaviour in response to the 'ghost of the predation past' has been suggested (Mehner *et al.*, 2007b). The main predator of vendace in Lake Stechlin, perch, occurs only in warm layers above the thermocline. However, visual contact with and chemical cues from predators may enhance activity and hence add metabolic costs to the occurrence of vendace in the upper water layers (Huuskonen & Karjalainen, 1997; Hawlena & Schmitz, 2010). These extra costs may result in an equal or even superior benefit-cost ratio of migrating over warm-stationary fish. Accordingly, if DVM serves primarily as predation-avoidance behaviour, the achievable growth rates at high resource densities but cyclic temperatures may be comparable or are even higher than those for fish performing a non-migrating strategy with permanent occurrence in shallow and warm layers. Thus, DVM avoids the severe energetic constraints of exclusively living in safe, but cold habitats, and simultaneously reduces predatory mortality without compromising the metabolic balance. To explain DVM of fish in a particular aquatic system hence will require simultaneous consideration of bioenergetics in relation to abiotic gradients and the vertical distributions of predators and resources (Levy, 1990; Mehner *et al.*, 2010). Our results suggest that an explicit consideration of biochemical body composition is additionally required to understand ecology and population dynamics of migrating fish.

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