Effects of Elevated Temperature on Gene Expression, Energy Reserves and Cellular Energy Status in *Salmo trutta*

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Introduction

- Global warming poses a significant threat to aquatic ecosystems, especially in temperate freshwater habitats.
- Brown trout (*Salmo trutta*) in the Salmonidae family are highly vulnerable to rising water temperatures.
- By 2080, 64% of European streams may become uninhabitable for brown trout.
- This study explored the acclimatisation potential of brown trout by gradually exposing them to 20°C over a 21-day period.
- The acclimatisation potential was studied at three levels: molecular, metabolic and physiological. Results showed an increase in the expression of heat shock proteins as well as genes related to oxidative stress and apoptosis.
- The fish exhibited energetic stress, indicated by a reduction in energy reserves and cell energy status. Physiological stress was also evident, with the treatment group showing a suppression in growth compared to the control group.

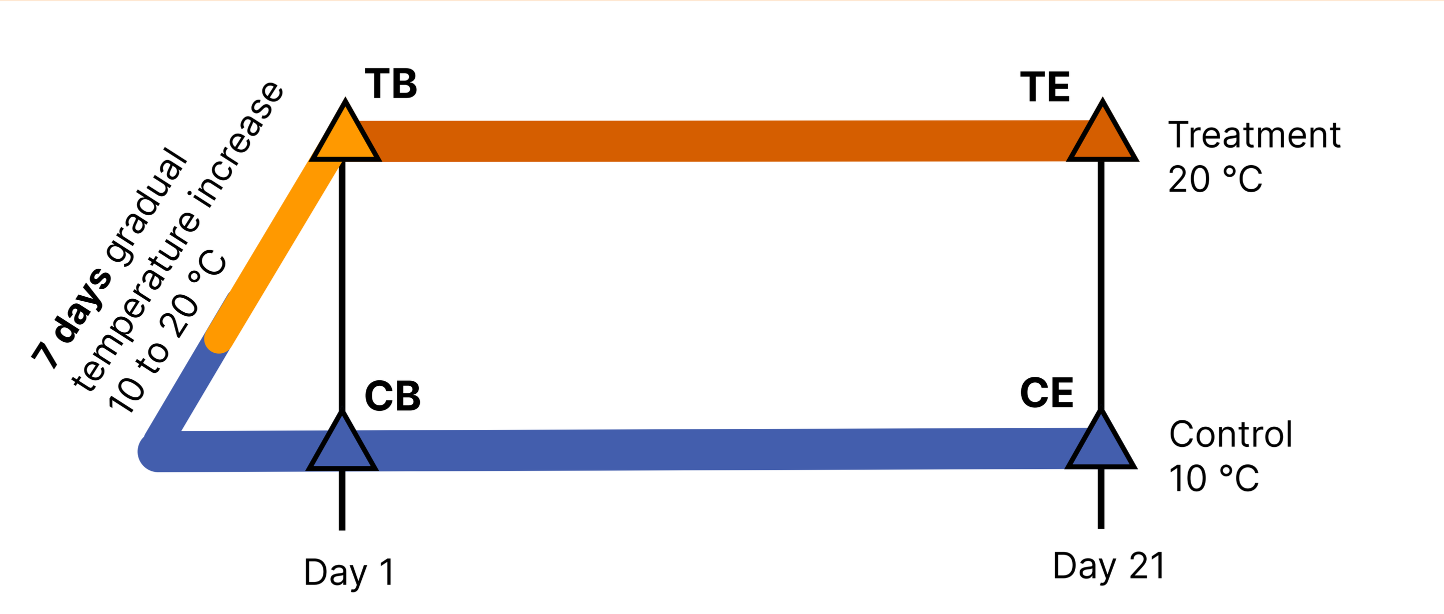
Hypothesis

- With increasing temperature, specific genes are up or downregulated to produce functional molecules that help mitigate the associated stressors.
- Temperature increases result in a reduction of energy reserves and cellular energy status as the fish utilises its energy reserves to mitigate the stressor.

Experimental design and Methodology

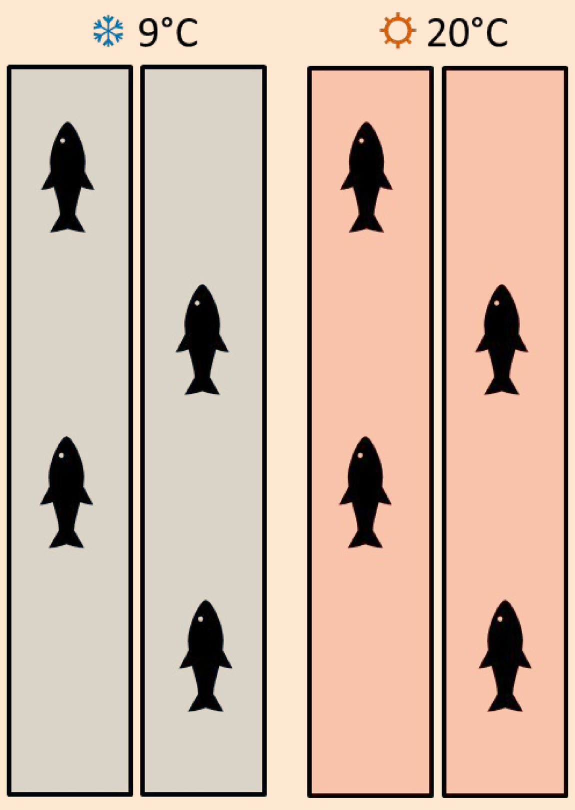
A

B



CB = Control beginning, **CE** = Control end, **TB** = Treatment beginning, **TE** = Treatment end

C



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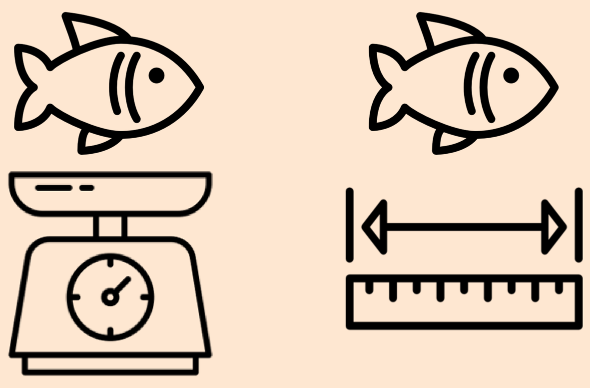

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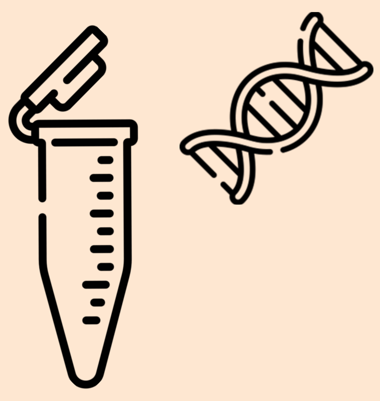

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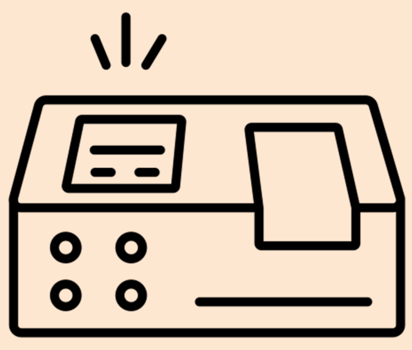

Fig. 1: **Experimental design:** Experimental site (Fish Farm Kreuzstein) (A), Experimental design and sampling points (B), Experimental set up (C); **Methods:** Morphometrics & indices calculation (D), Gene expression analysis from brown trout liver samples stored in RNAlater, (E) Metabolite analysis from brown trout liver samples stored in perchloric acid (F)

Results

	CB	CE	TB	TE	Effects
BW	3.5 ± 0,19a	5.5 ± 0,16b	3.7 ± 0,22a	4.3 ± 0,22b	43 % Supressed growth (CB-CE vs TB-TE)
HSI	1.49 ± 0.06a	1.54 ± 0.08a	1.36 ± 0.08a	1.26 ± 0.09a	-7.4 % Decrease (TB vs TE)
FCV	13.0 ± 0.04a	8.9 ± 0.04a	10.3 ± 0.02a	3.0 ± 0.02b	70 % Decrease (TB vs TE)
K	0.91 ± 0.02a	0.96 ± 0.02a	0.93 ± 0.02a	0.98 ± 0.02a	No significant effect

Table 1: Table indicating body weight (BW), hepatosomatic index (HSI), fat contet of viscera (FCV) and condition factor (K) for the control group at the beginning (CB) and the end (CE) as well as the treatment group at the beginning (TB) and the end (TE). Effect column shows the differences between selected treatments.

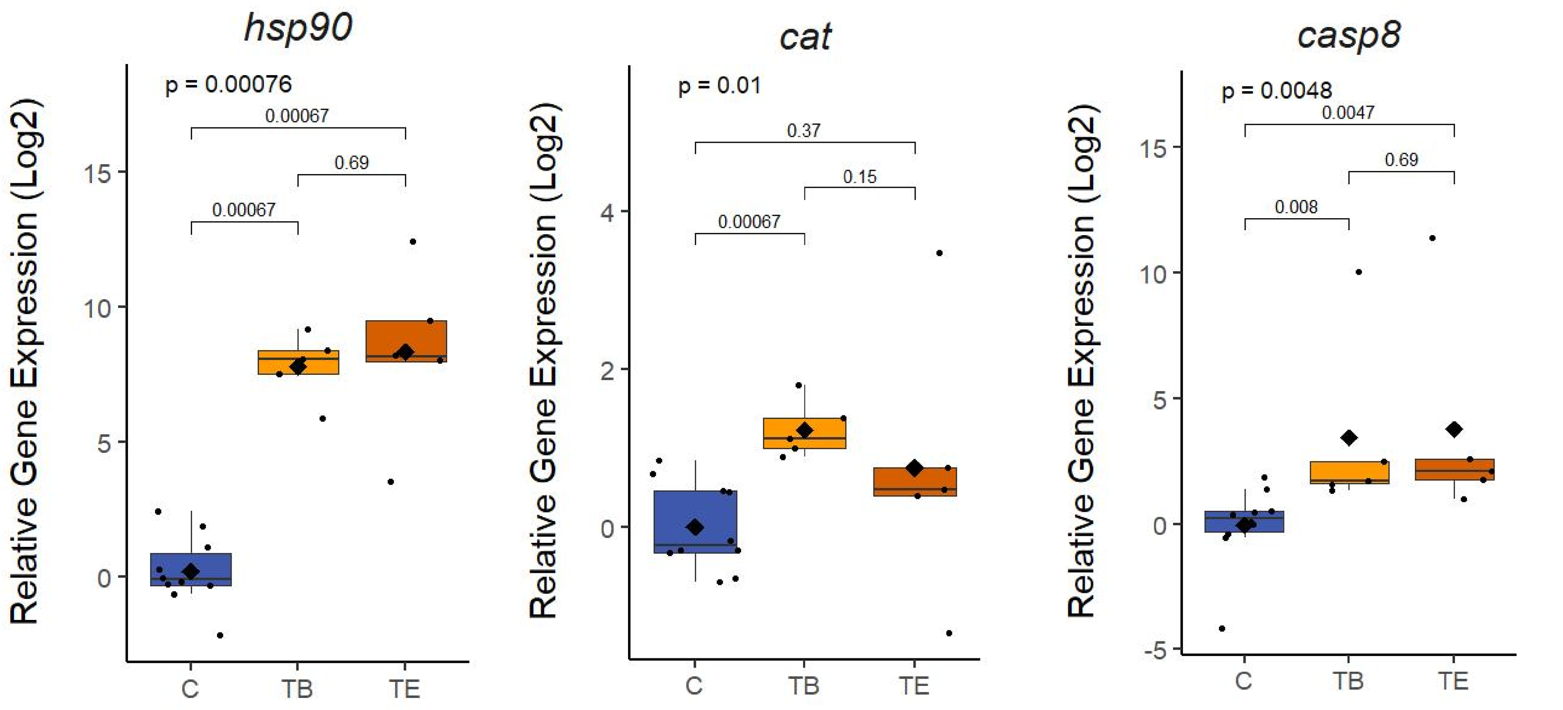

Fig. 2: Boxplots show the relative expression heat shock protein, *hsp90* (A), oxidative stress response gene *cat* (B), and apoptotic related function gene *casp8* (C). Data are presented as log2 relative expression values (n = 10, 5, 5 for C, TB, TE respectively). Kruskal Wallis H test revealed that there was a significant difference across some of the groups in *hsp90, cat* and *casp8*, with significance levels indicated over the bars atop the treatments. Black diamond indicates the mean.

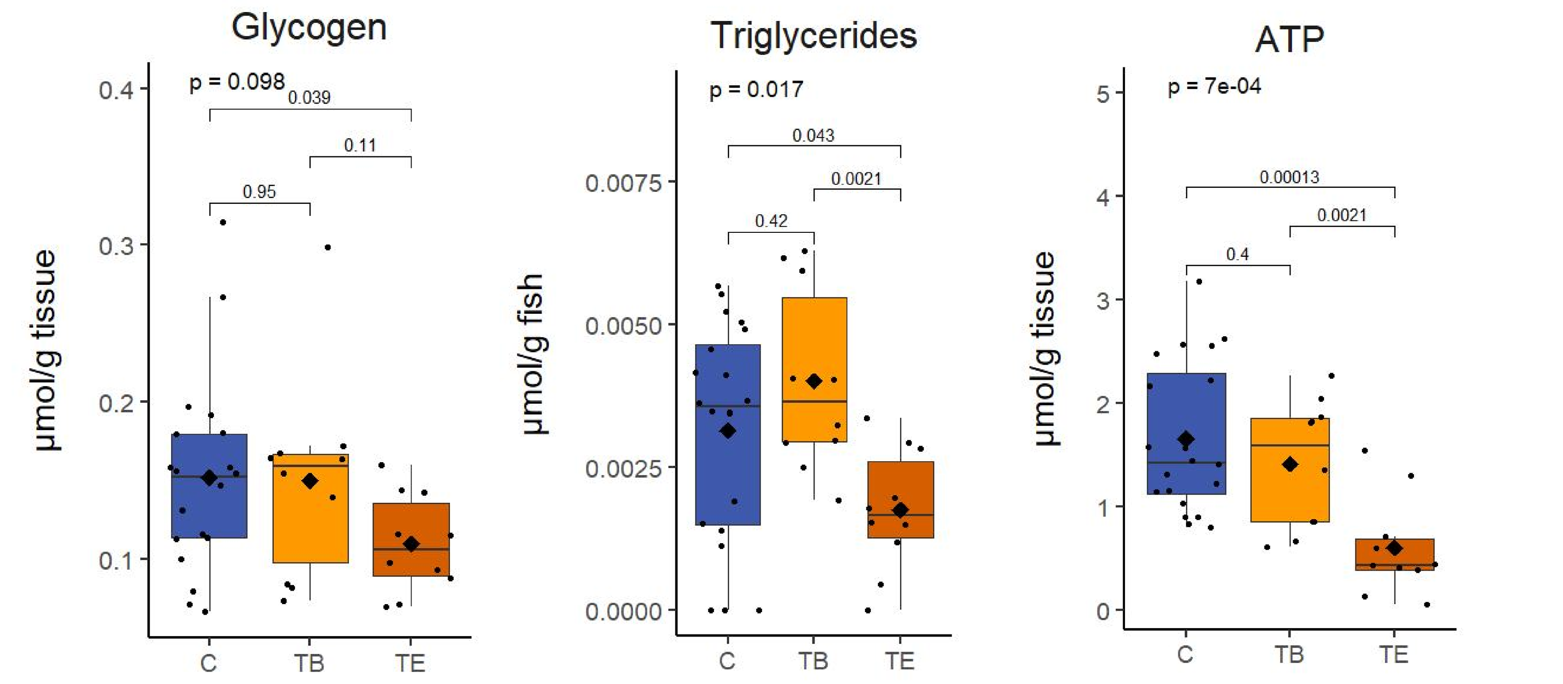

Fig. 3: Liver glycogen, triglyceride, ATP, were compared among three groups C, TB and TE (9°C – 20°C water temperature). Kruskal Wallis H test showed a significant decrease in all the metabolites from C and TB to TE. Black diamond indictaes the mean. (n = 20, 10, 10 for C, TB, TE respectively)

Discussion

Morphometrics and condition indices: Our results showed that there was a suppression in growth for the treatment group compared to the control group of 43%; this was attributed to an increase in metabolic rate, loss of appetite, and stress at elevated temperatures (Conde-Sieira et al., 2018). We also observed a 70% decrease in FCV (TB vs. TE), indicating that the fat depot in the viscera, which is the primary lipid storage in teleosts, was utilized during exposure to elevated temperatures; as these fats are the first to be mobilized in response to energy demand (Navarro & Gutiérrez, 1995).

Gene Expression: Gene expression analysis showed an instant upregulation of the investigated genes in the treatment group relative to the control group (Fig. 2), indicating that brown trout initiated cellular defence mechanism to mitigate protein denaturation (*hsp90*), cell and tissue damage by oxidation *(cat*), and programmed cell death (*casp8*).

Energy storage and Cell energy status: Finally, our results showed a decrease in the concentration of glycogen, triglycerides, and ATP, metabolites that are crucial for energy metabolism, from C to TB to TE (Fig. 3). This decrease is attributed to increased metabolic rates at higher temperatures and increased demand for metabolites for energy production to maintain homeostasis (Kullgren et al., 2013).

Conclusion

Given the eminent threat of global warming on aquatic ecosystems

- This study is essential for assessing the adaptive capacity of brown trout in a warming world.
- Our study showed that brown trout has acclimatisation capacity
- Our study also clearly showed that brown trout experienced significant energetic and physiological stress
- This stress may impede their long-term adaptation to rising water temperatures

References

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