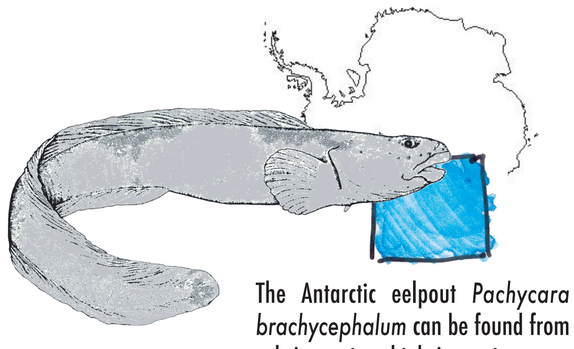
Uncoupled eelpouts -

the thermal sensitivity of UCP2 expression in Antarctic & boreal zoarcids



The Antarctic eelpout *Pachycara brachycephalum* can be found from sub-Antarctic to high Antarctic waters

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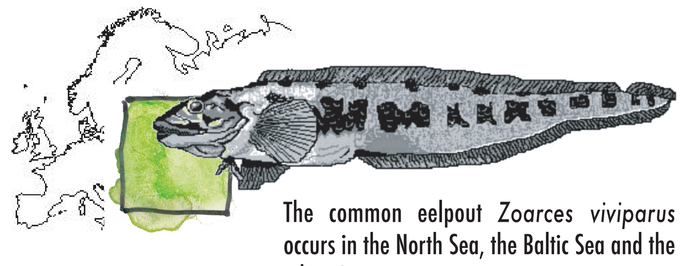


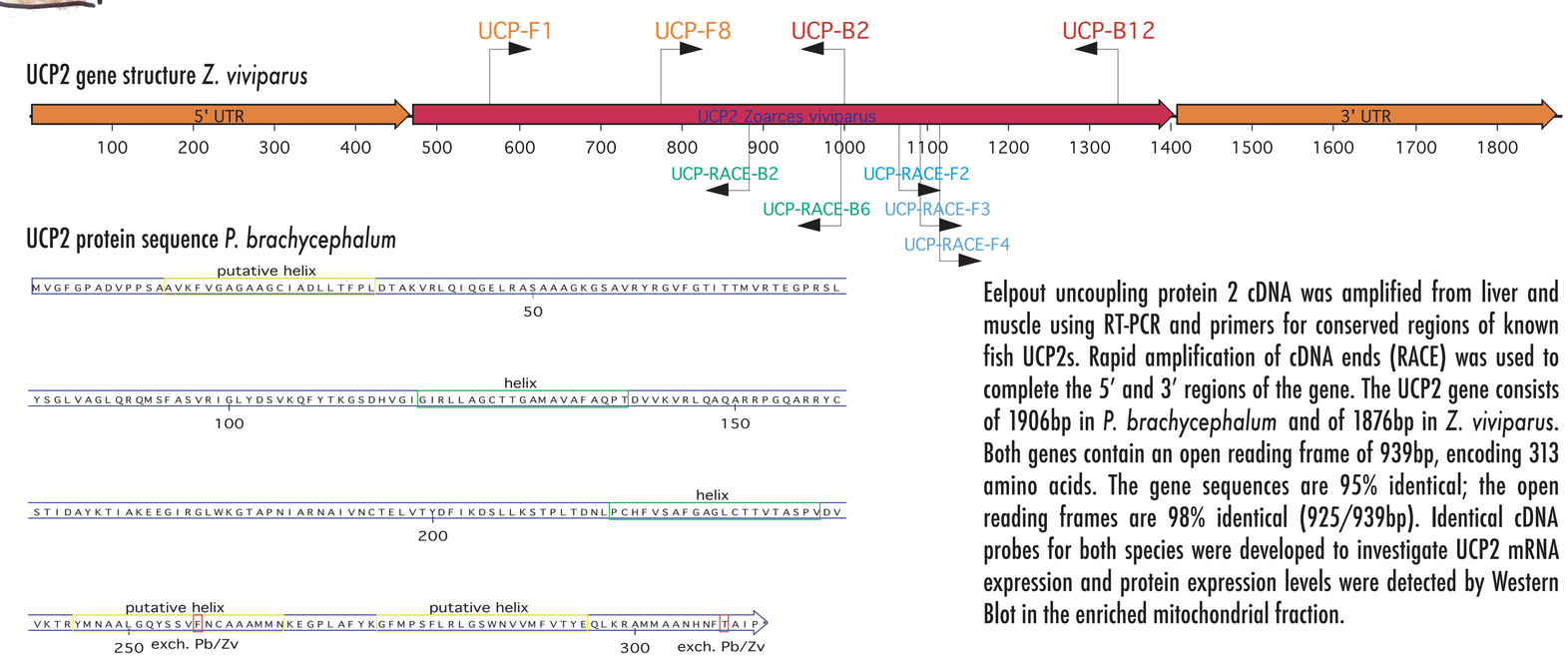
The common eelpout *Zoarces viviparus* occurs in the North Sea, the Baltic Sea and the White Sea

introduction

Mitochondrial uncoupling protein 1 (UCP1) is known to be crucial in thermoregulatory processes in various endothermic animals, and its homologues are widely distributed among vertebrates, invertebrates and plants[1]. Mitochondria are essential in the adaptation of poikilothermic animals to changing environmental temperature. The increase in mitochondrial densities frequently observed in cold adapted ectotherms may contribute to higher energetic demands through mitochondrial maintenance costs and the intrinsic energy dissipation through proton leakage.

To investigate whether uncoupling proteins are possibly involved in the thermal adaptation of ectothermic animals, we isolated and characterised the entire genes of UCP2 for two closely related zoarcid fish species from Antarctic (*Pachycara brachycephalum*) and boreal (*Zoarces viviparus*) waters.

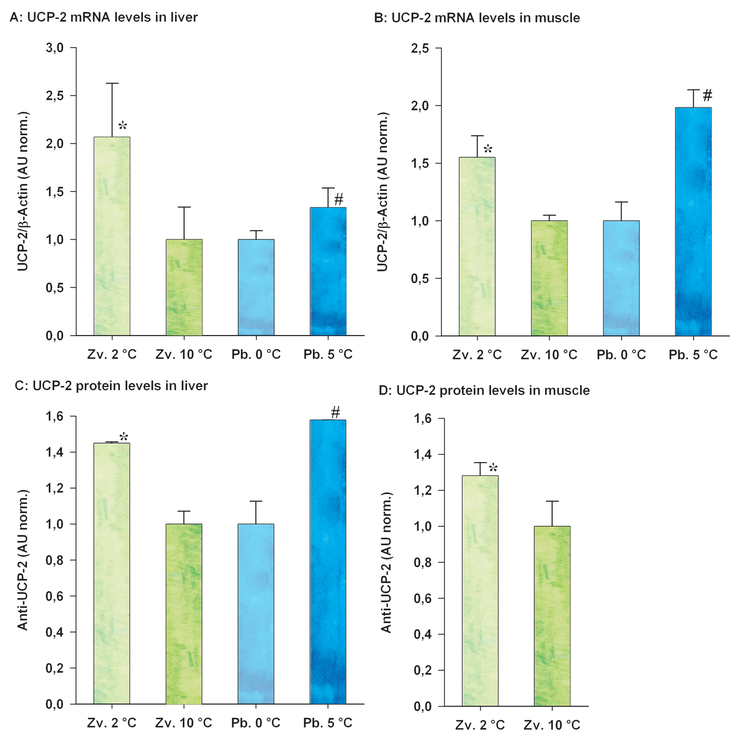
identification of the UCP2 genes



Eelpout uncoupling protein 2 cDNA was amplified from liver and muscle using RT-PCR and primers for conserved regions of known fish UCP2s. Rapid amplification of cDNA ends (RACE) was used to complete the 5' and 3' regions of the gene. The UCP2 gene consists of 1906bp in *P. brachycephalum* and of 1876bp in *Z. viviparus*. Both genes contain an open reading frame of 939bp, encoding 313 amino acids. The gene sequences are 95% identical; the open reading frames are 98% identical (925/939bp). Identical cDNA probes for both species were developed to investigate UCP2 mRNA expression and protein expression levels were detected by Western Blot in the enriched mitochondrial fraction.

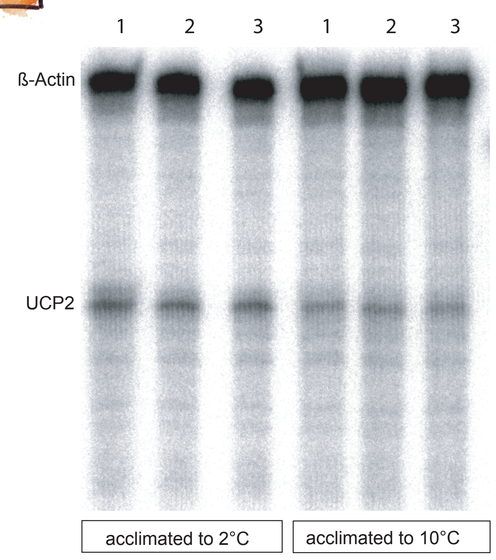
The proteins consist of 313 amino acids and show 99% identity between the two species (two amino acid exchanges (red)). Protein sequences of zoarcid UCP2 are 77% identical to rat UCP2 and 75-79% to those of zebrafish (*D. rerio*), carp (*C. carpio*) and red sea bream (*P. major*). As a membrane protein, UCP2 contains 5 putative transmembrane helices (yellow and green).

temperature dependent UCP2 expression



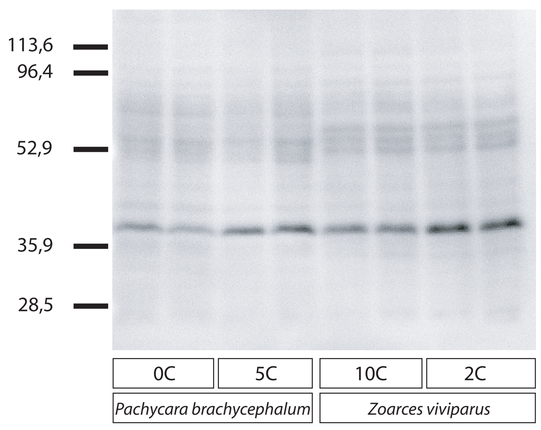
UCP2 mRNA and protein expression levels in liver and muscle of the two zoarcids *P. brachycephalum* (Pb) and *Z. viviparus* (Zv), acclimated to 0 and 5°C and 10 and 2°C, respectively. In both species, UCP2 mRNA and protein expression in muscle and liver tissue strongly increased above and below habitat temperatures.
A: mRNA expression in liver. B: mRNA expression in muscle. C: protein expression in liver. D: protein expression in muscle. *: significantly different from Zv. 10°C; #: significantly different from Pb. 0°C, (P=0.05). Error bars represent standard error of the mean (SEM).

expression at the mRNA level



Ribonuclease protection assay of liver RNA samples of *Z. viviparus*, acclimated to 2°C and 10°C. Each lane was run with 10µg RNA, lanes 1-3 represent triplicates of pooled RNA (n=5). The size of the protected beta actin fragment was 205bp, the length of the UCP2 fragment was 137bp. In cold acclimated animals, UCP2 is clearly more expressed.

expression at the protein level



Western Blot detection of UCP2 in enriched mitochondrial fractions of liver samples of the two zoarcids *P. brachycephalum* and *Z. viviparus*, acclimated to 0 and 5°C and 10 and 2°C, respectively. Each lane contained 50mg of protein pooled from five individuals, lanes were run in duplicates. The UCP2 antibody bound to a protein band of approximately 38kDa. UCP expression rose above and below habitat temperatures.

conclusions

In this study, we characterised zoarcid UCP2 and for the first time investigated temperature dependent UCP2 expression in fish. Upon cold and warm acclimation, we found different phenomena. Following cold acclimation, there was a general upregulation of UCP2 expression levels in the temperate common eelpout Z. *viviparus*, in line with evidence for cold-induced mitochondrial proliferation provided by other authors[2]. During warm acclimation of the cold adapted Antarctic eelpout *P. brachycephalum*, UCP2 expression underwent as yet undocumented changes: in muscle and liver tissue we found a putatively regulative increase in UCP2 levels, both at mRNA and protein levels.

Our findings suggest that in ectotherms UCP2 plays a role in the mitochondrial energy metabolism, especially at temperatures at the edge of the thermal tolerance range[3]. During thermal stress it may support control of the mitochondrial membrane potential and balance ROS formation and ATP production.

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