# Adaptation of ion regulatory capacities in fish gills under hypercapnic acidosis

Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gemeinschaft

Katrin Deigweiher, Hans-O. Pörtner, Magnus Lucassen

Marine Animal Physiology, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany.

Correspondence: katrin.deigweiher@awi.de

## Introduction

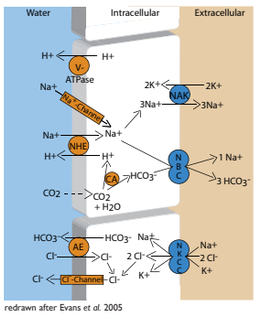
The currently rising $CO_2$ levels in the atmosphere and in marine surface waters combine with trends of increasing temperatures.

Simultaneous shifts in those factors will likely enhance sensitivity especially in stenothermal animals (reviewed in Pörtner *et al.* 2004).

The preservation of ion balance and the regulation of pH despite environmental fluctuations play a special role in this context, and support the maintenance of vital cellular functions. Therefore, it comprises a significant fraction of the energy budget.

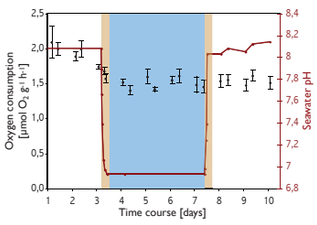
Here, we investigated the time course of acclimation to hypercapnia (10,000 ppm) in the benthic eelpout *Zoarces viviparus* at optimum temperature.

The contribution of different transporters to ion and pH regulation in the gills were studied on functional, translational and transcriptional levels.




redrawn after Evans *et al.* 2005

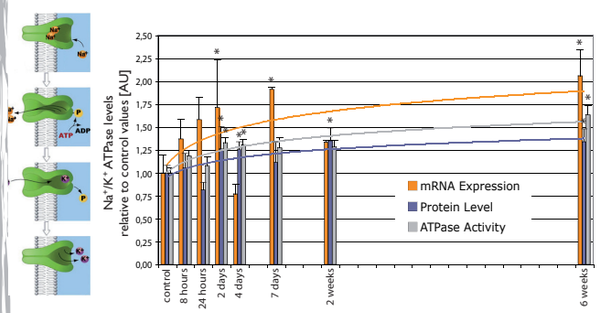
## Whole Animal Regulation Capacity




*Zoarces viviparus* shows a high capacity for pH and ion regulation, comparable to other studies on fish (reviewed in Ishimatsu *et al.* 2005).

Whole animal oxygen consumption remains constant during and after acute exposure to 10,000 ppm hypercapnic incubation and recovery (time course 3.5 - 4.0 - 3.0 days).

## Gill $Na^+/K^+$ ATPase Capacity

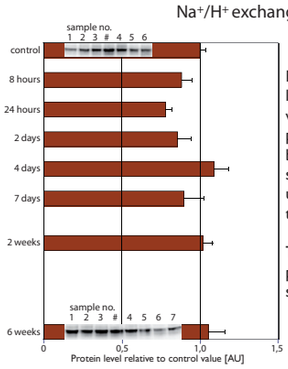



$Na^+/K^+$ ATPase regulation follows a similar pattern on different regulation levels.

Higher functional capacities are established by higher protein and mRNA levels.

## Ion Transporter Protein Levels

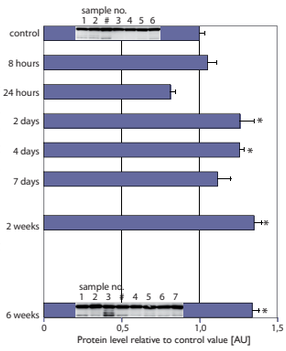
### $Na^+/H^+$ exchanger




$Na^+/H^+$ exchanger protein level shows a slight decrease within the first 2 days of hypercapnia incubation, followed by recovery after 4 days and stabilization at control level until the end of the 6 weeks trial.

The protein levels correspond to the respecitve transcript levels.

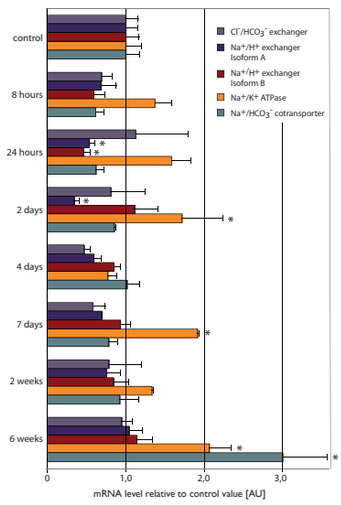
### $Na^+/K^+$ ATPase




$Na^+/K^+$ ATPase protein level increases significantly after 2 days of hypercapnia incubation and stabilizes to 135% of control level at the end of the 6 weeks time course.

This pattern is paralleled by increased transcript and activity levels.

## Ion Transporter mRNA Expression




Overall, the expression of the „passive" transporters is reduced during the early phase of acclimation, and is restored at the end.

The expression of the active $Na^+$ pump remains high during the early phase and doubles until the end of acclimation.

During early phase the $Na^+/HCO_3^-$ cotransporter behaves like the other passive transporters, but expression is about threefold at the end of acclimation.

## Experimental Procedure

Long term exposure to hypercapnia

Individuals of *Zoarces viviparus* were kept at optimum temperature (10°C) before and during exposure to 10,000 ppm hypercapnic incubation (corresponding pH of 6,9).

Groups of 8 - 10 fishes were sampled for each point of the time course.

Gill tissue was dissected and frozen immediatly in liquid nitrogen.

RNA was isolated and used for mRNA quantification of single ion transporters by Real-time PCR.

Tissue homogenates were prepared to determine $Na^+/K^+$ ATPase activity as well as $Na^+/H^+$ exchanger and $Na^+/K^+$ ATPase protein quantities by Western blotting.

## Conclusion & Prospects

Even though whole animal energy consumption is not affected by acute hypercapnic incubation, large rearrangements in all investigated gill transporters at the molecular level could be shown.

Since the protein levels of $Na^+/H^+$ exchanger and $Na^+/K^+$ ATPase follow their respective mRNA patterns, similar increments of $Na^+/HCO_3^-$ cotransporter may be predicted according to the observed higher mRNA levels.

Elevated $Na^+/K^+$ ATPase capacities might influence the energy budget of the euythermal species.

## Literature

Evans, D.H., Piermarini, P.M. & Choe, K.P. (2005). "The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste." *Physiological Reviews* **85**(1): 97-177.

Ishimatsu, A., Hayashi, M. & Lee, K.S. (2005). "Physiological effects on fishes in a high-$CO_2$- world." *Journal of Geophysical Research* **110**: C09S09

Pörtner, H.O., Langenbuch, M. & Reipschläger, A. (2004). "Biological impact of elevated $CO_2$ concentrations: lessons from animal physiology and earth history?" *Journal of Oceanography* **60**: 705-18.