



Insights into Marine Fish Physiology in a Changing World

From biochemical to behavioural effects

Karine Bresolin de Souza

Institutionen för biologi och miljövetenskap
Naturvetenskapliga fakulteten

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Abstract

Ocean acidification and global warming are largely caused by increased levels of atmospheric CO₂, and marine fish are exposed to both these stressors simultaneously. Although the effects of temperature on fish have been investigated over the last century, the effects of moderate CO₂ exposure and the combination of both stressors are not well-understood, especially long-term effects.

In **Papers I, II and III** we investigated the protein expression and biochemical parameters in gills, blood plasma, and liver of Atlantic halibut (*Hippoglossus hippoglossus*) exposed to temperatures of 5, 10, 12 (control), 14, 16, and 18 °C (impaired growth) in combination with control (400 µatm) or elevated CO₂ (1000 µatm) levels for 3 months.

Paper I shows the protein expression in gills and blood plasma of halibuts exposed to elevated CO₂ at 12 °C and 18 °C. Elevated CO₂ induced the regulation of immune system-related proteins in plasma of fish from both temperature treatments. Gills from fish exposed to elevated CO₂ at control temperature show modulation of energy metabolism proteins, as well as indications of increased cellular turnover and apoptosis signalling; while gills from fish exposed to both elevated CO₂ and elevated temperature indicate increased expression of energy metabolism proteins. In conclusion, moderate CO₂-driven acidification, alone and combined with increased temperature, can elicit biochemical changes that may affect fish health.

To further investigate the findings in **Paper I** we analysed non-specific immune components in blood plasma (**Paper II**), and examined the occurrence of oxidative stress in liver (**Paper III**) of Atlantic halibut exposed to elevated CO₂ at 5, 10, 12, 14, 16, and 18 °C. **Paper II** reveals that both measured immune components (lysozyme and complement system) had increased activities in response to elevated CO₂, which is consistent with the findings of **Paper I**. These changes represent an additional energetic cost for fish.

Paper III indicates the occurrence of oxidative stress, which can damage macromolecules such as DNA, membranes, and enzymes. Protein carbonyls were consistently higher in the elevated CO₂-treated fish at all studied temperatures, while the antioxidant enzymes did not show the same results, suggesting that the exposure to elevated CO₂ increased reactive oxygen species (ROS) formation, with consequent oxidative damage that bypasses the antioxidant defence system of the cells. The consequent oxidative stress might be connected to the increased expression of energy metabolism proteins seen in **Paper I**.

Paper IV provided an overview of elevated CO₂ effects at whole organism-level through behavioural studies. Elevated CO₂ exposure for 20 and 40 days caused several behavioural disturbances, including the reduction of boldness, exploratory behaviour, lateralization, and learning in the three-spined stickleback (*Gasterosteus aculeatus*). The effects were present throughout the exposure period and increased in effect size with exposure time. Given the severity of disturbances, our findings suggest that elevated CO₂ can pose a serious problem for sticklebacks.

This thesis provides significant insights into how marine fish can be affected by near-future elevated CO₂ and temperature. The CO₂ levels estimated to occur at the end of this century can pose physiological challenges to marine fish, and have the potential to negatively impact fish populations if acclimation fails to occur.

Keywords: ocean acidification, carbon dioxide, temperature, global warming, *Hippoglossus hippoglossus*, *Gasterosteus aculeatus*, teleost fish, gills, plasma, liver, immune system, energy metabolism enzymes, oxidative stress, proteomics, behaviour.