



Abstract

The Stress Response in Antarctic Fish: HPI Modulation, Cortisol Profiles, Interrenal Sensitivity, and Gene Expression of *Notothenia rossii* Acclimated to Temperature Challenges [†]

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Abstract: The Antarctic Ocean is one of the most extreme marine environments. Antarctic fishes evolved in stable cold thermal conditions (−1.9 °C to 2 °C) for roughly 20 million years, displaying structural and functional features resulting from adaptation or inherited from resilient ancestral species. Climate change forecast models show temperatures may increase at a relevant pace. As fish face a warmer future, their physiological ability to adapt is uncertain. We aimed at evaluating the capabilities of the hypothalamus–pituitary–interrenal (HPI) axis in Antarctic fish and show plasma cortisol profiles, expression of key genes, the sensitivity of the ex vivo interrenal tissue, and the responses to known modulators of the HPI axis in temperature-acclimated fish before and after stress. *Notothenia rossii* were collected from the waters of Admiralty and Maxwell bays, in King George Island, and transferred to an open circuit with ocean-pumped seawater. Upon acclimation, three sets of experiments were performed: (1) eight groups at 2 °C were injected with drugs involved in blockage or stimulation of cortisol release/action (saline, cortisol, dexamethasone, metyrapone, spironolactone, mifepristone) and then kept at control or transferred to 6 °C and sampled after 36 h; (2) fish at 2 °C were exposed to a standard stress test (SST: chasing+netting+1min air exposure), returned to the respective tank and sampled after 1, 4 and 24 h, while one undisturbed group served as control; (3) six groups were acclimated to 2, 5 and 8 °C for 10 days when the control group of each temperature was sacrificed. The other group received SST and was sacrificed 90 min after. Plasma and tissue samples were collected for cortisol and stress-related genes, and the interrenal was used in vitro to determine sensitivity to ACTH in a perfusion system with a continuous flow of oxygenated ringers, and 20 min fractions were collected for 240 min. Cortisol was measured via radioimmunoassay, while glucose and lactate were determined using colorimetric kits; gene expression was evaluated by qPCR. Manipulation of the HPI axis revealed that these fish show similar dynamics to those reported in temperate fish but with lower amplitude. After SST, cortisol peaked at 1–4 h and reduced to basal between 24 and 48 h. Temperature influenced the cortisol response to SST. At higher temperatures, cortisol levels in the non-stressed group were as high as in fish subjected to SST. Interrenal cells showed little response to ACTH in warm conditions, suggesting low sensitivity and/or exhaustion. Liver cortisol receptor genes were downregulated, possibly indicating a peripheral desensitization process that parallels HPI. These results show the ability to respond to stress at cold and mild temperatures but important impairments and substantial allostasis in warm or continuously increasing temperatures.



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