Getting fresh: the role of chemical and molecular chaperones in dogfish shark (*Squalus acanthias*) experiencing hypo-osmotic stress

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Proteins are protected from cellular stress by both chemical and molecular chaperones. Using the dogfish as a model, our goal was to determine if molecular chaperones are induced during a whole animal hypo-osmotic stress. We determined that sharks cope with this osmotic stress without the induction of the molecular chaperone, HSP70 and speculate that dogfish cells are protected by the presence of high levels of the chemical chaperone, TMAO during osmotic stress.

Cellular function is compromised when an organism experiences environmental stress that may result in protein unfolding or damage. To mitigate damage and to assist with protein folding, cells use molecular and/or chemical 'chaperones', compounds that interact directly or indirectly with proteins, performing cellular quality control. Marine elasmobranchs make use of both molecular (*e.g.,* heat shock proteins, HSPs) and chemical chaperones, trimethylamine oxide (TMAO), the latter a nitrogenous base that helps these animals osmoconform to their saltwater environments. Interestingly, we have *in vitro* data indicating that HSP70 is induced in dogfish red blood cells with hypo-osmotic stress, but only in cells with TMAO[1]. Here, we conducted *in vivo* experiments on dogfish shark experiencing hypo-osmotic stress in an effort to understand the coordination of molecular and chemical chaperones in the whole animal, where humoral factors may be influencing the cellular stress response.

Repeated blood samples were collected through a cannula inserted in the caudal artery (as approved by the MDIBL IACUC). After recovery from surgery for 24 h in 100% seawater (SW), animals were gradually exposed over 3 h to dilute SW (70%) where they were maintained for 24 h while swimming freely. We collected blood samples under control conditions (100% SW) and 0, 2, 8 and 24 h into the osmotic stress. We measured haematocrit, whole blood glucose, red blood cell HSPs levels using immunoblotting, and blood and SW osmolality. We are currently measuring intra- and extracellular urea and TMAO concentrations.

Based on our earlier *in vitro* work[1] we predicted that hypo-osmotic stress would cause a significant increase in HSP70. Our results to date are variable, but indicate that HSP70 is not significantly induced with this stress *in vivo* (Table 1; $p = 0.111$ repeated measures ANOVA). It is possible that intracellular TMAO, in addition to regulating osmoconformation, is offering sufficient cellular protection. Our forthcoming TMAO data will shed light on this possibility.

Table 1. Red blood cell HSP70 band density before (control) and during hypo-osmotic stress.

Time Point (h)	HSP70 (band density/µg soluble protein)
Control	3.83 ± 2.09
0	0.74 ± 0.95
2	6.76 ± 4.19
8	6.63 ± 3.10
24	0.97 ± 0.91

Values are mean ± SEM (n = 5)

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1. **Currie, S and Robertson, C.** Cellular and molecular chaperones in the red blood cells of the spiny dogfish, *Squalus acanthias*. *Bull. Mt. Desert Isl. Biol. Lab.* 49:58-59, 2010.