3. Environmental stress proteomics of two blue mussel (genus *Mytilus*) congeners

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The warm-adapted Mediterranean blue mussel species *Mytilus galloprovincialis* invaded southern California during the last century and has since replaced the cold-adapted native *M. trossulus* from its
southern range, possibly due to climate change. Furthermore, *M. galloprovincialis* is more sensitive to lower salinity levels than the native. Both, temperature and salinity changes have been hypothesized to contribute to the range shifts and limits. Using proteomics, we characterized the underpinnings of interspecific differences in thermal and salinity tolerance limits. We conducted several experiments: an acute heat stress experiments to 24 °C, 28 °C and 32 °C, followed by a 24 h recovery at 13 °C; a 4-week long temperature acclimation (7 °C, 13 °C and 19 °C) experiment and an acute hyposaline (1000, 850 and 700 mOsm seawater) exposure for 4 h followed by a 24 h recovery. Using gill tissue, we applied 2D gel electrophoresis and mass spectrometry to separate and identify proteins. The results suggest that acute heat stress triggers a shift from pro-oxidant NADH- to anti-oxidant NADPH-producing pathways to reduce the production of reactive oxygen species (ROS) and increase the cell's capacity for ROS scavenging. Temperature acclimation showed that *M. trossulus* induces molecular chaperones at 19 °C. Cold acclimation increased oxidative stress proteins and molecular chaperones in both congeners, although more so in *M. galloprovincialis*, suggesting a ROS-induced challenge to protein homeostasis at lower temperatures. The responses to hypo-salinity stress suggest that *M. galloprovincialis* experiences greater levels of oxidative stress at 850 mOsm and is unable to launch a proteomic response to 700 mOsm. These results suggest that proteomic changes to temperature favor range expansion by the invader under a warmer climate but lower tolerance towards hyposaline stress may limit its expansion into more northern latitudes with greater freshwater input.

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