



Highly polluted life history and acute heat stress, a hazardous mix for blue mussels

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ARTICLE INFO

Keywords:

Mytilus edulis
Life history
Stress on stress
Proteomic
Gills

ABSTRACT

Intertidal sessile organisms constitute throughout their life history unintended stress recorders. This study focuses on the impact of pollution on *Mytilus edulis* ability to cope with an additional stress. For this purpose, two acclimation stages to different temperatures were conducted before an acute stress exposure in mussels collected from a heavily polluted site. Gill proteomes were analyzed by 2DE and regulated proteins identified. Massive mortality was observed for organisms acclimated to colder temperatures. Despite this major difference, both groups shared a common response with a strong representation of proteoforms corresponding to “folding, sorting and degradation” processes. Nevertheless, surviving mussels exhibit a marked increase in protein degradation consistent with the observed decrease of cell defense proteins. Mussels acclimated to warmer temperature response is essentially characterized by an improved heat shock response. These results show the differential ability of mussels to face both pollution and acute heat stress, particularly for low-acclimated organisms.

1. Introduction

Intertidal environments are defined by habitats submitted to many biotic and abiotic modifications such as temperature, oxygen, food availability and salinity. These constraints linked to tidal flows are further exacerbated by anthropisation consequences such as pollution or Global Change. Assessing the impact of these cumulative stresses on marine organisms is a complex question to address (Todgham and Stillman, 2013). Hence, complementary strategies have been developed: field studies with biomonitoring networks (i.e. Mussel Watch - Goldberg, 1986; ICON - Hylland et al., 2017) and studies involving exposure of organisms to one or more pollutants in controlled conditions. Laboratory surveys also include stress on stress approaches that consist of applying a stress of any kind to an organism already undergoing a primary stress such as chemical contamination. Since Bayne (1986), it is well established that stress causes molecular, cellular and physiological alterations that reduce the animal's ability to cope with further environmental variations. So, the superimposed stress reveals the organism's state of health through its capacity to adapt. Stress on stress (SoS) studies may be compared to a tolerance test with mortality as an endpoint (Hellou and Law, 2003). Moreover SoS enabled the

development of a standardized biomarker that constitute an index of health, widely used in environmental surveys (Martínez-Gómez et al., 2017; Moore et al., 2006; Viarengo et al., 1995). This strategy can also be used to detect molecular changes through specific target analysis or omics strategies in order to improve the understanding of underlying adaptation mechanisms (Dallas et al., 2018; Politakis et al., 2018; Tomanek, 2014). In addition, in a context of Global Warming, marine ecosystems will be subjected to both increases in average temperatures and frequencies of heatwave episodes as detailed in the IPCC report (2014). Hence, thermal stress is particularly relevant since, while retaining its capacity to characterize the state of health of organisms, it also corresponds to an increasingly likely stress whose consequences must be considered (Doney et al., 2012; Dowd and Somero, 2013; Tomanek, 2012).

Blue mussels constitute an emblematic specie of marine coastal environments. As other filter-feeding bivalves, they are considered as good integrators of environmental constraints including environmental fluctuations and pollution (Gosling, 2015; Suárez-Ulloa et al., 2013). Being sessile bivalves with high capacities for bioaccumulation and a worldwide distribution, *Mytilus* sp. represent a species of choice for environmental studies (Goldberg and Bertine, 2000). Cross effects of

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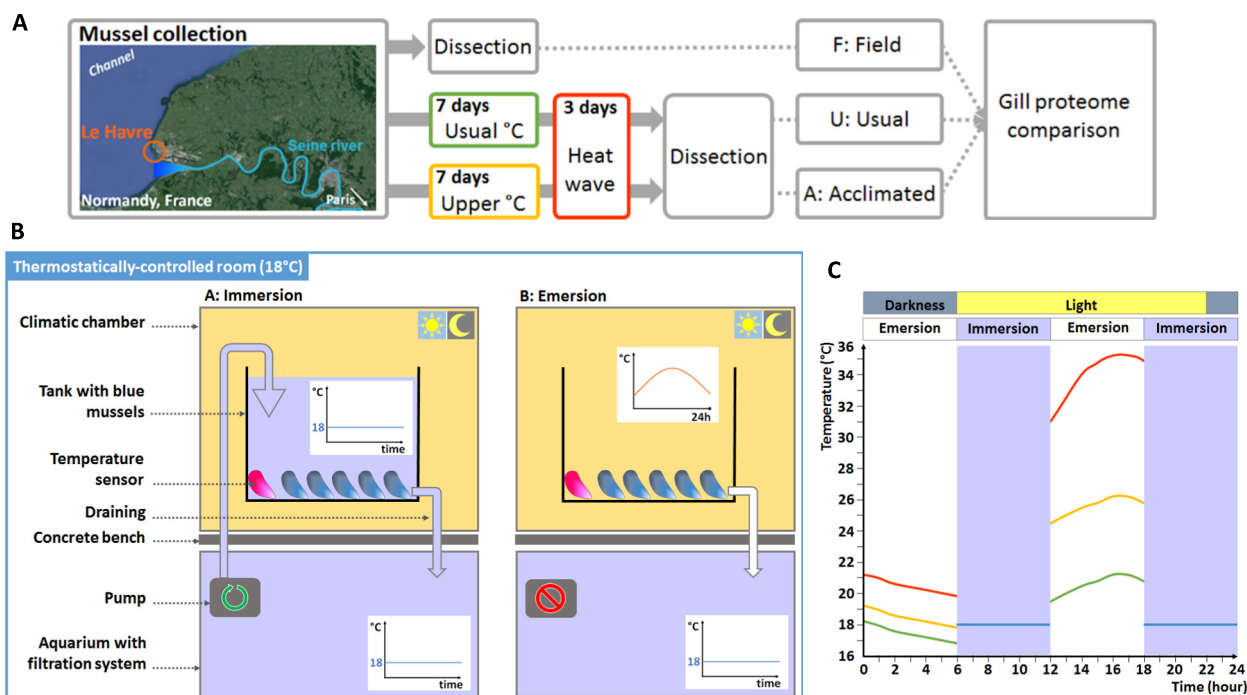


Fig. 1. Experimental design.

A. Collected mussels were either immediately sacrificed (field group, group F, grey) or placed in controlled conditions. Group U (usual, green) and group A (acclimated, yellow) were subjected to different air temperatures during 7 days and then exposed to an identical acute heat stress for 3 days. Proteome comparisons were conducted on gills. B. Microcosms allowed to reproduce tidal and day/night cycles. A biomimetic sensor was placed in the tank to monitor mussel temperature in real time. Water temperature was at 18° during the whole experiment while air temperature was driven by the climatic chamber. When immersed, mussels were subjected to 18 °C but at low tide, they were exposed to various air temperatures. C. Three types of daily air temperatures were developed. The first week, group U was at usual air temperatures ranging from 16.9 to 21.1 °C (green line) while group A was placed at higher temperatures ranging from 17.6 to 26.2 °C (yellow line). The 3 following days, both groups were submitted to an artificial heat-wave with temperatures ranging from 19.9 to 35.2 °C (red line). The third day at 6 pm animals were sacrificed.

temperature and pollution constitute an emerging topic and several studies pointed out the deleterious impact of both pollution and increasing temperature on survival (Gagné et al., 2007; Gunderson et al., 2016; Lannig et al., 2006). In an oyster study dedicated to joint thermal and cadmium stresses, Sokolova and collaborators demonstrated energy metabolism alterations associated with a mitochondrial disruption (Ivanina et al., 2015; Sokolova et al., 2004). In *Mytilus* gender, an integrative biomarker approach, the Index Biomarker Response (Beliaeff and Burgeot, 2002), revealed strong alterations in the metabolic status in *M. galloprovincialis* issuing from contaminated sites, especially in summer (Kamel et al., 2014). Temperature alone is known to trigger numerous regulations at the proteome level, such as HSPs induction or HIF over-expression (Fields et al., 2012; Péden et al., 2016; Tomanek and Zuzow, 2010). However, among studies dealing with impacts of multiple stresses upon marine organisms, few address combined effects of temperature and contamination. Furthermore, most of them focus on a single pollutant so the organism life history complexity is little considered (Gunderson et al., 2016; Przeslawski et al., 2015).

Le Havre harbour is located in the Seine River estuary (North-West coast of France) and is polluted by contaminants originating from agricultural runoff, urban sewage and inputs from industrial factories. In 2016, this harbour was ranked the fifth largest container port in the North European zone and the second French port in tonnage. Monitoring studies revealed high levels of polyaromatic hydrocarbons (PAHs), polychlorobiphenyles (PCB) and heavy metals in both sediments and mussels tissues collected in Le Havre harbour (Cachot et al., 2006; Fernandes et al., 1997; Rocher et al., 2006). More recently, the ICON project (Hylland et al., 2017) selected the Seine estuary as the French pilot area in the channel-North sea. This international workshop explored the relationships between pollution level and impact on

marine organisms through the study of a battery of biomarkers. The Seine estuary encompasses the entire area from Villerville (49.242 N, 0.074E) to Cap de la Hève (49.305 N, 0.409E) and includes Le Havre harbour. The whole area is under the influence of the Seine river plume. Once again high levels of PCBs, PAHs and heavy metals were pointed out (Burgeot et al., 2017) and detected at all levels: sediment, water and biota. Le Havre harbour was therefore defined as a heavily polluted area. Concerning blue mussels, several biomarkers were studied: lysosomal membrane stability, resistance to air exposure, condition index and scope for growth. Burgeot and collaborators concluded that lysosomal membrane stability was the most sensitive biomarker and defined the blue mussel status from the Seine estuary as “highly stressed”.

Understanding mechanisms involved in stress resistance may be addressed through molecular analyses. Indeed, biological processes are directly dependent of proteins which can be modified (i.e. phosphorylation, acetylation, oxidations and other post-translational modifications), so that the state of an organism is essentially reflected in its proteome (Feder and Walser, 2005; Mann and Jensen, 2003). The proteomic approach aims to a comprehensive, quantitative and qualitative description of protein expression and its changes under physiological or environmental conditions (Suárez-Ulloa et al., 2013; Tomanek, 2011). In this context, the two-dimensional electrophoresis allows to separate thousands of proteins and study their abundance at a time (Rabilloud et al., 2010). Moreover, 2DE is a well-suited method for non-model organisms whose genomes are partially known and to address post-translational modifications (Martins-de-Souza, 2018; Rogowska-Wrzęsinska et al., 2013). Hence, over the past ten years, several studies devoted to *Mytilus* and mainly based on 2DE were published. They aimed to establish a proteomic mapping (Rocher et al., 2015) and explore the impact of various stresses such as contaminants (Apraiz et al.,

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