Marine Environmental Research
July 2021, Volume 169 Pages 105393 (11p.)
https://doi.org/10.1016/j.marenvres.2021.105393
https://archimer.ifremer.fr/doc/00705/81742/

Molecular response of a sub-antarctic population of the blue mussel (*Mytilus edulis platensis*) to a moderate thermal stress

Bultelle F. [1, *], Boutet I. [2], Devin S. [3], Caza F. [4], St-Pierre Y. [4], Péden R. [1, 8], Brousseau P. [5], Chan P. [6], Vaudry D. [6, 7], Le Foll F [1], Fournier M. [5], Auffret Michel [9], Rocher B. [1]

[1] UMR-I 02 INERIS-URCA-ULH SEBIO / Environmental Stresses and Biomonitoring of Aquatic Ecosystems, FR CNRS 3730 Scale, Université Le Havre Normandie, F-76063, Le Havre Cedex, France
[2] Station Biologique de Roscoff CNRS, Laboratory Adaptation & Diversity in Marine Environment (UMR7144 CNRS-SU), Sorbonne Université, Roscoff, France
[3] UMR 7360 LIEC, Université Metz-Lorraine, France
[4] INRS-Institut Armand-Frappier, 531 Boul. des Prairies, Laval, Québec, H7V 1B7, Canada
[5] Institut des Sciences de la mer, Le Parc de la rivière Mitis, Sainte-Flavie, Québec, G0J 2L0, Canada
[6] Normandie Univ, UNIROUEN, Plateforme PISSARO, IRIB, 76821, Mont-Saint-Aignan, France
[7] Normandie Univ, UNIROUEN, INSERM U1239 DC2N, 76821, Mont-Saint-Aignan, France
[8] UMR-I 02 INERIS-URCA-ULH SEBIO / Environmental Stresses and Biomonitoring of Aquatic Ecosystems, Université de REIMS Champagne-Ardenne, Campus Moulin de la Housse, 51687, Reims, France
[9] UMR CNRS 6539-LEMAR/ Laboratoire des Sciences de l'Environnement Marin, Technopôle Brest-Iroise, 29280, Plouzané, France

* Corresponding author : F. Bultelle, email address : florence.buktelle@univ-lehavre.fr

isabelle.boulet@sb-roscoff.fr ; simon.devin@univ-lorraine.fr ; france.caza@iaf.inrs.ca ; yves.st-pierre@iaf.inrs.ca ; romain.peden@univ-reims.fr ; Pauline_Brousseau@uqar.ca ; philippe.chan@univ-rouen.fr ; david.vaudry@univ-rouen.fr ; frank.le-foll@univ.lehavre.fr; Michel_Fournier@uqar.ca ; Michel.Auffret@univ-brest.fr ; beatrice.rocher@univ-lehavre.fr

Abstract :

The Kerguelen Islands (49°26′S, 69°50′E) represent a unique environment due to their geographical isolation, which protects them from anthropogenic pollution. The ability of the endemic mussel, part of the Mytilus complex, to cope with moderate heat stress was explored using omic tools. Transcripts involved in six major metabolic functions were selected and the qRT-PCR data indicated mainly changes in aerobic and anaerobic energy metabolism and stress response. Proteomic comparisons revealed a typical stress response pattern with cytoskeleton modifications and elements suggesting increased energy metabolism. Results also suggest conservation of protein homeostasis by the long-lasting presence of HSP while a general decrease in transcription is observed. The overall findings are consistent with an adaptive response to moderate stresses in mussels in good physiological condition, i.e. living in a low-impact site,

and with the literature concerning this model species. Therefore, local blue mussels could be advantageously integrated into biomonitoring strategies, especially in the context of Global Change.

Highlights

► Kerguelen Islands: an opportunity to study animal adaptive capacities from a wildlife sanctuary. ► What is the ability of the endemic blue mussel to deal with heat stress? ► Observed changes in energy metabolism, cytoskeleton and lasting presence of HSPs. ► A new *Mytilus* to be enrolled in biomonitoring programs?

Keywords : Kerguelen island, Mytilus sp, Indicator species, Gills, Abiotic stress, Temperature, qRT-PCR, 2DE, Biomonitoring

1. Introduction

The sub-Antarctic islands are amongst the most isolated territories from any continental landmass and contain a number of marine, coastal habitats present at these latitudes. On one hand, due to rapid and drastic changes in climatic conditions at high latitudes, accurate assessments of the sensitivity and vulnerability of polar organisms must be achieved in order to reliably predict possible changes in those ecosystems. On the other hand, thanks to scientific projects conducted in this location for decades, observation and experimentation on sentinel species provide priceless opportunities to improve our knowledge of adaptive responses and ecophysiological strategies in invertebrates from naturally, highly variable environments, such as intertidal habitats (Caza et al., 2015; Suda et al., 2015; Guillaumot et al., 2020). In addition, the physicochemical quality of marine waters is much influenced by both hydrologic factors and atmospheric inputs. Hence, these isolated marine ecosystems have been found to face anthropogenic pressure, as trace contaminations were reported in aquatic habitats and biota (Carravieri et al., 2013; Jaffal et al., 2015).

Scientific consensus has been reached on Global Change and several scenarios predict significant average temperature increase toward 2100 (Intergovernmental Panel on Climate Change-IPCC, Pachauri et al., 2014; Pörtner et al., 2019). They anticipate not only a global warming, but also an increase of extreme events such as heat waves. One challenge we face is to decipher temperature impact on organisms when combined with pre-existing stress. Marine invertebrates have been widely used as bioindicators of coastal water quality, mainly for monitoring chemical pollution, in order to measure site contamination levels but also to assess biological effects on invertebrate communities (Auffret, 2005; Beyer et al., 2017). The intertidal belt of the Kerguelen archipelago is inhabited by numerous mussel beds. Two species share the same rocky shore habitat: the Antarctic sub-species of blue mussel, *Mytilus edulis platensis* (*M. edulis platensis*) and the ribbed mussel *Aulacomya atra*. Blue mussels are keystone species in coastal ecosystems distributed in many oceans as a result of genetic differentiation within smooth-shelled mussels, now grouped in the so-called *Mytilus* complex. In the Northern Hemisphere, the *M. edulis* complex of species is composed of three genetically well delineated taxa: *M. edulis*, *M. galloprovincialis* and *M. trossulus* (Gerard et al, 2008). The occurrence of blue mussels has also been reported in the Southern Hemisphere, as *M. galloprovincialis* and so-called "native Chilean smooth-shelled *Mytilus*" at the southern tip of the South American continent (Borsa et al, 2012). The latter species was assigned at a subspecific rank and named *M. edulis platensis* by these

authors. Kerguelen mussels, earlier described as *M. desolationis*, are now assigned as *M. edulis platensis* (Gerard et al, 2015). To our knowledge, there is no report relating the occurrence of other members of the genus *Mytilus* at the Kerguelen archipelago, nor was any introduction reported to date. The ecophysiological status of mussel populations remains largely unknown. In a recent study, Caza et al. (2015) compared the immune status of the two cohabiting mussel species and observed that responses to experimental abiotic changes differed between these species suggesting possible contrasted resilience to environmental stress.

The present study focused on stress ecophysiology of the endemic blue mussels collected from natural populations in response to temperature stress. Investigations in those osmo- and thermo-conformers were expected to produce valuable baseline biomarker signatures that would be relevant for monitoring both anthropogenic and climatic pressure on high latitudes and other pristine coastal ecosystems. Temperature variations were found to modify physiological processes such as respiration and nutrition, but also numerous molecular pathways, with species-specific thermal tolerance (Kültz, 2020; Somero, 2020). At the physiological level, small temperature variation increases respiration and nutrition processes, but when more stressful temperature occurs, metabolism shuts down with marked decrease of respiration rate.

In bivalve mollusks, respiration and nutrition are supported by the gills, an interface organ between the mussel and its environment. In the present work, gill tissues collected from mussels exposed to a mild thermal stress were analyzed by proteomic and transcriptomic tools using two-dimensional gel electrophoresis (2DE) and qPCR, respectively. On-gel 2DE constitutes an open approach to analyze complex proteomic patterns and detect differentially abundant proteoforms (DAPs) (Rabilloud et al., 2010; Lee et al., 2020). In parallel with the proteomic analyses, the mRNA expression of 51 genes was monitored to determine the impact of exposure to increased temperature on the expression pattern of individuals. These genes were chosen to cover different metabolic pathways (energetic metabolism, oxidative stress scavenging, stress response, detoxication, immune response and cell cycle regulation), on the basis of previous studies. Hence, molecular components involved in the process of adaptation and resistance to thermal stress are numerous and have been reviewed in several publications of interest (for examples, please see: Somero, 2010; Lockwood and Somero, 2011; Fields et al., 2012; Tomanek, 2012, 2014; Péden et al., 2016; Clark et al., 2017; Coppola et al., 2018; Feidantsis et al., 2020). In addition, we genotyped individuals (Glu-5' locus, Inoue et al.; 1995; Gerard et al., 2015) to

verify whether there is potential inter-individual variability in the response pattern to temperature change. In summary, this study aimed to investigate the ability of the endemic blue mussels to cope with a thermal stress.

2. Material and Methods

2.1. Site description and mussel collection

Kerguelen is a small, isolated archipelago in the Southern Indian Ocean characterized by a highly heterogeneous environment. The proximity of the polar front generates a temperature contrast between the northern and the southern coasts. The circumpolar, oceanic front creates two separated branches from the western side of the main island. Combined with high freshwater inputs from many rivers, waters in the northeastern part of the archipelago have relatively lower salinity, as in most locations at the mouth of many small coastal rivers.

Seawater temperatures at Kerguelen were obtained from the long-term monitoring program "Proteker" (Féral et al. 2016). It revealed that between 2012 and 2019 the mean annual temperature in the Baie du Morbihan where mussels were collected varied between 4.1 and 5.5°C at 5 m depth. The maximums observed ranged between 5.6 and 11.1°C during the austral summer while the minimums ranged between 1.0 and 4.6°C in the austral winter (http://www.proteker.net/-Thermorecorders-.html).

Mussels (*M. edulis platensis*) (65.23 ± 4.55 mm shell length) were collected from Kerguelen Archipelago (Bras-Laboureur: 049°27.897S / 069°66.086E) in December of 2013. The mussels were sampled at once in the intertidal zone and kept in 20L containers filled with clean, aerated seawater collected offshore at the sampling site. The water was oxygenated by aeration using a battery-operated portable air pump and organisms were maintained throughout the transport phase at a water temperature close to those measured on field (7.5°C).

Once at the Port-aux-Français laboratory (approximately 6h post-harvest), the mussels were divided in homogenous groups of 50 individuals per 50L aerated aquarium tanks containing filtered recirculating seawater maintained on a 12h:12h light cycle (Fig. 1). A 7-days acclimation period was conducted at 7.5°C, which corresponds to the water temperature observed in the field. A first sampling was then performed for gene expression analysis (T0-control and T0-test). After acclimation, one group of mussels was maintained at the field temperature while the other group was exposed to a mild thermal challenge. Specifically, water temperature was increased from 7.5 to 20°C over a period of 7 days

(+1.8°C per day). Finally, mussels were kept under their thermal conditions with daily follow up of temperature, salinity and pH until their sacrifice at T28 (group T28-control and group T28-test). For all sampling, gills were dissected out, immediately frozen in liquid nitrogen and stored at -80°C until analysis. Our experimental design is based on the individual as the sampling unit (and thus, as the replication level), and the level at which we want to assess variations in gene expression and proteomics.

2.2. Gene expression

2.2.1. RNA extraction and qRT-PCR analysis

Total RNA was extracted from gill tissue using TRI Reagent® solution (Ambion) according to the manufacturer's instructions (n=10 per condition). Three µg of total RNA were reverse transcribed using M-MLV reverse transcriptase (Promega, Madison, WI), random hexamers (Promega) and an anchor-oligo(dT) primer (5'-CGCTCTAGAACTAGTGGATCT$_{(17)}$-3'). The relative mRNA expression of 51 genes selected for their involvement in different metabolisms was estimated by real-time PCR amplification using specific primers (Table S1). A volume of 2.1 µl of each diluted reverse transcription product (1:100) was subjected to real-time PCR in a final volume of 5 µl containing 0.4µM of primers and 1X LightCycler® 480 SYBR green I master (Roche, Germany). The amplification was carried out as follows: initial enzyme activation at 95°C for 6 min, then 40 cycles of 95°C for 10 sec, hybridization at 60°C for 30 sec and elongation at 72°C for 30 sec. Calibration curves were obtained using serial dilution of a cDNA pool from 12 individuals. In this study, we used a fragment of 28S as a reference gene, because other commonly used reference genes (such as EF1α or actin) are often found regulated in stressed bivalves (Boutet et al., 2004; Tanguy et al., 2004; David et al., 2005). Relative expression of each gene was calculated according to comparative Ct method using the formula: $RQ = 2^{-\Delta Ct}$.

2.2.2. Statistical analysis

In order to assess modifications of transcriptomics response patterns according to time and temperature, all the dataset was analyzed by using a PLS-DA (Partial Least Square Discriminant Analysis, MixOmics package version 6.6.2., Le Cao et al. 2016). This method is appropriate for experimental designs with far more variables than individuals within each group, and when the dataset contains missing values. It allows identification of the gene expression levels that are the most suitable to separate exposure conditions, *i.e.* whose variations are characteristics of each condition.

Important genes were selected based on the VIP (Variable Importance in the Projection) values, with a threshold value of 1. Exposure conditions were then plotted on a factorial plane combining the two first axes of the PLS-DA, while variables were plotted on a correlation circle, giving the correlation of each variable with the axes of the PLS-DA. The significance of the exposure condition discrimination was assessed by a permutation test based on a cross-validation test (Westerhuis et al., 2010; Szymańska et al., 2012, RVAideMemoire package version 0.9-75). Here, the structure described by our classification variable (exposure condition) was analyzed using a "null model". In this model, gene expression data are randomized among the two groups, to generate a structure where chance is the only driver of the dataset. At each iteration, the observed and the random structure are compared, and the model is significant if the discrimination is better with the observed rather than with the random dataset. In a first step, the test was performed to define whether the built model was significant, hence that at least one group is significantly discriminated from the others. Once this first assessment done, a second test performs paired comparisons among the four experimental groups with an FDR (false detection rate) adjustment of p-values, to further explore gene expression differences between each group. All analyses were performed with RStudio version 1.1.463, with R version 3.6.3 (R Core Team 2000, RStudio Team 2016).

2.2.3. Glu-5′ genotyping

After extracting DNA from the gills using a standard phenol-chloroform-isoamyl alcohol extraction protocol (25:24:1), the locus *Glu-5′* was amplified with primers Me-15 (5'-CCAGTATACAAACCTGTGAAGAC-3') and Me-17 (5'-CTGGTGGATAATTTGTCTTTGC-3'). This locus is located at the 5′ extremity of exon Glu coding for an adhesive foot protein (Inoue et al. 1995; Rawson et al., 1996). This locus contains an insertion/deletion (indel) zone, whose amplification reveals three alleles: (T, E and G) that, respectively distinguish *M. trossulus*, *M. edulis*, and *M. galloprovincialis* in the Northern Hemisphere (Borsa et al., 1999). Here, the amplification of the *Glu-5'* exon produced 210-bp (allele E) and 160-bp (allele G) fragments, typical of, respectively, *M. edulis* and *M. galloprovincialis* from the Northern Hemisphere (Inoue et al., 1995), but allowing identification of local adaptations of *M. edulis platensis* in Kerguelen (Gerard et al, 2015). The genotypes at *Glu-5'* were determined from fragment-length variation on 2% agarose gels.

Differences in gene expression according to the genotype was studied through a PCA. Since the database contains missing values, a first step was to impute missing values using the imputePCA function of the missMDA package v1.18 (Josse and Huchon, 2013, 2016). Then a classical PCA was performed with the ade4 package v1.7-15 (Dray and Dufour, 2007). To confirm the absence of structure observed in the factorial plane, a MANOVA on the individuals coordinates on the two first axes of the PCA, with the exposure condition x genotype combination as independent variable was performed.

Since the experimental design was not equilibrated for genotype, since it's only a posteriori data, no systematic test was possible to compare gene expression at each condition and to test for interaction between exposure duration and temperature. However, some control can be performed. The tank effect at T0 was compared with the (EG) genotype. After checking for normality and homoscedasticity, we performed either a student t test or a Welch t test. With the same procedure, we tested the exposure duration effect on gene expression with the (EE) genotype for the control tank (n=3 for T0, n=4 for T28). Finally, the genotype effect was assessed at T0, in the test tank (n=3 for (EE) genotype and n=6 for (EG) genotype).

2.3. Proteome analysis and statistical treatment

Protein extraction and two-dimensional analysis were performed as previously described in Péden et al., 2016. Gills of mussels sacrificed at day 28 (two conditions: group T28-control and group T28-test, n=8) were ground using Precellys® Homogenizers and sonicated in a 1:4 ratio of lysis buffer (9 M urea, 2% CHAPS, 65 mM dithioerythritol, 0.02% pharmalyte 3-10NL, GE Healthcare and 16 $\mu g \cdot mL^{-1}$ aprotinin). Cellular debris was removed by centrifugation at 10000× *g* for 20 min at 20°C and protein concentrations of supernatants were determined according to the method of Bradford with bovine serum albumin as a standard. For 2D electrophoresis, 750 µg of proteins per sample were loaded on non-linear wide-range immobilized pH gradients (IPG strip pH 3-10, NL/18 cm, GE Healthcare). The IEF (isoelectrofocusing) was carried out with a horizontal electrophoresis apparatus (Multiphor, Amersham Pharmacia Biotech). The electric parameters were as follow: 500 V for 0.01 h (gradient), 500 V for 5 h, 3500 V for 5 h (gradient) and 3500 V for 9.5 h. After IEF, strips were incubated for 15 min in an equilibration buffer (0.5 M Tris buffer pH 6.8 with 6 M urea, 1% SDS, 26% glycerol) containing first, 30 mM dithiothreitol and second, 245 mM iodoacetamide. SDS-PAGE electrophoresis was performed with a Protean plus Dodeca-Cell (Bio-Rad) allowing a parallel run of 12 large gels (12% polyacrylamide, 20

cm x 20 cm x 1.5 cm). After migration, proteins were visualized by colloidal blue staining. Gels were scanned using the GS-800™ Calibrated Densitometer (Bio-Rad) and exported from Quantity One as raw file (16-bits, .tif). Only gels exhibiting highly reproducible patterns were selected for analysis (4 per condition). Images were analyzed using Delta 2D (Decodon, GmbH). With this software, a single composite image named the fusion gel was created using the group warping strategy to connect gel images through match vectors. After quality checking, a gel fusion of 957 spots was obtained. The spot boundaries detected on the fusion gel were subsequently transferred back to all the gels, establishing spot parameters. For each spot, the normalized volume was expressed as percentage of the total volume of all spots of the gel. Normalized volumes were compared between control group (group T28-control) and group of mussels exposed to 20°C (group T28-test) following a Student t-test. A total of 61 spots passed the $p<0.01$ cut-off and of these, 49 were determined to be correctly delimited on all individual gels and therefore were considered as significantly different.

2.4. MS/MS and protein identification

Spots of interest were excised manually from colloidal blue stained 2D gel and submitted to in gel digest by trypsin (Promega France). After digestion, peptides were dried on a speedvac, resuspended in 15 µL of 3% (v/v) acetonitrile and 0.1% (v/v) formic acid, and then analyzed with a nano-LC1200 system coupled to a Q-TOF 6520 mass spectrometer equipped with a nanospray source and an HPLC-chip cube interface (Agilent Technologies). A 30-min linear gradient (3-80% acetonitrile in 0.1% formic acid), at a flow rate of 370 nL/min, was used to separate peptides on polaris-HR-Chip C18 column (150 mm long x 75 µm inner diameter). Full autoMS1 scans from 200 to 1700 m/z and autoMS2 from 9 to 1700 m/z were recorded. In every cycle, a maximum of 5 precursors sort by charge state (2+ referred and single-charged ions excluded) were isolated and fragmented in the collision cell. Collision cell energy was automatically adjusted depending on the m/z. Active exclusion of these precursors was enabled after 2 spectra within 1 min, and the threshold for precursor selection was set to 4000 counts. A peak list for database searching was created by using Masshunter Workstation-Qualitative Analysis (version B.04.00; Agilent Technologies). For protein identification, MS/MS peak lists were extracted and analyzed with X!tandem pipeline (http://pappso.inrae.fr/). Mass spectra were investigated using protein databanks translated with TransDecoder from RNA-seq data of *Mytilus galloprovincialis* and *Crassostrea gigas* species (Moreira et al., 2015). The searches were performed with the following specific parameters:

enzyme specificity, trypsin; one missed cleavage permitted; variable modifications, methionine oxidation, cysteine carbamidomethylation; peptide mass tolerance, 10 ppm; fragment mass tolerance, 0.5 Da. As filter identification results, the following parameters were used: peptide Evalue, 0.05; minimum number of peptides per protein, 2; protein Evalue -4.0. Protein blast search was done against the non-redundant protein database from NCBI (https://blast.ncbi.nlm.nih.gov).

2.6. Protein network analysis

Association network analysis of proteins was conducted using STRING (https://string-db.org; Szklarczyk et al., 2019). STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database of known and predicted protein-protein interactions, including physical and functional associations. The data stem from computational prediction, from knowledge transfer between organisms, and from interactions aggregated from other databases. The interaction map was generated from the web-based search STRING v11.0 database (https://string-db.org/). Default settings are as follows: evidences were selected for meaning of network edges; all active interaction sources were used for linkage; minimum required interaction score was fixed at medium confidence (0.400). Network nodes represent proteins. Edges represent protein-protein associations, meant to be specific and meaningful, *i.e.* proteins jointly contribute to a shared function.

3. Results & discussion

The average temperature at Kerguelen depends on numerous factors, including depth, localization, seasons, currents, etc. In summer, the typical temperature at a 5m depth is approximately 7-8°C (Féral et al., 2016). In summer, during low tide, however, the temperature in mussel beds can reach more than 10-15°C, and possibly more in sunny conditions. The 20°C exposure condition is thus not an unrealistic scenario and is most likely to become a common event given the increase of seawater temperature at Kerguelen. The 28-day period is an experimental condition that more or less represents a semi-chronic exposure often used to study the effect of specific factors on the health status of mussels (Beaudry et al., 2016; Coppola et al., 2018; Lenz et al., 2018, etc.).

A recurrent debate exists within scientific literature on what is the appropriate level of replication in ecological and ecotoxicological studies (Bastos et al., 2013; Devin et al., 2014; Davies and Gray, 2015). Indeed, pseudoreplication can’t be avoided, and a tank effect can have occurred in our experiment.

However, given the tested hypothesis and the recorded biological responses, it's far more likely that it's the thermal stress that induced such effects.

3.1. Transcriptional analysis reveals metabolic adaptations

Our results indicate that mRNA expression profiles are significantly different between groups of mussels subjected or not to a temperature increase (Table 1 and Table S2 for significant and non-significant results, respectively). The gene expression levels allow a better discrimination than a null model, with a classification error rate of 0.56 (p=0.008). Such a high level of error rate is not surprising, since no differences were expected between the two T0 tanks (T0-control and T0-test), nor between T0-control and T28-control (absence of thermal stress).

Looking at the factorial plane (Fig. 2A) focused on genes with VIP > 1 (threshold considered as significant in the PLS analysis, Fig. 2B and Table 1), it appears that the ellipses corresponding to the groups of mussels maintained at field temperature (T0-control and T28-control) are superimposed (p=0.23), indicating that the expression profiles do not vary over time. For individuals of the test tank at T0, the superposition of the ellipses is also present with the control groups (T0-control and T0-test, p=0.59), evidencing that the initial physiological state of each group of mussels was the same and that the exposure time alone did not induce any modification in gene expression, while an increase in inter-individual variability is observed. Finally, after 28 days of exposure, a significant difference in gene expression level between the two-groups was evidenced (T28-control and T28-test, p=0.014). However, in relation to this variability, pairwise comparisons failed to evidence significant differences between-conditions. In order to identify the potential source of these variations, we hypothesized that an underlying genetic component could partly explain the increase in inter-individual variability in the group of mussels exposed to 20°C. In a previous study, potential correlations between the phosphoglucomutase genotype and the level of mRNA expression have been shown in the hydrothermal mussel *Bathymodiolus azoricus* exposed to different temperature increases (Boutet al., 2009). In this study, we genotyped the *Glu-5'* locus, which was also used to determine the fine genetic structure of the Kerguelen mussels (Gerard et al., 2015) and showed that the three different genotypes (EE, EG and GG) are represented in the four experimental groups (Table S3). In order to detect a potential difference in mRNA expression depending on the genotype of the individuals, a PCA was performed. The factorial plane evidenced no particular structure within the data, with random distribution of genotypes among

exposure conditions (Fig. 3). The MANOVA performed on the individuals with the exposure condition x genotype combination as independent variable was not significant (Wilks Lambda = 0.557, p=0.74). Other tests done to detect a potential effect of conditioning of individuals (tanks and exposure time) are not significant, showing that for a given condition individuals are impacted in the same way. Finally, the genotype effect was assessed at T0, in the test tank (n=3 for (EE) and n=6 for (EG)). In this last case, 5 significant differences were found between expression levels of the two genotypes: ATP synthase (p=0.002), Big defensin (p=0.008), HSP90 (p=0.037), mitMDH (p=0.046) and PGmut (p=0.043). Values were similar for the 46 other genes. As no mortality related to conditioning or temperature increase was observed over the duration of the experiment, this suggests that the mussels are capable of withstanding a relatively large temperature change. In their original environment, mussels are subjected to strong variations related to the tides (emersion, sudden temperature change, etc.) and their distribution on the intertidal zone is not only conditioned by these factors but also by the structure of the environment (rock vs. sand, waves, fresh water input, presence or absence of an algae bed). These constraints influence the genetic structuring of the mussels (detected with the nuclear marker *Glu-5'*) at the scale of the archipelago but also at the scale of a site (Gerard et al., 2015). Although this study is purely genetic and does not provide information on the physiology of these mussel populations, we can assume that individuals carrying different *Glu-5'* genotype have different responses to environmental variations.

Increase of water temperature is known to induce changes at mRNA expression level as a part of cellular stress response (CSR) (Evans and Hofmann, 2012), including genes related to various metabolism processes in order to maintain cell functions (Kültz, 2005; Lockwood et al., 2010, 2015; Somero, 2010, 2020). In the present study, the expression of 51 genes involved in different metabolism pathways was analyzed (Table S1) and among them, 17 showed significant variations in expression between groups of mussels exposed to 7.5°C or 20°C at T28 (Table 1). Interestingly, the expression of most of them decreased after exposure to 20°C and only 4 had higher expression in the same individuals. More generally, when all the genes are observed (including those with no significant differences in expression), mRNA expression decreases after exposure to 20°C. Even if the differences are not significant on the global analysis, it can nevertheless be noted that the lack of significance at this level is partially explained by the inter-individual variability observed previously (Fig. 2). The decrease in gene expression and more generally in metabolism in response to stress (temperature, hypoxia, etc.) has been particularly well described in marine organisms (Storey and Storey, 2004; Anestis et al., 2007,

2008; Boutet et al., 2009) and is presented as an essential mechanism during hibernation, estivation, oxygen deprivation or periods of intense heat stress (see Storey and Storey, 2004 for review). In the case of Kerguelen mussels, exposure to 20°C, although considered a moderate stress, seems to affect the metabolism at the transcriptional level to limit energy cost. At the global level, genes coding for proteins involved in energy metabolism (aerobic and anaerobic) seem to be strongly impacted: 6 have a significant decrease in expression (*glyc phos*, *aconitase*, *TPI*, *octDH*, *enolase* and *aldolase*) and 1 an increase (*ATP synth*) (Table 1), and the other 19 genes involved in energy metabolism show a decrease in expression although not significant. Our results with ATP synthase subunit α are consistent with the fact that response to stress impacts organism metabolism according to its duration and/or intensity and that mussels have to cope with energy demand through ATP production to maintain organism and cell functions (Sokolova, 2013). ATP synthase is particularly involved in this mechanism and is actively regulated at both transcriptomic and proteomic levels. Moreover, the anaerobic metabolic pathways commonly involved in the response to thermal and hypoxic stress in coastal organisms (opine pathways for example, Harcet et al., 2013; Han et al., 2017), are here regulated (significantly or not) in the sense of a decrease in mRNA expression. Indeed, while *LDH* and *cytMDH* expression did not vary significantly, *OctDH* mRNA is significantly less expressed in mussels exposed to 20°C. It can be assumed that these enzymes are regulated at post-transcriptional level to respond to environmental constraints, as is the case here for cytMDH which shows an increase in the amount of protein (see section 3.2).

In response to an increase in temperature, the expression of some other selected varies significantly: *Hsp60* (stress response), *mt20* (detoxification) and *HFI* (O_2 sensing) are overexpressed in individuals exposed to 20°C. Several studies have shown a regulation of *Hsp60* mRNA expression in marine bivalves in response to increased temperature, without fully understanding its direct involvement in this response (Snyder et al., 2001; Ding et al., 2018). The other members of the HSP family studied here show either a significant decrease in expression (HSP24 and HSP90) or an absence of variation (HSC71, HSP22 and HSP78). In this work, it would appear that stress proteins are also regulated at the post-transcriptional level.

Interestingly, the MT10 and MT20 metallothionein system is antagonistically regulated, with *mt10* showing a decrease in expression while *mt20* increases in the gills of mussels exposed to 20°C. Although MTs are mainly involved in protection against metals or oxidative conditions (Le et al., 2016), an increase in the level of *mt20* expression has already been shown in thermally challenged mussels

(Hamer et al., 2008; Gourou et al., 2010; Feidantsis et al., 2020). On the other hand, *mt10* has so far rather been considered as having no expression variations (constitutive form as opposed to the inducible form MT20, Dondero et al., 2005; Raspor et al., 2006; Gourou et al., 2010). However, several studies have reported variations in the expression of *mt10* (often an increase) in response to different stresses (Banni et al., 2014; Lacroix et al., 2014; Rossi et al., 2016). It thus appears that Kerguelen mussels have a different response to *mt10* gene expression than other mussels (especially from the northern hemisphere) characterized by a decrease of expression.

Under natural conditions, an increase in temperature reduces the amount of available oxygen, thereby inducing the risk of hypoxia-driven stress response for organisms, which have higher metabolic demand (Vaquer-Sunyer et al., 2008; Breitburg et al., 2018; Roman et al., 2019). During the experiment, the mussels were in constant immersion and the water was continuously aerated; no hypoxic events were thus caused by the temperature increase in the tanks. The regulation of genes involved in oxygen sensing is therefore not linked to a decrease in available oxygen but to the temperature increase itself. In this work, the expression profile of the 5 genes coding for proteins involved in the response to oxidative stress and O_2 sensing is variable: indeed, only Hypoxia-inducible factor 1 α inhibitor (*HFI*) shows a significant increase in its expression, while *CuZnSOD*, *perox 6* and *P-hydrox* show a non-significant decrease and catalase increases non-significantly. These genes have been used as biomarkers in other studies and show significant variations in their level of expression (Banni et al., 2014). The activation pathways by HIF-1α, although acting promptly during a hypoxic event (Hochachka and Lutz, 2001; Kawabe et al., 2012; Cai et al., 2014; Giannetto et al., 2015), seem to be regulated here by an increase in temperature. However, it is difficult to determine the exact role of HFI in the regulation of the gene cascades involved in the response of mussels. Indeed, HIF-1 α is involved in the regulation of many metabolic pathways under stress conditions, notably glycolysis (Hochachka and Lutz, 2001) and its regulation by an inhibitor at the transcriptional level is complex to relate to the overall response of the organism.

3.2. Proteomic analysis pinpoints a marked recruitment of stress proteins.

In ecotoxicology, 2DE has been widely used to study *Mytilus* species responses to various stresses including temperature, mainly in *M. galloprovincialis*, *M. californianus* or *M. trossulus* (Tomanek and Zuzow, 2010; Ji et al., 2013; Gomes et al., 2014; Xu et al., 2016) but also in *M. edulis* (Apraiz et al.,

2006; Sheehan and McDonagh, 2008; Letendre et al., 2011; Hu et al., 2014; Péden et al., 2016; Péden et al., 2018). We thus conducted a 2DE proteomic analysis of gills collected from mussels maintained at 7.5°C and 20°C.

Eight gels displaying highly reproducible patterns were kept for further analyses (4 per condition). The analysis of the 2DE gel images using Delta 2D (Decodon) produced a final dataset of 957 proteoforms. Analyses revealed that 49 of them were significantly differentially abundant with a 0.01 p-value, of which 37 exhibited higher abundance and 12 were less abundant compared to control (Table 2 and Fig. S1). Most of the proteoforms were up-regulated (67%) and 37 out of 49 were identified by mass spectrometry (Table S4). The overall response corresponds to limited modifications of the gill proteome in terms of number of DAPs and fold changes. Those elements suggest there is no cellular homeostasis disruption in the thermal stress group, only adjustments (Brose et al., 2012).

The interaction map of the 37 aforementioned DAPs was generated from the web-based search STRING database (Fig. 4). The resulting network of protein-protein interaction (PPI) exhibited an enrichment PPI p-value lower than1.10^{-16}, indicating significantly more interactions than randomly expected. In particular, Cellular response to heat stress is the first Reactome Pathways of the network with a false discovery rate of $1.03\ 10^{-7}$ and a cluster of 7 proteins (surrounded in bold in Fig. 4 and Table 2), all presented an increase in abundance in gills of T28-test mussels as compared to the control group: HSP90 A1 (spot 1536), HSP90 B1 (spot 1454), HSC 71 (spot 1608), HSPA 5 (spot 1552), HSP70, spot 1599, HSP56 (spot 1756) and 14-3-3 protein epsilon (spot 2194). All of these proteins, with the exception of the 14-3-3 protein epsilon, belong to the highly conserved Heat Shock Protein family (HSP) which participates in folding and allows protein preservation or renaturation after stress-induced alteration. Upregulation of members of this family is commonly observed during adaptive response to various stresses (Buckley et al., 2001; Kültz, 2003; Hartl et al., 2011; Brose et al., 2012; Powers and Balch, 2013; Kültz, 2020; Somero, 2020). 14-3-3 proteins are adapter proteins implicated in the regulation of a large spectrum of signaling pathways, including glucose metabolism, apoptosis, cell cycle progression, autophagy and cell motility. Other proteoforms involved in response to various stresses were also modified: peptidyl-prolyl cis-trans isomerase B (PPIB, spot 2241), heat shock protein 40 (DNAJB11, spot 1917), heat shock protein 75 kDa, mitochondrial (TRAP1, spot 1588) and a peroxiredoxin (PRDX, spot 2349), the latter being the only one with a decreased abundance. Moreover, the neutral αglucosidase AB is a non-HSP family proteoform involved in folding and quality control in endoplasmic

425 reticulum and was upregulated. Interestingly, we also observed over-expression of a galectin (spot
426 1656). Galectins belong to the multi-functional lectin family and may act as stress sensors. For instance,
427 galectins are involved in autophagy and apoptosis and contribute to innate immune response in
428 invertebrates (Bai et al., 2017). Taken together, these results indicate that exposure of mussels at 20°C
429 was sufficient to induce a mild but significant stress response.
430 In addition to variations in stress proteins, we paid particular attention to proteins involved in cytoskeletal
431 rearrangement and cell trafficking (Fig. 4). Indeed, cytoskeleton is often targeted under stress (Magi et
432 al., 2008; Letendre et al., 2011; Jayasundara et al., 2015; Madeira et al., 2020). Our results showed a
433 significant increase in abundance of β-actin (spot 1913) and tubulin (spot 1721). Expression of other
434 cytoskeletal proteins included an actin filament bundling (fascin, spot 1738), a membrane-cytoskeleton
435 linker protein (radixin, spot 1557), and a protein involved in polymerization and depolymerization of actin
436 filaments (66KDa stress protein, spot 1669). These results are consistent with data from previous
437 studies showing that a temperature stress increases ciliary activity, filtration function and respiratory
438 process in gills of *Mytilus edulis* (Tomanek, 2012). Those changes are accompanied by cell network
439 remodeling. Thus, the cellular traffic category is represented by the sorting nexin 6-like (upregulation,
440 spot 1851) which participates in protein recycling and also in retrograde transport of cargo proteins from
441 endosomes to the trans-Golgi network. This response remains moderate but indicates architectural and
442 transport cellular redesign for adaptation to thermal exposure (Fields et al., 2012). Finally, a wide range
443 of cytoskeletal proteins were observed among the identified DAPs, including other proteins involved in
444 assembly and maintenance of epithelial cells such as tropomodulin 3 (spot 1973), gelsolin (spot 1909)
445 and a predicted centrosomal protein of 135 kDa (spot 1437), the latter being the only one to show a
446 decrease in abundance in comparison with the controls. As discussed above, changes in the
447 cytoskeleton have already been identified in studies examining heat stress in mussels, and in addition
448 to adaptations to cell stress, they may reflect cell proliferation requirements.
449 Facing thermal stress implies an energy cost that requires cellular energy mobilization (Tomanek, 2012;
450 Somero, 2020). Our findings provide several clues to such demands (Table 2 and Fig. 4): firstly, a rise
451 of cytosolic malate dehydrogenase (spot 2078); secondly, an increase in several mitochondrial actors,
452 namely a NADH ubiquinone oxidoreductase 75kDa (core subunit of respiratory chain complex I, spot
453 1578), a succinate dehydrogenase [ubiquinone] flavoprotein subunit (major catalytic subunit of
454 respiratory chain complex II, spot 1616), a glutamate dehydrogenase (mitochondrial matrix enzyme

involved in glutamine anaplerosis by catalyzing the oxidative deamination of glutamate to alpha-ketoglutarate and ammonia, spot 1735) and prohibitin (spot 2244), mainly involved in mitochondria biogenesis and activity (Signorile et al., 2019). These results suggest an increase in mitochondrial metabolism, *i.e.* an enhanced energy supply capacity. This point will be further discussed in the following section.

3.3. Omics data comparison is consistent with enhanced energy supply supporting a persistent cellular stress response.

Although qRT-PCR and proteomic analyses were conducted independently, it is attractive to compare them in order to seek consistency and gain a clearer insight into the cellular processes involved in the response to heat stress.

In this study, a point-by-point comparison results in a significant but expected discrepancy. Hence, only 4 actors are common to both data sets (HSP90, HSC71, HSPA5 and cytosolic malate dehydrogenase); none of them exhibited an identical variation (*i.e.* up/down regulation). Two elements can be raised. First, discrepancy between transcriptomic and proteomic responses has been frequently observed and is fairly widely accepted (Romero et al., 2019). This phenomenon is linked to regulation mechanisms that occur between pre-RNA synthesis and mature protein production (post-transcriptional and post-translational regulations). Moreover, degradation can modulate protein turnover. Second, the size of the two sets of data are not similar because they were not obtained using the same strategy, with gene expression from selected targets whereas 2DE proteomics is a non-targeted approach.

As mentioned above, our study presents different results for candidates followed in transcriptomics and proteomics. However, the integration of the two levels of data can lead to interesting hypotheses. Synthetically, we observed a protein response to heat stress at the protein level, certainly related to the need to maintain protein homeostasis under elevated temperature conditions. At the transcriptomic and proteomic levels, energy metabolism appears to be stimulated, probably to ensure the energy cost of sustaining the chronic heat stress response. It is important to note that HSP protein expression, while often induced in acute stress responses, can also be maintained in more mild or low-level chronic types of stress to accomplish its protective functions (Pei et al., 2012; Ortega et al., 2019; Zhan et al., 2019). This observation is reinforced by the increased abundance of the two proteins, TRAP1 (spot 1588) and prohibitin (spot 2244), which both participate in the protection and maintenance of mitochondrial

function. Furthermore, the qPCR results suggest a general decrease in transcription, which is consistent with a lasting presence of chaperones specialized in the response to thermal stress and, more broadly, to cellular stress. The high level of stress chaperones after 28 days of exposure may therefore reflect less protein degradation. Since the protein turnover is the result of a balance between synthesis and degradation, by reducing degradation in parallel with a decrease in transcription, there is conservation of the general level of proteins, and thereby, of protein homeostasis. This hypothesis is consistent with the absence of any proteasomal component in the DAPs identified at the proteomic level. The decreased abundance of two proteins implicated in post-transcriptional regulation process strengthens this hypothesis: namely, RNA-binding protein 8A (spot 2377), component of the spliceosome required for pre-mRNA splicing and the heterogeneous nuclear ribonucleoprotein L (spot 1661), involved in the formation, packaging, processing, and function of mRNAs.

4. Conclusion

Taken together, our results indicate that mussels from the Kerguelen islands have developed an adaptive response to temperature stress with three main characteristics: i) a long-lasting HSP response supported by ii) increase in energy capacities and iii) cytoskeleton modulations underlying cellular scaffolding and trafficking processes. The response remains moderate, which corresponds to mussels in good physiological condition, *i.e.* living in a low impact site, exposed to slight thermal stress.

Blue mussels are sentinel species used all over the world and there would be a particular interest in integrating subarctic mussels in environmental studies: thanks to their low level of contamination, they constitute a well-preserved equivalent to conventionally monitored populations living in degraded environments. Finally, this would also be an opportunity to gain insight into the impact of solely Global Change on intertidal organisms and test the following hypothesis and its consequences: the gradual temperature rise might lead to adaptation where basal levels of proteins implicated in heat stress response are higher, conferring a greater tolerance to stress at the cost of higher energy demand.

Acknowledgements

This work was supported by a grant from both ANR and NSERC Agencies (joined France-Canada project) as a part of the research program IPOC (Interactions between POllution and Climate changes:

515 Development of improved monitoring strategy, ANR-12-ISV7-0004-01, CRSNG-STPGP-430539-2012,
516 2013-2016, https://www-iuem.univ-brest.fr/ipoc/fr).

517

518 Mussel exposures were performed at Port-aux-Français Station (Kerguelen Islands, Southern and
519 Antarctic French Territory) and were funded and logistically supported by the French Polar Institute
520 (IPEV) (program N°409 IMMUNOTOXKER). The authors would like to thank all the personnel from the
521 IPEV and the Terres Australes et Antarctiques Françaises (TAAF) for their help and hospitality during
522 the research mission in the Kerguelen archipelago.

523

524 **References**

525 Anestis, A., Lazou, A., Pörtner, H.O., Michaelidis, B., 2007. Behavioral, metabolic, and molecular stress
526 responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient
527 temperature. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, 911-921.
528 https://doi.org/10.1152/ajpregu.00124.

529 Anestis, A., Pörtner, H.O., Lazou, A., Michaelidis, B., 2008. Metabolic and molecular stress responses
530 of sublittoral bearded horse mussel *Modiolus barbatus* to warming sea water: implications for vertical
531 zonation. J. Exp. Biol. 211, 2889-2898. https://doi.org/10.1242/jeb.016782.

532 Apraiz, I., Mi, J., Cristobal, S., 2006. Identification of proteomic signatures of exposure to marine
533 pollutants in mussels (*Mytilus edulis*). Mol. Cell. Proteomics MCP 5, 1274–1285.
534 https://doi.org/10.1074/mcp.M500333-MCP200

535 Auffret, M., 2005. Bivalves as Models for Marine Immunotoxicology, in: Investigative Immunotoxicology.
536 pp. 29–48. https://doi.org/10.1201/9781420036817.ch3

537 Bai, Z., Zhao, L., Chen, X., Li, Q., Li, J., 2017. A galectin contributes to the innate immune recognition
538 and elimination of pathogens in the freshwater mussel *Hyriopsis cumingii*. Dev. Comp. Immunol. 73,
539 36–45. https://doi.org/10.1016/j.dci.2017.03.008

540 Banni, M., Hajer, A., Sforzini, S., Oliveri, C., Boussetta, H., Viarengo, A., 2014. Transcriptional
541 expression levels and biochemical markers of oxidative stress in *Mytilus galloprovincialis* exposed to

542 nickel and heat stress. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 160, 23-29.
543 https://doi:10.1016/j.cbpc.2013.11.005

544 Bastos, A.C., Monaghan, K.A., Pestana, J.L.T., Lillebø, A.I., Loureiro S., 2013. A comment on the
545 Editorial “Replication in aquatic biology: the result is often pseudoreplication” Aquat. Toxicol. 126: 467-
546 470. https://doi.org/10.1016/j.aquatox.2012.11.003

547 Beaudry A., Fortier M., Masson S., Auffret M., Brousseau P., Fournier M., 2016. Effect of temperature
548 on immunocompetence of the blue mussel (*Mytilus edulis*). J Xenobiot.10;6(1) 5889. https://doi:
549 10.4081/xeno.2016.5889

550 Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Bråte, I.L.N., Schøyen, M., 2017.
551 Blue mussels (*Mytilus edulis spp*.) as sentinel organisms in coastal pollution monitoring: A review. Mar.
552 Environ. Res. 130, 338–365. https://doi.org/10.1016/j.marenvres.2017.07.024

553 Borsa, P., Daguin, C., Ramos Caetano, S., Bonhomme, F. 1999. Nuclear-DNA evidence that
554 northeasten Atlantic *Mytilus trossulus* mussels carry *M. edulis* genes. J. Molluscan Stud. 35, 524-527.

555 Borsa, P., Rolland, V., Daguin-Thiébaut, C. 2012. Genetics and taxonomy of Chilean smooth-shelled
556 mussels, *Mytilus* spp. (Bivalvia: Mytilidae). Comptes rendus biologies, 335(1), 51-61.

557 Boutet, I., Tanguy, A., Le Guen, D., Piccino, P., Hourdez, S., Legendre, P., Jollivet, D., 2009. Global
558 depression in gene expression as a response to rapid thermal changes in vent mussels. Proc. Roy. Soc.
559 B. 276, 3071-3079. https://doi.org/10.1098/rspb.2009.0503

560 Boutet, I., Tanguy, A., Moraga, D., 2004. Response of the Pacific oyster *Crassostrea gigas* to
561 hydrocarbon contamination under experimental conditions. Gene. 329, 147-157.
562 https://doi.org/10.1016/j.gene.2003.12.027

563 Boutet, I., Tanguy, A., Le Guen, D., Piccino, P., Hourdez, S., Legendre, P., Jollivet, D., 2009. Global
564 depression in gene expression as a response to rapid thermal changes in vent mussels. Proc. Roy. Soc.
565 B. 276, 3071-3079. https://doi.org/10.1098/rspb.2009.0503

566 Breitburg D., Levin L.A., Oschlies A., Grégoire M., Chavez F.P., Conley D.J., Garçon V., Gilbert D.,
567 Gutiérrez D., Isensee K., Jacinto G.S., 2008. Declining oxygen in the global ocean and coastal waters.
568 Science. 359, 6371-6382. https:// doi.org/10.1126/science.aam7240

569 Brose, R.D., Shin, G., McGuinness, M.C., Schneidereith, T., Purvis, S., Dong, G.X., Keefer, J., Spencer,
570 F., Smith, K.D., 2012. Activation of the stress proteome as a mechanism for small molecule therapeutics.
571 Hum. Mol. Genet. 21, 4237–4252. https://doi.org/10.1093/hmg/dds247

572 Buckley, B.A., Owen, M.E., Hofmann, G.E., 2001. Adjusting the thermostat: the threshold induction
573 temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of
574 thermal history. J. Exp. Biol. 204, 3571–3579.

575 Cai, X., Huang, Y., Zhang, X., Wang, S., Zou, Z., Wang, G., Wang, Y., Zhang, Z., 2014. Cloning,
576 characterization, hypoxia and heat shock response of hypoxia inducible factor-1 (HIF-1) from the small
577 abalone *Haliotis diversicolor*. Gene. 534, 256-264. https://doi.org/10.1016/j.gene.2013.10.048.

578 Carravieri, A., Bustamante, P., Churlaud, C., & Cherel, Y., 2013. Penguins as bioindicators of mercury
579 contamination in the Southern Ocean: birds from the Kerguelen Islands as a case study. Science of the
580 total environment, 454, 141-148. https://doi.org/10.1016/j.scitotenv.2013.02.060

581 Caza, F., Betoulle, S., Auffret, M., Brousseau, P., Fournier, M., St-Pierre, Y., 2015. Comparative analysis
582 of hemocyte properties from *Mytilus edulis desolationis* and *Aulacomya ater* in the Kerguelen Islands.
583 Mar. Environ. Res. 110, 174–182. https://doi.org/10.1016/j.marenvres.2015.09.003

584 Clark M.S., Sommer U., Sihra J.K., Thorne M.A.S., Morley S.A., King M., Viant M.R., Peck L., 2017.
585 Biodiversity in marine invertebrate responses to acute warming revealed by a comparative multi-omics
586 approach. Global Change Biology. 23, 318-330. https://doi.org/10.1111/gcb.13357

587 Coppola F., Henriques B., Soares A.M., Figueira E., Pereira E., Freitas R., 2018. Influence of
588 temperature rise on the recovery capacity of *Mytilus galloprovincialis* exposed to mercury pollution. Ecol.
589 Indic. 1;93:1060-9. https://doi.org/10.1016/j.ecolind.2018.05.077

590 David, E., Tanguy, A., Pichavant, K., Moraga, D., 2005. Response of the Pacific oyster *Crassostrea*
591 *gigas* to hypoxia exposure under experimental conditions. FEBS J. 272, 5635-52.
592 https://doi.org/10.1111/j.1742-4658.2005.04960.x

593 Davies, G.M., Gray, A., 2015. Don't let spurious accusations of pseudoreplication limit our ability to learn
594 from natural experiments (and other messy kinds of ecological monitoring). Ecol Evol. 5: 5295-5304.
595 https://doi.org/10.1002/ece3.1782

596 Devin, S., Giambérini, L., Pain-Devin, S., 2014. Variation in variance means more than mean variations:
597 What does variability tell us about population health status? Environ Int. 73, 282-287.
598 https://doi.org/10.1016/j.envint.2014.08.002

599 Ding, J., Li, J., Yang, D., Yang, F., Nie, H., Huo, Z., Yan, X., 2018. Molecular characteristics of a novel
600 HSP60 gene and its differential expression in Manila clams (*Ruditapes philippinarum*) under thermal
601 and hypotonic stress. Cell Stress Chaperones. 23, 179-187. https://doi.org/10.1007/s12192-017-0796-
602 7.

603 Dondero, F., Piacentini, L., Banni, M., Rebelo, M., Burlando, B., Viarengo, A., 2005. Quantitative PCR
604 analysis of two molluscan metallothionein genes unveils differential expression and regulation. Gene
605 345, 259-270. https://doi.org/10.1016/j.gene.2004.11.031.

606 Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists.
607 Journal of Statistical Software. 22: 1-20

608 Evans, T.G., Hofmann, G.E., 2012. Defining the limits of physiological plasticity: how gene expression
609 can assess and predict the consequences of ocean change. Philos. Trans. R. Soc. B Biol. Sci. 367,
610 1733–1745. https://doi.org/10.1098/rstb.2012.0019

611 Feidantsis, K., Giantsis, I.A., Vratsistas, A., Makri, S., Pappa, A.Z., Drosopoulou, E., Anestis, A.,
612 Mavridou, E., Exadactylos, A., Vafidis, D., Michaelidis B., 2020. Correlation between intermediary
613 metabolism, Hsp gene expression, and oxidative stress-related proteins in long-term thermal-stressed
614 *Mytilus galloprovincialis.* Am. J. Physiol. Regul. Integr. Comp. Physiol. 319, R264-R281.
615 https://doi.org/10.1152/ajpregu.00066.2020.

616 Féral, J. P, Saucède T., Poulin E., Marschal C., Marty G., Roca J.C., Motreuil S, Beurier J.P., 2016.
617 PROTEKER: Implementation of a submarine observatory at the Kerguelen Islands (Southern Ocean).
618 Underwater Technology 34(1), 3-10. https://doi:10.3723/ut.34.003.

619 Fields, P.A., Zuzow, M.J., Tomanek, L., 2012. Proteomic responses of blue mussel (*Mytilus*) congeners
620 to temperature acclimation. J. Exp. Biol. 215, 1106–1116. https://doi.org/10.1242/jeb.062273

621 Gérard, K., Bierne, N., Borsa, P., Chenuil, A., Féral, J.-P., 2008. Pleistocene separation of mitochondrial
622 lineages of *Mytilus spp*. mussels from Northern and Southern Hemispheres and strong genetic

623 differentiation among southern populations. Mol. Phylogenet. Evol. 49, 84–91.
624 https://doi.org/10.1016/j.ympev.2008.07.006

625 Gérard, K., Roby, C., Bierne, N., Borsa, P., Féral, J.-P., Chenuil, A., 2015. Does natural selection explain
626 the fine scale genetic structure at the nuclear exon Glu-5' in blue mussels from Kerguelen? Ecol. Evol.
627 5, 1456–1473. https://doi.org/10.1002/ece3.1421

628 Giannetto, A., Maisano, M., Cappello, T., Oliva, S., Parrino, V., Natalotto, A., De Marco, G., Barberi, C.,
629 Romeo, O., Mauceri, A., Fasulo, S., 2015. Hypoxia-inducible factor α and Hif-prolyl Hydroxylase
630 characterization and gene expression in short-time air-exposed *Mytilus galloprovincialis*. Mar.
631 Biotechnol. 17, 768–781. https://doi.org/10.1007/s10126-015-9655-7

632 Gomes, T., Chora, S., Pereira, C.G., Cardoso, C., Bebianno, M.J., 2014. Proteomic response of mussels
633 *Mytilus galloprovincialis* exposed to CuO NPs and Cu^{2+}: an exploratory biomarker discovery. Aquat.
634 Toxicol. Amst. Neth. 155, 327–336. https://doi.org/10.1016/j.aquatox.2014.07.015

635 Gourgou, E., Aggeli, I.-K., Beis, I., Gaitanaki, C., 2010. Hyperthermia-induced Hsp70 and MT20
636 transcriptional upregulation are mediated by p38-MAPK and JNKs in *Mytilus galloprovincialis* (Lamarck);
637 a pro-survival response. J. Exp. Biol. 213, 347–357. https://doi.org/10.1242/jeb.036277

638 Guillaumot, C., Saucède, T., Morley, S. A., Augustine, S., Danis, B., Kooijman, S., 2020. Can DEB
639 models infer metabolic differences between intertidal and subtidal morphotypes of the Antarctic limpet
640 *Nacella concinna* (Strebel, 1908)? Ecological Modelling, 430, 109088.
641 https://doi.org/10.1016/j.ecolmodel.2020.109088

642 Hamer, B., Jaksić, Z., Pavicić-Hamer, D., Perić, L., Medaković, D., Ivanković, D., Pavicić, J., Zilberberg,
643 C., Schröder, H.C., Müller, W.E.G., Smodlaka, N., Batel, R., 2008. Effect of hypoosmotic stress by low
644 salinity acclimation of Mediterranean mussels *Mytilus galloprovincialis* on biological parameters used
645 for pollution assessment. Aquat. Toxicol. Amst. Neth. 89, 137–151.
646 https://doi.org/10.1016/j.aquatox.2008.06.015

647 Han, G., Zhang, S., Dong, Y., 2017. Anaerobic metabolism and thermal tolerance: The importance of
648 opine pathways on survival of a gastropod after cardiac dysfunction. Integr Zool. 12, 361–370.
649 https://doi.org/10.1111/1749-4877.12229

650 Harcet, M., Perina, D., Pleše, B., 2013. Opine dehydrogenases in marine invertebrates. Biochem.
651 Genet. 51, 666-676. https://doi.org/10.1007/s10528-013-9596-7.

652 Hartl, F.U., Bracher, A., Hayer-Hartl, M., 2011. Molecular chaperones in protein folding and proteostasis.
653 Nature 475, 324–332. https://doi.org/10.1038/nature10317

654 Hochachka, P.W., Lutz, P.L., 2001. Mechanism, origin, and evolution of anoxia tolerance in animals
655 Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 130, 435-459. https://doi.org/10.1016/S1096-
656 4959(01)00408-0

657 Hu, W., Culloty, S., Darmody, G., Lynch, S., Davenport, J., Ramirez-Garcia, S., Dawson, K.A., Lynch,
658 I., Blasco, J., Sheehan, D., 2014. Toxicity of copper oxide nanoparticles in the blue mussel, *Mytilus*
659 *edulis*: a redox proteomic investigation. Chemosphere 108, 289–299.
660 https://doi.org/10.1016/j.chemosphere.2014.01.054

661 Inoue, K., Waite, J.H., Matsuoka, M., Odo, S., Harayama, S., 1995. Interspecific variations in adhesive
662 protein sequences of *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus*. Biol. Bull. 189, 370-375.
663 https://doi.org/10.2307/1542155.

664 Jaffal, A., Betoulle, S., Biagianti-Risbourg, S., Terreau, A., Sanchez, W., & Paris-Palacios, S., 2015.
665 Heavy metal contamination and hepatic toxicological responses in brown trout (*Salmo trutta*) from the
666 Kerguelen Islands. Polar Research, 34, 22784-98. https://doi.org/10.3402/polar.v34.22784

667 Jayasundara N., Tomanek L., Dowd W.W., Somero G.N., 2015. Proteomic analysis of cardiac response
668 to thermal acclimation in the eurythermal goby fish *Gillichthys mirabilis*. J. Exp. Biol. 218, 1359-1372.
669 https://doi.org/10.1242/jeb.118760

670 Ji, C., Wu, H., Wei, L., Zhao, J., Wang, Q., Lu, H., 2013. Responses of *Mytilus galloprovincialis* to
671 bacterial challenges by metabolomics and proteomics. Fish Shellfish Immunol. 35, 489–498.
672 https://doi.org/10.1016/j.fsi.2013.05.009

673 Josse, J., Husson, F., 2013. Handling missing values in exploratory multivariate data analysis methods.
674 Journal de la SFdS. 153 (2), 79-99.

675 Josse, J., Husson, F., 2016. missMDA. A Package for Handling Missing Values in Multivariate Data
676 Analysis. Journal of Statistical Software, 70 (1), pp 1-31 <doi:10.18637/jss.v070.i01

677 Kawabe, S., Yokoyama, Y., 2012. Role of Hypoxia-Inducible Factor α in response to hypoxia and heat
678 shock in the Pacific oyster *Crassostrea gigas*. Mar. Biotechnol. 14, 106–119.
679 https://doi.10.1007/s10126-011-9394-3.

680 Kültz, D., 2020. Evolution of cellular stress response mechanisms. J. Exp. Zool. Part A Ecol. Integr.
681 Physiol. https://doi.org/10.1002/jez.2347

682 Kültz, D., 2005. Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol.
683 67, 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635

684 Kültz, D., 2003. Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous
685 function. J. Exp. Biol. 206, 3119–3124. https://doi.org/10.1242/jeb.00549

686 Lacroix, C., Coquillé, V., Guyomarch, J., Auffret, M., Moraga, M., 2014. A selection of reference genes
687 and early-warning mRNA biomarkersfor environmental monitoring using *Mytilus spp*. as sentinel
688 species. Mar. Pol. Bull. 86, 304-313. https://doi.org/10.1016/j.marpolbul.2014.06.049

689 Le, T.T.Y., Zimmermann, S., Sures, B., 2016. How does the metallothionein induction in bivalves meet
690 the criteria for biomarkers of metal exposure? Environ. Pollut. Barking Essex 1987 212, 257–268.
691 https://doi.org/10.1016/j.envpol.2016.01.070

692 Le Cao K.H., Rohart F., Gonzalez I., Dejean S., Gautier B., Bartolo F., Monget P., Coquery J., Yao F.,
693 Liquet B. (2016). mixOmics: Omics Data Integration Project. R package version 6.1.1. https://CRAN.R-
694 project.org/package=mixOmics

695 Lee, P.Y., Saraygord-Afshari, N., Low, T.Y., 2020. The evolution of two-dimensional gel electrophoresis
696 - from proteomics to emerging alternative applications. J. Chroma. Part A 1615, 460763-72.
697 https://doi.org/10.1016/j.chroma.2019.460763

698 Letendre, J., Dupont-Rouzeyrol, M., Hanquet, A.-C., Durand, F., Budzinski, H., Chan, P., Vaudry, D.,
699 Rocher, B., 2011. Impact of toxicant exposure on the proteomic response to intertidal condition in *Mytilus*
700 *edulis*. Comp. Biochem. Physiol. Part D Genomics Proteomics 6, 357–369.
701 https://doi.org/10.1016/j.cbd.2011.08.002

702 Lenz M., Yasser A., Canning-Clode J., Díaz E., Eichhorn S., Fabritzek A.G., da Gama B.A., Garcia M.,
703 von Juterzenka K., Kraufvelin P., Machura S., Oberschelp L., Paiva F., Penna M.A., Ribeiro F.V., Thiel

M., Wohlgemuth D., Zamani N.P., Wahl M., 2018. Heat challenges can enhance population tolerance to thermal stress in mussels: a potential mechanism by which ship transport can increase species invasiveness. Biol. Invasions;20(11):3107-22. https://doi.org/10.1007/s10530-018-1762-8

Lockwood, B.L., Connor, K.M., Gracey, A.Y., 2015. The environmentally tuned transcriptomes of *Mytilus* mussels. J. Exp. Biol. 218, 1822–1833. https://doi.org/10.1242/jeb.118190

Lockwood, B.L., Somero, G.N., 2011. Transcriptomic responses to salinity stress in invasive and native blue mussels (genus *Mytilus*). Mol. Ecol. 20, 517–529. https://doi.org/10.1111/j.1365-294X.2010.04973.x

Lockwood, B.L., Sanders, J.G., Somero, G.N., 2010. Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. J. Exp. Biol. 213, 3548–3558. https://doi.org/10.1242/jeb.046094

Madeira D., Araújo J.E., Madeira C., Mendonça V., Vitorino R., Vinagre C., Diniz M.S., 2020. Seasonal proteome variation in intertidal shrimps under a natural setting: Connecting molecular networks with environmental fluctuations. Science of the Total Environment. 703, 134957. https://doi.org 10.1016/j.scitotenv.2019.134957

Magi, E., Liscio, C., Pistarino, E., Santamaria, B., Di Carro, M., Tiso, M., Scaloni, A., Renzone, G., Cosulich, M.E., 2008. Interdisciplinary study for the evaluation of biochemical alterations on mussel *Mytilus galloprovincialis* exposed to a tributyltin-polluted area. Anal. Bioanal. Chem. 391, 671–678. https://doi.org/10.1007/s00216-008-2055-3

Moreira, R., Pereiro, P., Canchaya, C., Posada, D., Figueras, A., Novoa, B., 2015. RNA-Seq in *Mytilus galloprovincialis*: comparative transcriptomics and expression profiles among different tissues. BMC Genomics 16, 728. https://doi.org/10.1186/s12864-015-1817-5

Ortega E., Gálvez I., Martín-Cordero L., 2019. Extracellular Hsp70 and low-grade inflammation-and stress-related pathologies. in Heat Shock Proteins and Stress (pp. 13-38). Springer, Cham.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., Church, J.A., Clarke, L., Dahe, Q., Dasgupta, P., Dubash, N.K., Edenhofer, O., Elgizouli, I., Field, C.B., Forster, P., Friedlingstein, P., Fuglestvedt, J., Gomez-Echeverri, L., Hallegatte, S., Hegerl, G., Howden, M., Jiang, K., Jimenez

731 Cisneroz, B., Kattsov, V., Lee, H., Mach, K.J., Marotzke, J., Mastrandrea, M.D., Meyer, L., Minx, J.,
732 Mulugetta, Y., O'Brien, K., Oppenheimer, M., Pereira, J.J., Pichs-Madruga, R., Plattner, G.-K., Pörtner,
733 H.-O., Power, S.B., Preston, B., Ravindranath, N.H., Reisinger, A., Riahi, K., Rusticucci, M., Scholes,
734 R., Seyboth, K., Sokona, Y., Stavins, R., Stocker, T.F., Tschakert, P., van Vuuren, D., van Ypserle, J.-
735 P., 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the
736 Fifth Assessment Report of the Intergovernmental Panel on Climate Change, EPIC3Geneva,
737 Switzerland, IPCC, 151 p., pp. 151, ISBN: 978-92-9169-143-2. IPCC, Geneva, Switzerland.

738 Péden, R., Rocher, B., Chan, P., Vaudry, D., Poret, A., Olivier, S., Le Foll, F., Bultelle, F., 2018. Highly
739 polluted life history and acute heat stress, a hazardous mix for blue mussels. Mar. Pollut. Bull. 135, 594–
740 606. https://doi.org/10.1016/j.marpolbul.2018.07.066

741 Péden, R., Rocher, B., Chan, P., Vaudry, D., Poret, A., Olivier, S., Le Foll, F., Bultelle, F., 2016.
742 Consequences of acclimation on the resistance to acute thermal stress: Proteomic focus on mussels
743 from pristine site. Mar. Environ. Res. 121, 64–73. https://doi.org/10.1016/j.marenvres.2016.02.006

744 Pei Y., Wu Y., Qin Y., 2012. Effects of chronic heat stress on the expressions of heat shock proteins 60,
745 70, 90, A2, and HSC70 in the rabbit testis. Cell Stress Chaperones;17(1):81-7. https://doi:
746 10.1007/s12192-011-0287-1

747 Pörtner, H.-O., Roberts, D., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck,
748 K., Alegría, A., Nicolai, M., Okem, A., Petzold, J., Rama, B., Weyer, N., (eds) 2019. IPCC 2019: IPCC
749 Special Report on the Ocean and Cryosphere in a Changing Climate. IPCC, Geneva, Switzerland. In
750 press. https://www.ipcc.ch/srocc/

751 Powers, E.T., Balch, W.E., 2013. Diversity in the origins of proteostasis networks--a driver for protein
752 function in evolution. Nat. Rev. Mol. Cell Biol. 14, 237–248. https://doi.org/10.1038/nrm3542

753 R Core Team (2020). R: A language and environment for statistical computing. R Foundation for
754 Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

755 RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL
756 http://www.rstudio.com/.

Rabilloud, T., Chevallet, M., Luche, S., Lelong, C., 2010. Two-dimensional gel electrophoresis in proteomics: Past, present and future. J. Proteomics 73, 2064–2077. https://doi.org/10.1016/j.jprot.2010.05.016

Raspor, B., Dragun, Z., Erk, M., Ivankovic, D., Pavicic, J., 2004. Is the digestive gland of *Mytilus galloprovincialis* a tissue of choice for estimating cadmium exposure by means of metallothioneins? Sci. Total Environ. 333, 99-108. https://doi.org/10.1016/j.scitotenv.2004.05.008.

Rawson, P.D., Joyner, K.L., Meetze, K., Hilbish T.J., 1996. Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity 77, 599-607.

Roman M.R., Brandt S.B., Houde E.D., Pierson J.J., 2019. Interactive effects of hypoxia and temperature on coastal pelagic zooplankton and fish. Frontiers in Marine Science 6, 139. https://doi.org/ 10.3389/fmars.2019.00139

Romero M.R., Pérez-Figueroa A., Carrera M., Swanson W.J., Skibinskie D.O.F., Diz A.P., 2019. RNA-seq coupled to proteomic analysis reveals high sperm proteome variation between two closely related marine mussel species. J. Proteomics, 192: 169-87. https://doi.org/10.1016/j.jprot.2018.08.020

Rossi, F., Palombella, S., Pirrone, C., Mancini, G., Bernardini, G., Gornati, R., 2016. Evaluation of tissue morphology and gene expression as biomarkers of pollution in mussel *Mytilus galloprovincialis* caging experiment. Aquat. Toxicol. 181, 57-66. https://doi.org/10.1016/j.aquatox.2016.10.018

Sheehan, D., McDonagh, B., 2008. Oxidative stress and bivalves: a proteomic approach. Invertebrate Survival Journal (2008) 5: 110-123

Signorile, A., Sgaramella, G., Bellomo, F., De Rasmo, D., 2019. Prohibitins: A Critical Role in Mitochondrial Functions and Implication in Diseases. Cell. 71. https://doi.org10.3390/cells8010071

Snyder, M.J., Girvetz, E., Mulder, E.P., 2001. Induction of marine mollusc stress proteins by chemical or physical stress. Arch. Environ. Contam. Toxicol. 41, 22–29. https://doi.org/10.1007/s002440010217

Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr. Comp. Biol. 53, 597–608. https://doi.org/10.1093/icb/ict028

Somero, G.N., 2020. The cellular stress response and temperature: Function, regulation, and evolution. J. Exp. Zool. Part A Ecol. Integr. Physiol. https://doi.org/10.1002/jez.2344

Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers." J. Exp. Biol. 213, 912–920. https://doi.org/10.1242/jeb.037473

Storey, B.K., Storey, J.M., 2004. Metabolic rate depression in animals: transcriptional and translational controls. Biol Rev Camb Philos Soc. 79, 207-33. https://doi: 10.1017/s1464793103006195

Suda, C. N., Vani, G. S., de Oliveira, M. F., Rodrigues, E., Lavrado, H. P., 2015. The biology and ecology of the Antarctic limpet *Nacella concinna*. Polar Biology. 38, 1949-1969. https://doi.org/10.1007/s00300-015-1789-6

Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., Jensen, L.J., Mering, C.V., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 8; 47(D1):D607-D613. https://doi: 10.1093/nar/gky1131.

Szymańska, E., Saccenti, E., Smilde, A.K., Westerhuis, J.A., 2012. Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies. Metabolomics Off. J. Metabolomic Soc. 8, 3–16. https://doi.org/10.1007/s11306-011-0330-3

Tanguy, A., Guo, X., Ford, S.E., 2004. Discovery of genes expressed in response to *Perkinsus marinus* challenge in Eastern (*Crassostrea virginica*) and Pacific (*C. gigas*) oysters. Gene. 338, 121-131. https://doi.org/10.1016/j.gene.2004.05.019

Tomanek, L., 2014. Proteomics to study adaptations in marine organisms to environmental stress. Journal of Proteomics. 105, 92-106. https://doi.org/10.1016/j.jprot.2014.04.009

Tomanek, L., 2012. Environmental proteomics of the mussel *Mytilus*: implications for tolerance to stress and change in limits of biogeographic ranges in response to climate change. Integr. Comp. Biol. 52, 648–664. https://doi.org/10.1093/icb/ics114

808 Tomanek, L., Zuzow, M.J., 2010. The proteomic response of the mussel congeners *Mytilus*
809 *galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits and
810 metabolic costs of thermal stress. J. Exp. Biol. 213, 3559–3574. https://doi.org/10.1242/jeb.041228

811 Vaquer-Sunyer R., Duarte C.M., 2008. Thresholds of hypoxia for marine biodiversity. Proceedings of
812 the National Academy of Sciences. 7;105, 15452-7. https://doi.org/10.1073/pnas.0803833105

813 Westerhuis, J.A., van Velzen, E.J.J., Hoefsloot, H.C.J., Smilde, A.K., 2010. Multivariate paired data
814 analysis: multilevel PLSDA versus OPLSDA. Metabolomics Off. J. Metabolomic Soc. 6, 119–128.
815 https://doi.org/10.1007/s11306-009-0185-z

816 Xu, L., Peng, X., Yu, D., Ji, C., Zhao, J., Wu, H., 2016. Proteomic responses reveal the differential effects
817 induced by cadmium in mussels *Mytilus galloprovincialis* at early life stages. Fish Shellfish Immunol. 55,
818 510–515. https://doi.org/10.1016/j.fsi.2016.06.017

819 Zhan Y., Li J., Sun J., Zhang W., Li Y., Cui D., Hu W., Chang Y., 2019. The Impact of Chronic Heat
820 Stress on the Growth, Survival, Feeding, and Differential Gene Expression in the Sea Urchin
821 *Strongylocentrotus intermedius*. Front Genet; 10:30. https:// doi: 10.3389/fgene.2019.00301

822 **Figure captions**

823 **Figure 1: Experimental design.**

824 Control tank water was maintained at 7.5°C. Test tank water temperature ranged from 7.5°C at T0 to
825 20°C at T28. Mussels were sampled at T0 and T28 for gene expression (Gene exp., n=10) and at T28
826 for 2DE analysis (Proteom., n=8).

827

828 **Figure 2: Gene selection based on the VIP (Variable Importance in the Projection) values.**

829 The PLS-DA (Partial Least Square Discriminant Analysis) was performed on the relative gene
830 expressions of 51 selected genes in the 37 exposed individuals (10 for each conditions à 7.5°C, 9 at T0
831 for 20°C and 8 at T28 for 20°C). **A.** Exposure conditions were plotted on the factorial plane combining
832 the two first axes of the PLS-DA, explaining 60% of the total variance. Ellipses correspond to a 95%
833 confidence interval for individual distribution exposed in each condition. Lines connect each data point
834 (individual location on the factorial plane) to the gravity center of the exposure condition; **B.** Variables
835 were plotted on a correlation circle, giving the correlation of each variable with the axes of the PLS-DA.

836

837 **Figure 3: Distribution of genotypes among exposure conditions.**

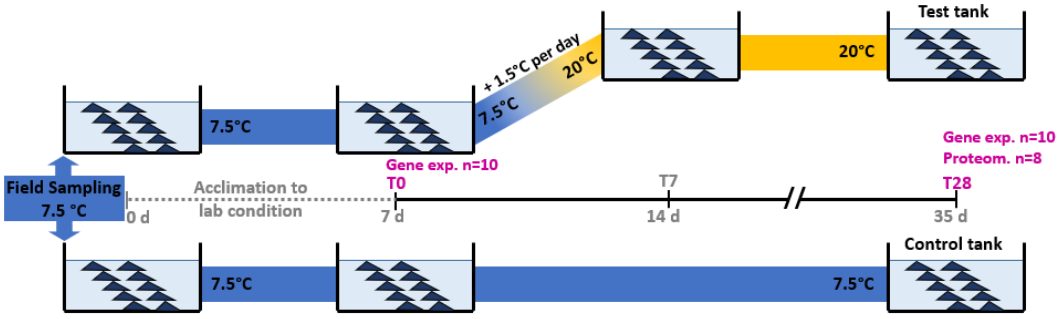
838 Factorial plane on the two first axes of the PCA (Principal Component Analysis) on the individual x gene
839 expression table (37 lines, 51 rows). Individuals are grouped according to the exposure time and
840 temperature. UNK refers to the individual whose genotype is unknown.

841

842 **Figure 4: Global protein interaction of the gill proteome of *Mytilus edulis platensis* under mild**
843 **stress.**

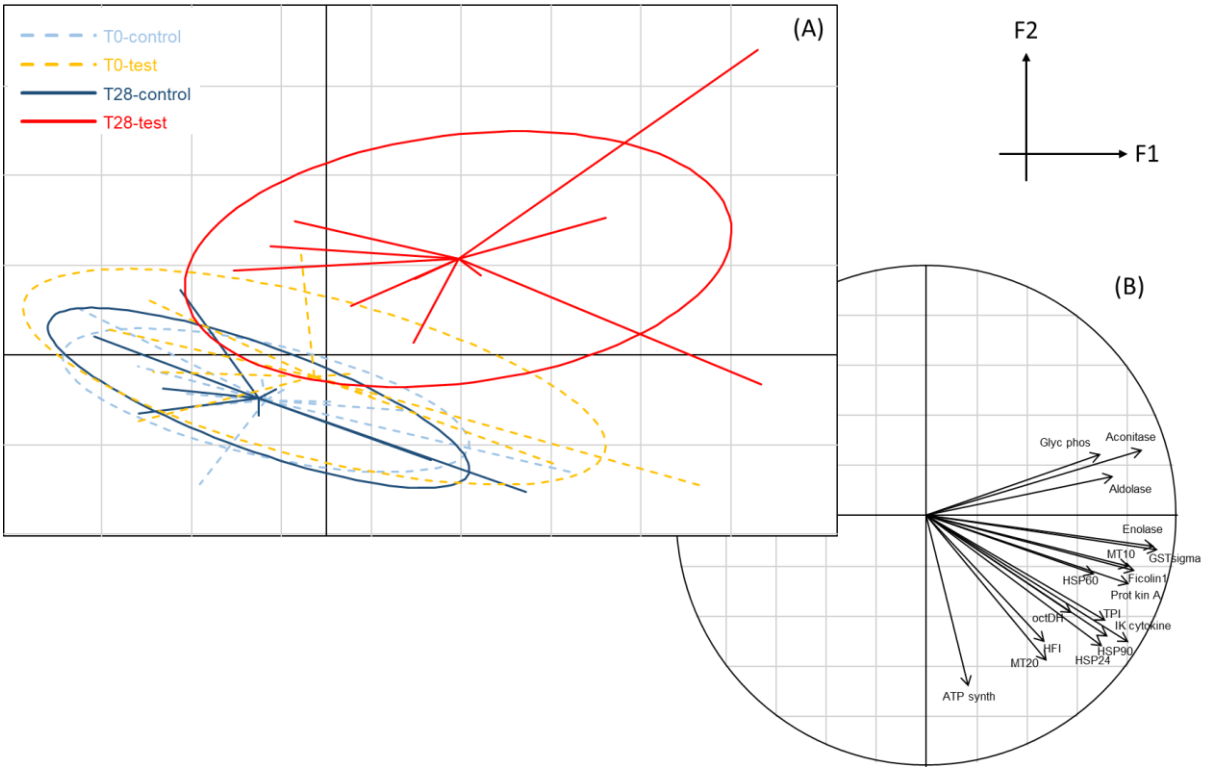
844 Protein-protein interactions predicted using STRING database for the differentially abundant proteins
845 (DAP). Nodes are the DAPs described in Table 2. Interactions are shown by the lines which connect
846 each node. Black circled nodes represent protein involved in the "Cellular Response to Heat Stress"
847 reactome pathway.

848



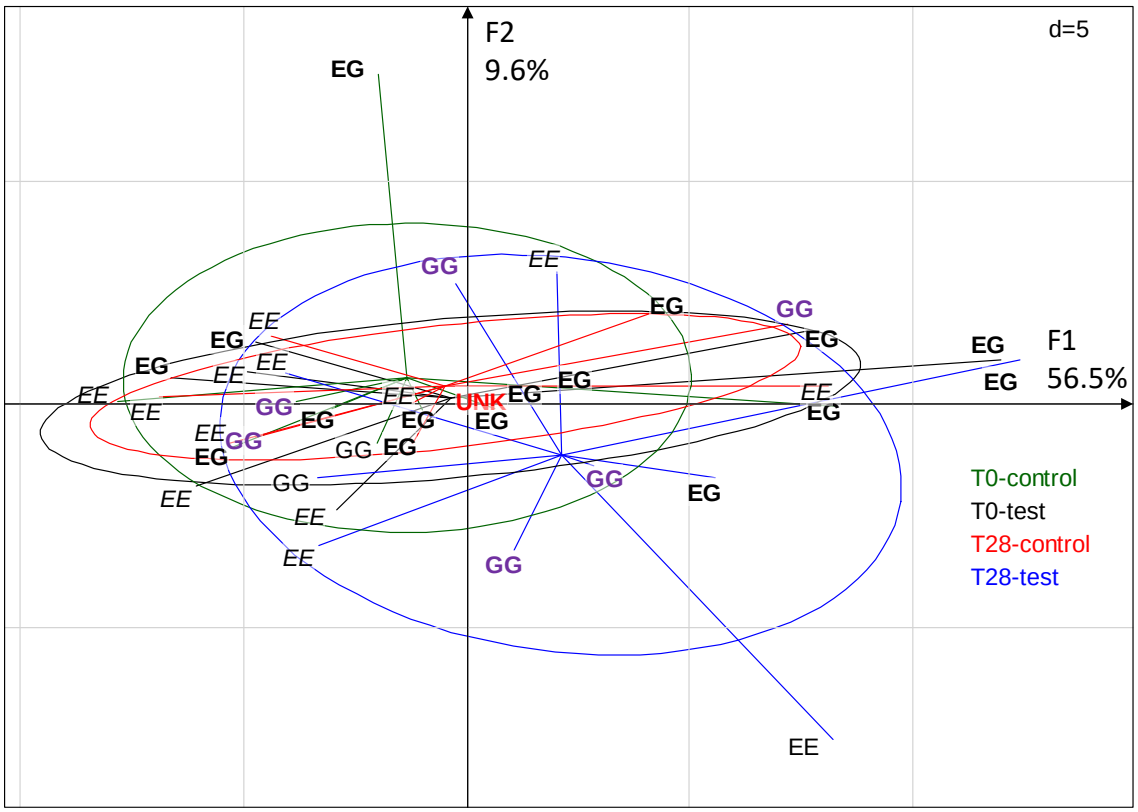
849

850 Fig. 1



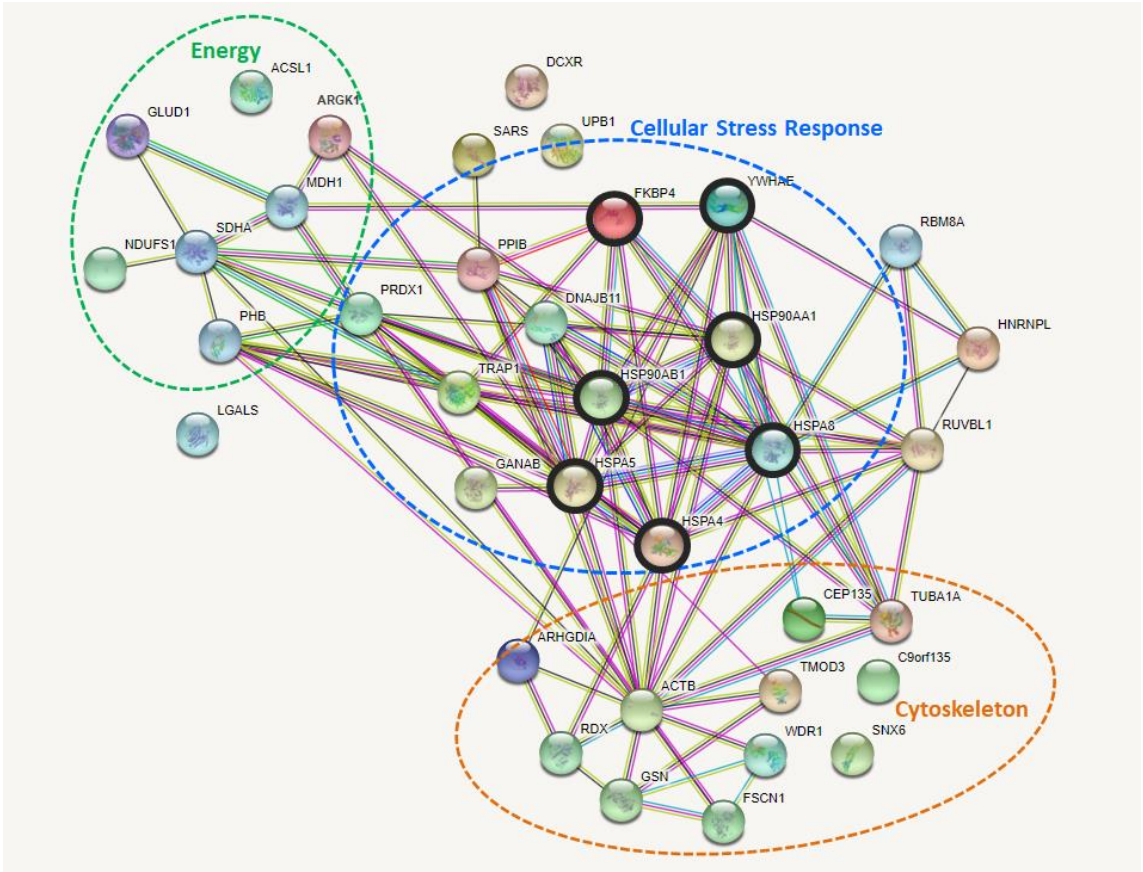
851

852 Fig. 2



853

854 Fig. 3



855

856 Fig. 4

Table 1. Values of mRNA expression of the different genes in gills of control individuals and exposed to thermal stress (exposed 20°C). VIP values calculated in PLS analysis. Only significant results are presented (VIP>1). Non-significant results are shown in Table S2.

Gene	Control 7.5°C	Exposed 20°C	VIP
MT20	-1,65	2,4	2,22
ATP synth	-1,07	1,45	1,7
Glyc phos	1,45	-3,72	1,45
Aconitase	1,54	-5,62	1,44
IK cytokine	1,26	1,11	1,36
HSP60	-1,75	-1,21	1,33
HFI	1,28	1,52	1,33
TPI	1,72	1,29	1,31
Ficolin 1	1,39	-1,21	1,29
Prot kin A	1,16	-1,14	1,19
HSP24	2,57	1,37	1,19
MT10	1,68	-1,17	1,17
OctDH	1,64	1,1	1,15
HSP90	1,04	-1,15	1,14
Enolase	1,18	-1,31	1,1
Aldolase	1,61	-1,57	1,09
GSTsigma	-1,19	-4,32	1,05

857

Spot Number	obs Mr (Da)	obs pI	Ratio	Identification	Abbreviation
ID1600	72686	5.78	260%		
ID2378	21109	5.33	234%		
ID1557	82031	5.33	183%	Radixin	RDX
ID1448	119923	5.78	176%	Neutral alpha-glucosidase AB	GANAB
ID1973	38812	4.97	166%	Tropomodulin-3	TMOD3
ID1796	50618	5.78	145%		
ID1699	60397	6.76	136%	Serine-tRNA ligase, cytoplasmic	SARS 1
ID1756	55609	5.32	132%	**Heat shock protein 56**	**FKBB4**
ID1632	69964	5.79	124%		
ID1616	71464	5.56	114%	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA
ID1536	88992	5.00	106%	**Heat shock protein 90 A1**	**HSP90AA1**
ID1851	46233	6.05	106%	Sorting nexin 6	SNX6
ID1599	73619	5.58	85%	**Heat shock protein 70**	**HSPA4**
ID1656	69872	5.05	83%	Galectin	LGALS
ID1588	76523	5.34	82%	Heat shock protein 75 kDa, mitochondrial	TRAP1
ID1669	63860	6.77	80%	Actin-interacting protein 1,66 kDa stress protein	WDR1
ID1578	77961	5.32	75%	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	NDUFS1
ID1587	74975	6.05	70%	Long-chain-fatty-acid--CoA ligase 1	ACSL1
ID2045	34710	8.00	69%		
ID1735	55712	7.10	61%	Glutamate dehydrogenase, mitochondrial	GLUD1
ID1785	49985	6.05	53%	RuvB-like 1	RUVBL1
ID1909	42203	4.83	52%	Gelsolin	GSN
ID1454	110405	4.94	42%	**Heat shock protein 90 B1**	**HSP90AB1**
ID1917	41315	5.60	41%	Heat shock protein 40	DNAJB11
ID1552	78605	4.98	41%	**Heat shock protein 70 protein 5**	**HSPA5**
ID2244	25927	5.33	41%	Prohibitin	PBH
ID1738	55602	5.85	40%	Fascin	FSCN1
ID1608	69940	5.28	39%	**Heat shock cognate 71**	**HSPA8**
ID2287	23857	5.15	35%	Rho GDP-dissociation inhibitor 1	ARHGDIA
ID2001	36624	6.00	32%		
ID2078	33100	6.00	31%	Malate dehydrogenase, cytoplasmic	mdh1
ID1721	53457	5.18	29%	Tubulin alpha-1 A chain	TUBA1A
ID1913	39362	5.32	25%	Actin beta chain	ACTB
ID2194	27756	4.56	21%	**14-3-3 protein epsilon**	**YWHAE**
ID1911	41744	6.30	-30%	Beta-ureidopropionase	UPB1
ID2009	36800	5.60	-36%		
ID2016	35479	8.00	-37%	Arginine kinase-like protein-1	ARGK1
ID2054	34759	5.60	-43%		
ID2377	20994	4.99	-46%	RNA-binding protein 8A	RBM8A
ID2030	35626	5.60	-48%		
ID2238	25992	7.50	-52%	L-Xylulose reductase	DCXR
ID2230	26288	6.15	-52%	C9orf135 protein	C9orf135
ID2349	22468	7.63	-53%	Peroxiredoxin	PRDX
ID2241	26073	7.00	-54%	Peptidyl-prolyl cis-trans isomerase B	PPIB
ID2393	21064	8.00	-60%	Unnamed protein product	
ID2360	21674	5.27	-64%		
ID2222	26845	5.70	-66%		
ID1437	136826	5.56	-68%	Centrosomal protein of 135 kDa, predicted	CEP135
ID1661	67272	7.41	-69%	Heterogeneous nuclear ribonucleoprotein L	HNRNPL

Table 2. Differentially abundant proteins (p<0.01) after thermal stress exposure

Spot numbers as assigned in Figure S1. Ratio correspond to variation in % (T28-test/T28-control). Idenfications were performed by Xtandem analyses on spectra obtained by tandem mass spectrometry (For details see Table S3). Mr: molecular mass; pI: isoelectric point; obs: observed value on gel.