Cold-adapted amphipod species upon heat stress: proteomic responses and their correlation with transcriptomic responses

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Abstract

The cellular heat shock response (HSR) comprises transcriptomic and proteomic reactions to thermal stress. It was here addressed, how the proteomic, together with the transcriptomic HSR, relate to the thermal sensitivities of three cold-adapted but differently thermo-sensitive freshwater amphipod species. The proteomes of thermosensitive *Eulimnogammarus verrucosus* and thermotolerant *Eulimnogammarus cyaneus*, both endemic to Lake Baikal, and of thermotolerant Holarctic *Gammarus lacustris* were investigated upon 24 h exposure to the species-specific 10% lethal temperatures (LT10). Furthermore, correlations of heat stress induced changes in proteomes (this study) and transcriptomes (previous study with identical experimental design) were examined. Proteomes indicated that the HSR activated processes encompassed (i) proteostasis maintenance, (ii) maintenance of cell adhesion, (iii) oxygen transport, (iv) antioxidant response, and (v) regulation of protein synthesis. Thermo-sensitive *E. verrucosus* showed the most pronounced proteomic HSR and the lowest correlation of transcriptomic and proteomic HSRs. For proteins related to translation (ribosomal proteins, elongation factors), transcriptomic and proteomic changes were inconsistent: transcripts were downregulated in many cases, with levels of corresponding proteins remaining unchanged. In the *Eulimnogammarus* species, levels of hemocyanin protein but not transcript were increased upon heat stress, suggesting a HSR also directed to enhance oxygen transport.

Thermosensitive *E. verrucosus* showed the most pronounced relocation of transcription/translation activity to proteostasis maintenance, which may indicate that the general species-specific stability of protein structure could be a fundamental determinant of thermotolerance. By combining transcriptomic and proteomic response data, this study provides a comprehensive picture of the cellular HSR components in the studied amphipods.

Keywords

heat shock response; proteome-transcriptome comparison; cold-adaptation; Lake Baikal; *Eulimnogammarus cyaneus*; *Eulimnogammarus verrucosus*; *Gammarus lacustris*

Introduction

Temperatures exceeding the physiological optimum of cells and organisms cause heat stress, which can have a detrimental impact on protein homeostasis: high temperatures lead to the formation of mis-/unfolded proteins[1,2]. In addition to disrupting protein homeostasis, heat stress causes oxidative stress[3], cell cycle arrest[1,4], changes in cellular membrane fluidity[1], and lysosomal membrane permeabilization[5]. The cellular response to heat stress is called heat shock response (HSR). Cells activate various HSR-related proteins upon heat stress, such as the so-called heat shock proteins (Hsps), molecular chaperones that prevent the formation of non-specific protein aggregates and thereby sustain protein homeostasis[6]. Studies on the HSR mainly focus on the regulation of molecular chaperones, although the HSR also includes the upregulation of proteins involved in translation, ubiquitin-dependent catabolic processes, and trehalose biosynthesis[2,7].

The present study is based on a previous study on the HSR transcriptomes of the here studied species (*Eulimnogammarus verrucosus*, *Eulimnogammarus cyaneus*, *Gammarus lacustris*)[15]. The transcriptomic responses of the species to thermal stress indicated a redirection of resources from normal, maintenance-related anabolic towards cell defensive processes. Thus, the most pronounced upregulation was seen for genes associated with the GO term

“response to stress”; genes of the GO term “translation” were downregulated[15]. In this study, we investigated the HSR proteomes of these freshwater amphipod species to obtain more detailed insights into the functional components involved in those amphipods´ HSR.

The studied amphipods are cold water species with different stress adaptation strategies. Two of the species, *E. verrucosus* and *E. cyaneus*, are endemic to Lake Baikal in Eastern Siberia, one of the world’s largest freshwater lakes and UNESCO world heritage site. Lake Baikal is a biodiversity hotspot with a high degree of endemism: 80% of the approximately 2600 animal species inhabiting the lake are endemic.[16] *Gammarus lacustris* is an amphipod occurring throughout the Holarctic and is found in waters in the Lake Baikal region, but it only occurs in waters that are not inhabited by species of Baikal’s endemic fauna[17]. *Eulimnogammarus verrucosus*, *E. cyaneus* and *G. lacustris* differ with regard to their thermal sensitivities. In the field, *E. verrucosus* is found in the littoral zone in water with comparatively cold temperatures; the species migrates to deeper, cooler water when water temperatures in the littoral rise during summer[19,20]. In contrast, the also littoral *E. cyaneus* always stays in the shallow water close to the water edge; temperatures in its habitat can fluctuate considerably up to the lake’s maximum water temperature during summer[21]. The different physiological thermal sensitivities of the species were explored by determining species-specific pejus temperatures (Tp) based on oxygen consumption and respiration in laboratory experiments. *Eulimnogammarus verrucosus* showed the highest thermal sensitivity (Tp = 10.6 °C) followed by *E. cyaneus* (Tp = 19.1 °C), and *G. lacustris* (Tp = 21.1 °C)[20]. Also, 50% lethal temperatures (LT50) calculated from the mortalities of amphipods incubated at different temperatures indicated that *E. verrucosus* is thermally more sensitive than the other species: LT50 values were 25.2 °C, 26.2 °C, and 27.2 °C for *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively[15]. Along those lines, *E. verrucosus* showed, compared to *E. cyaneus* and *G. lacustris*, the most pronounced transcriptomic response to thermal stress treatment[15]. In contrast, transcriptomic response data indicated acclimation of *E. cyaneus* to the increased temperature within the exposure time: transcriptomic

responses were less pronounced after 24 h than after 3 h heat stress treatment. *Gammarus lacustris* showed the least pronounced transcriptomic HSR, suggesting that it is more thermotolerant than the other two species[15].

The HSR comprises both transcript and protein expression level changes and the relationship between heat stress-induced transcriptomic and proteomic changes was addressed in a number of studies[7,8]. Changes in transcript levels by a trigger were found to not necessarily concur with changes in corresponding protein levels and may not be suitable to be used as indicators for protein abundance changes[9,10]. This observation was confirmed by studies on whole transcriptome and proteome datasets, showing that mRNA expression levels only partly determine the abundance of corresponding proteins[7,11]. The divergence in changes of transcript and corresponding protein levels is due to the complex regulatory processes of transcription and translation: parameters, such as alternative splicing, mRNA concentration and stability, translational efficiency, post-translational modification, and protein turnover rates influence protein abundance[12]. Moreover, due to the temporal succession of the transcription and translation processes, the correlation of transcript and protein levels at a particular time point can be low: mRNA levels may be comparatively briefly elevated, whereas an increase in the levels of the corresponding protein is delayed and may be more extended in time[13,14]. The correlation of transcriptomic and proteomic responses to treatment has been shown to be influenced by stress: with increased stress levels, transcriptomic and proteomic levels were found to be more inconsistent[7]. Combining transcriptomics and proteomics will therefore enable a more comprehensive understanding of the HSR-related cellular processes in a species.

In this study, the proteomic responses of *E. verrucosus*, *E. cyaneus*, and *G. lacustris* upon heat stress treatment were examined to obtain insights into the HSR of these species beyond the transcriptomic response (refer to Drozdova et al., 2019)[15]. Furthermore, transcriptomic and proteomic data were compared to identify concordances and differences between the transcriptomic and proteomic HSR in these species. The proteomic data obtained here was

analyzed based on the transcriptomic data from Drozdova et al. (2019)[15]. It was furthermore examined to which degree the transcriptomic (Drozdova et al., 2019)[15] and the proteomic HSR in the studied species concur based on correlation analyses. Comparison of transcriptomic and proteomic data from the different experiments was possible because all experimental procedures and parameters (amphipod species and populations, animal sampling, sampling sites, sampling month, maintenance and acclimatization of sampled amphipods in the lab, temperature treatments and controls, duration of exposures) were identical in both the previous transcriptomic[15] and the here presented proteomic studies.

Materials and Methods

Sampling and experimental design

Eulimnogammarus verrucosus (Gerstfeldt, 1858), *Eulimnogammarus cyaneus* (Dybowsky, 1874), and *Gammarus lacustris* Sars, 1863 were collected in August 2018 with a hand-net (kick sampling) in the littoral zone of Lake Baikal at water depths of 0.5-1.5 m close to the Biological Station of Irkutsk State University in the Bolshie Koty settlement ("Scuba diver's pier"; 51° 54'11.67"N, 105° 4'7.61"E; *E. verrucosus*, *E. cyaneus*) and in "Lake №14" in the vicinity of Bolshie Koty (51° 55'14.39"N, 105° 4'19.48"E; *G. lacustris*). Water temperatures during sampling were 18 and 17.5 °C in Lake Baikal and in "Lake №14", respectively. The sampling sites were the same as for the amphipods examined in the previous transcriptomic study (sampling in August 2013)[15]. A population genetics study on the here examined amphipod species in Lake Baikal showed that amphipods collected at the sampling site "Scuba diver's pier" on consecutive years were genetically from the same populations[22]. This indicates that the amphipods sampled for the transcriptomic[15] and the present proteomic studies were from the same populations.

After sampling, amphipods were directly brought to the lab and placed into 2 L aquariums with well-aerated water from Lake Baikal at 6 °C. Amphipods were acclimated to laboratory

conditions for 25 d. *Eulimnogammarus verrucosus* and *E. cyaneus* were fed *ad libitum* with a mix of ground dried amphipods, algae, aquatic plants, and detritus collected from the Baikal littoral; *G. lacustris* was fed *ad libitum* with leaves and detritus collected from Lake №14 and a commercial food mix consisting of dried *Gammarus sp.* (Barrom, Barnaul, Russia). Upon acclimatization, 8, 15, and 21 individuals of *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively, were shock-frozen in liquid nitrogen (first control group). The other animals were either subjected to a 24 h heat stress treatment by placing them into tanks with water at approximately 24.7±0.1 °C (~LT10 for all three species[15]; 10, 14, and 16 individuals of *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively) or transferred to new tanks with water at 6 °C as a second control group (5, 16, and 18 individuals of *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively). The animals from treatment and control were then shock-frozen in liquid nitrogen; a replicate consisted of a single *E. verrucous* individual, 2-3 *E. cyaneus* and 1-3 *G. lacustris* individuals pooled to obtain sufficient tissue for protein extraction. Different pool sizes were due to different species-specific body sizes of the studied animals. The numbers of replicates included in the analyses were: *E. verrucosus*: 8 replicates in the heat stress exposure; 8 - first control group, 5 - second control group; *E. cyaneus*: 6 - heat stress exposure; 7 - first control group; 4 - second control group; *G. lacustris*: 6 - heat stress exposure; 10 - first control group; 6 - second control group.

Protein extraction, TMT labeling, and MS/MS analysis

The frozen animals were ground to a fine powder with a pestle and a mortar pre-chilled with liquid nitrogen in 50-100 µl extraction buffer (100 mM ammonium bicarbonate with protease inhibitors) with 2-6 µl 10 % PMSF added. The ground tissue was incubated on ice for at least 15 min. Samples were centrifuged at 7000 rpm for 15 min at 4 °C. The supernatant was mixed with a lysis buffer (200 mM ammonium bicarbonate, 8 % SDS, 50 mM TCEP, and protease inhibitors) at a ratio of 1:1 and incubated at 95 °C for 3 min. The protein concentration in the extracts was measured using the Pierce™ 660nm Protein Assay Kit (Thermo Fisher Scientific).

For sample preparation and TMT labeling, paramagnetic carboxylate-coated beads were used according to the SP3 protocol[23,24]. Per sample, 20 µg of protein lysate was used. The sample preparation procedure included reduction with 20 mM TCEP for 1 h at 55 °C, alkylation with 34 mM IAA for 30 min at room temperature in the dark, and digestion with trypsin in a 1:50 ratio (µg trypsin to µg protein) for 16 h at 37 °C. Tandem mass tag (TMT)-labeling (TMT-10-plex, Thermo Scientific, Waltham, MA) was applied for multiplexed protein identification and quantitative proteome analysis. Digested proteins were incubated with 0.08 mg TMT labeling reagent for 1 h at room temperature. After reaction quenching, the respective samples were pooled (Table S8). Subsequently, peptide sample clean-up with acetonitrile and sample fractionation were performed as described before[24].

Samples were analyzed on a nano-UPLC system (Ultimate 3000, Dionex, USA), coupled online *via* a chip-based ESI source (Nanomate, Advion, USA) to a mass spectrometer (QExactive, Thermo Scientific, USA). Extracts were run over a trapping column at 5 µL/min by using 98 % water, 2 % ACN, and 0.05 % trifluoroacetic acid (Acclaim PepMap 100 C18, 3 µm, nanoViper, 75 µm × 5 cm, Thermo Fisher, Germany), peptides were separated on a reversed-phase column (Acclaim PepMap 100 C18, 3 µm, nanoViper, 75 µm × 25 cm, Thermo Fisher, Germany) with a flow rate of 300 nL/min. Solvent A of the mobile phase was 0.1 % formic acid in water; solvent B was 80 % ACN and 0.08 % formic acid in water. The parameters of the analytical HPLC gradient were set up as in [24]. The MS specter was set to the range from 350 to 1550 m/z. Full MS spectra were obtained in the Orbitrap at a resolution of 120,000 with a maximum fill time of 120 ms. The top 10 precursor ions were selected from each MS with an isolation window of 0.7 m/z; then, the ions were fragmented using higher energy collision-induced dissociation (normalized collision energy of 34). Fragment ion spectra were recorded at resolutions of 60,000 with an AGC target value of 2×10^3 and a maximum injection time of 120 ms.

Transcriptome de novo assembly, differential expression analysis, and construction of protein databases

The raw RNA sequencing data from *E. verrucosus*, *E. cyaneus* and *G. lacustris* from 3 h and 24 h heat stress exposures (24.7±0.1 °C for *E. verrucosus*, *E. cyaneus*, and *G. lacustris*) and the corresponding controls were taken from BioProject **PRJNA505233** from the NCBI database (Table S7)[15].

The here re-assembled *E. verrucosus*, *E. cyaneus*, and *G. lacustris* transcriptomic data from Drozdova et al. (2019)[15] were used to identify proteins in the proteomes of the amphipods. Transcriptome reassembly was performed with the RNA seq data obtained from the amphipods from the 24 h heat stress treatment and the 6 °C control from Drozdova et al. (2019)[15]. Drozdova et al. (2019)[15] used Trinity version v2.4.0 for transcriptome assembly. The here used version of the *de novo* transcriptome assembler rnaSPAdes, not available for transcriptome analysis in Drozdova et al. (2019)[15], was shown to assemble DNA reads more accurately than other assemblers; rnaSPAdes assembled contigs contain 5x less misassembled sequences than when Trinity is used[25]. Accuracy of the *de novo* assembly of transcript reads is particularly important, as no reference genomes are available for the here studied species. rnaSPAdes was run with the --ss fr parameter that indicates the strand specificity of the libraries. The qualities of the transcript assemblies performed with rnaSPAdes (v3.14.1) and Trinity (v2.11.0) were compared. For a shorter running time, the comparison was done with the RNA seq reads only from one species (*E. cyaneus*). Assembly completeness analysis was performed with the Benchmarking Universal Single-Copy Orthologs (BUSCO) tool[26] v4.1.4 using the arthropoda_odb10 dataset. It showed that rnaSPAdes generated a higher percentage of complete and less duplicated contigs than Trinity (Fig. S1; 69.2% *vs* 64.7% of complete and single-copy orthologues, for rnaSPAdes and Trinity, respectively). In addition, the *hsp70* sequence in the transcriptome assembly obtained with rnaSPAdes showed greater concordance with a verified reference *hsp70* sequence (NCBI no. JN704343) than the Trinity assembled

sequence; percent concordances were 99.69% with rnaSPAdes and 88.82% with Trinity assembled sequences. Proteome analyses were therefore performed with transcriptomes re-assembled with rnaSPAdes.

To avoid redundant transcripts, we clustered assembled contigs using cd-hit-est[27] v4.8.1 with -c 0.95 parameter (sequence identity threshold) and -n 10 parameter (word length). TransRate[28] v1.0.3 was used to filter out misassembled or incomplete contigs from non-redundant assemblies. Only contigs > 199 bp were used in the analyses. Sequence data were subjected to filtration as described before[43] to exclude sequences from prokaryotic symbionts/commensales/pathogens. Taxonomic assignment of each contig was done with DIAMOND[29] v2.0.4 with its sensitive mode (with parameter –sensitive) against the NCBI non-redundant protein sequence database of Oct 10, 2020; contigs assigned to Metazoa were regarded as from amphipods, and only those were kept for further analyzes.

To analyze transcript abundance, we utilized the align_and_estimate_abundance.pl script from the Trinity toolkit[30] v2.9.1. Alignment-free quantification was performed with salmon[31] v1.1.0 as an abundance estimation method. Differential expression (DE) analysis was performed with DESeq2[32] v1.26.0 (Tables S4, S5, S6). Finally, the fold change shrinkage was carried out using an adaptive shrinkage estimator from the apeglm v1.12.0 package[33].

Candidate protein-coding regions were identified with TransDecoder[34] v5.5.0 within the transcriptome assembly. Based on the thus identified coding regions, protein sequences were predicted and added to a predicted protein database.

Predicted open reading frames were annotated with the eggNOG-mapper v2 web server[35] and DIAMOND.

Peptide assembly, data normalization, and analyses of differential protein abundance, and correlations of protein and transcript levels

Obtained MS raw data were processed using MaxQuant v1.6.17.0 with the isobaric matching between runs and peptide-spectrum match level normalization[36]. The parameters of the

MaxQuant run can be found in Material S1. The database search was performed against a customized database based on the obtained transcriptome assemblies (see above) of the reads from all three species filtered with cd-hit v4.8.1 with sequence identity threshold parameter -c 1 to avoid redundant sequences. The database can be found in Material S2. The numbers of identified proteins were 1933, 2145, and 1870 for *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively. The minimal length of the peptide was set to 7 amino acids. Only proteins with at least two identified peptides were included in the analysis. Since MaxQuant default parameters require at least one unique peptide for identification, all filtered proteins contain at least one unique peptide. Proteins with data from less than three replicates in at least one condition were excluded from the analyses. Filtering according to these criteria resulted in 1653, 1845, and 1470 proteins for *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively.

For further analysis, corrected reporter ion intensities were used. The data analysis was performed with in-house R scripts (see Data Accessibility section). We performed sample loading (SL) normalization of the data[37] in two steps: (i) calculation of the normalization factor for each channel as the ratio between its sum protein intensity and the mean sum protein intensity across all channels; (ii) division of the channel intensities by the normalization factor.

Differential abundance (DA) analysis was performed using edgeR[38] v3.32.1 (Tables S1, S2, S3). Values of zero were replaced by missing values (NAs) as it is impossible to decipher the origin of a value of zero: zero can appear either because of the absence of protein in a sample or because of the selectivity of data-dependent acquisition. For each number of missing observations, we performed DA analysis separately, as the edgeR package forbids data with missing values. Benjamini-Hochberg false discovery p-value adjustment was applied to obtained p-values. DA proteins were defined as those with a Benjamini-Hochberg false discovery rate (FDR) ≤ 0.05. To increase method robustness, the two control groups (first and second (parallel) control groups; see description above) were combined and treated as one

control group in the differential abundance analysis. Protein abundances in the two control groups were found not to be significantly different.

The association of DA proteins with cellular processes was performed based on information from the literature. Upregulated and downregulated DE transcripts with unchanged levels of the corresponding proteins were characterized with significantly enriched gene ontology (GO) terms determined using the R package topGO[39] version 2.42.0.

Correlations of transcriptomes and proteomes were calculated with the log2 of the fold changes (log2(FC); heat stress treatment *vs* control) in abundance/expression levels of proteins/transcripts. Pearson correlation coefficients were determined with stats (R package) version 4.0.3 with the “pearson” method. Correlation analyses included either DA proteins for which corresponding transcripts were DE (Fig. 7) or all proteins for which corresponding transcripts were identified (Fig. S6).

Experimental Design and Statistical Rationale

In total, 60 biological samples were analyzed across three amphipod species. Each species was represented by 6-8 heat stress exposure replicates and 9-16 control replicates. The exact number of replicates for each species is described in the appropriate section. The number of replicates acquired, including biological replicates, and statistical methods used for analysis are described. Differential abundance analysis was performed using the edgeR package. Benjamini-Hochberg false discovery p-value adjustment was applied; adjusted p-values ≤ 0.05 were considered statistically significant.

Results and Discussion

Total numbers of identified transcripts and proteins in amphipods

Numbers of transcripts in here re-assembled transcriptomes from *E. verrucosus*, *E. cyaneus*, and *G. lacustris* were 27,773, 31,114, and 34,984, respectively, and numbers of proteins from the species were 1,653, 1,845, and 1,470, respectively.

Differentially abundant (DA) proteins in amphipod HSR proteomes

Fold changes in abundance were calculated for all identified proteins in the proteomes of the animals from the heat stress treatment (LT10-24 h) *vs* the parallel controls (6°C); proteins were classified as DA when FDR ≤ 0.05. Differential abundances upon heat stress treatment were seen for 38 (18 up- and 20 down-regulated), 15 (7 up- and 8 down-regulated), and 7 (5 up- and 2 down-regulated) proteins for *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively (Tables 2, S1, S2, S3). The identified DA proteins were from processes related to (**i**) maintenance of proteostasis, (**ii**) maintenance of cell adhesion, (**iii**) oxygen transport, (**iv**) antioxidant response, and (**v**) regulation of protein synthesis.

Proteomic responses to the heat stress treatment were overall similar in the three studied amphipod species but most pronounced in *E. verrucosus*. Although the LT10 (24 h) values were close or identical for the studied species (see above), the response amplitude was largest for *E. verrucosus*, indicating that this species responded most sensitively to the heat stress treatment. This is in line with the outcomes of previous physiological and molecular studies that showed that *E. verrucosus* is, in comparison to the other examined species, considerably more thermosensitive[20,40,41,42]. Yet, similar short-term LT10 values for all three studied species indicate that *E. verrucosus* is similarly capable of withstanding temporary heat stress as the other, more thermo-tolerant species. *Eulimnogammarus verrucosus* may thus be adapted to tolerate transient peaks within the strong water temperature fluctuations occurring in the Baikal littoral during summer[21,43].

Proteins involved in the maintenance of proteostasis (e.g. heat shock proteins) and of cell adhesion (e.g. fasciclin) were found to be increasingly abundant in animals of all three species upon heat stress; abundances of proteins associated with oxygen transport were increased in the endemic Baikal amphipods *E. verrucosus* and *E. cyaneus* from heat stress treatments, but not in *G. lacustris*.

(i) Maintenance of proteostasis:

A number of proteins acting as molecular chaperones, the major players in the HSR, were found to be upregulated in all three studied species upon heat stress, including proteins from the Hsp70 (HSPA1A, Hsc70-4) and the small heat shock protein (HSPB6) families. Proteins from the Hsp90 family, HSP90-alpha and Hsp83, were upregulated in *G. lacustris* and *E. cyaneus* but not in *E. verrucosus* from the heat stress treatment. *Eulimnogammarus cyaneus* additionally displayed upregulation of peptidyl-prolyl cis-trans isomerase FKBP4, shown to act as a co-chaperone[44]. Strikingly, *G. lacustris* from the heat stress treatment showed upregulation of the 14-3-3zeta protein. The 14-3-3 proteins, ubiquitous in all eukaryotes, are functionally diverse; they are involved in regulating protein activity and signaling in cells[45]. A function of the 14-3-3zeta protein that could potentially be relevant within this study is related to the finding that it acts as a molecular chaperone; however, the mechanism by which it prevents aggregation of proteins is poorly understood[46,47].

In *E. verrucosus* from the heat stress treatment, several proteins involved in proteostasis, beyond the Hsps described above, showed changed abundances: Upregulation was seen for (1) proteasome subunit beta, the part of the catalytic unit of the 20S proteasome degrading irreversibly damaged proteins; (2) PITH-domain containing protein, which stimulates the proteasome activity[48]; and (3) cytosolic α-mannosidase (Man2C1). This enzyme plays a crucial role in the catabolism of cytosolic-free oligomannosides released from unfolded and degraded glycoproteins[49,50]. Furthermore, two proteins that both were annotated as peptidase c1a (top listed upon a blastp[51] search) and as cathepsin B (second blastp hit) were downregulated in *E. verrucosus* from the heat stress treatment. Cathepsin B, a member of the peptidase c1a family, triggers apoptosis after its release from lysosomes due to heat-induced lysosomal membrane permeabilization[3]. Downregulation of cathepsin B may be a response mechanism that counteracts the increase of the cellular cathepsin B upon its heat-induced release that would initiate intracellular proteolysis and programmed cell death.

(ii) Maintenance of cell adhesion:

The abundance of fasciclin was significantly increased in all three species upon heat stress (FDR < 0.05 for *E. verrucosus* and *G. lacustris*; FDR < 0.09 for *E. cyaneus*). Fasciclin is an extracellular protein that modulates cell adhesion and migration of epithelial cells; it was shown that impaired cell adhesion sensitizes cells to prolonged proteotoxic stress[52]. Thus, heat stress activated upregulation of fasciclin can be considered a cellular response maintaining cell adhesion, which mitigates proteotoxic stress.

In *E. verrucosus* from the heat stress treatment, we also observed upregulation of UDP-glucose 6-dehydrogenase involved in the biosynthesis of glycosaminoglycans (GAGs), such as chondroitin sulfate and heparan sulfate, which both are involved in cell adhesion[53,54]. Furthermore, arylsulfatase B, a lysosomal protein degrading GAGs[55], was upregulated upon heat stress. Thus, the upregulation of arylsulfatase B might be linked to an increased turnover rate of GAGs.

(iii) Oxygen transport:

Hemocyanin was significantly upregulated in *E. verrucosus* and *E. cyaneus* during heat stress. This is consistent with a previous proteome study with *E. cyaneus*, in which animals were subjected to a heat stress treatment (25.5 °C for 1 h) followed by recovery at 6.5 °C for 3 h[56]. Upregulation of hemocyanin, which in the hemolymph of arthropods functions as an oxygen transporter, indicates a response to an increased need for oxygen due to increased metabolism at a higher temperature.

(iv) Antioxidant response:

Superoxide dismutase (SOD), a member of the cellular antioxidant system eliminating reactive oxygen species (ROS)[57], was upregulated in *E. verrucosus* upon heat stress. Upregulation of the enzyme may be related to increased levels of ROS caused by heat stress[58].

(v) Regulation of protein synthesis:

The eukaryotic translation initiation factor 2 subunit 1 (eIF2α), a subunit of the eIF2 protein, was downregulated in *E. verrucosus* from the heat stress treatment (Table S1). Downregulation

of eIF2α may result in a lower protein synthesis rate[59]. It may thus be a cellular reaction leading to a decreased amount of misfolded protein caused by heat stress. EIF2 is the main component of the ternary complex comprising eIF2, GTP, and Met-charged initiator tRNA ($tRNA_i^{Met}$). Methionyl-tRNA synthetase (MARS), downregulated in *E. verrucosus* upon heat stress (Table S1), produces $tRNA_i^{Met}$ [60]. The ternary complex is a key regulator of translation during stress[61]. Along those lines, the abundance of eIF5-mimic protein 2 (Bzw1) was increased in *E. verrucosus* from the heat stress treatment. This protein initiates the transcription of the chromatin component histone H4[62]. Chromatin generally limits the accessibility of transcription factors to the DNA[63]. An increase in histone H4 coincides with the general downregulation of transcription activity in this species upon heat stress[15]. Moreover, tarbp2, a component of an RNA-induced gene silencing complex (RISC) subunit, was upregulated in *E. verrucosus* from the heat stress treatment. The RISC enables gene silencing on the post-transcriptional level[64]. The upregulation of the RISC-loading complex subunit tarbp2 may contribute to the apparent downregulation of transcription activity, indicated by decreased expression of a high number of transcripts in *E. verrucosus* upon heat stress exposure[15].

A differential abundance of proteins from this functional group may be seen in the context of metabolic repression, a response of *E. verrucosus* to thermal stress[41]. Furthermore, these findings are in line with the downregulation of proteins involved in translation in *S. cerevisiae* under severe heat stress[7].

Relationships of transcript expression levels and protein abundances

Based on the here re-assembled RNAseq data from Drozdova et al. (2019)[15], DE transcripts in transcriptomes from animals from thermal stress treatments were redetermined. Corresponding to the here applied significance level for DA proteins in the proteome analysis (FDR ≤ 0.05), this significance level was also applied to determine DE transcripts (Table 1); numbers of DE transcripts are therefore higher here than in Drozdova et al. (2019)[15], where a more stringent significance level was used (FDR ≤ 0.001). As found for the proteomic response,

the transcriptomic response to heat stress was among the studied species most pronounced in *E. verrucosus*.

The following six cases regarding transcript expression levels (data from Drozdova et al. (2019)[15]) and protein abundances can be distinguished (Fig. 1, 2, 3):

Case 1. Up/downregulation of transcripts and corresponding up/downregulation of corresponding protein levels

Corresponding changes in both transcript and protein levels indicate protein level changes largely driven by the regulation of the corresponding transcript expression levels. Upregulation of both transcript and corresponding protein levels upon heat stress was detected for all DA proteins from the Hsp family and for fasciclin in the three here studied amphipod species (Fig. 1A, 2A, 3A).

Case 2. Unchanged transcript and upregulation of corresponding protein levels

Increased protein but unchanged corresponding transcript levels may be due to: the time-shift between the responses on the transcriptional and the translational levels; post-transcriptional regulation of protein abundance, e.g., by preferential processing of certain transcripts by ribosomes[65]; post-translational regulation of protein abundance by decreased protein ubiquitination and a consequently lower rate of protein degradation[66]. From the three studied species, most such responses on the transcriptomic and proteomic levels were seen for *E. verrucosus* (Fig. 1A) and in both *Eulimnogammarus* species for hemocyanin subunits (Fig. 1A, 2A).

Case 3. Unchanged transcript and downregulation of corresponding protein levels

Decreased protein abundance with unchanged corresponding transcript levels may indicate a protein half-life that, upon heat stress, is shortened. A reason for this may be changes in the affinity of proteins to the ubiquitin-proteasome system that alters protein degradation rates. Among the down-regulated proteins with unchanged transcript levels, there are four peptidases

and two members of the translation initiation system - eIF2α and methionyl-tRNA synthetase MARS, key regulators of translation, which may be associated with an overall decrease in protein levels.

Case 4. Downregulation of transcript and upregulation of corresponding protein levels

This case was detected only in *E. verrucosus*. It may be due to: (a) short-term upregulation of transcript upon heat stress, followed by a rapid decay of the mRNA; and a delayed upregulation of the protein, remaining at an increased level for a more extended time as the protein decay proceeds more slowly; (b) downregulation of transcript as a result of gene expression regulation or increased mRNA decay upon heat stress and a lower rate of protein degradation due to decreased protein ubiquitination[12,67]. This mRNA/protein level relationship was observed for proteasome subunit beta, RISC-loading complex subunit tarbp2, serine-threonine kinase receptor-associated protein, and superoxide dismutase (Fig. 1A). The increase in abundance of these proteins during heat stress suggests an important role in the HSR.

Case 5. Upregulation of transcript and unchanged corresponding protein levels

Transcript upregulation with the corresponding protein level unchanged (Fig. 4, 5, 6) can be explained by a higher turnover rate of proteins upon heat stress. It was shown before that the higher expression of some genes, especially of those encoding stress-protective proteins, compensates for shortened protein half-life under heat stress[7]. Indeed, in three studied species, we observed upregulation of transcripts without changes in protein level for GO terms protein folding (GO:0006457) and response to stress (GO:0006950; Table 2). This includes transcripts encoding proteins from the Hsp family; activator of 90 kDa heat shock protein ATPase; and the mitochondrial outer membrane protein Tom34 (a cytosolic cochaperone of the Hsp90/Hsp70 protein complex). In the *Eulimnogammarus* species, we detected upregulation of Hsc70-interacting protein st13; stress-induced-phosphoprotein 1 (Hop), which is known as a co-chaperone of HSP90AA1[68]; and subunits of T-complex protein Ring Complex (TRiC) involved in folding of about 10% of proteins in eukaryotic cells (Fig. 4, 5)[69]. In *E. verrucosus*, the

transcript encoding cytosolic α-mannosidase Man2C1 was upregulated without change of the corresponding protein level (Fig. 4). As mentioned above, this protein is involved in the catabolism of cytosolic free oligomannosides released from unfolded and degraded glycoproteins[49,50]. Furthermore, the transcript encoding ER degradation-enhancing alpha-mannosidase Edem1 was upregulated (Fig. 4). As Man2C1, this protein plays a role in the degradation of misfolded glycoproteins[70].

For all three studied amphipods, we observed upregulation of transcripts coding for elements of the ubiquitination machinery: polyubiquitin Ubc, calcyclin-binding protein (CACYBP), and ubiquilin-1 (FDR = 1.06e-16, with fold change = 2.2) in *E. verrucosus*; E3 ubiquitin ligase and COP9 signalosome subunit 1 in *E. cyaneus*; polyubiquitin Ubc and ubiquitin-protein ligase (HECT domain protein) in *G. lacustris*. Increased activity of the ubiquitination machinery during heat stress is an important component of the cellular HSR[2,7].

In all three here studied species, a transcript was upregulated upon heat stress that, according to a blast search, could be annotated as “Zinc finger MYND-type” or as “SET domain-containing protein SmydA-8”, containing the MYND domain (E-value for both hits < 5e-324). SmydA is an arthropod-specific histone lysine methyltransferase regulating gene expression; its fundamental function was shown by its knockdown in *D. melanogaster*[71]. Smyd methyltransferase is an S-adenosyl-L-methionine-dependent type of methyltransferase[72]. In *E. verrucosus*, the transcript encoding S-adenosylmethionine synthase (MAT1A) was upregulated; MAT1A catalyzes the formation of S-adenosyl-L-methionine, a cofactor of histone lysine methyltransferase (Fig. 4). On the protein level, decreases in levels of S-adenosyl-L-methionine-dependent methyltransferases were found in *E. cyaneus*, *G. lacustris* (Fig. 2B and 3B), and *E. verrucosus* (FDR = 0.098; Table S1) from heat stress treatments. Transcript upregulation of SmydA upon heat stress in all studied species indicates that this gene is a gene expression regulator, promoting the HSR in amphipods. Transcript downregulation of other methyltransferases may indicate that they are not involved in the cellular HSR.

Furthermore, in the context of gene expression regulation upon heat stress, the transcript encoding the arginine/serine-rich splicing factor was upregulated in *E. cyaneus* and *G. lacustris* (Fig. 5 and 6), with the level of the corresponding protein unchanged. Arginine/serine-rich splicing factors play a pivotal role in regulating alternative splicing[73]. In this context, it could be assumed that under heat stress the comparatively thermoresistant *E. cyaneus* and *G. lacustris* produce fewer thermally unstable splice variants of proteins.

Transcripts encoding the ion transport proteins sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1) were found to be upregulated in heat-stressed *E. verrucosus* (Fig. 4). This upregulation of ion transport proteins may be seen as a response compensating the disruption of the ion balance at cellular membranes related to heat stress in *E. verrucosus*. The disruption in ion balance due to acute heat stress was previously shown in arthropods[74,75]. The observed upregulation of the leucyl aminopeptidase lap-2 transcript (Fig. 4) may be also seen in this context. This enzyme processes peptides setting free single amino acids, which may maintain the cellular osmotic balance[76]. Heat stress was previously shown to cause upregulation of lap-2 transcript in blue mussels[77].

In *E. verrucosus* upon heat stress, upregulation of a transcript encoding solute carrier family 2 facilitated glucose transporter member 1 was found, also known as Glucose transporter 1 (GLUT1; Fig. 4). GLUT1 is a membrane protein mediating glucose transport into the cell[78]. The species may have upregulated the transcript to maintain the GLUT1 protein level upon heat stress to sustain the cellular glucose supply.

In *G. lacustris*, upon heat stress, several transcripts involved in energy metabolism were upregulated: very long-chain specific acyl-CoA dehydrogenase (ACADVL) catalyzing the first step of mitochondrial fatty acid beta-oxidation; succinate-CoA ligase subunit beta (SUCLG2), member of the tricarboxylic acid cycle; cytosolic phosphoenolpyruvate carboxykinase (Pepck), the rate-limiting enzyme in gluconeogenesis; and glyoxalase 1 (GLOD4) involved in the detoxification of methylglyoxal, a byproduct of glycolysis. These data show that compensation

for the degradation of proteins involved in providing energy as a consequence of heat stress can be an important component of the cellular HSR.

Case 6. Downregulation of transcript and unchanged corresponding protein levels

This case (also refer to Fig. S3-S5) may occur if there is a comparatively strong time delay between the transcriptomic and proteomic responses to the heat stress impact. A response on the protein level may thus occur at a later time point.

In the thermosensitive *E. verrucosus*, downregulated transcripts with unchanged corresponding protein abundances encode proteins involved in translation (GO:0006412) and peptide metabolic processes (GO:0006518; Table 2). Transcripts related to translation were not downregulated in the more thermotolerant *E. cyaneus* and *G. lacustris*. This corresponds with the finding that levels of proteins involved in translation were decreased upon a high level of heat stress but not upon mild heat stress[7]. In *E. cyaneus* and *G. lacustris* from the heat stress treatment, transcripts from GO terms RNA processing (GO:0006396), RNA splicing *via* transesterification reactions (GO:0000375), and negative regulation of RNA metabolic process (GO:0051253) were downregulated (Table 2). This response in *E. cyaneus* and *G. lacustris* but not in *E. verrucosus* may also be related to a comparatively higher thermotolerance of the first two species. A decrease in the levels of transcripts involved in RNA processing may occur as part of the cellular HSR in order to avoid competition with mRNA encoding HSR proteins at the ribosome[7,79].

A number of other transcripts were downregulated in *E. verrucosus* from the heat stress treatment (Fig. S3), with protein levels unchanged. They encoded proteins involved in energy production, including mitochondrial ATP synthase subunit alpha (ATP5A1), mitochondrial ATP synthase subunit O (Oscp; FDR = 0.0022, fold change = 1.6), ADP-ATP carrier protein (sesB; FDR < 0.0013, fold change = 28), enolase (Eno), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and cytosolic phosphoenolpyruvate carboxykinase (Pepck; FDR = 0.0003, fold change = 1.87). As described above, for the heat stress caused regulation of proteins related to

protein synthesis, decreased levels of these transcripts may also be related to a heat stress caused by metabolic depression in *E. verrucosus*.

Correlations of HSR transcriptomes and proteomes

The correlation of the log2 fold changes of transcript and protein levels in animals from the 24 h heat stress exposures *vs* the respective controls was highest for *E. cyaneus* (Pearson correlation coefficient (R) = 0.872, $p < 2 \cdot 10^{-11}$), followed by *G. lacustris* (R = 0.6772, $p < 2 \cdot 10^{-6}$) and *E. verrucosus* (R = 0.2763, p < 0.03) (Fig. 7A and S6A). The number of cases of significant decreases of transcript levels but unchanged or increased abundances of the corresponding proteins was particularly high for *E. verrucosus* (Fig. 7A and S6A). The stronger trend of a heat stress caused overall downregulation of transcription (Fig. S6A) *vs* translation in *E. verrucosus* may be due to comparatively low metabolic rates[20] and a consequently slow protein turnover in this species. The low metabolic rate of *E. verrucosus* is according to the metabolic scaling theory related to body size[80]; body sizes of *E. verrucosus* considerably exceed those of *E. cyaneus* and *G. lacustris*[81].

For *E. verrucosus*, the correlation coefficient of the transcript level changes upon 3 h [15] (Fig. 7B and S6B) and the protein level changes upon 24 h heat stress exposures was R = 0.396 (p < 0.005), which is 1.73-fold higher than the correlation coefficient of transcriptomic and proteomic responses both upon 24 h heat stress exposure. For *E. cyaneus*, in contrast, the correlation coefficient of the transcript level changes upon 3 h and the protein level changes upon 24 h heat stress exposure (R = 0.2415, p = 0.255) was only one-third of the correlation coefficient of the transcript/protein level changes each upon 24 h heat stress exposure. This indicates differences in both species in timing of the HSR transcription and translation processes upon heat stress. A reason for this may be the different metabolic rates of the species under heat stress conditions, which are in comparison higher in *E. cyaneus*[20]. For *G. lacustris*, the correlation coefficient of the transcript upon 3 h and protein level changes upon 24 h heat stress exposure (R = 0.5392, p <

0.04) was similar to the correlation coefficient of the transcript and protein level changes in this species upon 24 h heat stress exposure. A reason for similar correlations of both 3 h and 24 h heat stress transcriptomes with the 24 h heat stress proteome may be that *G. lacustris*, unlike both Baikal species, reaches a steady state in stress response already after 3 h[15].

Conclusions

This study shows that the proteomic HSR in the here studied cold-adapted amphipod species is complex, comprising cellular activities that maintain cellular homeostasis on different levels. In the studied species, the proteomic HSR comprised activation of (i) proteostasis maintenance, (ii) antioxidant response, (iii) oxygen transport, and (iv) cell adhesion maintenance, as well as deactivation of (v) protein synthesis, indicate a boost of processes that enhance stabilization of cellular structures and cellular supply with oxygen and nutrients and reduce anabolism in the heat-stressed organisms (Fig. 8). In contrast, the transcriptomic HSR particularly in *E. verrucosus* was found to be associated with a decrease of the levels of transcription/translation related transcripts. Together, this all can be seen as a switch of cellular processes into “emergency mode”, enabling the species to tolerate the effects of heat stress that primarily destabilize the organism’s biological macromolecules. This shows that by combining transcriptomic and proteomic HSR data a more comprehensive picture of the cellular HSR components in the studied amphipods can be obtained. Our data revealed that the proteomic responses to heat stress in the different species showed the same general patterns with specific patterns observed only for the most thermosensitive species. Thus, the proteomic HSR, most pronounced in *E. verrucosus*, suggests the highest stress level at LT10 (24 h) in this species among the studied species. It is assumed that due to metabolic depression, occurring as a response to stress on both transcriptomic and proteomic levels in *E. verrucosus*, the mortality rate was kept comparatively low despite a comparatively high stress level. Among the studied species, the diverging responses on the transcriptomic and proteomic levels became evident by

the lowest correlation coefficient in *E. verrucosus*. The marked proteomic response could indicate that the proteome has significant buffering capacities, maintaining cellular homeostasis when there is a stress-related decrease of transcriptomic activity. The processes regulating transcription and translation can be confounded by increased heat stress, which may occur from different reactions of cellular processes to heat stress-related responses, such as metabolic depression. Thermosensitive *E. verrucosus* showed the most pronounced relocation of transcription/translation activity to proteostasis maintenance, which may indicate that the general species-specific stability of protein structure could be a fundamental determinant of thermotolerance.

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Data Availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[82] partner repository with the dataset identifier **PXD031000** and **10.6019/PXD031000**. In-house scripts for data analysis are available at https://github.com/PolinaLip/ProteomeTranscriptomeComparison_BaikalAmphipods.

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Tables

Table 1. Numbers of up- and downregulated DE transcripts and DA proteins in HSR transcriptomes and proteomes in the studied amphipods. RNAseq data are from Drozdova et al. (2019)[15] and were re-analyzed.

Species	Transcriptome (24 h HS exposure)			Transcriptome (3 h HS exposure)			Proteome (24 h HS exposure)		
	Total*	Up*	Down*	Total*	Up*	Down*	Total*	Up*	Down*
E. verrucosus	4,044	1,292	2,752	1,385	569	817	38	18	20
E. cyaneus	1,663	583	1,080	1,257	817	789	15	7	8
G. lacustris	1,517	683	834	165	110	55	7	5	2

* - DE transcripts and DA proteins with FDR ≤ 0.05; HS - heat shock

Table 2. GO terms enriched (p-value ≤ 0.05) in the upregulated or downregulated DE transcripts with unchanged levels of corresponding proteins (cases 5 and 6). The listed GO terms relate to the “Biological process” group.

Species	**Upregulated**	**Downregulated**
E. verrucosus	● protein folding ● response to abiotic stimulus ● response to heat ● negative regulation of metabolic process ● negative regulation of gene expression ● response to stress	● peptide metabolic process ● cellular nitrogen compound biosynthetic process ● translation ● cellular response to oxidative stress
E. cyaneus	● protein folding ● regulation of immune system process ● response to stress	● RNA processing ● RNA splicing, via transesterification reactions

G. lacustris	● response to heat ● protein folding ● regulation of transcription by RNA polymerase II	● negative regulation of RNA metabolic process ● DNA-templated transcription

Figure legends

Figure 1. Levels of transcripts and the corresponding upregulated (**A**) and downregulated (**B**) DA proteins (FDR ≤ 0.05) in *E. verrucosus* from the heat stress treatment *vs* the control; C1 - case 1, C2 - case 2, C3 - case 3, C4 - case 4 (refer to paragraph "*Relationships of transcript expression levels and protein abundances*").

Figure 2. Levels of the DE transcripts and of the corresponding upregulated (**A**) and downregulated (**B**) DA proteins (FDR ≤ 0.05) in *E. cyaneus* from the heat stress treatment *vs* the control; C1 - case 1, C2 - case 2, C3 - case 3 (refer to paragraph "*Relationships of transcript expression levels and protein abundances*").

Figure 3. Levels of the DE transcripts and of the corresponding upregulated (**A**) and downregulated (**B**) DA proteins (FDR ≤ 0.05) in *G. lacustris* proteins from the heat stress treatment *vs* the control; C1 - case 1, C2 - case 2, C3 - case 3 (refer to paragraph "*Relationships of transcript expression levels and protein abundances*").

Figure 4. Levels of upregulated transcripts (FDR ≤ 0.001) and of corresponding proteins with unchanged abundances (case 5, refer to paragraph "*Relationships of transcript expression levels and protein abundances*") in *E. verrucosus* from the heat stress treatment *vs* the control. Only the most substantial transcript level changes (fold changes > 3) are depicted.

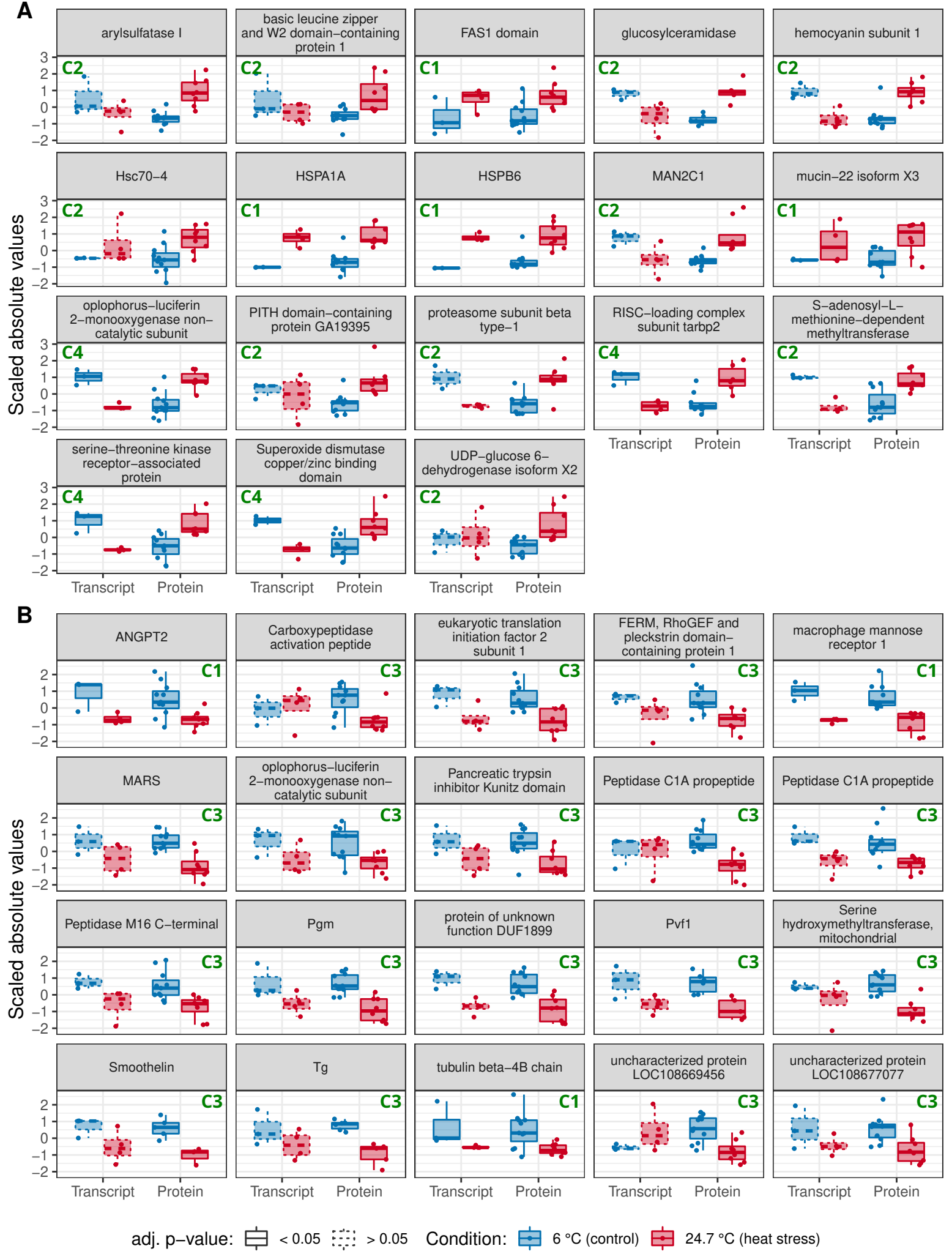
Figure 5. Upregulated transcript levels (FDR ≤ 0.01) with unchanged abundances (case 5, refer to paragraph "*Relationships of transcript expression levels and protein abundances*") of the corresponding proteins in *E. cyaneus* from the heat stress treatment *vs* the control.

Figure 6. Upregulated transcript levels (FDR ≤ 0.01) with unchanged abundances (case 5, refer to paragraph "*Relationships of transcript expression levels and protein abundances*") of the corresponding proteins in *G. lacustris* from the heat stress treatment *vs* the control. Only the most substantial transcript level changes (fold changes > 2) are depicted.

Figure 7. Correlations between the log2 fold changes (FC) of transcript and protein levels in the here studied amphipods upon heat stress treatment *vs* control. **A.** Proteomic *vs* transcriptomic log2(FC), both from animals from 24 h exposures to LT10 (24 h); **B.** Proteomic log2(FC) from animals from 24 h exposures to LT10 (24 h) *vs* transcriptomic log2(FC) from animals from 3 h exposures to LT10 (24 h). Only data for DE transcripts and DA proteins were included in the analyses. Here re-analyzed transcriptome data are from Drozdova et al. (2019)[15]; R - Pearson correlation coefficient.

Figure 8. Scheme illustrating the HSR in the here studied cold-adapted amphipod species on the cellular level. An overview is given on the transcript expression level/protein abundance changes and related cellular processes. Names of proteins are shown that were either differently abundant and/or the corresponding transcripts were differentially expressed in one or more of the studied amphipod species. Names with asterisk are transcripts upregulated with protein abundance unchanged; names without asterisk are DA proteins. Names in bold letters indicate proteins/transcripts found as DA/DE in more than one species and are therefore considered as general components of the HSR; plain letters – proteins/transcripts detected as DA/DE only for *E. verrucosus*; PTR – post-transcriptional regulation, eIF2α – eukaryotic translation initiation factor 2a, Ecy – *E. cyaneus*, Eve – *E. verrucosus*, Gla – *G. lacustris*, Hsp – heat shock protein, Man – mannosidase, MARS – methionyl-tRNA synthetase, GAGs – glycosaminoglycans, RISC – RNA-induced gene silencing complex, ROS – reactive oxygen species, Bzw1 – eIF5-mimic protein 2, SOD – superoxide dismutase, tarbp – TAR RNA binding protein, AHSA1 – Activator of 90 kDa heat shock protein ATPase, Tom34 – mitochondrial outer membrane protein, Hop – stress-induced-phosphoprotein 1, TRiC – T-complex protein Ring

Complex, Ubc – polyubiquitin, Ubr – E3 ubiquitin ligase, SmydA – arthropod-specific histone lysine methyltransferase, MAT1A – S-adenosylmethionine synthase, ATP1A1 – ATPase subunit alpha-1, PMCA – calcium-transporting ATPase, lap-2 – leucyl aminopeptidase, Man2C – cytosolic α-mannosidase, Edem1 – ER degradation-enhancing alpha-mannosidase.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C2
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C2
FAS1 domain
C1
glucosylceramidase
C2
hemocyanin subunit 1
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Hsc70−4
C2
HSPA1A
C1
HSPB6
C1
MAN2C1
C2
mucin−22 isoform X3
C1
oplophorus−luciferin 2−monooxygenase non−catalytic subunit
C4
PITH domain−containing protein GA19395
C2
proteasome subunit beta type−1
C2
RISC−loading complex subunit tarbp2
C4
S−adenosyl−L−methionine−dependent methyltransferase
C2
serine−threonine kinase receptor−associated protein
C4
Superoxide dismutase copper/zinc binding domain
C4
UDP−glucose 6−dehydrogenase isoform X2
C2
Scaled absolute values
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2
1
0
−1
−2
Transcript
Protein
B
ANGPT2
C1
Carboxypeptidase activation peptide
C3
eukaryotic translation initiation factor 2 subunit 1
C3
FERM, RhoGEF and pleckstrin domain−containing protein 1
C3
macrophage mannose receptor 1
C1
MARS
C3
oplophorus−luciferin 2−monooxygenase non−catalytic subunit
C3
Pancreatic trypsin inhibitor Kunitz domain
C3
Peptidase C1A propeptide
C3
Peptidase C1A propeptide
C3
Peptidase M16 C−terminal
C3
Pgm
C3
protein of unknown function DUF1899
C3
Pvf1
C3
Serine hydroxymethyltransferase, mitochondrial
C3
Smoothelin
C3
Tg
C3
tubulin beta−4B chain
C1
uncharacterized protein LOC108669456
C3
uncharacterized protein LOC108677077
C3
Scaled absolute values
2
1
0
−1
−2
Transcript
Protein
adj. p−value:
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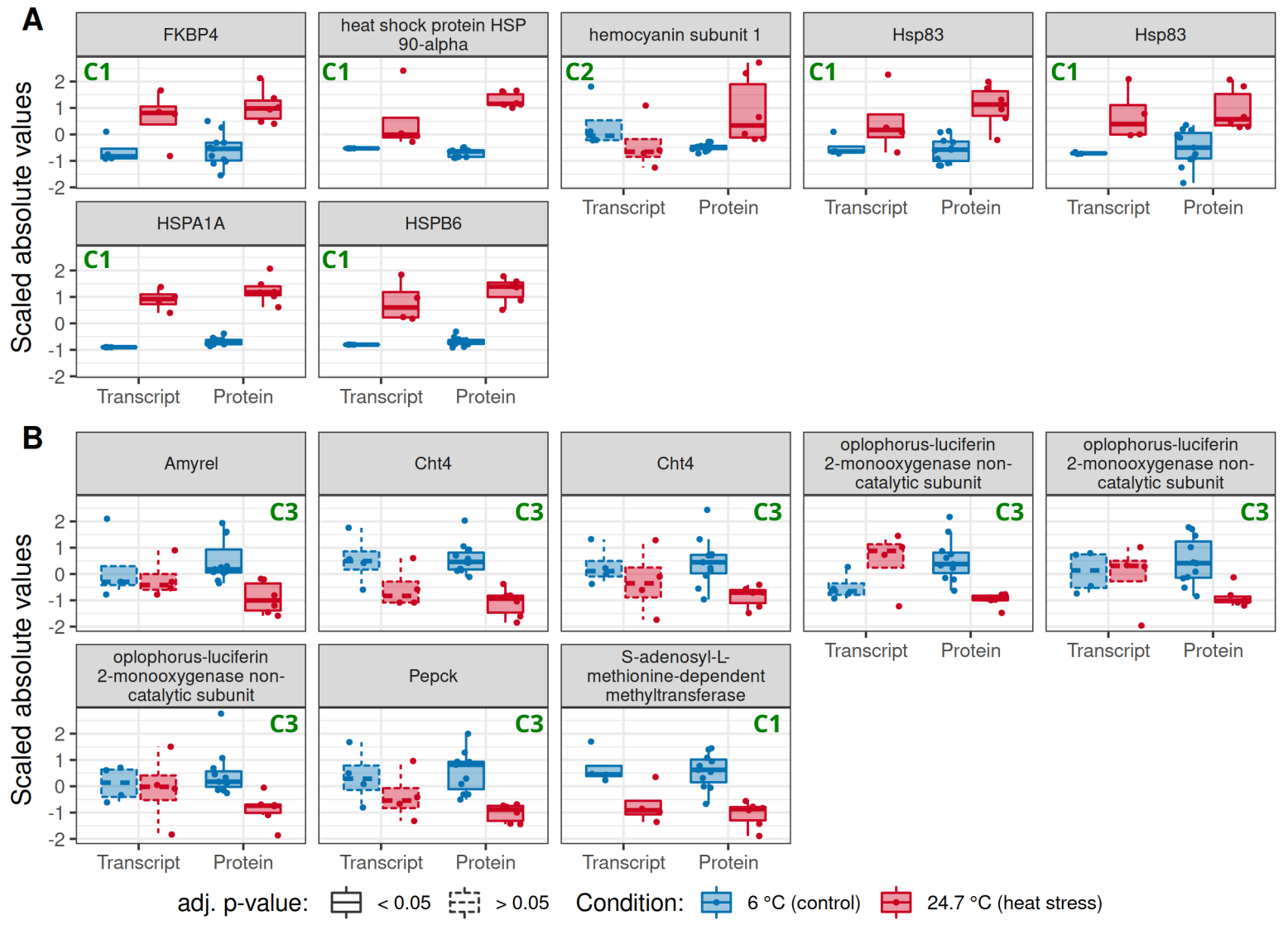
> 0.05
Condition:
6 °C (control)
24.7 °C (heat stress)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Hsp83
Hsp83
HSPA1A
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C1
C1
C2
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C1
C1
C1
Scaled absolute values
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-1
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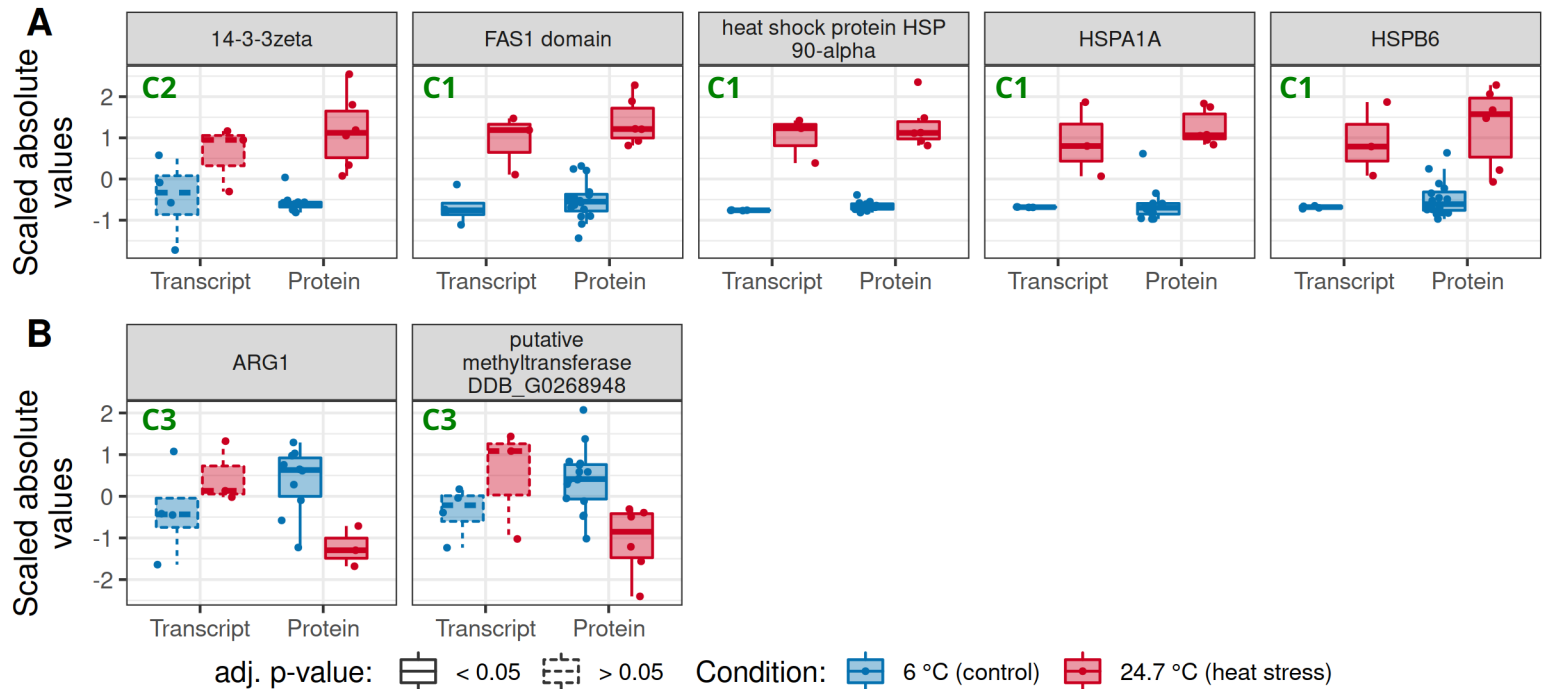
Transcript
Protein
B
Amyrel
Cht4
Cht4
oplophorus-luciferin 2-monooxygenase non-catalytic subunit
oplophorus-luciferin 2-monooxygenase non-catalytic subunit
oplophorus-luciferin 2-monooxygenase non-catalytic subunit
Pepck
S-adenosyl-L-methionine-dependent methyltransferase
C3
C3
C3
C3
C3
C3
C3
C1
adj. p-value:
< 0.05
> 0.05
Condition:
6 °C (control)
24.7 °C (heat stress)


A
B
Scaled absolute values
14-3-3zeta
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heat shock protein HSP 90-alpha
HSPA1A
HSPB6
ARG1
putative methyltransferase DDB_G0268948
C1
C2
C3
Transcript
Protein
adj. p-value:
< 0.05
> 0.05
Condition:
6 °C (control)
24.7 °C (heat stress)


97 kDa heat shock protein
ABC transporter type 1 transmembrane domain
activator of 90 kDa heat shock protein ATPase homolog 1
Alcohol dehydrogenase N-terminal
ATP1A1
CACYBP
CCT2
CCT4
CCT6A
Chaperone DnaJ
Cht4
dnaJ homolog subfamily A member 2
Edem1
FKBP4
flocculation protein FLO11
Hop
Hsc70-3
Hsp83
HSP90AB1
HSP90B1
HSPA1A
HSPB6
HSPD1
lap-2
lqf
MAN2C1
MAT1A
Membrane attack complex component/perforin (MACPF) domain
Membrane attack complex component/perforin (MACPF) domain
myosin heavy chain kinase B
PLA2G4A
probable protein disulfide-isomerase A6
Protein of unknown function DUF227
Serpin domain
solute carrier family 2, facilitated glucose transporter member 1
st13
Sushi/SCR/CCP domain
Tom34
Ubc
von Willebrand factor type A
VWA5A
Zinc finger MYND-type
Scaled absolute values
2
0
-2
Transcript
Protein
Condition:
6 °C (control)
24.6 °C (heat stress)
adj. p-value:
< 0.05
> 0.05


97 kDa heat shock protein
activator of 90 kDa heat shock protein ATPase homolog 1
Band 3 cytoplasmic domain
Calponin repeat
CCT6A
CCT8L2
Chaperone DnaJ
COP9 signalosome complex subunit 1
dnaJ homolog subfamily A member 2
Droj2
E3 ubiquitin ligase domain of unknown function DUF913
emb
FAS1 domain
Gelsolin domain
GstT2
Heat shock protein 70 family
Hop
Hsc70-4
hsp70-binding protein 1
HSP90B1
HSPA1A
HSPD1
Membrane attack complex component/perforin (MACPF) domain
MZB1
nuclear migration protein nudC
P58IPK
Pdi
Phosphatidylinositol-specific phospholipase C X domain
probable splicing factor, arginine/ serine-rich 6
RISC-loading complex subunit tarbp2
st13
Tom34
von Willebrand factor type A
Zinc finger MYND-type
Scaled absolute values
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1
0
-1
-2
Transcript
Protein
Condition:
6 °C (control)
24.6 °C (heat stress)
adj. p-value:
< 0.05
> 0.05

97 kDa heat shock protein
ACADVL
activator of 90 kDa heat shock protein ATPase homolog 1
Chaperone DnaJ
GLOD4
HECT domain
Hsc70-3
Hsp83
HSPA1A
lap-2
mle
myosin heavy chain kinase B
Pepck
Phosphatidylinositol-specific phospholipase C X domain
PMM1
probable protein disulfide-isomerase A6
probable splicing factor, arginine/ serine-rich 6
RNA recognition motif domain
RNA recognition motif domain
Spectrin repeat
SUCLG2
Tom34
TROVE2
Ubc
Zinc finger MYND-type
Scaled absolute values
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6 °C (control)
24.6 °C (heat stress)
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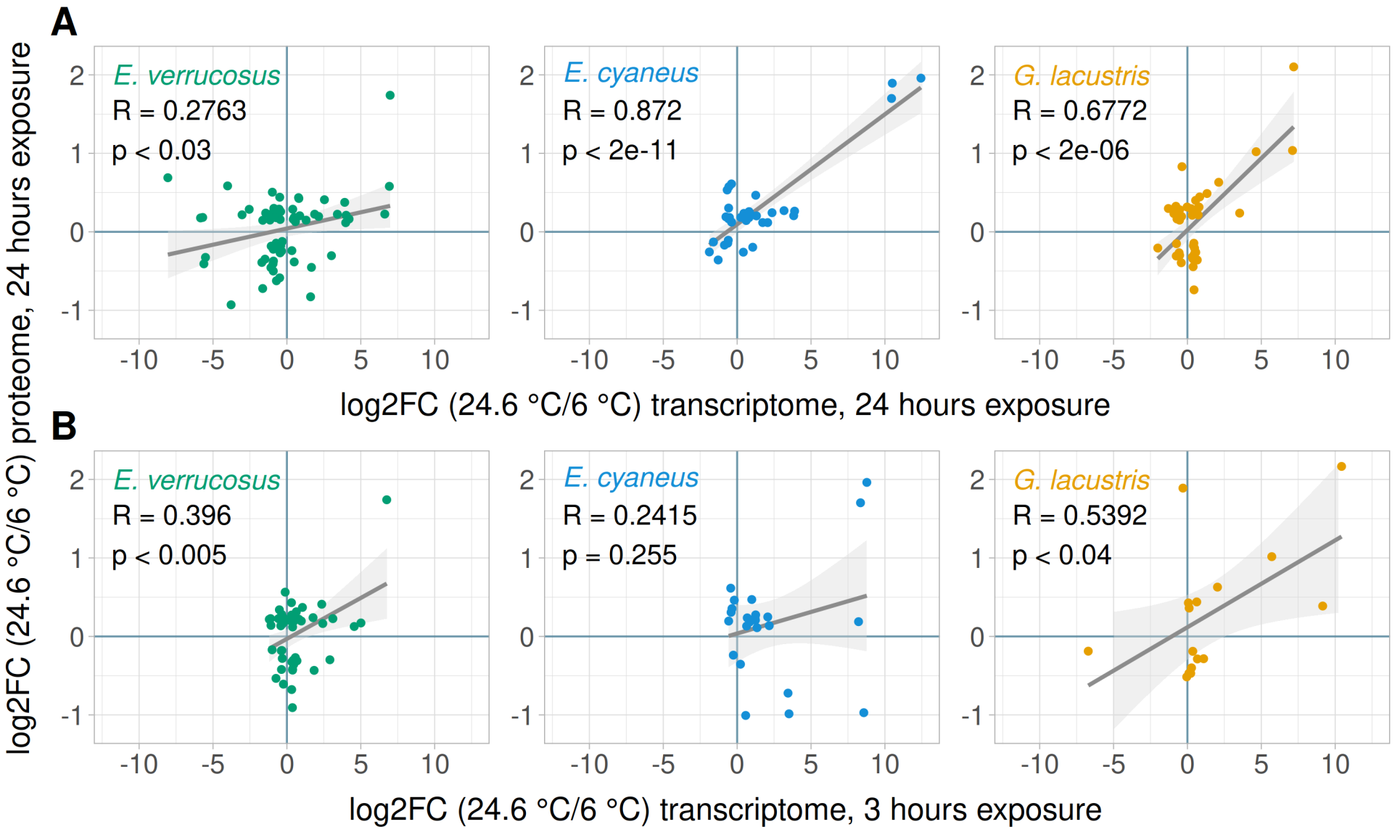
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E. verrucosus
R = 0.2763
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E. cyaneus
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G. lacustris
R = 0.6772
p < 2e-06
log2FC (24.6 °C/6 °C) transcriptome, 24 hours exposure
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E. verrucosus
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p < 0.005
E. cyaneus
R = 0.2415
p = 0.255
G. lacustris
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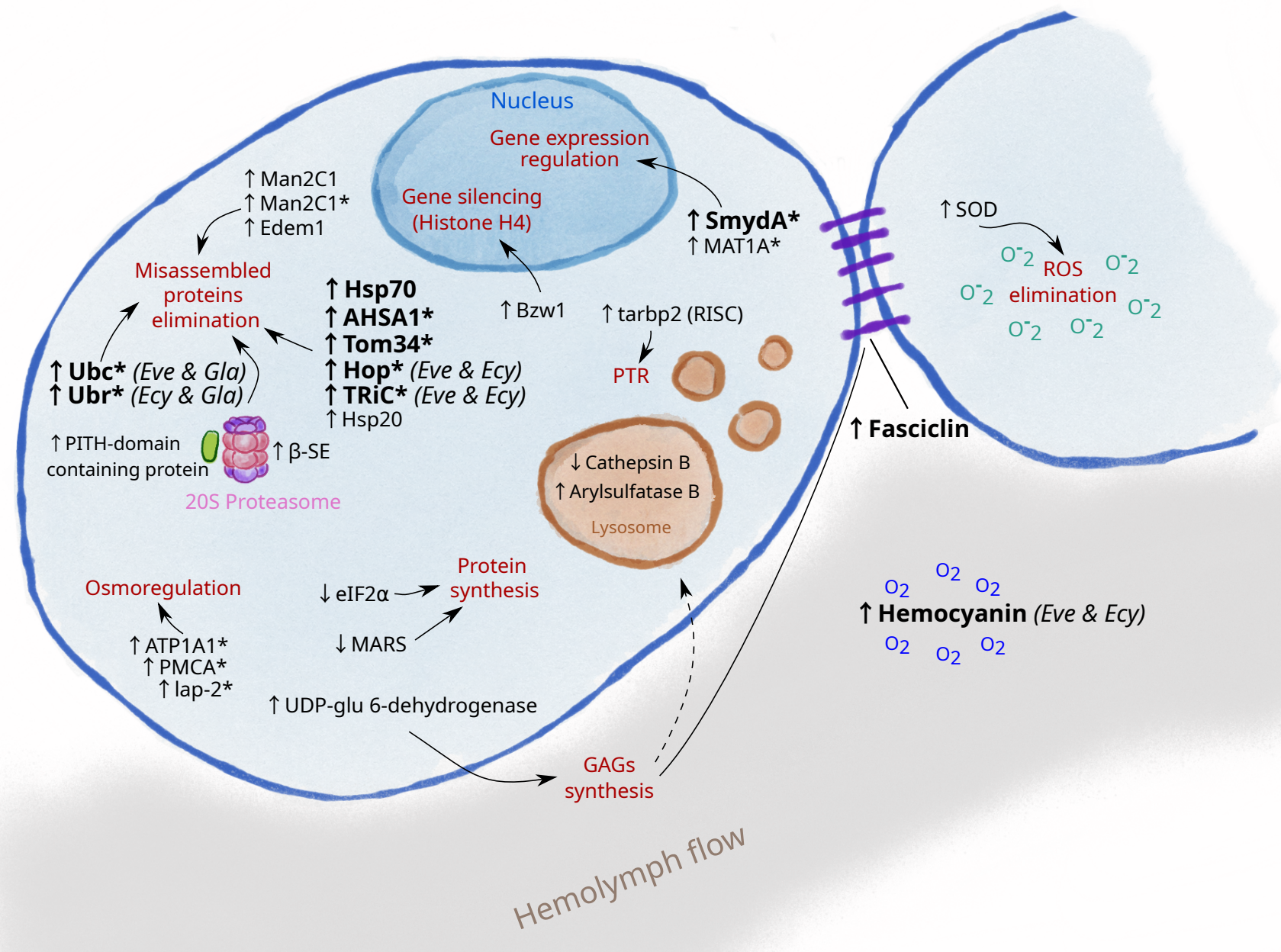
log2FC (24.6 °C/6 °C) proteome, 24 hours exposure


Nucleus
Gene expression regulation
Gene silencing (Histone H4)
↑ Man2C1
↑ Man2C1*
↑ Edem1
↑ SmydA*
↑ MAT1A*
↑ SOD
O-2
ROS elimination
Misassembled proteins elimination
↑ Hsp70
↑ AHSA1*
↑ Tom34*
↑ Hop* (Eve & Ecy)
↑ TRiC* (Eve & Ecy)
↑ Hsp20
↑ Bzw1
↑ tarbp2 (RISC)
PTR
↑ Ubc* (Eve & Gla)
↑ Ubr* (Ecy & Gla)
↑ PITH-domain containing protein
↑ β-SE
20S Proteasome
↓ Cathepsin B
↑ Arylsulfatase B
Lysosome
↑ Fasciclin
Osmoregulation
↑ ATP1A1*
↑ PMCA*
↑ lap-2*
↓ eIF2α
↓ MARS
Protein synthesis
O2
↑ Hemocyanin (Eve & Ecy)
↑ UDP-glu 6-dehydrogenase
GAGs synthesis
Hemolymph flow