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Synthesis of transcriptomic studies reveals a core response to heat stress in abalone (genus *Haliotis*)

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Abstract

Background As climate change causes marine heat waves to become more intense and frequent, marine species increasingly suffer from heat stress. This stress can result in reduced growth, disrupted breeding cycles, vulnerability to diseases and pathogens, and increased mortality rates. Abalone (genus *Haliotis*) are an ecologically significant group of marine gastropods and are among the most highly valued seafood products. However, heat stress events have had devastating impacts on both farmed and wild populations. Members of this genus are among the most susceptible marine species to climate change impacts, with over 40% of all abalone species listed as threatened with extinction. This has motivated researchers to explore the genetics linked to heat stress in abalone. A substantial portion of publicly available studies has employed transcriptomic approaches to investigate abalone genetic response to heat stress. However, to date, no meta-analysis has been conducted to determine the common response to heat stress (i.e. the core response) across the genus. This study uses a standardized bioinformatic pipeline to reanalyze and compare publicly available RNA-seq datasets from different heat stress studies on abalone.

Results Nine publicly available RNA-seq datasets from nine different heat-stress studies on abalone from seven different abalone species and three hybrids were included in the meta-analysis. We identified a core set of 74 differentially expressed genes (DEGs) in response to heat stress in at least seven out of nine studies. This core set of DEGs mainly included genes associated with alternative splicing, heat shock proteins (HSPs), Ubiquitin–Proteasome System (UPS), and other protein folding and protein processing pathways.

Conclusions The detection of a consistent set of genes that respond to heat stress across various studies, despite differences in experimental design (e.g. stress intensity, species studied—geographical distribution, preferred temperature range, etc.), strengthens our proposal that these genes are key elements of the heat stress response in abalone. The identification of the core response to heat stress in abalone lays an important foundation for future research. Ultimately, this study will aid conservation efforts and aquaculture through the identification of resilient populations, genetic-based breeding programs, possible manipulations such as early exposure to stress, gene editing and the use of immunostimulants to enhance thermal tolerance.

Keyword Climate change, Gene expression, Meta-analysis, RNA-seq, Thermal stress

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Background

Abalone (*Haliotis* spp.) are marine gastropods belonging to the family Haliotidae. Members of this genus are distributed worldwide, along rocky shores and coral reefs, across tropical and temperate waters [1, 2]. In addition to their ecological, historical, and cultural importance [3–5], abalone are one of the most highly prized seafood products, underpinning valuable wild-harvest and aquaculture industries in many countries [6, 7]. However, most abalone fisheries are fully or overexploited, and currently, more than 95% of the world's abalone is produced in aquaculture [7]. However, abalone species are at risk due to climate change-induced ocean warming and marine heat waves (MHWs), causing mass mortalities of abalone in the wild, as well as ongoing heat-stress-related losses in aquaculture [8, 9]. As a result of the significant decline in wild abalone populations [6, 10], the IUCN Red List of Threatened Species lists 44% of abalone species as threatened with extinction [11–13].

MHWs have been observed worldwide and are expected to increase in intensity and frequency with ongoing climate change [14]. Over the past 100 years, the annual number of MHW days has increased by 54% [15], and mass mortalities (of marine organisms) associated with marine heat waves have been reported worldwide [16–18]. Mortality events that occur during the summer months (likely heat-related) are often referred to as "summer mortalities", a phenomenon described in the 1940 s for the Pacific Oyster [19, 20] and other marine organisms [21]. Abalone are also greatly affected by this phenomenon, and farmers often report higher abalone mortalities over the summer months [22]. During the summer of 2010–2011, the Western Australian coast experienced seawater temperatures up to 6 °C above the average. This led to a devastating 99% mortality rate of the wild Roe's abalone (*Haliotis roei*) stocks in Kalbarri, Western Australia [23–26]. Similarly, the 2016 mortality event of wild abalone near the coast of Tasmania led to smaller catches and reduced quotas [27]. These events have great economic impacts on the abalone industry, resulting in a significant drop in production and loss of income. The temperate abalone species *Haliotis laevigata* (Greenlip abalone) and *Haliotis rubra* (Blacklip abalone) are the most extensively farmed species in Australia. These species have been identified as particularly susceptible to the impacts of climate change, making them highly vulnerable [28]. Due to the severe impact of summer mortality on abalone populations, the Australian government initiated the *Abalone Aquaculture Subprogram* [22]. In America, MHWs in 2014–2016, combined with the loss of kelp forests and overfishing, are believed to have caused the mass mortality of *Haliotis rufescens* populations and a widespread decrease of *Haliotis sorenseni, Haliotis corrugata, Haliotis cracherodii, Haliotis fulgens, Haliotis kamtschatkana* and *Haliotis walallensis* all along the west coast, from Mexico to Canada [11, 29]. These seven abalone species are listed as Critically Endangered (CR) in the IUCN Red List of Threatened Species [11–13].

While some marine organisms can avoid warmer waters by horizontal and/or vertical migration (swimming or diving), others, such as abalone, lack this capability and are restricted in their movement and mobility. Thus, abalone are subject to exposure to prolonged heat stress. Considering their limited movement abilities and narrow dispersal patterns [30, 31], abalone are likely to possess some genetic mechanisms that provide them with defensive elements to respond to thermal stress and, potentially, survive. It has been reported that there is a genetic basis for resilience to thermal stress in marine invertebrates [32], such as the Pacific oyster [33, 34], the Greenlip abalone (*H. laevigata*) [35–37], the Pacific abalone (*H. discus hannai*) [38] and its hybrid [39].

In the past decade, the availability of genetic data (specifically annotated abalone genomes and transcriptomes) and omics tools has allowed researchers to examine changes in gene expression associated with response to heat stress. Indeed, heat stress is the most well-studied stress factor in abalone [40]. The molecular response to heat stress is most likely not associated with only one gene but rather with a more complex network of genes regulated at different levels (epigenetic, transcriptional, post-transcriptional, translational, and post-translational). Also, the levels of gene expression are highly related to the intensity and magnitude of thermal stress [41]. Mechanisms such as transcriptomic plasticity allow organisms to respond rapidly and effectively to environmental changes [42]. Organisms can enhance their survival chances by "switching on" or "switching off" specific genes in response to stressors [43] such as temperature-related stress [44, 45] and salinity [46, 47]. For example, during heat stress, proteins are misfolded and aggregate in the cell. Certain levels of misfolded aggregated proteins might be toxic and result in mortality. However, this unwanted aggregation in the cell can be avoided by 1) the refolding of these proteins or 2) the breakdown into smaller molecules (i.e. smaller chains of amino acids) that can be transported out of the cell. These lifesaving regulations of protein misfolding are associated with the upregulation of Heat Shock Protein genes (HSP) and genes related to the Ubiquitin–Proteasome System (UPS) [48, 49]. Thus, an abalone individual that can efficiently regulate the transcription of these genes might be able to increase the probability of its survival. A study done on Greenlip abalone revealed differences in gene expression between summer mortality resilient and susceptible

individuals and suggested the role of transposable elements and their association with epigenetic mechanisms [37]. Other transcriptomic differences were linked with metabolism and immune process [36]. Other mechanisms, such as the regulation of metabolic pathways, were also suggested as a key element in a case study on the heat tolerance of abalone [50]. In hybrid abalone exhibiting thermal heterosis, there is a higher frequency of alternative splicing events under heat stress compared to their parent species, suggesting that the increased splicing diversity may enhance thermal tolerance in hybrids by producing a range of protein isoforms that support stress adaptation [39].

This paper focuses on the transcriptomic response to heat stress in abalone. It provides a meta-analysis of RNA-Seq data from nine different studies across seven different abalone species and three hybrids [37, 39, 51–57]. The range of available transcriptomic data associated with heat stress in abalone derives from studies with different experimental designs (i.e. intensity and magnitude of stress, tissue type and *Haliotis* species) that most likely influence the findings since gene expression is highly context-dependent [58]. The latter provides the opportunity to identify a set of genes that were universal in their expression patterns regardless of the species or any other experimental factor. By integrating and comparing multiple transcriptomic data sets and synthesising knowledge from other relevant studies, the current paper suggests genes and pathways that may be the core response to heat stress in abalone. Hopefully, findings from this study will aid abalone aquaculture and conservation efforts and provide a basis for future studies.

Methods

The overall workflow of this meta-analysis is summarised in Fig. 1 and documented in detail in an associated GitHub repository (http://github.com/roybarkan2020/Abalone-RNAseq-meta-analysis).

RNA-Seq data collection

Nine studies were compiled in this meta-analysis (Table 1). These studies were identified by searching Google Scholar (during October 2023) for peer-reviewed publications using the keywords: #RNA-seq, #Abalone, #Transcriptomics, #Heat-stress, #*Haliotis*, and #Thermal-stress. The raw RNA-seq data and the associated metadata for each study were downloaded from The National Center for Biotechnology Information (NCBI) and the China National GeneBank DataBase (CNGBdb). The associated publication for each study was reviewed to ensure the study met the eligibility criteria: (1) the study was performed on abalone species or a hybrid, (2) the study was done to examine gene expression under heat stress, (3) the gene expression analysis was carried out using RNA-seq data. Additionally, metadata for each study was obtained to provide all associated information for each sample. The studies' experimental design and metadata were reviewed thoroughly to ensure all BioSamples suit the aim of this meta-analysis and to exclude BioSamples that may introduce bias (e.g. RNA-seq data from individuals who simultaneously experienced multiple stressors, such as high temperature and salinity). Finally, 150 samples (control and heat-stressed) from nine different studies provided 300 FASTQ files (paired reads), which were downloaded from the NCBI Sequence Read Archive (SRA) and the CNGBdb. For a detailed list of samples used for this meta-analysis, along with their associated metadata, see Table S1 -Additional file 1.

Differential gene expression analysis

All datasets with biological replicates were analysed using the same pipeline (i.e. data processing, bioinformatic tools, tool parameters, cut-offs and thresholds). For two datasets [51, 55] (PRJNA481417 and PRJNA453554) with no biological replicates, the final analysis step (identification of differentially expressed genes) was performed based on fold change values alone since it was not possible to estimate a comparable p-value. Briefly, once data collection was completed (as above), quality control reports were generated using FastQC [59] and MultiQC [60] tools. Based on QC reports, trimming was performed using Trimmomatic [61]. Following the QC stage for all datasets, the data was mapped to a single reference transcriptome using Bowtie2 [62]. To increase comparability and since reference assemblies and annotations were not available for each abalone species in the analysis, the transcriptome of the red abalone, *H. rufescens* [63], was selected as a common reference since it also provided the overall highest average mapping rate (86%) across species (Table S2 -Additional file 1). Mapped reads for each sample were used to generate gene-level read counts using the RSEM software package [64]. Raw read counts from each dataset were imported into DESeq2 [65] and normalized to account for library size. The design formula was set to include the factors of interest (e.g., treatment) to model the experimental conditions. Genes with low counts were filtered, and a negative binomial distribution was used to estimate dispersion and fit generalized linear models for each gene. Statistical significance was assessed using the Wald test, and p-values were adjusted using the Benjamini–Hochberg method. Genes with an adjusted p-value < 0.05 were considered differentially expressed. For the two datasets with no biological replicates (PRJNA453554 and PRJNA481417), low gene counts were first removed.

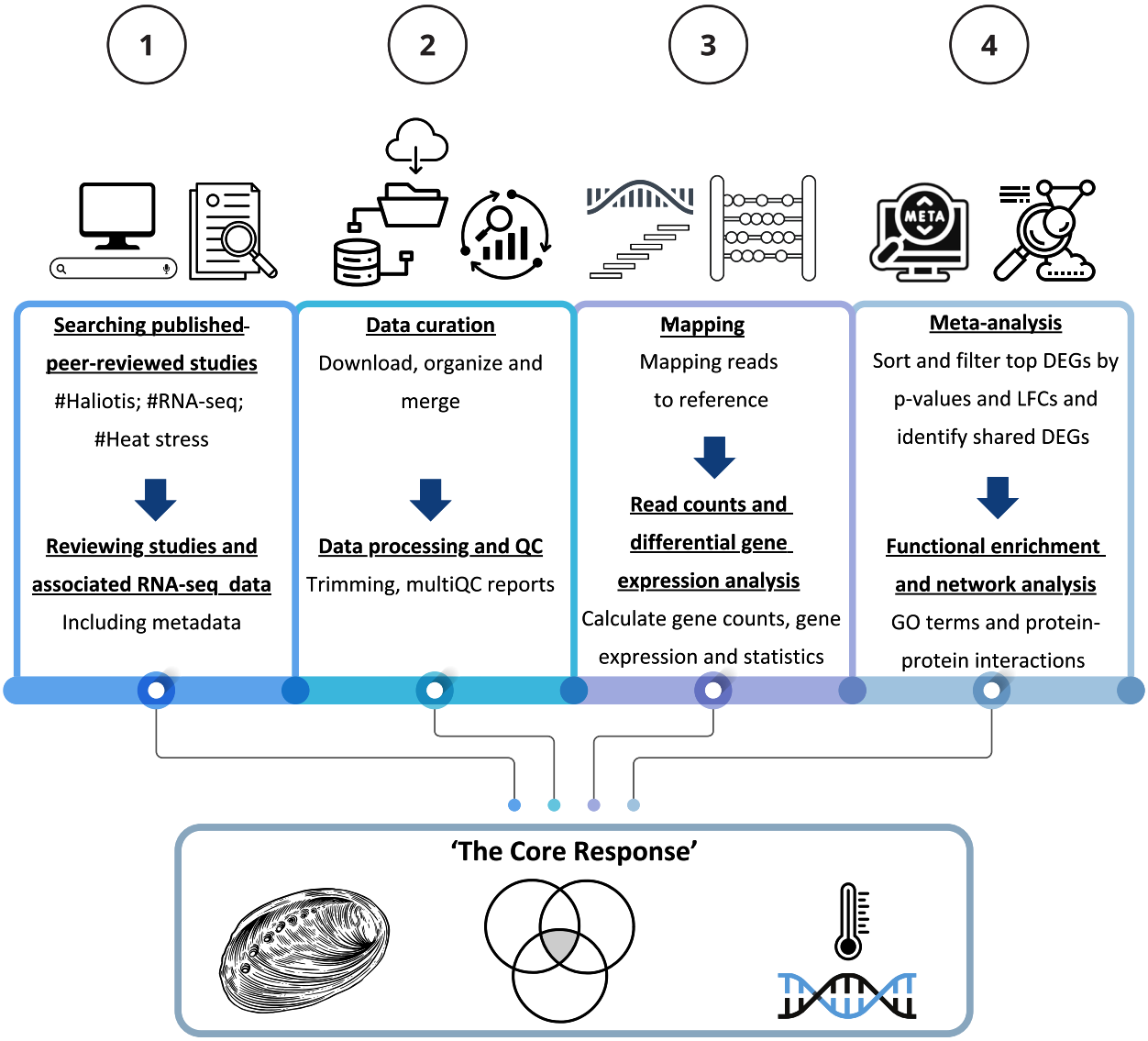

Fig. 1 General description of the meta-analysis workflow

Then, the filtered gene counts of each study were used to calculate the change in expression using log2 fold change (LFC) per gene. For the other datasets (with biological replicates), the DESeq2 results output of each study was used to obtain only results with statistical significance (padj < 0.05), which were then sorted by the adjusted *p*-value. Filtered and sorted gene expression results from all nine studies were merged by the gene name for comparison. The criteria for genes to be included in the 'core response genes' was to be identified in at least 7 out of 9 studies as statistically significant DEGs (padj < 0.05) or with |LFC| > 1 (where *p*-values cannot be calculated). Variance Stabilizing Transformation (VST) was applied to correct for size factors and normalization factors. The transformed data were then used for Principal Component Analysis (PCA), visualization, and cluster identification.

Functional enrichment and network analysis

All genes listed in the 'core response genes' were functionally annotated using g:Profiler [66] (interoperable web service for functional enrichment analysis and gene identifier mapping). Protein–protein interactions (functional and physical associations) were identified using STRING [67], and the results were filtered with *p*-values adjusted for multiple testing within each category through the Benjamini–Hochberg correction. *H. rufescens* was set as the defined organism reference,

Table 1 Summary of all RNA-seq studies investigating heat stress in Haliotis species and hybrids included in the meta-analysis

Species	Study (BioProject)	Control temp (°C)	Max temp (°C)	Δtemp (°C)	Max. duration of stress (hr)	Tissue
H. discus hannai	Kim et al. (2021) [56] PRJNA597237	20	30	10	24	Hepatopancreas
H. discus hannai	Kyeong et al. (2020) [52] PRJNA557314	20	30	10	1	Muscle, Mantle, Gills, and Blood
H. discus hannai	Zhou Wu et al. (2023) [57] CNP0003705	17	28	11	48	Hemolymph
H. discus hannai X *H. gigantea*	Xiao et al. (2021) [39] PRJNA721743	20	30	10	2	Gills
H. discus hannai X *H. fulgens*	Zhang et al. (2022) [54] PRJNA853707	18	32	14	24	Gills
H. diversicolor	Zhang et al. (2019) [51] PRJNA481417	25	31	6	96	Hemolymph
H. fulgens	Tripp-Valdez et al. (2019) [55] PRJNA453554	18	32	14	12	Pooled—Gills, Mantle and Hepatopancreas
H. laevigata	Shiel et al. (2017) [37] PRJNA286263	18.5	21	2.5	72	Tentacle
H. rufescens X *H. corrugata*	Tripp-Valdez et al. (2021) [53] PRJNA600240	18	22	4	2352	Whole organism

which was available in g:Profiler database. However, for STRING, the *H. rufescens* proteome and annotations were uploaded to the tool database to allow its availability as a reference organism (STRING proteome Id: STRG0 A18 FSP). The significance threshold of padj < 0.01 was used to assign Gene Ontology (GO) [68, 69] terms, Kyoto Encyclopedia of Genes and Genomes (KEGG) [70], and Reactome Pathways [71]. The 'core response genes' were also used as input to retrieve gene symbols via the BLAST-based homology approach. NetworkAnalyst [72], a comprehensive gene expression profiling via network visual analytics tool, was used to identify subnetworks within the core response genes by mapping these genes to the corresponding molecular interaction database. In addition, the R package for weighted correlation network analysis [73] (WGCNA) was used to study correlation patterns across genes, construct a gene network of co-expressed genes and detect any clusters (modules) in the network. This analysis was conducted by merging the normalized gene counts from all studies based on treatment, allowing the data to be treated as a unified dataset. This approach provided an independent method for identifying genes (across all studies) with highly correlated expression patterns. By utilizing eigengenes, we identified differentially expressed modules between the control and heat-stressed groups while excluding any other experimental factors, such as species and stress intensity. The networks generated with WGCNA were loaded into Cytoscape [74] version 3.10.1 for visualization.

Results

Nine studies passed the selection process according to the eligibility criteria described in the Methods section. The studies are listed in Table 1. For a detailed list of samples used for this meta-analysis, see Table S1 -Additional file 1.

Differential gene expression analysis

A total of 74 genes were found to be differentially expressed in at least seven (> 75%) of the studies (Fig. 2). This grouping, which we refer to as the 'core response genes' involved in the transcriptomic response to heat

(See figure on next page.)

Fig. 2 The core response to heat stress in abalone. The x-axis represents the number of studies (study count) in which a specific 'core response gene' was identified as DEG. The left side (x < 0) of the figure represents downregulated genes compared to the control group. The right side of the figure (x > 0) represents upregulated genes compared to the control group. Each colour represents a different study (as detailed in the figure legend). The left y-axis represents the gene name, which was significant in its expression compared to the control group. The right y-axis represents the description of the gene. The star symbol, grouping the top 15 genes, represents genes shared among eight out of nine studies (padj < 0.05)

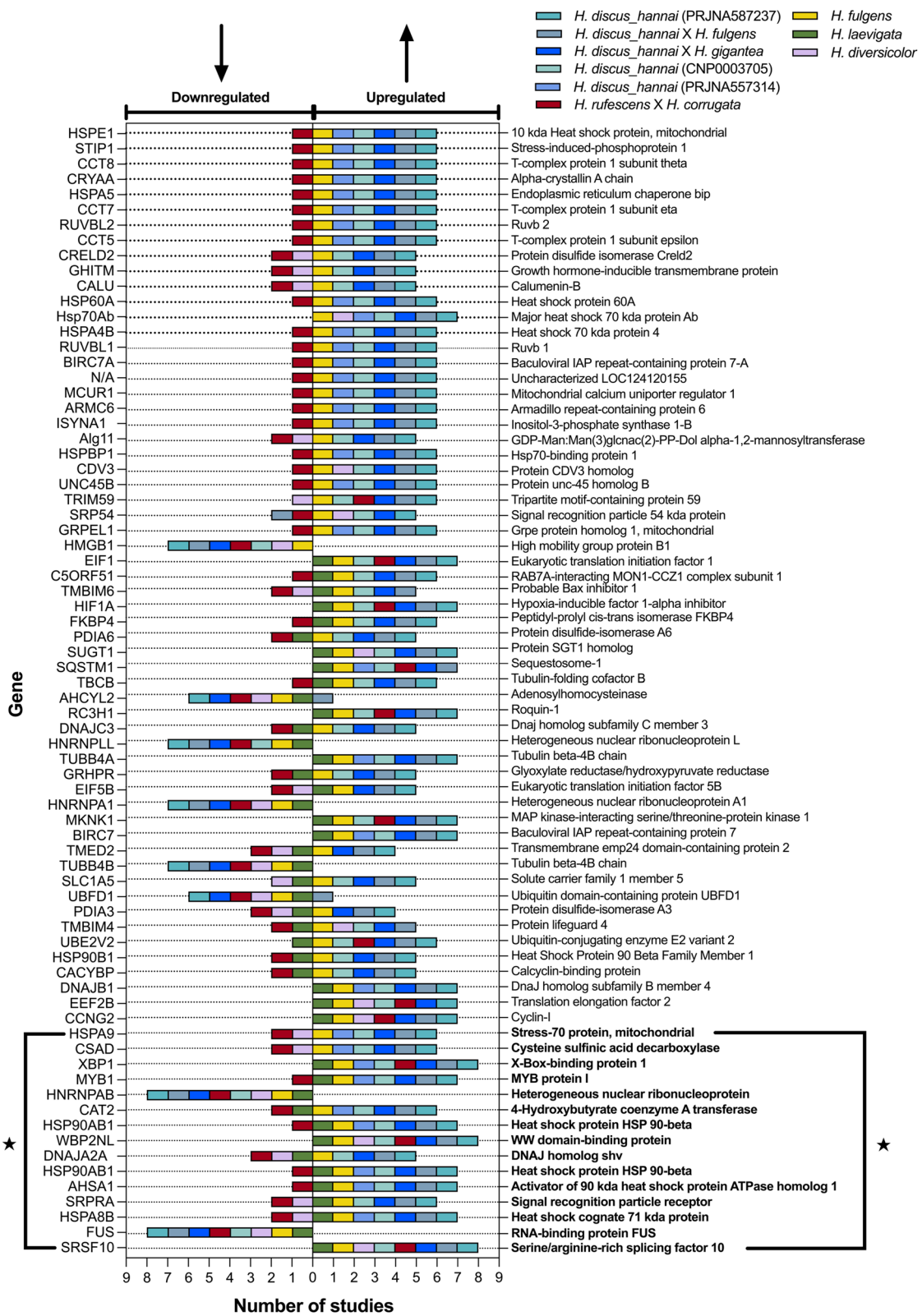

Fig. 2 (See legend on previous page.)

stress, included 15 genes that were shared among eight studies (padj <0.05) and 59 genes that were shared among seven studies (Table S3 – Additional file 1). In both core gene sets, the direction of change (negative or positive log2 FC; Fig. 3; Table S3 – Additional file 1) in response to heat stress was remarkably consistent across studies. However, in most of these cases, the same study [53] (BioProject PRJNA600240), which was done on *H. rufescens* X *H. corrugata* hybrid, dissented from the majority in the direction of expression (i.e. downregulation of genes that were upregulated in the other studies).

Most core genes were expressed at higher levels under heat stress, and this was consistently true across studies. Of the 74 genes in the core set, 50 were expressed at higher levels under heat stress (i.e. upregulated) in at least six studies. Of these, 15 genes were upregulated in all studies in which they were identified as significant DEGs.SRSF10, WBP2 NL and XBP1 were upregulated in all of the eight studies in which they were identified as DEGs. Other genes, such as CCNG2, HIF1 A, EIF1, BIRC7, HSP70 Ab, DnaJB1/HSP40 and others, were upregulated in all of the seven studies in which they were identified as DEGs. Although far fewer genes were found to be downregulated under heat stress, these were also highly consistent across studies with FUS, HNRNPAB, TUBB4B, HNRNPA1, HNRNPLL, and HMGB1 downregulated in every study.

HSPs made up over 15% of the core response genes. The only study with a relatively lower number of HSP genes as DEGs (only two out of 11 HSP genes) was carried out on *H. diversicolor* [51]. This study also had the lowest number of DEGs common with the other studies.

Where possible, principal component analysis (PCA) of gene count data was conducted to test the effect of other experimental factors (i.e. tissue type or species). PRJNA721743 [39] (comprising *H. discus hannai*, *H.*

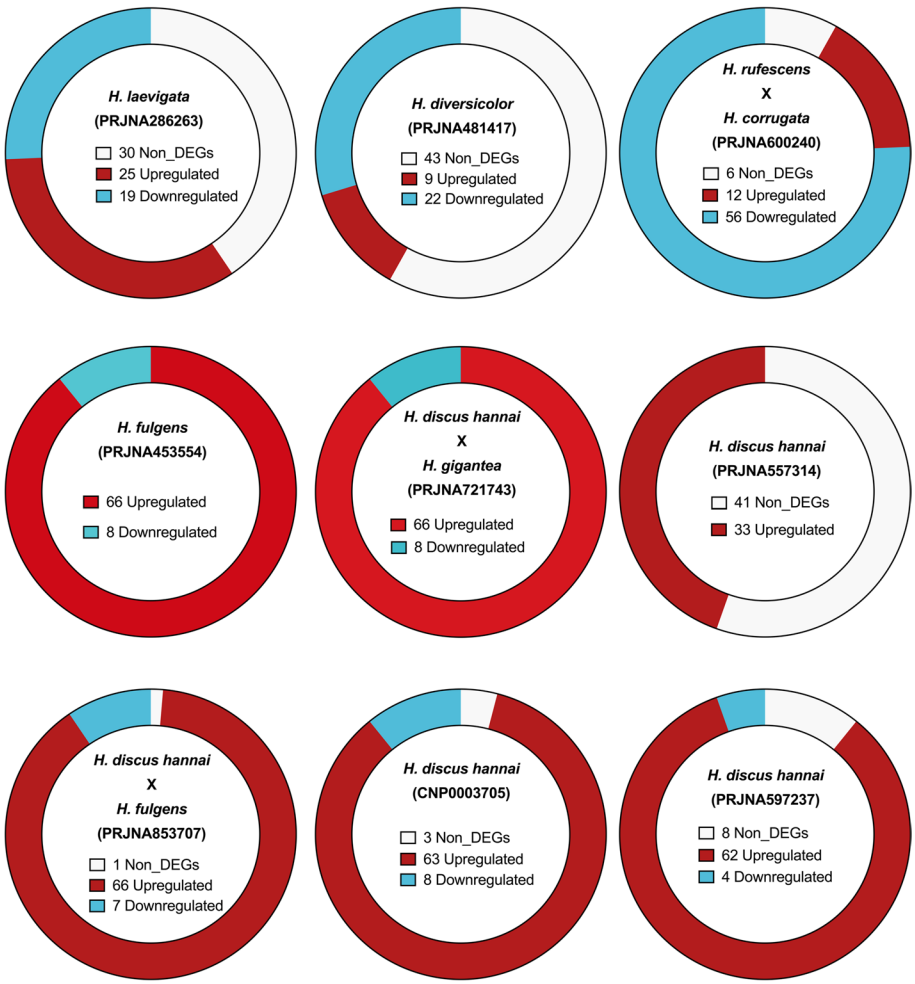

Fig. 3 Study-specific core response summary and expression pattern. Each of the nine charts represents a single study (species and BioProject are labelled in the centre of each chart). Within each chart, the portions are coloured to indicate the number of 'core response genes' (out of the total 74 'core response genes') that were not identified in the specific study (white), identified as upregulated (red) or downregulated (blue)

gigantea and their hybrid) presented a clear clustering by treatment along PC1 with an additional data clustering by the species along PC2 (Fig. 4A). The PCA of study PRJNA557314 [52] revealed a more significant effect of tissue type compared to the effect of actual stress (Fig. 4B).

Functional enrichment and network analysis

The overall core gene set (74 genes) was enriched in GO terms associated with protein folding and protein processing (Fig. 5), specifically, ten heat shock protein (HSP) genes (i.e. HSP60, HSP70 and HSP90) and genes related to the ubiquitin–proteasome pathway. The most enriched GO term was 'Protein Folding' (GO:0006457), which is associated with 24 of the 'core response genes' ($p < 0.001$), followed by other protein folding-related, RNA regulation, and stress-response GO terms (Table S4 – Additional file 1). STRING network analysis of the 'core response genes' (Table S8 – Additional file 1) revealed three highly enriched clusters (PPI enrichment p-value < 1.0e-16; presented in Fig. 6). The STRING network comprises 74 nodes (74 'core response' genes/predicted proteins) with 211 edges (predicted functional associations). Each of the three clusters contained a distinct set of HSPs, which were associated with cluster-specific proteins. The main cluster (Fig. 6, orange cluster) included five HSPs (HSPA8B, HSP60 A, HSPE1, HSP70 AB and HSP90 AB1) in association with 15 other proteins related to protein folding, such as activator of HSP90 ATPase activity-1 protein (AHSA1), three chaperonin-containing TCP1 complex proteins (CCT5, CCT7 and CCT8), RuvBL1/RuvBL2 complex proteins, Calcyclin binding protein (CACYBP) and Ubiquitin-conjugating enzyme E2. The second cluster (Fig. 6, blue cluster), which was also associated with protein folding processes, included two HSPs (HSPA5, HSP90B1), two protein disulfide isomerase family A members (PDIA3 and PDIA6), three DnaJ family members (DnaJB1, DnaJA2 A and DnaJC3), X Box Binding Protein-1 (XBP1), Calumenin (calcium-binding protein CALU), transmembrane BAX Inhibitor motif containing-6 (TMBIM6) and the transmembrane P24 trafficking protein-2 (TMED2). The third cluster (Fig. 6, purple cluster) consisted of four HSPs (HSPA9, HSPA4B, HSPE1, and HSPBP1) in interaction with the two signal recognition particle (SRP) components (SRPRA and SRP54), the alpha-A crystallin protein (CRYAA), protein SGT1 homolog (SUGT1 and GrpE protein homolog-1 (GRPEL1).

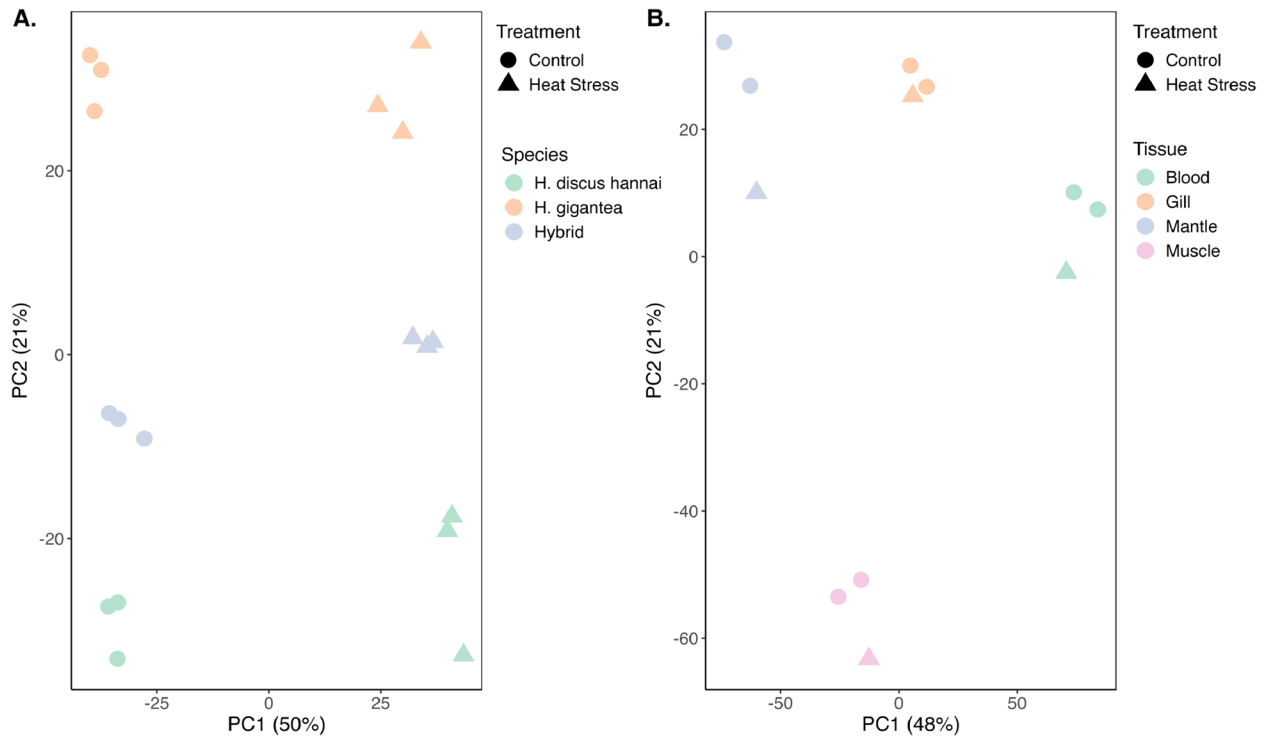

Fig. 4 Principal component analysis (PCA) of control and heat-stressed abalone. (A) PCA of the normalized count data (BioProject PRJNA721743, *H. discus hannai*, *H. gigantea* and their hybrid). Different colours represent the species/hybrid, and the shapes represent the treatment. (B) PCA of the normalized count data (BioProject PRJNA557314, *H. discus hannai*). Different colours represent the tissue type, and the shapes represent the treatment. Each axis represents a principal component (PC) and the percentage (%) of data variation it explains

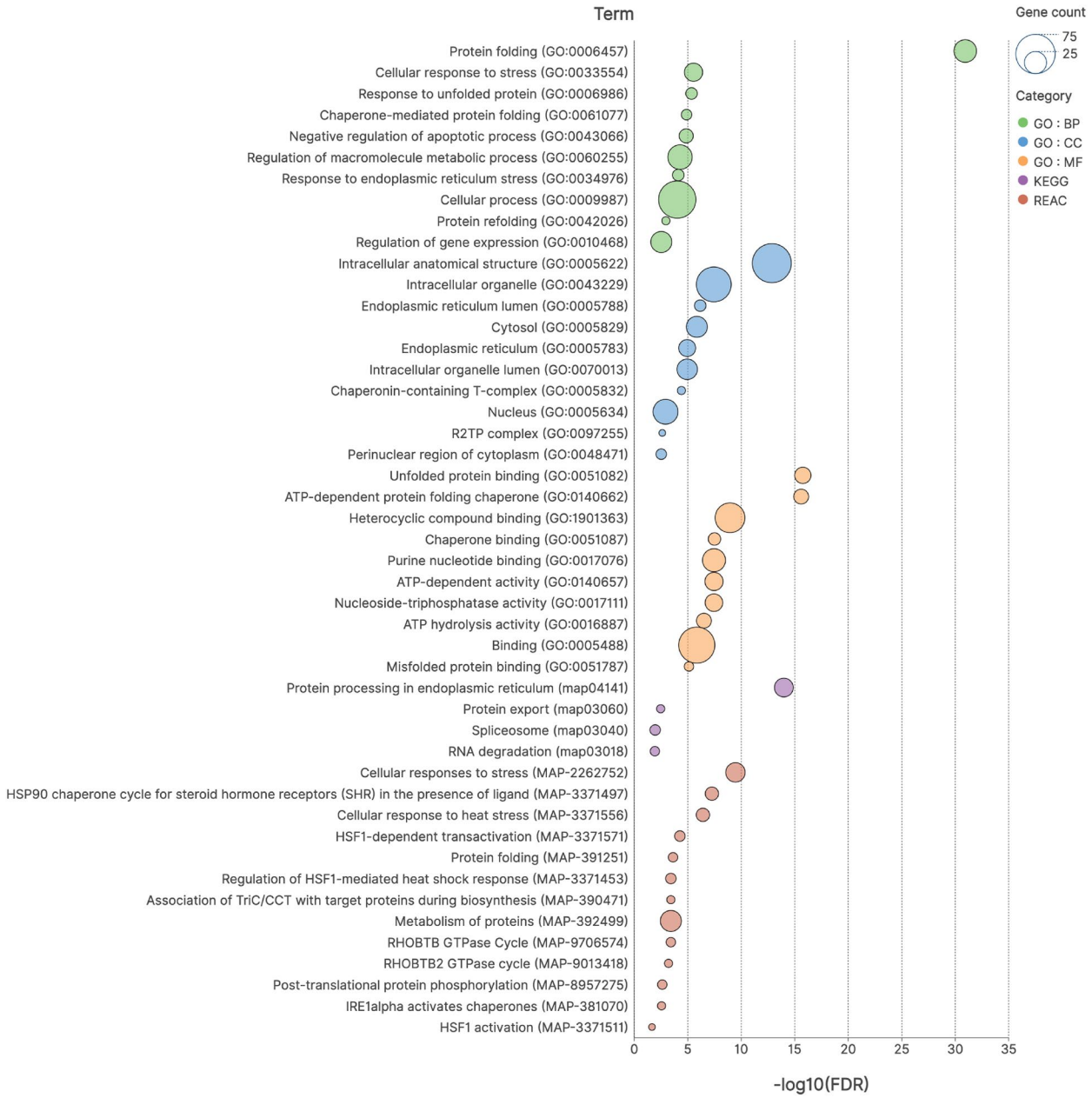

Fig. 5 Top significant terms and pathways associated with the 74 'core response' genes. The y-axis represents the terms associated with each gene cluster. The x-axis represents the statistical significance level as a negative log False Discovery Rate (-log10(FDR)). The size of the circle indicates the count of genes clustered into the same specific term. Each colour represents one of the terms/pathways databases – Gene Ontology Biological Process (GO: BP – in green), Cellular Component (GO: CC – in blue), Molecular Function (GO: MF—in orange), Kyoto Encyclopedia of Genes and Genomes (KEGG – in purple), and Reactome Pathway Database (REAC – in red)

WGCNA of gene counts of all studies revealed a single highly significant module across treatments (Figure S1 – Additional file 2; $p < 0.001$). This module consisted of 77 co-expressed genes, which were all downregulated in the control individuals compared to the heat-stressed individuals. These genes were mostly assigned to protein folding (GO:0006457, $p < 0.0001$), ATP-dependent protein folding chaperone (GO:0140662, $p < 0.0001$), unfolded protein binding (GO:0051082, $p < 0.0001$) and other protein folding and processing pathways (Table S5 – Additional file 1).

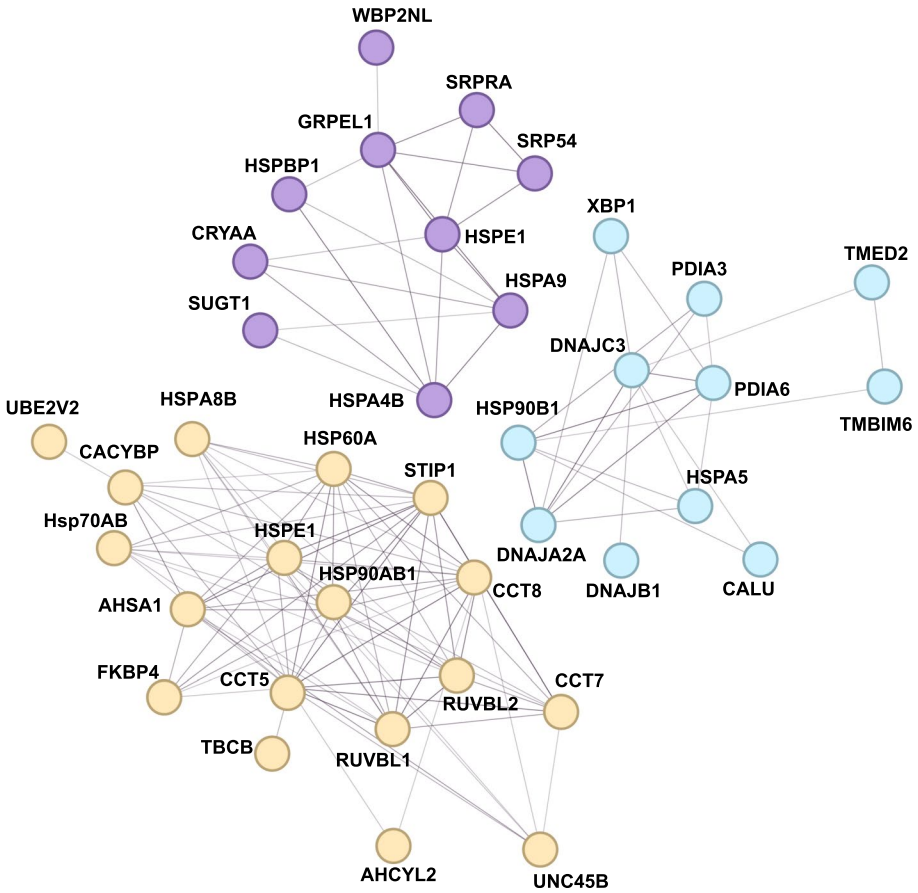

Fig. 6 The core response STRING network. Each circle (node) represents a single 'core response' protein-coding gene. The connecting lines (edges) represent predicted functional and physical associations. Only the high confidence score interactions are presented within the three main clusters: orange (18 protein-coding genes), blue (11 protein-coding genes), and purple (10 protein-coding genes)

Discussion

Using RNA-Seq data from multiple publicly available studies that differ in their experimental design and conditions, allowed the identification of DEGs that play a universal role in the genetic mechanism that abalone employ to deal with heat stress. Although gene expression is often highly individualistic and strongly associated with factors such as taxa, species, tissue type, life stage, environmental conditions, duration of stress and more [58, 75], meta-analysis across all available datasets enabled identification of 74 differentially expressed genes shared across heat stress studies of abalone, which we term the 'core response'.

Genes associated with the ubiquitin–proteasome system (UPS) and protein folding processes (e.g. HSPs) were a universal part of the core response to heat stress in abalone, suggesting the major role of these pathways regardless of any other experimental factor. This was further supported by STRING analysis (PPI), which identified three highly connected clusters. All clusters were primarily associated with protein folding, with a high proportion of heat shock proteins (HSPs) interacting with other proteins. The ubiquitin–proteasome pathway is responsible for the detection, degradation, and evacuation of misfolded proteins [76–78]. Under thermal stress, proteins are misfolded at a higher rate than in ambient conditions. Accumulation of misfolded proteins can result in mortality [49, 79–81]. Monitoring protein integrity, labelling misfolded proteins, and transporting them out of the cell can prevent the accumulation of protein in the cell and allow the survival of the organism [82, 83]. UPS is highly conserved in all taxa. In notothenioid fishes, the expression of genes involved in the ubiquitin–proteasome pathway is responsible for temperature compensation [84]. In the marine environment, it was found to be a significant factor in the response to heat stress in sweet kelp (*Saccharina japonica*) [85], smooth cauliflower coral (*Stylophora pistillata*) [86], silverfish (*Trachinotus ovatus*) [87], longjaw mudsucker (*Gillichthys mirabilis*) [88], saltwater mussels (*Mytilus* sp.) [89]

and others [90]. It is important to mention that these are significant mechanisms under other stressors across taxa (e.g. salinity, oxygen levels, cancer) [80, 91, 92].

Other genes that were found to be universal in response to heat stress in abalone have been reported as stress-related genes in other organisms. The serine/arginine-rich splicing factor gene (SRSF) plays an extremely important role in the stress response as part of the splicing process of precursor RNA [93, 94]. Alternative splicing may be one of the mechanisms that allow abalone to handle heat stress. The latter was also described in a study conducted on coral reef fish during a marine heatwave event [95] and heat-stress experiments on rainbow trout [96], redside dace [97] and catfish [98]. Alternative splicing plays a pivotal role as a crucial regulatory mechanism in response to diverse stresses. Emerging from a common pre-mRNA precursor, alternative splicing events enable swift adaptations in the abundance and functionality of vital stress-response components [94].

Out of the 74 genes we defined as the core response, only six genes were downregulated in all the studies in which they were identified as significant DEGs. Four of these genes were heterogeneous nuclear ribonucleoprotein-related (HnRNP; i.e. HNRNPAB, FUS, HNRNPLL and HNENPA1). The heterogeneous nuclear ribonucleoprotein genes regulate stress response through alternative splicing and inhibition of stress-related molecular agents [99, 100]. For example, by inhibiting heat shock-induced transcriptional activity of heat shock factors, such as reduced transcription of HSP genes [101]. HnRNP and serine/arginine-rich splicing genes were also identified as DEGs with a similar expression pattern in all tested tissue types of the Pacific White Shrimp (*Litopenaeus vannamei*) under heat stress [102]. A study completed on 18 RNA-seq datasets of salinity stress in rainbow trout (*Oncorhynchus mykiss*) also identified HnRNP genes as a key element in stress response. It concluded that these genes play a regulatory role, affecting downstream target genes that actively participate in the response to salinity changes in the gill tissues [103].

Members of the HSP family, specifically HSP70 and HSP90, were differentially expressed among most studies. These genes are part of the global response to heat stress in all organisms across the Tree of Life, with only certain features that are species-specific [104]. Interestingly, none of these genes were detected as DEGs in *H. diversicolor* in both the original study [51] and this meta-analysis. Moreover, *H. diversicolor* shared the lowest number of DEGs with other species in this study. This suggests that, overall, this species applies a relatively different response to heat stress compared to other *Haliotis* members. Genetically, *H. diversicolor* is relatively phylogenetically distinct from most *Haliotis* species in this meta-analysis [105–107]. In addition, *H. diversicolor* populations are distributed in warmer waters compared to the other *Haliotis* species included in the meta-analysis. However, previous studies have found that the expression of HSPs was relatively high in the haemocytes of *H. diversicolor* compared to other tissue types [108, 109], suggesting that the tissue type may not be the reason why HSP genes were not detected in *H. diversicolor*. A possible factor that might explain the fact that HSPs were not identified as DEGs in *H. diversicolor* in both the meta-analysis and the original study associated with this dataset is the duration of stress. A previous study (which was excluded from the meta-analysis because it was done using qRT-PCR) carried out on *H. diversicolor* under heat stress measured the relative expression of Heat Shock Cognate 70 (HSC70), a gene that is highly similar to the HSP70 in its sequence and its role [110], found the smallest difference in relative expression between the heat-stressed abalone to the control group after 96 h [109], the same time in which sampling took place in the *H. diversicolor* study included in the meta-analysis. Addressing the variations in the response to heat stress in the meta-analysis and confirming whether these variations are due to technical factors in the experimental design or biological and genetic factors is statistically challenging and requires further research. This may be clarified further by experiments that test the genetic response to heat stress (specifically gene expression) in 1) tropical abalone species, 2) different stress intensities within the same abalone species, 3) stress duration, and other experimental factors on the same species (e.g. differences among tissues, age, etc.).

The hybrid of *H. rufescens* X *H. corrugata* [53] exhibited 31 genes (42%) from the core response that were downregulated, whilst these were found to be upregulated in all other studies included in the meta-analysis. One of the main differences in the experimental design of this study was the stress duration (98 days of heat stress), with a relatively moderate increase in temperature (18 °C to 22 °C) compared to other studies in this meta-analysis (Table 1). This might suggest that moderate stress intensity, over time, could result in higher tolerance, which can shift the genetic response due to heat priming. Heat priming or thermal priming is a process mainly described in plants in which an organism experiences early heat stress, which results in improved tolerance to future heat-stress events [111–113]. Although this is an interesting finding, and the time at which the abalone were under stress was 50-fold longer than the average time in all other studies, we cannot be certain that this is one (or the only) explanation. Two other possibilities as to why the hybrid of *H. rufescens* X *H. corrugata* [53] study showed such an opposite pattern of expression are 1) the

study included a pool of multiple tissues, which might be masking the possibility of one tissue type that is overrepresented (i.e. overall, the genes are upregulated in most tissues but highly downregulated in other tissues, which may affect the final, overall, expression pattern). 2) The study's "heat-stress temperature" (22 °C) is actually closer to the optimal temperature of *H. corrugata* (24.5 °C) [114] than to the study's "control temperature" (18 °C). This can also explain the higher growth at 22 °C (than at 18 °C) that the individuals presented in the hybrid of *H. rufescens* X *H. corrugata* as suggested by the authors that conducted the study. The authors also add that it is possible that *H. corrugata's* genetic contribution was greater than that of *H. rufescens*, which results in a higher thermal optima of the hybrid [53].

Heat stress at various levels of intensity or duration can cause negative effects on abalone reproduction, growth, feeding behaviour, immunity, and more [115–117]. For the aquaculture industry, these negative effects result in impaired feeding efficiency and digestibility, susceptibility to diseases and pathogens, lower reproductive performances, and loss of stock [118, 119]. With that being said, it is also known that some individuals possess genetically based resistance to thermal stress [35–38].

Conclusion

The current study presents the core transcriptomic response to heat stress shared among multiple abalone species. By providing a core set of genes, this study sets the stage for creating solutions to the negative effects of heat stress in abalone, which could result in new uses of methods and technological advancements in future studies and conservation. For aquaculture, future attempts to provide higher tolerance to heat stress in abalone by manipulating a set of genes through thermal priming, gene editing techniques, selective breeding and/or use of immunostimulants (e.g. polycytidylic acid) could improve stock survival in aquaculture operations. Focusing on HSPs, HNRNPs, and other genes responsible for protein folding, processing, and regulation via splicing events, which are found to be ubiquitous elements in response to heat stress in abalone, offers a more targeted approach. Ultimately, this study provides knowledge that can contribute to aquaculture and conservation during increased exposure and frequency of MHWs and high temperatures due to climate change.

Supplementary Information

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Additional file 1

Additional file 2

Additional file 3

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Statements and declarations
The authors declare no conflicts of interest.

Authors' contributions
All authors contributed to the conceptualization of the study. R.B. collected the data, while J.S., I.C., and S.W. co-supervised the work and analysis. J.S. and I.C. provided guidance on the bioinformatics pipeline. R.B. conducted the data and statistical analyses, which were validated by J.S., I.C., and S.W. All authors discussed and reviewed each section of the manuscript and contributed to its final version.

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Data availability
All of the data used for the meta-analysis is cited in the text and included in the reference section. In addition, studies associated with this data and BioProject accessions are listed in Table 1. RNA-seq data (raw sequencing data only) used for the analysis and the reference genome repository (used for the analysis) are available using the following links:
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA597237
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA557314
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA721743
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA853707
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA481417
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA453554
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA286263
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA600240
https://db.cngb.org/search/project/CNP0003705/
The code used for this analysis is publicly available on GitHub: https://github.com/roybarkan2020/Abalone-RNAseq-meta-analysis.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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