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Transcriptome responses in copepods *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* exposed to phenanthrene and benzo[*a*]pyrene

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ABSTRACT

Arctic and sub-arctic pelagic organisms can be exposed to effluents and spills from offshore petroleum-related activities and thus it is important to understand how they respond to crude oil related contaminants such as polycyclic aromatic hydrocarbons (PAHs). The copepod species *Calanus finmarchicus, Calanus glacialis* and *Calanus hyperboreus* represent key links in the arctic marine food web. We performed a transcriptome analysis of the three species exposed to phenanthrene (Phe) and benzo[*a*]pyrene (BaP) representing low and high molecular weight PAHs, respectively. Differential expression of several genes involved in many cellular pathways was observed after 72 h exposure to Phe (0.1 μM) and BaP (0.1 μM). In *C. finmarchicus* and *C. glacialis*, the exposure resulted in up-regulation of genes encoding enzymes in xenobiotic biotransformation, particularly the phase II cytosolic sulfonation system that include 3′-phosphoadenosine 5′-phosphosulfate synthase (PAPSS) and sulfotransferases (SULTs). The sulfonation pathway genes were more strongly induced by BaP than Phe in *C. finmarchicus* and *C. glacialis* but were not affected in *C. hyperboreus*. However, a larger number of genes and pathways were modulated in *C. hyperboreus* by the PAHs including genes encoding xenobiotic biotransformation and lipid metabolism enzymes, suggesting stronger responses in this species. The results suggest that the cytosolic sulfonation is a major phase II conjugation pathway for PAHs in *C. finmarchicus* and *C. glacialis*. Some of the biotransformation systems affected are known to be involved in metabolism of endogenous compounds such as ecdysteroids, which may suggest potential interference with physiological and developmental processes of the copepod species.

1. Introduction

With the warming Arctic and retreating sea ice allowing more human activities, the marine arctic ecosystems are increasingly at risk of exposure to pollution, particularly from offshore petroleum extraction-related industries. Zooplankton in arctic cold-waters have adaptations to environmental factors such as seasonal variability in feeding that is reflected in higher body lipid storage, and such factors should be considered in risk assessment of contaminant effects (Borgå et al., 2004). Therefore, it is important to understand how arctic copepod species respond to exposures to contaminants such as the highly lipophilic polycyclic aromatic hydrocarbons (PAHs) that may contaminate the marine environment due to offshore petroleum-related activities in the region (Beyer et al., 2020).

The copepod species *Calanus finmarchicus, Calanus glacialis* and *Calanus hyperboreus* are key links in energy transfers from primary producers to higher-level predators such as fish, seabirds and marine mammals (Falk-Petersen et al., 2009; Wassmann et al., 2006). These species represent the most abundant zooplankton biomass in the region, and their core distribution areas are in the water masses of the Atlantic (*C. finmarchicus*), the Arctic shelf (*C. glacialis*) and the Arctic basin (*C. hyperboreus*) (Falk-Petersen et al., 2009; Søreide et al., 2008). Although *C. finmarchicus* is of Atlantic origin, it is transported by the inflow of Atlantic water masses and therefore also found in the Arctic

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Ocean (Falk-Petersen et al., 2009). Because of the typical association of the three species with Atlantic (*C. finmarchicus*) and Arctic (*C. glacialis* and *C. hyperboreus*) waters, they are considered as biological indicators of water masses in assessing effects of climate change (Hays et al., 2005; Kwasniewski et al., 2003; Wassmann et al., 2015; Wassmann et al., 2006). In the Barents Sea, *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* have a life span of 1 year, 1–2 years and 2–5 years, respectively (Falk-Petersen et al., 2009; Falk-Petersen et al., 2007). In terms of body size size, *C. hyperboreus* is the largest and *C. finmarchicusis* the smallest of the three (Falk-Petersen et al., 2007). These species also differ in lipid content and composition of lipid classes which may affect their physiology (Falk-Petersen et al., 2007; Scott et al., 2000), and their capacity to accumulate lipophilic contaminants (Ruus et al., 2021). Short-term exposures to PAH compounds or crude oil have shown that PAHs can accumulate and cause potentially harmful effects in copepods (Agersted et al., 2018; Brussaard et al., 2016; Cailleaud et al., 2009; Jensen et al., 2012; Skottene et al., 2019a). For example, a recent report suggests that short-term exposure (for 5 days) to water-soluble fraction (WSF) of crude oil may affect the timing of diapause termination through its negative effects on utilization of lipid stores in *C. finmarchicus* and *C. glacialis* (Skottene et al., 2019a). Oxidative-stress responses were observed in *C. finmarchicus* exposed to water-accommodated fraction of crude oil (Soloperto et al., 2022). A long-term exposure of *C. hyperboreus* to pyrene during overwintering resulted in reduced feeding and lipid accumulation (Toxværd et al., 2019).

Studies suggest that copepods can mobilize the chemical defense systems and metabolize PAHs through biotransformation (Berrojalbiz et al., 2009; Han et al., 2015; Jensen et al., 2012; Tarrant et al., 2019). The chemical defensome in animals mainly constitutes a set of genes encoding phase I functionalization enzymes such as cytochrome P450 monooxygenases, phase II conjugation enzymes such as sulfotransferases and glutathione S-transferases (GSTs), and phase III efflux transporters (Goldstone et al., 2006). The genes and gene families of the chemical defensome are largely conserved between vertebrates and invertebrates including crustaceans, with some exceptions such as the absence of the xenobiotic-ligand dependent activation of the aryl hydrocarbon receptor (AHR) signaling pathway in invertebrates (Goldstone et al., 2006; Hahn et al., 2017; Han et al., 2017; Yadetie et al., 2012). Genes and enzymes that are induced by pollutants such as crude oil PAH components are commonly used as biomarkers of pollutant exposure. In copepods, expression analysis of genes for biomarker proteins such as CYPs and GSTs have been used to study responses to PAH compounds or WSF of crude oil (Han et al., 2017; Hansen et al., 2013; Hansen et al., 2008; Hansen et al., 2009; Lauritano et al., 2021). More recently, global transcriptomic approaches have been used in copepod species in physiological and toxicological studies (Tarrant et al., 2019). Although selected biomarkers of chemical stress have been characterized in the arctic calanoid copepods *C. finmarchicus* and *C. glacialis* (Tarrant et al., 2019), mapping global transcriptome responses is important to further explore biotransformation systems responding to PAHs exposure. These results may give further insights into the biotransformation systems of *Calanus* species and provide potential biomarkers for monitoring pollution such as oil-spill in the Arctic region.

PAHs are widely considered as the most toxic components of crude oil that may contaminate the aquatic environment (Hylland, 2006; Pampanin and Sydnes, 2013). The dominant PAHs in crude oil and produced water discharges are composed of lower molecular weight (MW) compounds such as the 3-ring phenanthrene (Phe) that are considered less toxic compared to high MW PAHs such as the 5-ring benzo[*a*]pyrene (BaP) (Hylland, 2006). In vertebrates, the heavier PAHs such as BaP are more toxic largely because they can activate the AHR pathway inducing CYP1 family enzymes that may generate more toxic metabolites (Honda and Suzuki, 2020; Hylland, 2006). Although induction of CYP1 genes is absent in invertebrates and they have a lower capacity of phase I biotransformation (Stegeman and Lech, 1991), some other CYP family genes are induced by PAHs and crude oil components in copepods (Tarrant et al., 2019). The relative contribution of the low MW and high MW PAHs in inducing biotransformation enzymes in copepods is not known.

The objective of this study was to characterize and compare the transcriptome responses in the three main *Calanus* species found in the Arctic region (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) following exposure to Phe and BaP, focusing on biotransformation systems.

2. Materials and methods

2.1. Sample collection

Calanus finmarchicus, *Calanus glacialis* and *Calanus hyperboreus* were collected during the Nansen Legacy Project Q3 sampling cruise on RV Kronprins Haakon in the northern Barents Sea in August 2019. The sampling transect ranged from the northern Barents Sea south of the Polar Front (76°N, 31°E) to the Arctic Ocean (82°N, 30°E) (Fig. 1). The three copepod species were captured with a Bongo Net (180 μm mesh size), or a WP-3 net (1000 μm mesh size) at the sampling stations P6 and P7 (see Fig. 1). First, *C. finmarchicus* was sampled at P6 (81.56°N, 31.52°E, bottom depth 856 m), then *C. glacialis* was also sampled later at P6 (81.57°N, 31.47°E, bottom depth 895 m), and *C. hyperboreus* was sampled at P7 (81.93°N, 29.15°E, bottom depth 3302 m) (Table 1).

The nets were hauled vertically from 10 m off the bottom to the surface at 0.5 ms^{-1}. The copepods were quickly transferred to a bucket with seawater and kept well diluted at 0–4 °C until sorting to species. Under the microscope (Leica MZ 16, Leica Microsystems), the copepods were hand-picked by pipette from ice cooled glass dishes and copepodite stage C5 were gently transferred into vials with filtered sea water and used in all experiments. *Calanus hyperboreus* was identified based on size and its taxonomical features (acute corners of prosome terminal segment in lateral view) as described before (Brodskii et al., 1983). The two species *C. finmarchicus* and *C. glacialis* were identified by measuring prosome length (PL) of individuals and following size classes described before (Kwasniewski et al., 2003). Since distinguishing between *C. finmarchicus* and *C. glacialis* based on morphological features can be difficult (Choquet et al., 2018; Hirche et al., 1994; Weydmann and Kwasniewski, 2008), individuals with border-line PL (close to 2.9 mm) were excluded. In addition, antenna pigmentation (redness), which is more prominent in live *C. glacialis* (Choquet et al., 2018; Nielsen et al., 2014) was assessed. Furthermore, based on recent studies that

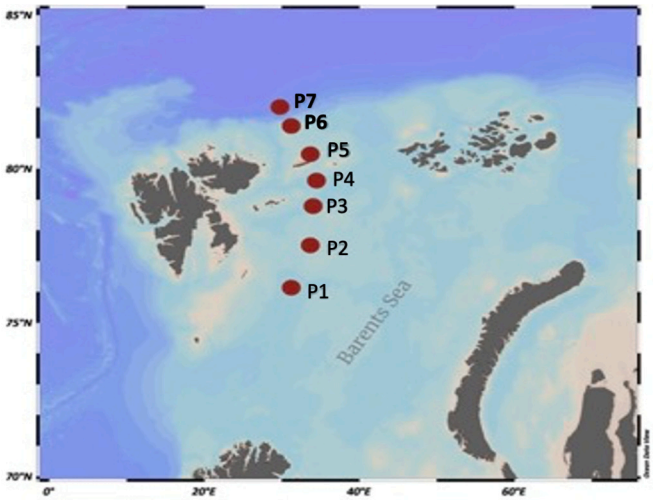

Fig. 1. Map of sampling sites. *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* were collected in the vicinity of the P6 and P7 stations (shown in bold) in the northern Barents Sea. The "process stations" P1-P7 are sites of longer stays for the cruise ship along the transect. This figure was created with Ocean Data View and http://Biorender.com (with permissions).

Table 1
Sampling and exposure data for *C. finmarchicus (Cf), C. glacialis (Cg)* and *C. hyperboreus (Ch)*.

Site	Location	Bottom depth	Species	Number of individuals per sample	Number of samples per group			Total number of individuals analyzed	Exposure time and temperature
					DMSO	Phe (0.1 μM)	BaP (0.1 μM)		
P6	81.56°N, 31.52°E	856 m	*Cf*	15	5	5	5	225	72 h, 3.5 °C
P6	81.57°N, 31.47°E	895 m	*Cg*	10	4	4	4	120	72 h, 3.5 °C
P7	81.93°N, 29.15°E	3302 m	*Ch*	10	4	5	4	140	72 h, 1.5 °C

recommend the use of molecular methods for correct identification of the two species (Choquet et al., 2017; Choquet et al., 2018; Gabrielsen et al., 2012) we validated species identification using diagnostic species-specific markers for *C. finmarchicus* and *C. glacialis* (Smolina et al., 2014).

2.2. Exposure

All chemicals, unless specified otherwise, were purchased from Sigma-Aldrich (Oslo, Norway). The exposure experiments were performed on board Nansen Legacy project cruise on RV Kronprins Haakon in the northern Barents Sea in August 2019. All animals were collected from a single site for each species and pooled before randomly assigning to groups for exposure (Table 1). We performed a pilot experiment using 0.001 to 10 μM BaP and Phe and chose 0.1 μM for both compound as a well-tolerated non-lethal concentration. The exposures were performed for 72 h in the dark at a temperature 3.5 °C for *C. finmarchicus* and *C. glacialis*, and 1.5 °C for *C. hyperboreus*, using solvent control (DMSO), 0.1 μM BaP or 0.1 μM Phe in filtered seawater (0.7 μm) (Table 1). Lower exposure temperature was chosen for *C. hyperboreus* because these animals were collected from Arctic waters of lower temperature. For each group, stage C5 animals (20, 11 and 10 individuals for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively) were kept in a 0.5 L glass flask with 0.4 L filtered seawater at the respective culture temperature for 72 h, without feeding. The 72 h exposure time was chosen based on the low ambient temperatures used and previous studies on biomarker responses in *C. finmarchicus* and *C. glacialis*, where 24–96 h exposures were used (Hansen et al., 2013; Hansen et al., 2011; Soloperto et al., 2022). For each treatment group, 4–5 replicate bottles ($n = 4$–5) were used as shown in Table 1. The animals were observed every 24 h and dead animals were removed. At the end of the experiment, all animals were alive except one in the vehicle control, two in the Phe group of *C. finmarchicus*, and one in the Phe group of *C. glacialis*. After 72 h, the animals were harvested with 180 μm filter, transferred to cryotubes and snap-frozen in liquid nitrogen for storage at −80 °C until RNA-extraction. The number of animals per group collected and frozen for RNA extraction was 15 for *C. finmarchicus* and 10 for *C. glacialis* and *C. hyperboreus* (Table 1). In total 225 (15 × 15), 120 (10 × 12) and 140 (10 × 14) animals were collected for RNA extraction for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively (Table 1).

2.3. RNA extraction

RNA extraction was performed for each sample that contains pooled 15 (for *C. finmarchicus)* or 10 individuals (for *C. glacialis* and *C. hyperboreus*) (Table 1) using TriReagent (Sigma) according to the manufacturer's instructions. RNA purity and quantity (260/280 and 260/230 was >1.8 for each sample, as determined spectrophotometrically using Nanodrop (ThermoFisher Scientific, USA) and the RNA integrity was further confirmed to be intact as assessed using agarose gel electrophoresis, and Agilent 2100 Bioanalyzer according to the manufacturer's protocols (Agilent Technologies, Palo Alto, CA, USA). The average amount of RNA obtained was 27, 37 and 50 μg for *C. finmarchicus, C. glacialis* and *C. hyperboreus*. RIN values could not be determined for the RNA-samples since in heat denatured total RNA, the 28S ribosomal RNA co-migrated with the 18S ribosomal RNA, as shown in copepods and other arthropods (Asai et al., 2015; McCarthy et al., 2015). In all the RNA samples strong 18S signals were observed and no signs of degradations were detected.

2.4. Library preparation for transcriptome sequencing

Library preparation and RNA sequencing was performed by Novogene Europe (Cambridge, UK). Briefly, for each sample, 1 μg total RNA was used for library preparation using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) according to manufacturer's protocols. Poly(A) + RNA was purified from total RNA using poly-T oligo-attached magnetic beads and fragmented using divalent cations under elevated temperature. First strand cDNA was synthesized using random hexamer primer and reverse transcriptase, followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. Size selected cDNA fragments (150–200 bp) were PCR amplified and sequenced to generate 150-bp paired-end sequences using Illumina NovaSeq 6000 platform.

2.5. Transcriptome assembly, quantification, annotation and differential expression analysis

For each species, raw RNA-seq reads were cleaned by 1) removing reads with adapter sequences, 2) removing reads where the occurrence of undetermined nucleotides N is >10%, 3) removing reads with low quality nucleotides (when over 50% of bases have Phred scores <5). Quality filtering of the raw RNA-seq reads was performed using Illumina's CASAVA v1.8. *De novo* transcriptome assembly was performed by combining all reads (from both control and exposed samples) per species. RNA-seq reads from a total of 225, 120, and 140 individuals were used to perform transcriptome assembly for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively (Table 1). There were 15, 12 and 14 samples for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively, with each sample containing pooled individuals (15 individuals for *C. finmarchicus* and 10 for *C. glacialis* and *C. hyperboreus*) (Table 1). Transcriptome assembly was performed using the trinity software (Grabherr et al., 2011) version 2.6.6 with the following parameters: minKmerCov = 3, min_glue = 4 and the defaults for other values. The CORSET software (Davidson and Oshlack, 2014) was used to remove redundancy by clustering the assembled Trinity transcripts. The longest transcript from each cluster was then selected as"Trinity gene", hereafter referred to as "unigene". All subsequent analyses were performed with the unigene reference assembly for each species. The quality of the assembled reference transcriptome for each species was assessed using BUSCO (Benchmarking Universal Single-Copy Orthologs) software (Seppey et al., 2019). For each species, the unigene assembly was annotated using BLAST+ (v2.2.28+) searches in the databases NT, NR, KO, SwissProt, Pfam, Go and KOG. Coding sequence (CDS) predictions were done by translating unigene sequences with BLAST hits in the NR and Swissprot databases, and further using ESTScan (version 3.0.3) to

predict coding regions of unigene sequences with no BLAST hits. The software versions and parameters used in these database searches are listed in Table S1.

After assembling reference transcriptome for each species, reads from individual samples (a pool of 10 or 15 individuals, see Table 1) were mapped to the respective unigene reference assembly and quantified using Bowtie2 (version bowtie2–2.2.2) and RSEM (version 1.2.28) (Li and Dewey, 2011). Unigene transcripts (Trinity genes) with FPKM (Fragments Per Kilobase of transcript sequence per Million base pairs sequenced) > 0.3 were considered expressed and used in down-stream analyses. For each species differential expression analysis between two groups (DMSO control and treated) was performed using the DESeq2 package (version 1.26.0) (Love et al., 2014), with negative binomial distribution model for *p*-value estimation and Benjamini and Hochberg's (BH) FDR correction for multiple testing. Unigenes with BH adjusted *p*-values <0.05, as determined by DESeq2 were considered differentially expressed genes (DEGs). Pathway analysis was performed using the list of DEGs. Hierarchical clustering (average linkage, mean = 0, variance = 1) was performed with log-transformed normalized-FPKM values and heatmaps were generated from the top discriminating genes (based on ANOVA *p*-values) in Qlucore Omics Explorer version 3.7 (Qlucore AB, Lund, Sweden). The RNA-seq raw and processed data files have been deposited and made publicly available in the ArrayExpress database at EMBL-EBI (http://www.ebi.ac.uk/arrayexpress) under accession numbers E-MTAB-10917, E-MTAB-10911 and E-MTAB-10918 for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively. The assembled transcriptome sequences have been submitted to ENA at EMBL-EBI and can be accessed under the accessions PRJEB51404 (TSA accession: HBXF01000001-HBXF01154909), PRJEB51407 (TSA accession: HBXE01000001-HBXE01111768) and PRJEB51409 (TSA accession: HBXD01000001-HBXD01093239), for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively. The submitted data for each species contain the raw sequences (Fastq files), processed data file: Fasta file of assembled contigs (AssembleTranscriptomeSeq.txt), raw count data (Readcount.txt), normalized count data (fpkm.txt) and results of differential expression analysis (DEA) with blast annotations (BaPvsDMSOdeaBlastAnnotation.txt and PhevsDMSOdeaBlastAnnotation.txt).

2.6. Functional enrichment analysis

Pathway analysis of differentially expressed genes was performed based on the Blast annotations (e-value $<1e^{-5}$) with the SwissProt database described above. Functional annotations for enrichment analyses based on human databases were available only for genes that received SwissProt annotations, and thus human based pathway enrichment analyses were limited to the SwissProt annotations. But additional analysis was performed using arthropod (*Drosophila melanogaster* and *Dapnia pulex* specific databases. The DEGs (Trinity genes/unigenes) were annotated by BLASTX with the best hits (E-value $< 1e^{-5}$) of the *D. melanogaster* protein database. BLASTX was performed with locally installed NCBI BLAST+ software (version 2.9.0) with the *Calanus* DEGs sequence queries against *D. melanogaster* proteins downloaded from https://www.uniprot.org (accessed last, May 22, 2021) and formatted as databases. Both Swiss-Prot (3632 entries) and TrEMBL (39,186 entries) of the Drosophila proteins were used. The DEGs were similarly annotated by BLASTX with the best hits (E-value $<1e^{-5}$) of the *D. pulex* protein database downloaded from Ensembl (https://metazoa.ensembl.org/index.html) (accessed last, June 20, 2021). For pathway and network enrichment analysis, gene symbols from annotations with other model organisms were converted to putative human orthologs for human-based enrichment or the Drosphila and Dapnia gene symbols were used for arthropod based analysis using Metascape (Zhou et al., 2019) and STRING protein-protein databases (Szklarczyk et al., 2015). Pathways, Gene Ontology terms and networks were considered significantly enriched at *p*-values corrected for multiple testing (adjusted *p*-values) < 0.05. Default settings were used for other parameters in Metascape and STRING enrichment analysis tools.

3. Results

3.1. Validation of species identification

Since we pooled several individuals per sample for each species and the assemblies were done using all sequences per species, we double-checked correct species identification using diagnostic species-specific transcript based nuclear insertion deletion (InDel) markers for *C. finmarchicus* and *C. glacialis* (T_461, T_595, T_1301, T_1338, T_1966, T_3133, T_4700, T_6474) (Smolina et al., 2014). Sequence analysis of our transcriptome assemblies (accessions: PRJEB51404, PRJEB51407 and PRJEB51409 for *C. finmarchicus, C. glacialis* and *C. hyperboreus*, respectively) using these 8 InDel markers for each of *C. finmarchicus* (accessions: KF913034, KF913035, KF913036, KF913037, KF913038, KF913039, KF913040, KF913041) and *C. glacialis* (accessions: KF913042, KF913043, KF913044, KF913045, KF913046, KF913047, KF913048, KF913049) in NCBI BLASTN showed no species cross-contamination, as detailed in Supplementary file 1.

3.2. Assembly quality assessment

The number of raw reads, clean reads and other quality measures are summarized for each of *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* samples sequenced (Table S2–4). In total 333, 253, and 288 million clean paired-end reads were used for transcriptome assembly of *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively (Table S2–4). Table 2 shows the transcriptome assembly statistics. The transcriptome

Table 2
Assembly statistics for unigene (Trinity gene) transcriptome in the three *Calanus* species.

Quality parameter	*C. finmarchicus*	*C. glacialis*	*C. hyperboreus*
Number of unigene transcripts	154,909	111,768	93,239
Minimum length (bp)	301	301	301
Mean length (bp)	717	883	955
Median length (bp)	507	579	614
Maximum length (bp)	24,603	20,201	26,329
N50 (bp)	810	1139	1279
N90 (bp)	365	405	421
Total nucleotides	111,030,452	98,699,962	88,997,455
BUSCO summary	Number and percentage of unigenes		
Complete (single)	662 (67.7%)	647 (66.2%)	814 (83.2%)
Complete (duplicated)	107 (10.9%)	281 (28.7%)	79 (8.1%)
Fragmented	175 (17.9%)	30 (3.1%)	56 (5.7%)
Missing	34 (3.5%)	20 (2.0%)	29 (3.0%)
Annotation by databases*	Number and percentage of unigenes annotated		
NR	73,171 (47.2%)	57,438 (51.4%)	41,658 (44.7%)
NT	9814 (6.3%)	8261 (7.4%)	4905 (5.3%)
KO	34,204 (22.1%)	27,558 (24.7%)	18,740 (20.1%)
SwissProt	47,664 (30.8%)	39,574 (35.4%)	27,861 (29.9%)
PFAM	63,649 (41.1%)	51,185 (45.8%)	39,379 (42.2%)
GO	63,629 (41.1%)	51,176 (45.8%)	39,371 (42.2%)
KOG	24,471 (15.8%)	21,733 (19.4%)	14,267 (15.3%)
Annotated in at least one database	92,412 (60.0%)	70,825 (63.4%)	53,058 (56.9%)

* Databases. NR: NCBI non-redundant protein sequences, NT: NCBI nucleotide sequences, KO: Kyoto Encyclopaedia of Genes and Genomes Orthologues, SwissProt: Curated Protein sequences, Pfam: Protein domains and families, GO: Gene Ontology, KOG: euKaryotic Orthologous Groups.

assembly resulted in 471,435, 290,408 and 258,271 assembled Trinity transcripts (unigenes) for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively (Table S5). The number of unigenes in the reference assembly was 154,909, 111,768 and 93,239 for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively (Table 2). As described above, for each species, the unigene assembly was used for all downstream analyses and the assembly statistics (Table 2) refers to this assembly. For comparison, the assembly statistics for both unigene and Trinity transcript assemblies are also provided in Table S5. The average mapping rates of the clean reads was 73%, 82% and 80% for *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*, respectively (Table S6–8). Thus, the *C. glacialis* and *C. hyperboreus* reads were well-represented, but the *C. finmarchicus* reads were of lower quality. The *C. finmarchicus* also showed lower quality in other assembly parameters. For example, it has the lowest N50 and highest number of fragmented transcripts of the three species (Table 2, Table S5). BUSCO analysis performed to assess completeness of the reference assemblies (Seppey et al., 2019) showed that the *C. hyperboreus* assembly was the best with (83.2%) complete single-copy BUSCOs compared to 67.7% and 66.2%, for *C. finmarchicus* and *C. glacialis*, respectively (Table 2). The *C. finmarchicus* reference assembly has the highest proportions of fragmented (17.9%) and missing (3.5%) BUSCOs among the three assemblies. Notably, the *C. hyperboreus* assembly has the highest proportion (28.7%) of complete duplicated BUSCOs. Complete BUSCOs (single-copy and duplicated) for *C. finmarchicus* (78.6%), *C. glacialis* (94.9%) and *C. hyperboreus* (91.3%) are comparable to BUSCOs for the recently published transcriptome assemblies for these species (Lenz et al., 2014; Lizano et al., 2022; Tarrant et al., 2014), although our assembles have also higher proportions of missing orthologs (Table 2).

The annotations of each of the unigene reference assemblies by the different databases mentioned above are given in Supplementary file 2 (*C. glacialis*), Supplementary file 3 (*C. finmarchicus*) and Supplementary file 4 (*C. hyperboreus*). To summarize, BLAST annotation against the NR database resulted in the highest percentage of annotated genes, followed by PFAM and GO (Table 2). In terms of annotation rate, the highest number of unigenes annotated by the different databases was that of the *C. finmarchicus* assembly, although the *C. glacialis* assembly had the highest proportions (Table 2). For example, for the NR database, 51.4%, 47.2% and 44.7% of the assembled transcripts were annotated for *C. glacialis*, *C. finmarchicus* and *C. hyperboreus*, respectively (Table 2). The predicted coding sequences (CDS) are summarized in Supplementary file 5 (*C. finmarchicus*), Supplementary file 6 (*C. glacialis*), Supplementary file 7 (*C. hyperboreus*). BLAST prediction of CDS resulted in hits for 33% (*C. finmarchicus*), 40% (*C. glacialis*) and 38% (*C. hyperboreus*) of the unigene reference assembly sequences (Supplementary file 5–7). ESTscan prediction of CDS resulted in 62%, 56% and 55% of the unigene reference assembly sequences *for C. glacialis*, *C. finmarchicus* and *C. hyperboreus*, respectively (Supplementary file 5–7).

3.3. Differentially expressed genes

3.3.1. Differentially expressed genes in C. finmarchicus and C. glacialis

In *C. finmarchicus* there were 344 and 235 differentially expressed genes (DEGs) in BaP and Phe exposed groups, respectively (Table S9, Table S10A and B). Of these, 44 were shared between the two exposures. In *C. glacialis*, there were 141 and 84 differentially expressed genes (DEGs), in BaP and Phe exposed groups, respectively, and of these 32 were common between the two exposure groups (Table S9, Table S10C and D). Thus, with larger number of genes affected, BaP appears to elicit stronger effects on gene expression in both species. The top distinct and shared differentially expressed genes in BaP and Phe exposed *C. finmarchicus* and *C. glacialis* are shown in Fig. 2 and Fig. 3, respectively.

The top list of up-regulated genes in BaP exposed *C. finmarchicus* is dominated by genes encoding the sulfonation pathway enzymes (3'-Phosphoadenosine 5'-Phosphosulfate Synthase/PAPSS, sulfotransferases/SULTs and 3′(2′),5′-bisphosphate nucleotidase 1/BPNT1) (Table S10A, Fig. 2). Notably, many genes encoding cuticle proteins were differentially expressed in BaP treated *C. finmarchicus* (Table S10A). In Phe exposed *C. finmarchicus*, fewer genes encoding the sulfonation pathway enzymes were significantly up-regulated (Table S10B). Some of the top up-regulated biotransformation related genes in Phe exposed *C. finmarchicus* are genes encoding drug transporters and other genes such as *CYP2C14* (Table S10B). Sulfonation pathway genes were also among the top affected in BaP and Phe exposed *C. glacialis* (Fig. 3, Table S10C and D).

3.3.2. DEGs in C. hyperboreus

Compared to the two other species, a higher number of genes was differentially expressed in PAH-exposed *C. hyperboreus* particularly in Phe treated groups with 490 and 2660 differentially expressed genes (DEGs), in BaP and Phe exposed groups, respectively (Table S9, Table S10E and F). Of these, 397 DEGs were common between the two exposures. Interestingly, the genes encoding phase II sulfonation enzymes such as sulfotransferases that dominated the top DEGs in *C. finmarchicus* and *C. glacialis* were not among the DEGs in *C. hyperboreus* (Table S10E and F). There were fewer genes (Trinity genes) in the reference assemblies annotated as PAPSS1/2 for *C. hyperboreus* (13), compared to *C. finmarchicus* (28) and *C. glacialis* (30) (Supplementary file 2–4). However, there is only a single PAPSS gene in lower animals such as *Caenorhabditis elegans* and two genes in vertebrates (Dejima et al., 2006). So, the differences in the number of annotated Trinity genes are likely due to redundancy and sequence variations. The number of Trinity genes annotated (NR database) as sulfotransferases for *C. finmarchicus* (276), *C. glacialis* (237), and *C. hyperboreus* (225) are comparable (Supplementary file 2–4). Similarly, the number of Trinity genes annotated as CYP is about 261, 224 and 146 in *C. finmarchicus, C. glacialis* and *C. hyperboreus* assemblies (Supplementary file 2–4). Many other biotransformation related genes are found in the DEGs lists from both BaP and Phe exposed *C. hyperboreus* (Fig. 4, Table S10E and F). For example, in Phe exposed *C. hyperboreus*, some *CYP* genes (e.g., *CYP2C44*) of phase I enzymes and phase II conjugating GST enzyme genes (*GST1*/GSTT1 and *GST4*/GSTT4) were up-regulated, and in BaP exposed groups, *CYP18A1* was up-regulated (Fig. 4, Table S10E and F). Among down-regulated biotransformation-related genes in BaP and Phe exposed animals, are also found genes encoding arylsulfatases ARSB, ARSI and ARSJ (Table S10E and F).

3.4. Pathway analysis

Pathway analysis was performed primarily using the human annotations, but additional analyses were also performed based the phylogenetically closer *D. melanogaster* and *D. pulex* genomes to capture arthropod and crustacean specific pathways. In general terms, the Drosophila and *D. pulex*-based analyses resulted in similar enriched pathways as the human-based analysis for some energy metabolism and most of the top biotransformation pathways in *C. finmarchicus* and *C. glacialis* (Fig. 5A-D, Fig. S1-8, Table S11A-D), but *C. hyperboreus* pathway enrichment profiles using the human based-analyses were largely different from the arthropod profiles (Fig. 5E and F, Fig. S9 and 10, Table S11E and F, Table S12A-F). The BLAST annotations showing putative orthologs of the DEGs are given in Table S13A-F and Table S14A-F for *D. melanogaster* and *D. pulex*, respectively.

3.4.1. Pathways affected in BaP and Phe exposed C. finmarchicus and C. glacialis

For the human-based analysis, the significantly enriched pathways as determined by Metascape tool (Zhou et al., 2019) in BaP and Phe exposed *C. finmarchicus* and *C. glacialis* are summarized in Fig. 5A-D, and detailed in supplementary data (Table S11A-D). In BaP and Phe exposed *C. finmarchicus* and *C. glacialis*, the top significantly enriched pathways are mainly related to biotransformation, especially, the *cytosolic*

sulfonation pathway (Fig. 5A, C and D, Table S11A, C and D), except for Phe treated *C. finmarchicus*, where the sulfonation pathway was not significantly enriched but pathways related to drug transport were in the top list (Fig. 5B, Table S11B).

Pathway analysis using the *D. melanogaster* and *D. pulex* orthologs in the STRING tools (Szklarczyk et al., 2015) resulted in enrichment of pathways largely similar to the human-based pathways such as phase II sulfonation similar with the enrichment analysis with the human orthologs (Fig. 5A-D, Fig. S1–8, Table S11A-D). Notable exceptions are the arthropod-specific pathways related to chitin metabolism and cuticle development enriched (for BaP exposed *C. finmarchicus*) only in the Drosophila and Daphnia-based analyses (Fig. S1A and B, Fig. S5A and B).

3.4.2. Pathways affected in BaP exposed C. hyperboreus

Many diverse significantly enriched pathways were detected in DEGs from BaP exposed *C. hyperboreus* in the human-based and arthropod-based analyses (Fig. 5E, Table S11E). The top enriched human-based pathways are related to pathways such as collagen catabolism, small molecule metabolism, cell signaling and immune response (Fig. 5E, Table S11E). Interestingly, in contrast to BaP exposed *C. finmarchicus* and *C. glacialis*, where sulfonation-related pathways dominated the top list (Table S11A and C), BaP exposure of *C. hyperboreus* did not result in significant enrichment of those pathways. A notable example among biotransformation genes is the up-regulation of the gene encoding the phase I enzyme *CYP18E1* (*D. melanogaster* ortholog) in BaP exposed *C. hyperboreus* (Table S10E). Comparing significantly enriched pathways, GO-terms and protein domains as annotated using the STRING database showed only limited similarity between human and arthropods (Drosophila and Daphnia) (Table S12A, C and E). However, as expected, there are significant overlaps of enriched KEGG and Interpro protein domains between Drosophila and Daphnia-based annotations (Fig. S9A and B). But the STRING human KEGG annotations were not shared with the arthropod annotations (Table S12A, C and E).

3.4.3. Pathways affected in Phe exposed C. hyperboreus

From the human-based Metascape pathway analysis, the significantly enriched pathways and GO terms in DEGs from Phe exposed *C. hyperboreus* are shown in Fig. 5F and Table S11F. The top list of pathways is dominated by genes encoding transport proteins and lipid metabolism. Among the long list, the top enriched biotransformation-related pathways that include drug transport, biological oxidations, sulfur compound metabolic process and lipid metabolism, partly overlap Phe effects in *C. finmarchicus* and *C. glacialis* (Table S11B and D, Table S11F). Genes encoding CYP enzymes also populated many pathways related to biotransformation including drug catabolic process, lipid and hormone metabolic process (Table S11F). The enriched NRF2 pathway is also populated by many phase II biotransformation-related genes such as GCLM, GSTM2, UGT2B7 and KEAP1, as well as several genes encoding Phase III drug transport proteins such as ABCC2, ABCC3, ABCC4 (Table S11F). Significantly enriched human, *D. melanogaster* and *D. pulex* pathways, GO-terms and protein domains as analyzed using STRING are shown in Table S12A-F. Comparison of the enriched KEGG pathways shows that some affected pathways (mostly related to energy metabolism) are common in the three organisms (Fig. S10). The Venn diagram shows that all except one *D. melanogaster* KEGG pathway are shared with *D. pulex* (Fig. S10), indicating more similarities within arthropods.

3.4.4. Comparison of DEGs and pathways affected in BaP and Phe exposed three Calanus species

Fig. 6 shows a heat map of pathways affected by the Phe and BaP in the three copepod species. Among the top pathways and processes, *sulfur compound metabolic process* and *biological oxidation* are affected by both compounds in the three species, except in BaP exposed *C. hyperboreus*. In general, the responses between *C. finmarchicus* and *C. glacialis* are more similar than the responses in *C. hyperboreus*. In these two species, fewer genes were affected by the PAHs than in *C. hyperboreus* and the top pathways affected are largely related to biotransformation, notably the sulfonation pathway (Fig. 5A, C and D). In contrast, the PAHs, particularly Phe modulated a much larger set of genes and more diverse pathways in *C. hyperboreus* than in the other two species (Fig. 6, Table S10E and F, Table S11E and F). Many pathways such as the metabolism of lipids were most affected in Phe exposed *C. hyperboreus* (Fig. 6).

3.4.5. Overview of biotransformation pathways in Calanus species exposed to BaP and Phe

As described in the previous sections, many genes encoding enzymes in phase I, II and III biotransformation pathways were induced in the three species. Among genes encoding for phase I enzymes, many *CYP* genes were up-regulated in the Phe and BaP exposed copepods in the current experiment. The genes *CYP2J3* and *CYP18E1* were up-regulated in BaP exposed *C. glacialis* and *C. hyperboreus*, respectively (Table S10C and E). The genes CYP6a13/*CYP6a21* and *CYP2I3* were up-regulated in Phe exposed *C. finmarchicus* (Table S1B). The *CYP* genes *CYP2C23*, *CYP6K1*, *CYP18A1*, *CYP12A5* and *CYP3A16* were up-regulated only in Phe exposed *C. hyperboreus* (Table S10F). Furthermore, in the Phe exposed *C. hyperboreus* many genes encoding phase II antioxidant enzymes and phase III transporters were up-regulated and the NRF2 pathway was enriched, suggesting the activation of the complete cascade of phase I-III biotransformation system (Table S11F).

In *C. finmarchicus* and *C. glacialis* exposed to Phe and BaP, activation of the cytosolic sulfonation system, where genes encoding successive enzymes catalyzing the cofactor PAPS synthesis (PAPSS1/PAPSS2), sulfotransferases (such as SULT1C2 and SULT1C4) and PAP degradation (BPNT1) were up-regulated in a coordinated manner (Table S10A, C and D). These enzymes dominate the top enriched sulfonation related pathways and networks of the Drosophila (Fig. S1, S3, S4) and Daphnia ortholog-based analyses (Fig. S5, S7 and S8). Examples of biotransformation genes up-regulated in PAH exposed *C. finmarchicus* and *C. glacialis* are presented in a simplified summary of biotransformation pathways in Fig. 7. In this summary, phase I CYP monooxygenase enzymes generate hydroxylated PAH substrates that are conjugated by phase II sulfotransferase enzymes of the cytosolic sulfonation pathways and excreted by the phase III drug transporters (Fig. 7).

4. Discussion

Although the three *Calanus* species can co-occur, each species is abundant in specific water masses, vary in life cycle duration, winter hibernation strategy, reproductive adaptations, and can be preyed on by different selective predators (Falk-Petersen et al., 2009; Karnovsky et al., 2003; Scott et al., 2000). These species also differ in size and total body lipid content and composition that may affect their accumulation of lipophilic contaminants such as Phe and BaP released during oil spills (Falk-Petersen et al., 2007; Ruus et al., 2021). In the current study, we generated *de novo* transcriptome assemblies and performed gene expression analyses in response to BaP and Phe exposure for three dominant *Calanus* species in Northern Barents Sea. The assemblies for *C. glacialis* and *C. hyperboreus* are of similar quality to the assemblies reported recently for these species (Lizano et al., 2022). However, the assembly for *C. finmarchicus* is of lower quality than previous assemblies for this species, PRJNA231164 (BUSCO complete: 86.7%) and PRJNA236528 (BUSCO complete: 90.2.%) (Lenz et al., 2014; Lizano et al., 2022; Tarrant et al., 2014). Species misidentification can happen in areas where *C. finmarchicus* and *C. glacialis* co-occur (Choquet et al., 2017; Choquet et al., 2018; Gabrielsen et al., 2012). In the Arctic waters where we performed sampling, morphological features (prosome length and redness of antennule) are more reliable parameters to distinguish these species (Choquet et al., 2018). However, since we pooled several individuals per sample for each assembly, we checked species

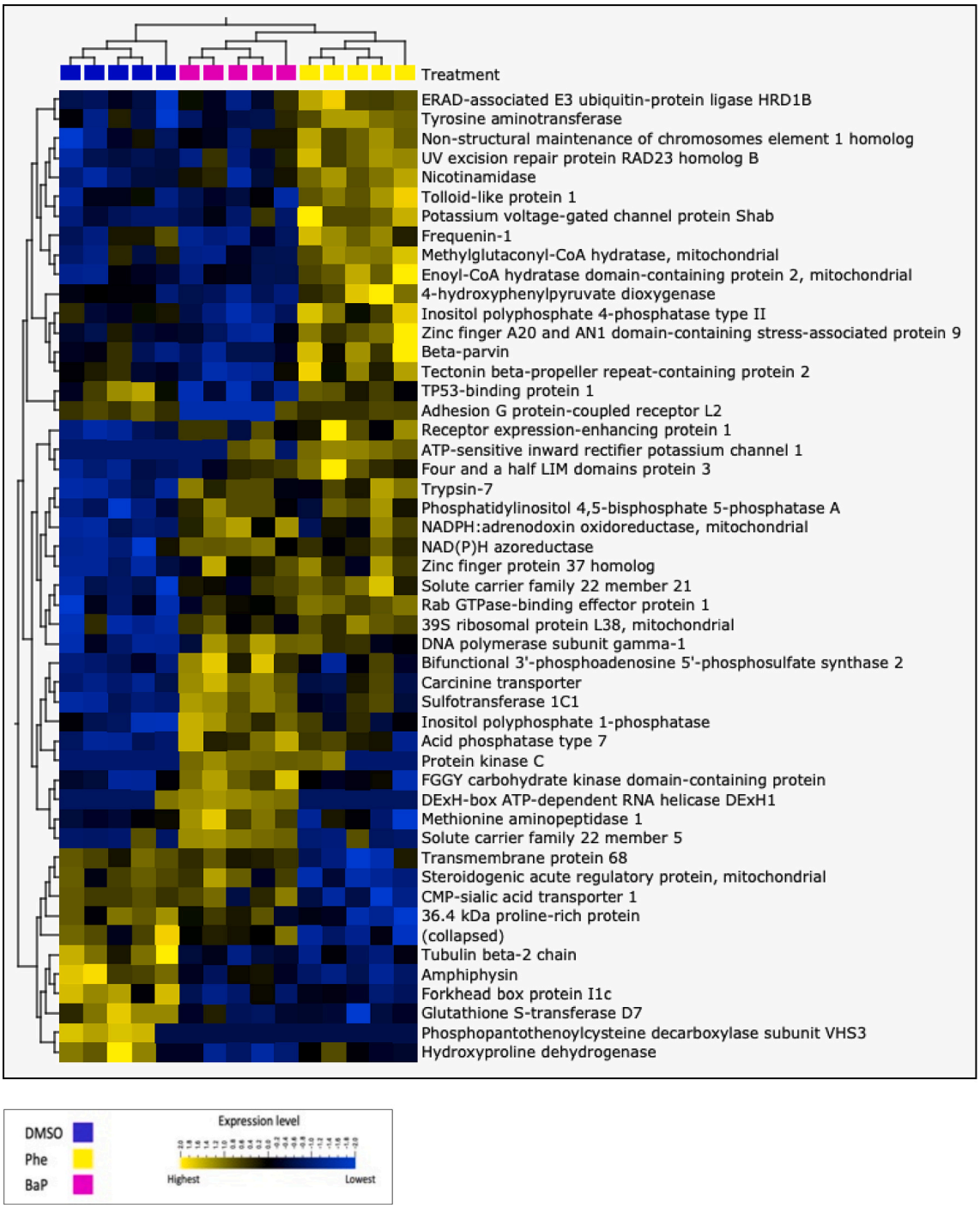

Fig. 2. Two-way hierarchical clustering analysis of differentially expressed genes in *C. finmarchicus* exposed to Phe and BaP. Log2-transformed normalized FPKM values in DMSO control, Phe (0.1 μM) and BaP (0.1 μM) exposed samples were used in two-way clustering based on top discriminating features (ANOVA, Qlucore Omics Explorer). Rows represent genes and columns represent samples. Yellow and blue color of the genes indicated by color bar at the bottom, represent high and low expression levels, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identification by comparing diagnostic species-specific markers for *C. finmarchicus* and *C. glacialis* (Smolina et al., 2014) with our assemblies and confirmed absence of cross-species contamination. Thus, our study reporting transcriptome assemblies, and their applications in transcriptome analysis contribute to the growing molecular tools and resources to study gene expression in response to various perturbations in the copepod species (Lauritano et al., 2021; Lenz et al., 2014; Lizano et al., 2022; Payton et al., 2022; Ramos et al., 2015; Roncalli et al., 2016; Smolina et al., 2014; Soloperto et al., 2022; Tarrant et al., 2019).

We performed a comparative transcriptome analysis following exposure to two compounds that represent the low MW (Phe) and high MW (BaP) crude oil related PAHs in the three abundant and important *Calanus* species in the Arctic marine ecosystems. The top affected pathways in Phe and BaP exposed *C. finmarchicus* and *C. glacialis* were dominated by genes encoding PAPSS and SULT family enzymes of phase II biotransformation pathways. Some *CYP* genes of the phase I biotransformation enzymes and other genes for phase II enzymes such as GST genes for Phase III transporters were also modulated in the two species. In *C. hyperboreus*, a longer list of genes and pathways were affected particularly by Phe, including *CYP* genes, antioxidant response

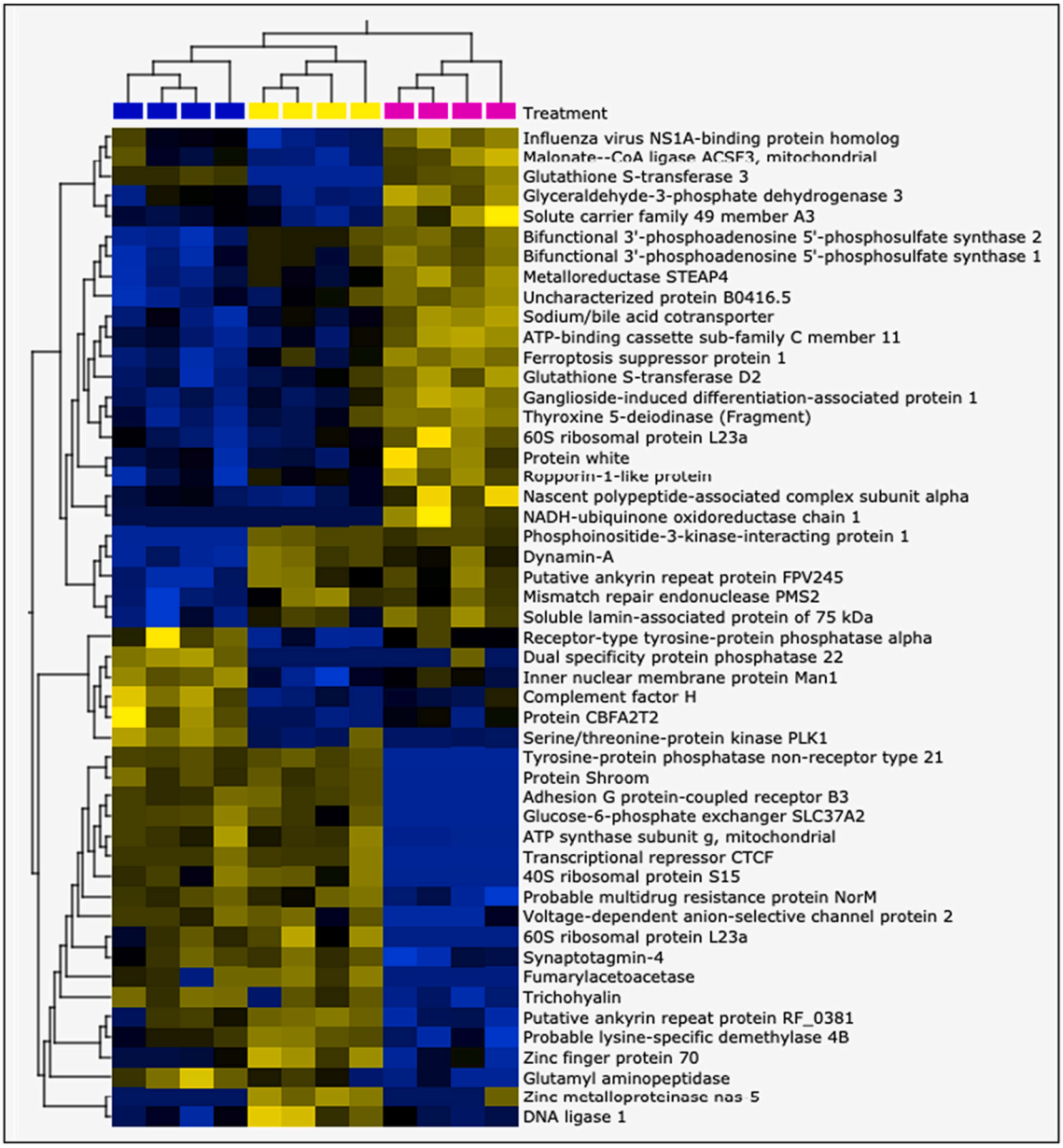

Fig. 3. Two-way hierarchical clustering analysis of differentially expressed genes in *C. glacialis* exposed to Phe and BaP. Log2-transformed FPKM values in DMSO control, Phe (0.1 μM) and BaP (0.1 μM) exposed animals were used in two-way clustering based on top discriminating features (ANOVA, Qlucore Omics Explorer). See Fig. 2 for color legend.

pathways and efflux transporters. In contrast to the two other species, the sulfonation pathways genes were not induced in PAH exposed *C. hyperboreus*. Thus, few largely similar biotransformation-related pathways dominated the top enriched pathways in *C. finmarchicus* and *C. glacialis*, while many diverse pathways (including biotransformation) were enriched in *C. hyperboreus* exposed to the Phe and BaP. More similar responses observed in *C. finmarchicus* and *C. glacialis* is not surprising since the two species have closer evolutionary relationships than *C. hyperboreus* (Lizano et al., 2022). Modulation of genes encoding biotransformation enzymes such as CYPs and GSTs have previously been studied and used as biomarkers to assess responses to PAHs and crude oil components in *C. finmarchicus* and *C. glacialis* (Hansen et al., 2011; Hansen et al., 2008; Hansen et al., 2009). In other arthropods, *Daphnia pulex* and *Daphnia magna*, the PAH pyrene and some pharmaceuticals have been shown to be conjugated by sulfonation, and CYP enzymes were also thought to be in involved in the phase I oxidation of these compounds (Ikenaka et al., 2006; Miller et al., 2017).

The sulfotransferase family enzymes are among the most important in Phase II biotransformation enzymes that catalyze sulfonation of a wide range of compounds including, hydroxylated PAHs, hydroxysteroids, alcohols and amino groups of arylamines (Gamage et al., 2006; Jancova et al., 2010; Kauffman, 2004). A coordinated up-regulation of genes encoding the set of enzymes involved in the cytosolic sulfonation pathway including PAPSS1/PAPSS2, SULTs and BPNT1 was observed in PAH exposed *C. finmarchicus* and *C. glacialis*. The sulfonation pathway involves the synthesis of a sulfate donor co-factor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) from sulfate and ATP molecules by the bifunctional (with sulfurylase and kinase activities) PAPS synthases (PAPSS1 and PAPSS2) (Gamage et al., 2006; Jancova et al., 2010; Kauffman, 2004). Sulfotransferases catalyze the transfer of a sulfate group from PAPS to the conjugation substrate. The PAP byproduct is then degraded by BPNT1, which is required to avoid a toxic accumulation of PAP (Hudson et al., 2013). Similar to our results here, a recent proteomics analysis showed that the enzymes of the sulfonation system

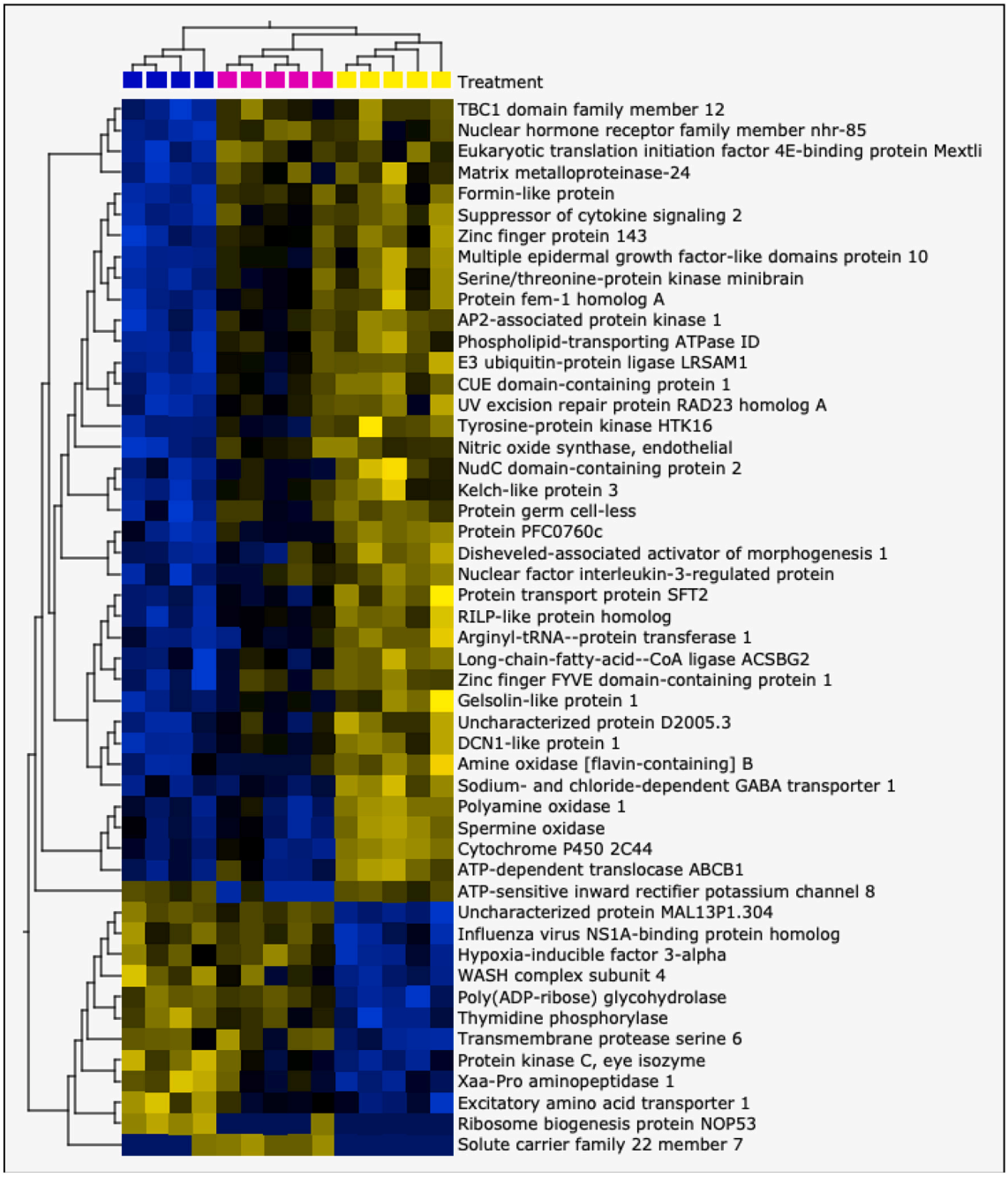

Fig. 4. Two-way hierarchical clustering analysis of differentially expressed genes in *C. hyperboreus* exposed to Phe and BaP. Log2-transformed FPKM values in DMSO control, Phe (0.1 μM) and BaP (0.1 μM) exposed animals were used in two-way clustering based on top discriminating features (ANOVA, Qlucore Omics Explorer). See Fig. 2 for color legend.

PAPSS, BPNT, sulfotransferases were coordinately up-regulated in *D. magna* after chronic exposure to polystyrene microplastic (Trotter et al., 2021). Thus, taken together, the coordinated up-regulation of the genes and enzymes of the cytosolic sulfonation pathway suggests that this enzyme system is a major detoxification pathway for PAHs and possibly other xenobiotic compounds, at least in arthropods.

Transcriptome responses to the PAH exposures in *C. hyperboreus* showed little similarity in terms of the set of genes and the top pathways affected compared to the two other calanoids. For example, the sulfonation pathway genes were not up-regulated in *C. hyperboreus*, although they were the top genes and pathway affected in the two other species. These differences in the transcriptome responses to the same compounds in *C. hyperboreus* compared to the other two species could also be attributed to the lower exposure temperature (1.5 °C for *C. hyperboreus* compared to 3.5 °C in *C. finmarchicus* and *C. glacialis*) and the higher lipid content in *C. hyperboreus*. Previously, temperature and lipid content have been shown to affect *GST* expression levels and toxicity in *C. glacialis* and *C. finmarchicus* exposed to crude oil (Hansen et al., 2011). However, the larger number of other pathways affected in *C. hyperboreus* especially in the Phe exposure suggests its higher sensitivity, which might be related to differences in lipid content and composition. *C. hyperboreus* has higher lipid content and a higher percentage of wax esters (WE) compared to the *C. finmarchicus* and *C. glacialis* (Scott et al., 2000). Of the storage lipids WE and triglycerides, the former has a higher tendency to accumulate lipophilic contaminants (Ruus et al., 2021). Many pathways related to lipid metabolism were also enriched in

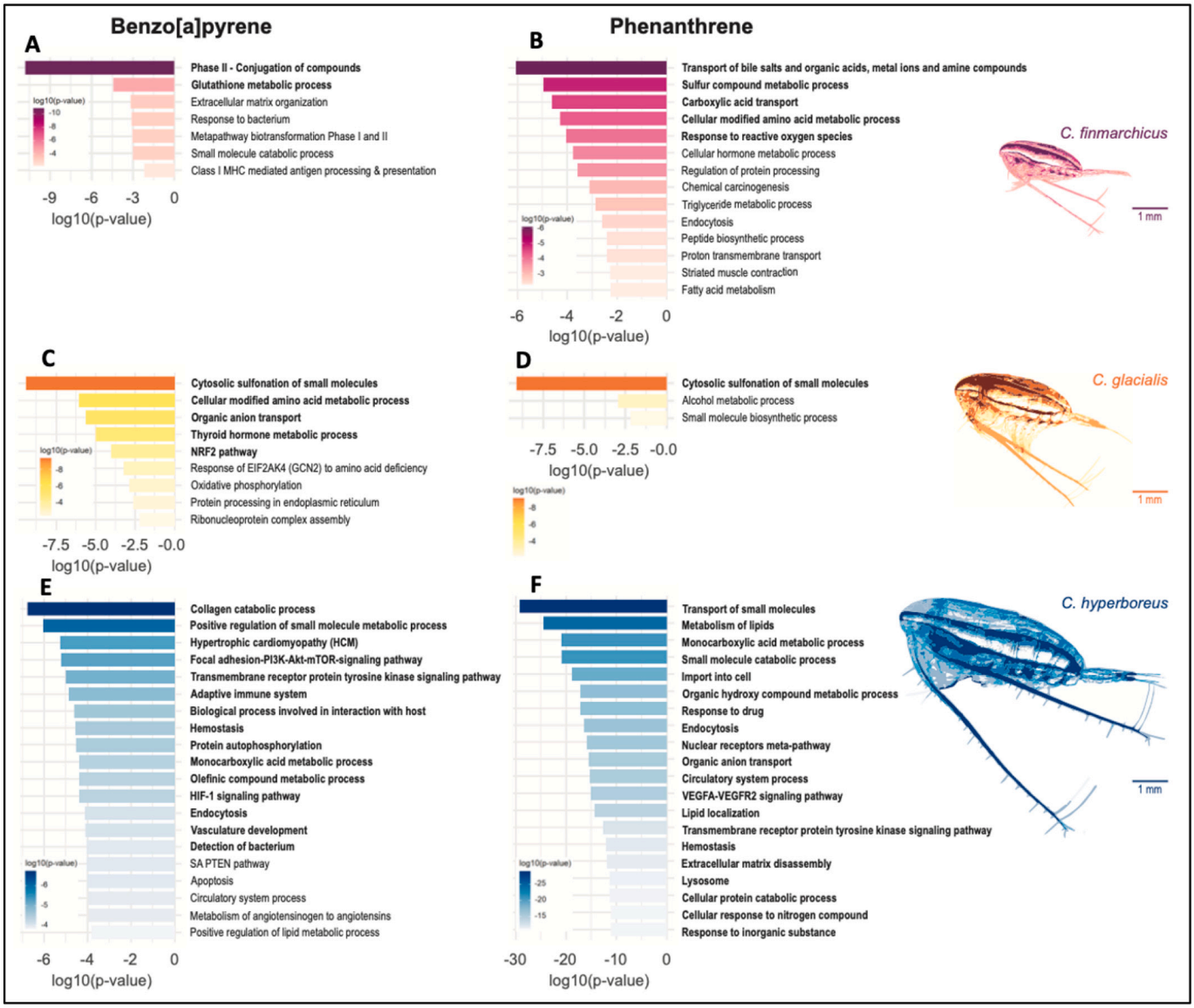

Fig. 5. Representative top enriched pathway and GO terms in BaP and Phe exposed *Calanus* species. Terms enriched in *C. finmarchicus* exposed to BaP (A) and Phe (B). Terms enriched in *C. glacialis* exposed to BaP (C) and Phe (D). Terms enriched in *C. hyperboreus* exposed to BaP (E) and Phe (F). Only the top significantly enriched representative terms in *C. hyperboreus* exposed to Phe are shown. For the representative terms shown in respective figures A-F, the details showing the full lists of significantly enriched pathways and GO terms including the constituent genes are given in Table S11A-F. Enrichment analysis was performed in Metascape using hypergeometric test with Benjamini-Hochberg *p*-value correction. Significantly enriched terms (padj. <0.05) are shown in bold font (A-F).

Phe exposed *C. hyperboreus* in the current study, suggesting effects on lipid metabolism. A recent study also showed that crude oil water soluble fraction exposure in *C. finmarchicus* and *C. glacialis* affected lipid metabolism (Skottene et al., 2019b). Further studies are needed to better understand gene expression responses and the possible ecotoxicological consequences in the arctic copepods.

In *C. finmarchicus* and *C. glacialis*, the high MW BaP had a stronger effect than the low MW Phe in up-regulating genes encoding enzymes in sulfonation pathways. The stronger effect of BaP appears consistent with the higher toxicity of heavier PAHs (Honda and Suzuki, 2020; Hylland, 2006). Despite differences in potency, the similar effects of Phe and BaP on the sulfonation pathway genes observed here might suggest more similar biotransformation mechanisms for both the low and high MW PAH compounds in these *Calanus* species. This appears to contrast with higher toxicity of BaP in vertebrates, which is attributed to induction of CYP1 enzymes that may generate more toxic metabolites (Honda and Suzuki, 2020; Hylland, 2006). Thus, the similar effects of Phe and BaP might be explained by the absence of AHR-dependent induction of CYP1 genes in invertebrates (Goldstone, 2008; Goldstone et al., 2006; Han et al., 2015; Yadetie et al., 2012), although it is possible that other phase I enzymes could generate toxic metabolites of PAHs. Indeed, some CYP genes were induced in the current experiment, and others have previously reported induction of CYP genes by BaP in the copepod *Tigriopus japonicus* (Han et al., 2017). The higher potency of BaP in inducing the sulfonation pathway genes may also be related to its higher bioaccumulation and lower elimination rates in *Calanus* (Jensen et al., 2012).

Pollutant induced biotransformation enzyme synthesis might interfere with the metabolism of endogenous compounds such as steroid hormones that may lead to disruption of homeostasis (You, 2004). The current study suggests that some of the genes for biotransformation enzymes induced may also target endogenous compounds such as hormones, that may result in disturbances of physiological functions and developmental timing (King-Jones and Thummel, 2005). For example, the CYP18A1 gene induced in Phe exposed *C. hyperboreus* is known to be involved in ecdysone metabolism in *D. melanogaster* (Guittard et al.,

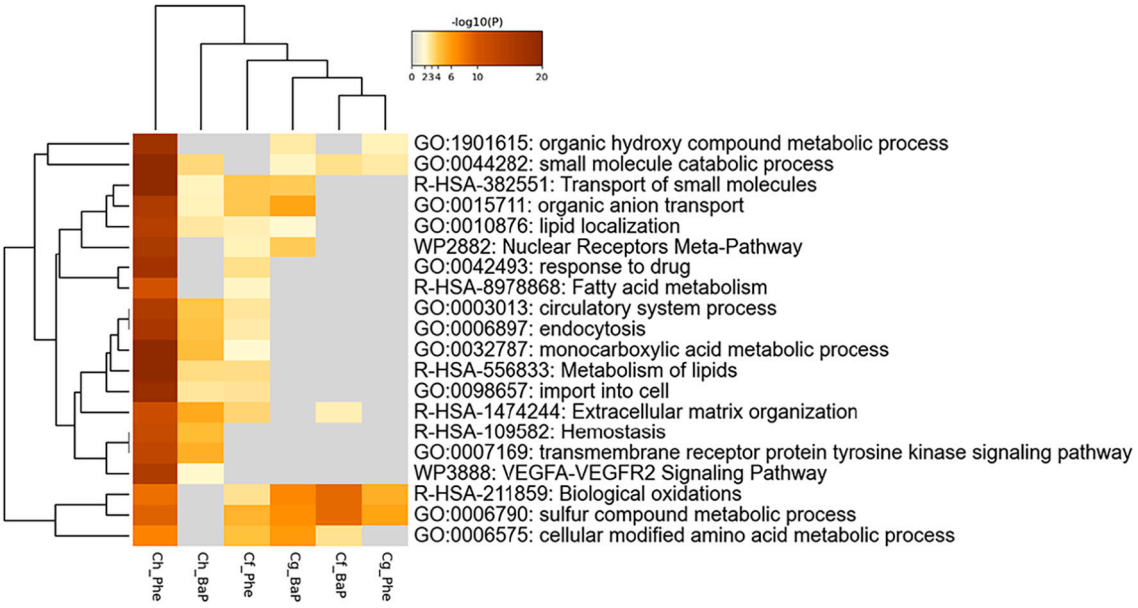

Fig. 6. Comparison of enriched representative pathways and processes in Phe and BaP exposed *C. finmarchicus* (*Cf*), *C. glacialis* (*Cg*) and *C. hyperboreus* (*Ch*). The heatmap colored by *p*-values shows shared pathways, with grey cells indicating a lack of enrichment. Enrichment analysis was performed in Metascape using hypergeometric test with Benjamini-Hochberg *p*-value correction.

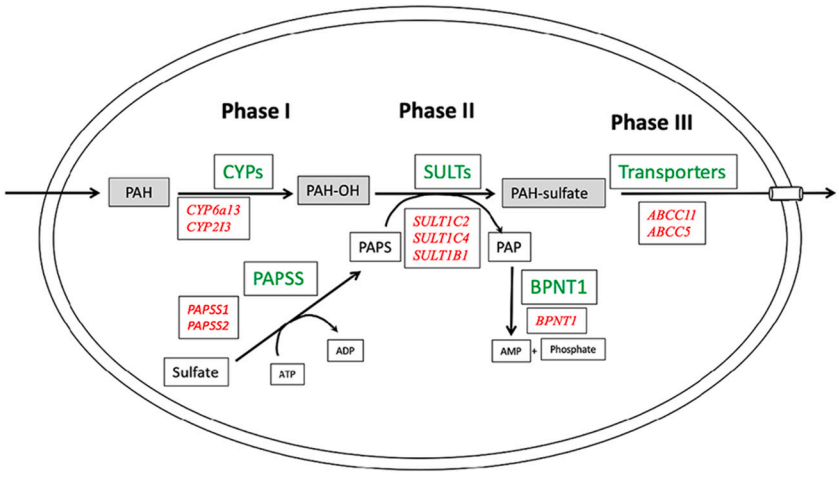

Fig. 7. A proposed simplified PAH biotransformation pathway showing examples of genes up-regulated in detoxification pathways in PAH (Phe and BaP) exposed *C. finmarchicus* and *C. glacialis*. Enzymes or enzyme families encoded by the up-regulated genes in Phase I, II and III biotransformation processes are shown in green, and examples of the individual up-regulated genes are shown in red. *CYP6a13: Cytochrome P450 6a13, CYP2I3: Cytochrome P450 2I3, PAPSS1: 3'-Phosphoadenosine 5'-Phosphosulfate Synthase 1, PAPSS2: 3'-Phosphoadenosine 5'-Phosphosulfate Synthase 2, SULT1C2: Sulfotransferase 1C2, SULT1C4: Sulfotransferase 1C4, SULT1B1: Sulfotransferase 1B1, SULT1A3: Sulfotransferase 1A3, BPNT1: 3′(2′),5′-bisphosphate nucleotidase 1, ABCC11: ATP-binding cassette transporter C11, ABCC5: ATP-binding cassette transporter C5*.

2011). Indeed, steroid metabolism was among the pathways enriched in Phe exposed *C. hyperboreus*. Many genes encoding cuticle proteins were down regulated in BaP treated *C. finmarchicus* (Table S11A). Of these, a gene encoding a putative ortholog of the Drosophila Ecdysone-dependent cuticle protein (Edg78E) was most strongly down-regulated. Ecdysone is known to regulate many processes such as cuticle formation in the arthropods (Beckstead et al., 2005; King-Jones and Thummel, 2005). The possible effects and mechanisms of PAHs and crude oil exposure on development and hormone metabolism in *Calanus* should be further investigated.

5. Conclusion

In this study, we assessed the transcriptome responses in three abundant arctic copepod species exposed to two PAH compounds found in crude oil contaminants. Our study represents the first global transcriptome responses to Phe and BaP exposure in the arctic *Calanus* species and has resulted in identification of potential biomarkers that could be useful for future monitoring of crude oil pollution in the region. In particular, genes encoding the enzymes PAPSS and SULTs were highly up-regulated in a coordinated manner suggesting that sulfonation is a major PAH detoxification pathway in *C. finmarchicus* and *C. glacialis*. In *C. finmarchicus* and *C. glacialis*, the high MW BaP elicited stronger effects than the low MW Phe on sulfonation pathway genes suggesting higher toxicity of the former. Stronger transcriptome responses with large number of affected genes observed in *C. hyperboreus* suggests its higher sensitivity to PAH toxicity compared to *C. finmarchicus* and *C. glacialis*.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2022.100981.

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CRediT authorship contribution statement

Fekadu Yadetie: Conceptualization, Formal analysis, Investigation,

Methodology, Visualization, Writing – original draft, Writing – review & editing. **Nadja R. Brun:** Conceptualization, Investigation, Methodology, Visualization, Writing – review & editing. **Julia Giebichenstein:** Conceptualization, Methodology, Writing – review & editing. **Katarzyna Dmoch:** Methodology, Writing – review & editing. **Ketil Hylland:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Katrine Borgå:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Odd André Karlsen:** Funding acquisition, Writing – review & editing. **Anders Goksøyr:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The RNA-seq raw and processed data as well as the assembled transcriptome sequences have been deposited and made publicly available in EMBL-EBI databases. Accessions have been provided in the text.

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