This is the final draft of the contribution published as:

Shatilina, Z., Drozdova, P., Bedulina, D., Rivarola-Duarte, L., **Schreiber, S.**, Otto, C., Jühling, F., **Aulhorn, S.**, **Busch, W.**, Lubyaga, Y., Kondrateva, E., Pobezhimova, T., Jakob, L., Lucassen, M., Sartoris, F.J., **Hackermüller, J.**, Pörtner, H.-O., Stadler, P.F., **Luckenbach, T.**, Timofeyev, M. (2020):
Transcriptome-level effects of the model organic pollutant phenanthrene and its solvent acetone in three amphipod species
Comp. Biochem. Physiol. D-Genomics Proteomics **33** , art. 100630

The publisher's version is available at:

http://dx.doi.org/10.1016/j.cbd.2019.100630

Transcriptome-level effects of the model organic pollutant phenanthrene and its solvent acetone in three amphipod species

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Abstract

Polyaromatic hydrocarbons (PAH) are common pollutants of water ecosystems originating from incineration processes and contamination with mineral oil. Water solubility of PAHs is generally low; for toxicity tests with aquatic organisms, they are therefore usually dissolved in organic solvents. Here we examined the effects of a typical model PAH, phenanthrene, and a solvent, acetone, on amphipods as relevant aquatic invertebrate models. Two of these species, *Eulimnogammarus verrucosus* and *Eulimnogammarus cyaneus*, are common endemics of the oligotrophic and pristine Lake Baikal, while one, *Gammarus lacustris*, is widespread throughout the Holarctic and inhabits smaller and more eutrophic water bodies in the Baikal area. Neither solvent nor phenanthrene caused mortality at the applied concentrations, but both substances affected gene expression in all species. Differential gene expression was more profound in the species from Lake Baikal than in the Holarctic species. Moreover, in one of the Baikal species, *E. cyaneus*, we found that many known components of the cellular xenobiotic detoxification system reacted to the treatments. Finally, we detected a negative relationship between changes in transcript abundances in response to the solvent and phenanthrene. This mixture effect, weaker than the impact by a single mixture component, needs further exploration.

Keywords: Baikal; Amphipoda; phenanthrene; acetone; transcriptome

Introduction

Polyaromatic hydrocarbons (PAH) are important pollutants of water and soil ecosystems (Ghosal et al., 2016). These substances come from natural sources, such as wildfires, and anthropogenic ones, such as gas- and oil-related industries. In natural environments, PAHs are primarily found in soil, sediments, and water environments (Renjarajan et al., 2015). PAHs are highly toxic and can have mutagenic and cancerogenic effects (Abdel-Shafy and Mansour, 2016). Thus, PAHs accumulated in soil and water bodies pose a threat to these ecosystems, and to humans due to bioaccumulation in food products (Yakan et al., 2013; Bansal et al., 2017).

Phenanthrene, listed among the 16 priority pollutants by the US Environmental Protection Agency (US-EPA, 2010), is considered non-cancerogenic (Rengarajan et al., 2015), but it is one of the most toxic PAHs for invertebrates (Yakan et al., 2013). Phenanthrene is also a model PAH compound; the toxicological effects of phenanthrene have been examined in various groups of organisms such as plants, fungi and animal species of both terrestrial (soil) and aquatic environments (Sans-Lazaro et al., 2008; Deng and Zeng, 2017). Transcriptome responses to phenanthrene, which could provide insight into the cellular response to the exposure, were investigated in a number of species including terrestrial and aquatic arthropods, such as the springtail *Folsomia candida* (Nota et al., 2009), larvae of the midge *Chironomus riparius* (Marinkovic et al., 2012), the cladoceran *Daphnia magna* (Antczak et al., 2013) and the amphipod *Amphelisca abdita* (Biales et al., 2013).

Testing PAHs in aquatic organisms is challenging, as many of these compounds are hydrophobic. Thus, organic solvents are often used to facilitate the dilution of PAHs in water. Typical organic solvents are acetone, dimethylsulfoxide (DMSO), dimethylformamide, ethanol, methanol, and triethylene glycol (Hutchinson et al., 2006; Marquis et al., 2006). The challenge when using solvents

is to apply them at concentrations that will not evoke any biological effects. For example, the US EPA recommends not to exceed the maximum concentration of acetone of 0.05% in basic static tests and 0.01% in flow-through tests (US EPA, 1975). The American Society for Testing and Materials (ASTM) recommends using the solvents diluted at 0.05%, respectively (ASTM, 1998).

Solvent controls are usually used to determine the biological effect of the solvent (Marquis et al., 2006). The impact of the pollution is frequently measured in terms of survival, development, behavior, particular enzymes of xenobiotic biotransformation, or stress response components such as Hsp70 levels (Hallare et al., 2006; Haap et al., 2008; Selderslaghs et al., 2009; David et al., 2012; Huang et al., 2018). Adverse effects of solvents differ depending on the test organism and the tested parameter. For example, from three different solvents (acetone, DMSO, and ethanol), ethanol had the strongest effect on the development of *Danio rerio* embryos but the least pronounced effect on Hsp70 protein levels, and the minimal concentrations of solvents that led to responses differed as well (Hallare et al., 2006).

Transcriptomic analyses provide a comprehensive view of the sublethal responses to acetone exposure. The effects of acetone at sublethal concentrations have been investigated in various soil and aquatic invertebrates where it was found to cause profound effects on gene expression (van Ommen Kloeke et al., 2012; Novais and Amorim, 2015; Ried et al., 2017; Poley et al., 2018).

Lake Baikal in southeastern Siberia, the largest and oldest lake on Earth, is a treasure trove for biologists because of its biodiversity (Moore et al., 2009). Among various taxa, the amphipod fauna (Amphipoda, Crustacea) of the lake is especially rich, comprising over 350 species and subspecies. Lake Baikal amphipods are part of benthic communities at all water depths of Baikal, and some species also inhabit the only outflow of the lake, the Angara River. In contrast to this situation, there

105 is only one amphipod species in different water bodies in the vicinity of Baikal, *Gammarus lacustris*
106 Sars, 1863, which does not inhabit open Lake Baikal (Takhteev et al., 2015).

107 Baikal is also a very important source of drinking water (Potemkina et al., 2018). It was declared
108 a UNESCO heritage site in 1996, and the UNESCO committee noted the value of the lake for
109 evolutionary science as well as its enormous freshwater reserve and raised concerns over pollution
110 issues (UNESCO). Among organic pollutants, PAHs are indeed an important concern, and
111 phenanthrene accounts for a significant proportion of PAHs in Baikal sediments (Ok et al., 2013).
112 PAHs enter Baikal from both natural (in places of natural oil seeps, as a result of wildfires) and
113 anthropogenic sources (use of water transport, railway, industry, residential coal-fired, and oil-fired
114 boilers) (Semenov et al., 2018). The average concentration of phenanthrene in the Baikal water is
115 1.28 ng/L (maximum 1.42 in the Southern basin and minimum 1.09 ng/L in the Northern basin).
116 However, it can increase due to tributaries: the average concentration of phenanthrene in the rivers at
117 the western coast (Buguldeika, Goloustnaya, Krestovka) is 12.46 ng/L, while in the rivers of
118 Khamar-Daban ridge (Utulik, Khara-Murin, Snezhnaya, Pereemnaya) it equals 6.78 ng/L (Semenov
119 et al., 2018). As both solvent and toxicant can have species-specific effects, there may be specific
120 minimal effects and maximum permissible concentrations of a chemical for the Lake Baikal fauna.

121 So far, only one study on PAHs effects on Baikal amphipods is available. In amphipods exposed
122 to phenanthrene dissolved in the water for 1–24 h *abcb1* and *hsp70* transcription was up- or
123 downregulated at different time points (Pavlichenko et al., 2015). In this study, phenanthrene at 1
124 mg/L was dissolved in the water using acetone (final concentration of 20 μL/L) as a solvent; the
125 solvent control contained acetone at 20 μL/L only. Other effects of phenanthrene and also of solvents
126 on Baikal amphipods have so far not been explored. Therefore, we here aimed to study the responses

127 on the transcriptome levels to the model PAH pollutant phenanthrene and its solvent acetone in two
128 endemic Baikal species, *Eulimnogammarus verrucosus* (Gersft.) and *Eulimnogammarus cyaneus*
129 (Dyb.), as well as in the Holarctic species *Gammarus lacustris* Sars.

130 **Materials and methods**

131 *Animals and experiments*

132 *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *Eulimnogammarus cyaneus* (Dybowsky,
133 1874) were collected in August 2013 in the Lake Baikal littoral zone near the Bolshie Koty village
134 (51°54'11.67"N 105°4'7.61"E); *Gammarus lacustris* Sars, 1863 was collected in a former gold
135 mining pond nearby ("Lake 14"; 51°55'14.39"N, 105°4'19.48"E). All animals were pre-acclimated
136 for one week at 6 °C in well-aerated Baikal water and were fed *ad libitum* with dried and ground
137 invertebrates and algae from the Baikal littoral. Adult animals were used for experiments. The body
138 lengths of adult individuals of *E. verrucosus* were 30 (±3) mm; of *E. cyaneus* 14 (±1) mm; and of
139 *G. lacustris* 14 (±2) mm, according to (Timofeyev et al., 2001). Water was exchanged every three to
140 four days. No mortality was observed during acclimation. The EU Directive 2010/63/EU for the care
141 of animals was followed.

142 *Experimental setup*

143 Exposure experiments were carried out in glass vessels in a volume of 1 L water from Lake
144 Baikal (6 °C). The water was aerated during the exposures over glass pipettes submerged in the water.
145 Along with the 1 mg/L phenanthrene (nominal concentration) treatment, a water control and a
146 solvent control (0.1% acetone) were set up. Actual phenanthrene concentrations in exposure
147 solutions at 0 h (start of the exposure) and at 24 h (end of the exposure) were quantified using

high-performance liquid chromatography (HPLC). Phenanthrene concentrations ranged between 472 and 630 µg/L at 0 h and between 28 and 331 µg/L at 24 h (see SI for detailed information). Acetone was used as a solvent for phenanthrene as it was expected to evaporate in the aerated water quickly, and it was previously shown to cause comparatively subtle biological effects (Hallare et al., 2006). Animals were placed into vessels immediately after adding solvent or phenanthrene.

The number of animals per vessel depended on the mean animal size of each species (see above; 5 individuals per vessel for *E. verrucosus*, 10 individuals for *E. cyaneus*, and 12 individuals for *G. lacustris*. Four independent replicates for each species were carried out. Exposures were for 3 and 24 h, and animals were shock-frozen in liquid nitrogen after this period of time. No mortality was observed during the exposure.

RNA extraction, library preparation, sequencing, and bioinformatic procedures

Extraction, sequencing, quality control, and *de novo* assembly procedures are described in detail elsewhere (Drozdova et al., 2019). Briefly, mRNA was extracted from one individual of *E. verrucosus* per treatment/control replicate and from pools of five individuals of *E. cyaneus* or three individuals of *G. lacustris* (miRNeasy/Oligotex mRNA Mini kits; Qiagen). The number of animals per pool was dictated by the animal size (see above). Sequencing libraries were created with the Epicentre ScriptSeq v2 kit according to the manufacturer's recommendations. Two to four biological replicates (sequencing libraries) were obtained for each combination of species and condition (Table S1). The *de novo* transcriptome assemblies were obtained with Trinity (Grabherr et al., 2011) (GenBank: **GHHK00000000.1**, **GHHW00000000.1**, and **GHHU00000000.1** for *E. verrucosus*, *E. cyaneus*, and *G. lacustris*). Annotation was performed with diamond (Buchfink et al., 2014) and FunctionAnnotator (Chen et al., 2017). The assemblies were additionally filtered to

remove potential contamination from symbiotic and parasitic organisms (Drozdova *et al*., 2019).

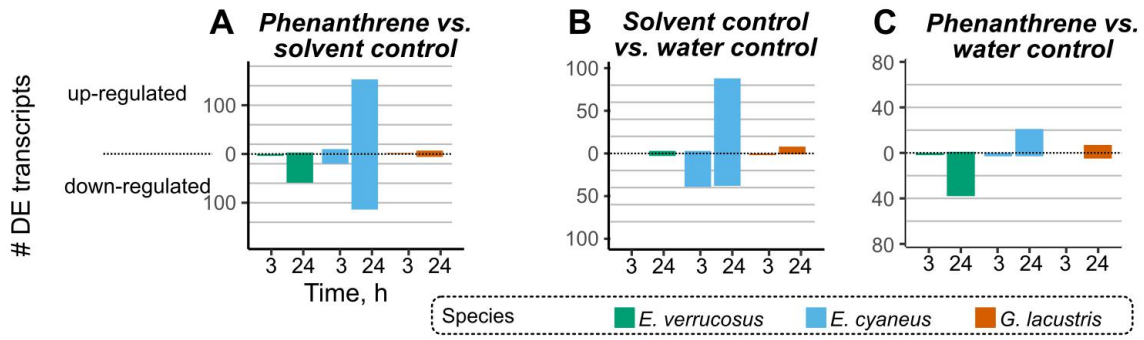
The Illumina sequencing reads were mapped to the assemblies with either the bowtie2/RSEM pipeline (Li and Dewey, 2011; Langmead and Salzberg, 2012) or salmon (Patro et al., 2017), and differential expression was quantified with either the edgeR (Robinson et al., 2010) or the DESeq2 (Love et al., 2014) package for the R statistical environment (R Core Team, 2017). In total, four combinations of abundance estimation and differential expression analysis methods were applied using the scripts provided by Trinity (Haas et al., 2013). The transcripts that were assigned a p-value < 0.05 and absolute $\log_2$ fold change > 1 (i.e., at least two-fold change) by each pipeline, were considered differentially expressed (DE). Overrepresentation analysis of gene ontology (GO) terms was performed with the topGO package (Alexa and Rahnenführer, 2009) for R. All the code used for data analysis is available from GitHub (Drozdova, 2019).

Results

General features of differential expression

To estimate differential expression, we analyzed differential expression of transcripts in the three possible comparisons: solvent control *vs*. water control; phenanthrene treatment *vs.* solvent control; phenanthrene treatment *vs.* water control. Each phenanthrene sample contained acetone as solvent.

The overall results of the differential expression analysis are shown in Fig. 1, and all differentially expressed genes are listed in Table S2.



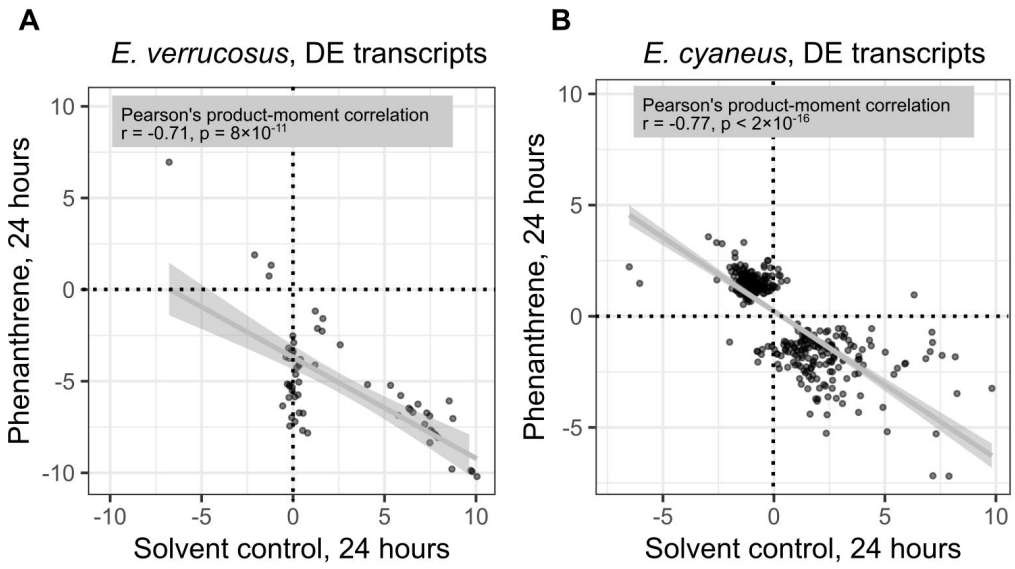
188

189 Fig. 1. Overview of n the numbers of differentially expressed (DE) up- and downregulated genes in
190 the amphipod species from the different conditions.

191

192 Comparisons of DE data from the different conditions revealed two differences. First, we
193 observed a stronger response (measured as the total number of DE genes) in the *Eulimmogammarus*
194 species as compared to *G. lacustris* in all three comparisons. Second, the impact of the solvent alone
195 appeared to be more pronounced than the effect of phenanthrene and solvent in the phenanthrene
196 treatment (Fig. 1).

197 To analyze potential mechanisms of compensation between the solvent and phenanthrene, we
198 further explored the relationship between phenanthrene- and acetone-induced changes after 24-h
199 exposures: the expression changes were generally greater at this time point (Fig. 1). Log_2 fold change
200 values for each transcript differentially expressed in at least one of the conditions were plotted (Fig. 2.
201 For both *Eulimogammarus* species, the correlation coefficients computed for the DE transcripts were
202 below -0.7 (Fig. 2), indicating a strong negative relationship. The same tendency was observed for
203 *G. lacustris* and in all investigated species when all transcripts were included in the analysis (Fig.
204 S1).



205

206 Fig. 2. Correlation between changes in gene expression for the DE genes of *E. verrucosus* (A) and
207 *E. cyaneus* (B). Log_2 fold change is shown along the axes.

208

209 *Functional groups of transcripts affected by solvent control or phenanthrene*

210 In the solvent control, a prevalent down-regulation of transcripts could be seen already after 3 h
211 in all species (Fig. 1B). Manual analysis of differentially expressed genes (Table S2) and
212 automatized analysis of overrepresented gene ontology terms (Table S3) revealed that different
213 functions were affected in the different species. In particular, we observed down-regulation of
214 proteolysis-related transcripts in *E. verrucosus* from the solvent control and in *E. cyaneus* from the
215 phenanthrene treatment after 24 h. This pattern of changes was not observed in other control or
216 treatment groups (Fig. 3). Interestingly, oxidation-reduction processes-related transcripts were
217 generally down-regulated in response to the solvent, while transposon-related transcripts were
218 down-regulated in response to phenanthrene in *E. cyaneus*.

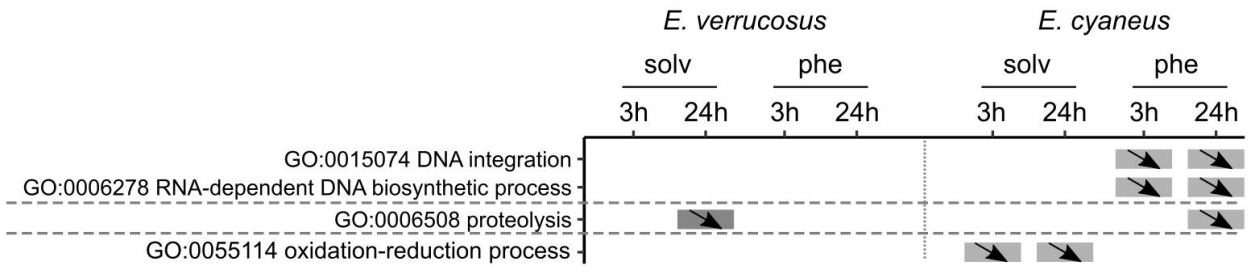


Fig. 3. Gene ontology (GO) terms overrepresented in the phenanthrene treatments and in the solvent controls. The arrows depict the direction of expression changes (up- or down-regulation). Only GO terms registered for at least two groups of samples are shown. The full list of GO terms and associated genes can be found in Table S3. Solv – solvent control vs. water control; phe – phenanthrene vs. solvent control.

Responses of particular genes known to react to the studied stressors

In addition to overrepresented functional groups, we used our data to search for known genes that may participate in response to the tested toxic substances. Generally, xenobiotics are converted to more water-soluble metabolites by addition of a hydroxyl group at phase 1 (one of the enzymes is cytochrome P450), and then a water-soluble endogenous molecule is conjugated to the metabolite during phase 2 (one of the enzymes is glutathione S-transferase, GST) (Dam et al., 2008, Walker, 2008, Ren et al., 2015). Finally, the solubilized metabolites get excreted by ABC transporter proteins (Vache et al., 2007).

In *E. cyaneus*, we registered that cytochrome P450 (cytP450) 2J2-like gene expression was inhibited in the presence of phenanthrene, whereas the transcription of another cytP450-encoding transcript, annotated as 18a1-like, was induced (Fig. 4A). At the same time, the cytochrome P450 2L1-like encoding transcript was up-regulated in response to the solvent in *G. lacustris.* We also saw a diverse response of GSTs, which were mostly down-regulated in response to the solvent and up-regulated in response to phenanthrene (Fig. 4A). Among ABC transporters, only an

ABCG-subfamily-like transcript reacted to the solvent, and none reacted to phenanthrene (Fig. 4B).

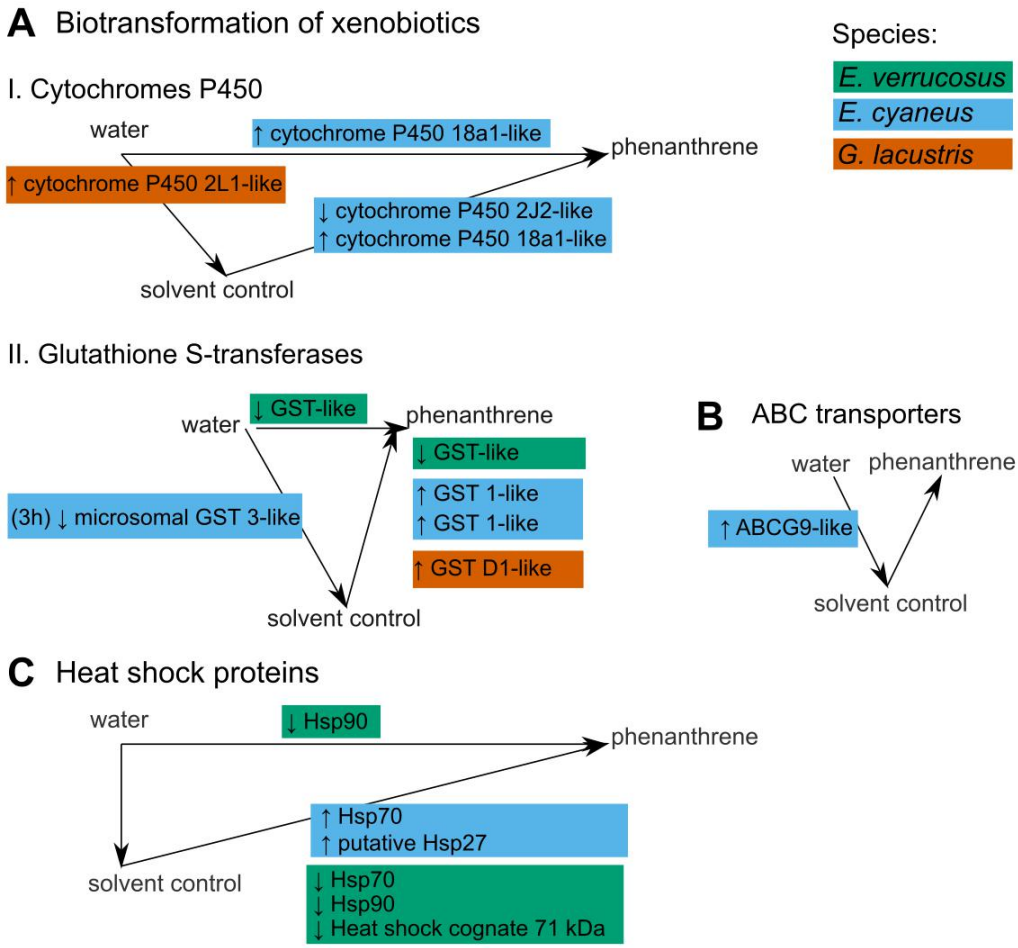


Fig. 4. Transcripts of the indicated groups and changes in their expression in response to the treatments. Most DE transcripts were found in the 24-h exposures; those found in 3-h exposures are specifically indicated (3 h).

In addition, heat shock proteins have been shown to react to xenobiotics (Weis, 2015). We did not register any change in *hsp* expression in response to the solvent treatment, while in response to phenanthrene treatment several *hsp* transcripts were up-regulated in *E. cyaneus* and, surprisingly, down-regulated in *E. verrucosus* (Fig. 4C). Interestingly, expression of one of these transcripts was significantly different between water control and phenanthrene treatment, meaning that in the solvent treatment it had a sub-threshold change.

Discussion

252 *Influence of solvent and phenanthrene on the survival of amphipods*

253 No mortality occurred in any of the studied amphipod species in the solvent controls and
254 phenanthrene treatments indicating that acetone and phenanthrene concentrations were at sublethal
255 levels.

256 Lethal concentrations of acetone were determined for a range of aquatic organisms including
257 crustaceans (Hutchinson et al., 2006). The 50% lethality concentration (LC_{50}) over 48 h for acetone
258 was more than 9 g/L (i.e., about 1.2%) for *D. magna* and about 8 g/L (about 1%) for another
259 daphniid, *Ceriodaphnia dubia* (Cowgill and Milazzo, 1991). For embryos of the decapod
260 *Palaemonetes pugio*, four and twelve-day LC_{50} values were close to 7 g/L (0.9%); 4 g/L was
261 determined as the maximal safe concentration in this study (Rayburn et al., 1997). Sublethal effects
262 were shown for 0.01% acetone, which affected the sex ratios and reproductive strategies in *D. magna*
263 (Zhang and Baer, 2000). Thus, lethal and sublethal effect concentrations of acetone for these
264 crustaceans differ by two orders of magnitude.

265 The literature on acetone toxicity for amphipod species is scarce. There is information that
266 acetone in water at 1:10,000 (ten-fold lower than the acetone concentration in our solvent control)
267 caused 2.5% mortality within 24 h and 12.5% mortality within 48 h in another gammarid amphipod,
268 *Gammarus mucronatus*. However, in this experiment a water control, necessary to determine the
269 background mortality rate, was missing (Ruber et al., 1983). For another amphipod species,
270 *Gammarus fasciatus*, it was found that a 96-h exposure in a 100 mg/L acetone solution (~0.01%)
271 resulted in less than 50% mortality (Ewell et al., 1986).

272 Regarding lethal phenanthrene concentrations, literature data for seawater amphipod species
273 exist: the LC_{50} (48 h) values for phenanthrene were 173.85 µg/L for *Gammarus aequicauda*, 147.64

274 µg/L for *Gammarus locusta*, and 215.20 µg/L for *Corophium multisetosum* with >80% survival in the
275 control conditions (Sanz-Lázaro et al., 2008). The measured phenanthrene concentrations in the
276 exposure water at 0 and 24 h were 48 % and 84 % below the nominal concentration of 1 mg/L (refer
277 to the table in the Text S1), but these concentrations were in the same range as the reported lethal
278 concentrations. Yet, the absence of mortality in our experiments indicates that the studied amphipod
279 species were less sensitive to phenanthrene than the species for which LC50 values had previously
280 been determined.

281 *Transcriptome-level effects of acetone*

282 Transcriptome-wide effects of acetone treatment have been analyzed in several invertebrates
283 (Fig. 5). For example, in the springtail *F. candida* treatment with acetone-spiked soil (1:1 w/w), even
284 after acetone was allowed to evaporate overnight, triggered significant changes in expression of 130
285 genes (van Ommen Kloeke et al., 2012). In the oligochaete *Enchytraeus albidus*, acetone-spiked soil
286 also changed the expression of approximately 130 genes, most of which had no functional annotation
287 (Novais and Amorim, 2015). Finally, in the aquatic parasitic copepod *Lepeophtheirus salmonis*,
288 more closely related to amphipods than the other species, 0.35% acetone triggered changes in the
289 expression of about 300 genes if compared with a seawater control; among those genes were genes
290 encoding different classes of molecular chaperones such as *hsp40* (*dnaJ*), *hsp70*, *hsp90* and
291 ribosomal proteins (Poley et al., 2018).

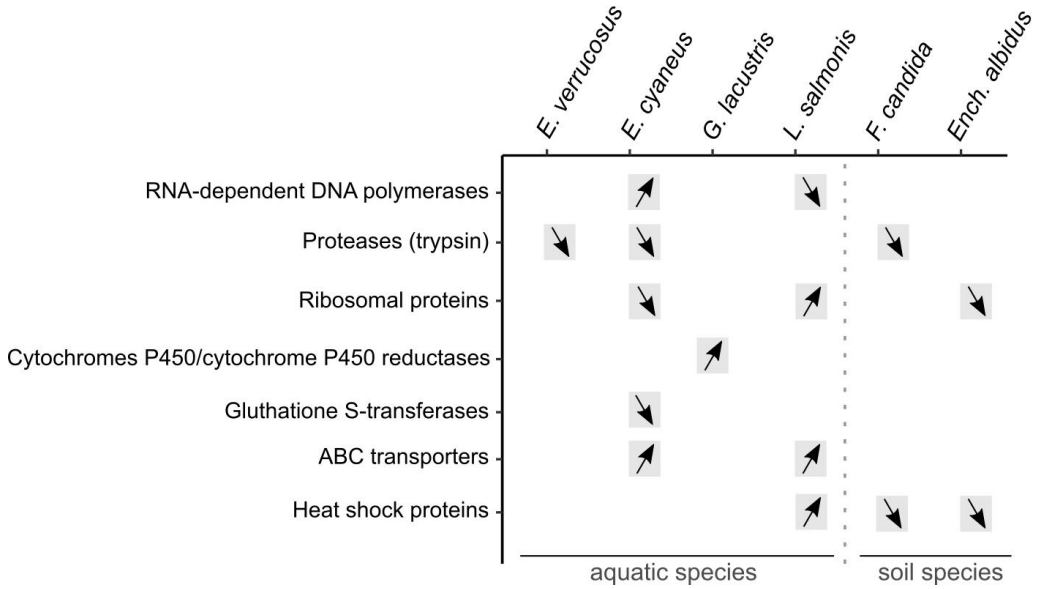


Fig. 5. Transcriptomic responses to the solvent (acetone) control compared to a water control in different species. The data for *L. salmonis*, *F. candida*, and *Ench. alibidus* are taken from Poley et al. (2018); van Ommen Kloeke et al. (2012); Novais and Amorim (2015), respectively.

Our data had some similarities with each of the published datasets but did not show full correspondence to any of them (Fig. 5). Interestingly, in two cases ABC-transporters (ABCG subfamily) were up-regulated. The responses of ribosomal protein genes were diverse in the different species. A cytochrome P450-encoding gene was differentially expressed only in *E. cyaneus*. It is interesting to note that it was annotated as 2J2-like; the human CYP2E ortholog had been found to respond to acetone (Walker, 2008). The acetone concentrations of 0.1 % or approximately 0.8 g/L in solvent controls and phenanthrene treatments in our experiments are orders of magnitude below the reported lethal acetone concentrations but in the range of sublethal effects (see above). This indicates that aquatic invertebrates are generally highly sensitive to low acetone concentrations in the water.

Transcriptome-level effects of phenanthrene

The transcriptome-level responses in the amphipods studied here indicate sublethal effects of phenanthrene on the animals at the applied concentration. Phenanthrene effects on gene expression

have previously been investigated in several species including the collembolan *F. candida* (Rota et al., 2009), an oligochaete *Ench. crypticus* (Roeflos et al., 2016), and the midge *C. riparius* (Marinkovic et al., 2012) (Fig. 6). In the case of *Ench. crypticus* (Roeflos et al., 2016), more genes were down-regulated than up-regulated; in the other organisms, numbers of down- and up-regulated genes were similar.

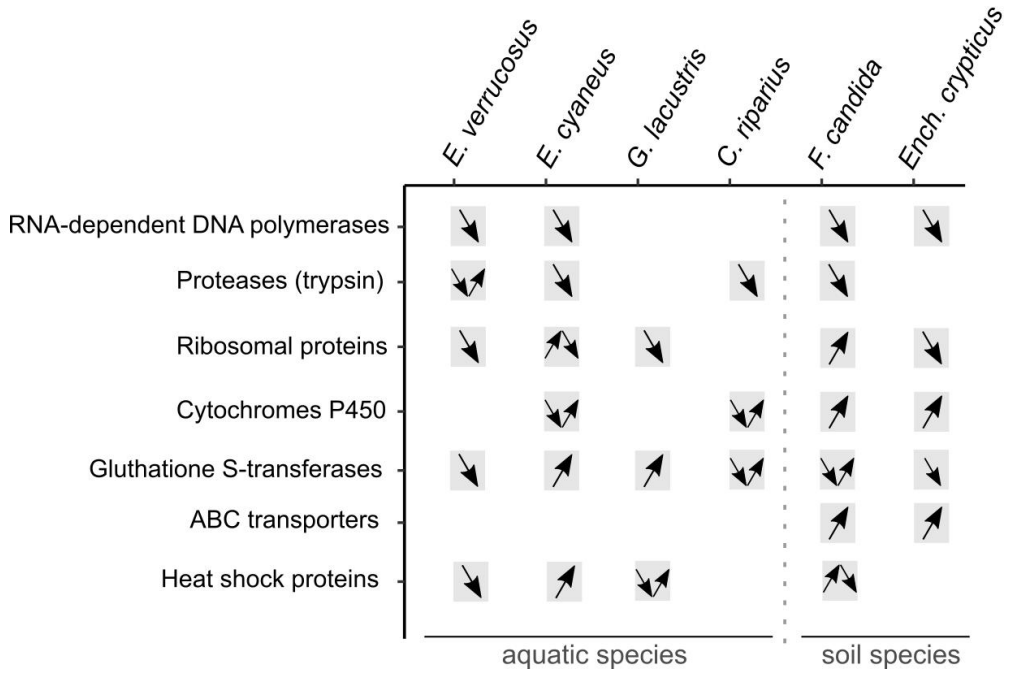


Fig. 6. Transcriptomic response to phenanthrene (phenanthrene+solvent compared to solvent) in different species. The data for *C. riparius*, *F. candida*, and *Ench. crypticus* are taken from Marinkovic et al. (2012); Rota et al. (2009); Roeflos et al. (2016), respectively.

The gene expression changes in response to phenanthrene exposure were quite similar for most species (Fig. 6). For some reason, RNA-directed RNA polymerases were down-regulated in four out of six species, trypsin-like enzymes were down-regulated in three species. However, cytochrome P450, GSTs, and heat shock protein genes were affected in the majority of species but showed inconsistent responses with both up- and down-regulated transcripts. In some cases, but not in amphipods, ABC transporters were up-regulated.

Earlier, effects of toxicants on the activity of enzymes of the xenobiotic biotransformation pathways in Baikal amphipods were studied. The exposure of littoral Baikal amphipods (*Gmelinoides fasciatus* and *Pallasea cancelloides*) to humic substances resulted in a slight decrease or in no changes of GST activity (Timofeyev, Steinberg, 2006). Additionally, a reduction of multixenobiotic resistance transporter activity by both natural organic matter and cadmium in Baikal littoral amphipods (*E. verrucosus* and *E. cyaneus*) was shown (Timofeyev et al., 2007).

Relationship between the responses to acetone and phenanthrene

We observed a clear negative correlation between the responses to acetone (compared to water) and to phenanthrene (compared to acetone). The effect of phenanthrene looks like partial reversion of the effect of acetone. It is known that the interaction of chemicals can change their effect (Green, Wheeler, 2013). As phenanthrene is not water-soluble but is soluble in acetone, a possible explanation for this mitigating effect of phenanthrene could be that the addition of phenanthrene removes some of the acetone molecules from the reaction mixture. As a consequence, less acetone would be taken up by the animals in the phenanthrene treatment.

Difference in responses of different species

We found that the responses of Baikal endemic amphipods to acetone were generally more pronounced than the response of the Holarctic *G. lacustris*. There are several possible reasons for this difference. First, it is possible that the Holarctic species, dwelling in the conditions of increased levels of organic pollutants (Kozhova, Izmest'eva, 1998), is pre-adapted to organic substances. For example, it was found that when exposed to humic compounds in the water that *hsp70* transcript

levels in the endemic Baikal species were decreased while they were increased in *G. lacustris* (Protopopova et al., 2014). Second, it is worth noting that the preacclimation and experimental conditions (Lake Baikal water at 6 °C) might have been far from optimal (i.e. the habitat) conditions for this species, as it does not inhabit open Lake Baikal (Takhteev et al., 2015), has a preferred temperature of 15-16 °C (Timofeyev et al., 2001). It may even hibernate at 6-7 °C, as animals caught at such temperatures were found inactive in decomposing leaves (Jakob et al., 2016). Thus, it is possible that the comparatively little response in *G. lacustris* on the transcriptome level was due to a decrease of the metabolic rates and concomitantly of cellular processes at the experimental temperature.

Conclusions

In this work, we found that short term exposures (3/24 h) to sublethal concentrations of the PAH phenanthrene lead to transcriptome-wide responses in the amphipods *E. cyaneus* and *E. verrucosus* endemic to Lake Baikal and the Holarctic *G. lacustris*. The differences in responses across species were substantial. Species-specific stress response capacities and species-specific rates of uptake of the chemical from the water may play a role; a contributing factor for the differences may be different species-specific physiological rates at the exposure temperature (6 °C). The high numbers of DE transcripts in the solvent control indicate strong effects by the solvent acetone and, surprisingly, differences to controls were more pronounced than in the phenanthrene treatment (also containing the solvent acetone). This suggests that acetone causes effects appearing to be mitigated or compensated by phenanthrene. This mixture effect, weaker than the effect by a single mixture component, needs further exploration.

Acknowledgments

We are grateful to Denis Axenov-Gribanov, Kseniya Vereshchagina, Ekaterina Madyarova and other members of the Biosystems adaptations lab for help and advice, and to Steve Hoffmann for help with data analysis and critical reading of the manuscript.

Funding: This research was supported by the Russian Science Foundation / Helmholtz Association of German Research Centers (RSF grant number 18-44-06201).

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