

Short-Chain Chlorinated Paraffin Effects on the Expression of Key Genes of *Gammarus pulex* Exposed at Two Temperatures



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Introduction:

- Short-chain chlorinated paraffins (SCCPs) = **ubiquitous pollutants** found in air, sediments, water, biota => included in **Stockholm Convention** (2017).
- **Persistent, bioaccumulative and toxic (PBT)**, readily bioaccumulates + biomagnifies.
- Present in surface waters at **concentrations in the ng or µg/L range** => **what are the sublethal effects on exposed organisms ?**
- Freshwater amphipod crustacean *Gammarus pulex* studied to determine SCCP effects at relevant concentrations (**10, 100 and 1000 ng/L**).
- **Measuring mRNA levels of genes** for immune defences, moult-related endocrine cycle, osmoregulation, apoptosis, oxygen transport, antioxidant & antitoxic defences (Table 1).
- **Two temperature ranges** = simulate cooler and warmer climates = potential **interactions** of heat-related stress and toxicity of SCCPs?

Materials & methods:

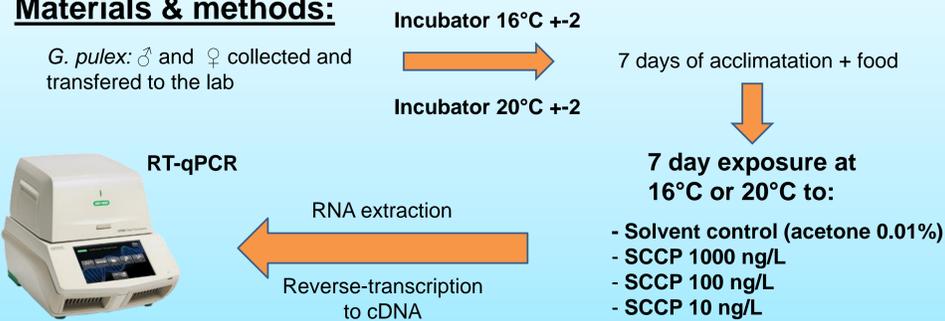


Table 1: list of studied genes, their biological functions, and their abbreviated names.

Biological functions	Genes
Respiration	Hemocyanin (HC)
Osmoregulation	Na/K ATPase (NaK)
Apoptosis	Caspase 3 (Casp3)
Immune System	Prophenoloxidase (ProPO)
Endocrine System	Moult-inhibiting hormone (MIH) Ecdysone receptor (EcR) Farnesoic acid O-methyltransferase (Famet) Methylfarnesoate epoxidase (CYP15A1)
Oxidative Stress	Glutathione-S-transferase (GST) Selenium-dependant glutathione-S-transferase (SeGPX) Catalase (CAT) Mn superoxide dismutase (MnSOD) Cu/Zn superoxide dismutase (CuZnSOD)
General Stress	Heatshock protein 70 (HSP70) Thioredoxin (THX) Thioredoxin reductase (THX red)

+1 0 -1

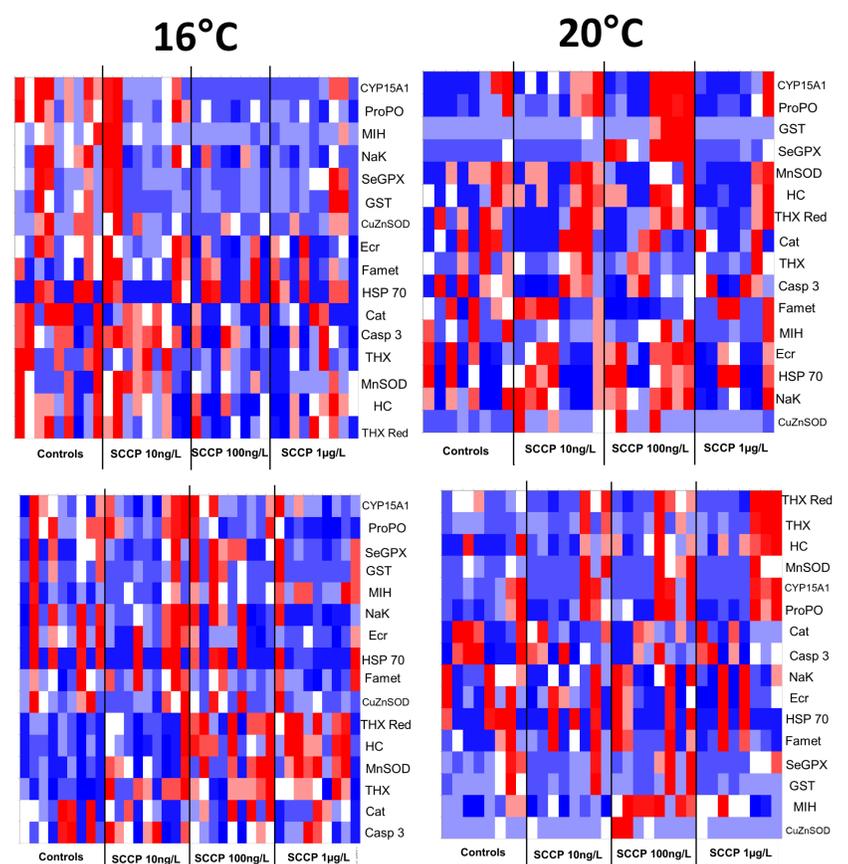


Fig. 1: heatmaps of mRNA levels for the studied genes. Red = higher level of mRNA compared to controls, blue = lower level. Genes regrouped by similarity of mRNA expression patterns.

Females

Males

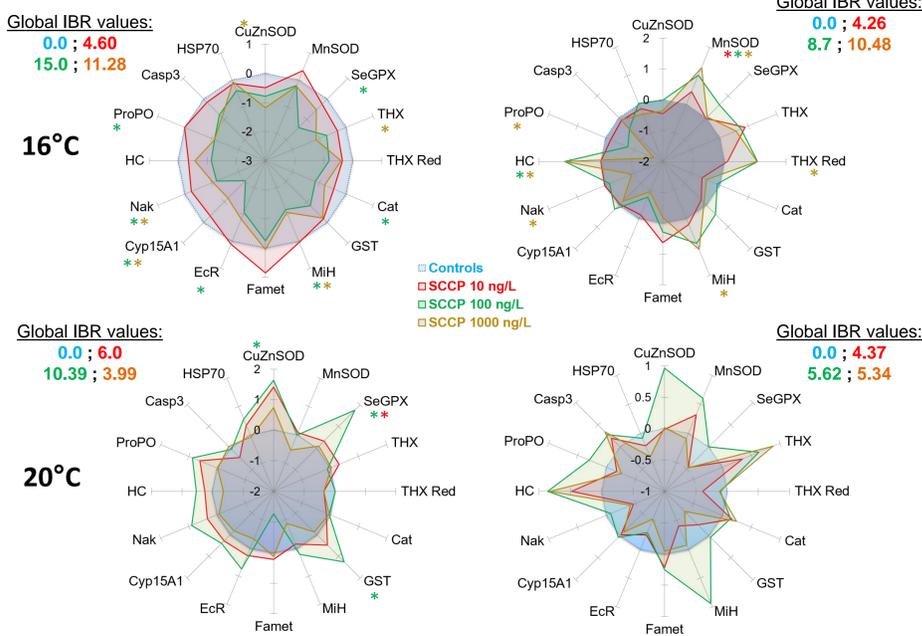


Fig. 2: radar plots of the integrated biomarker response (IBR) values of the studied genes after a 7-day SCCP exposure, compared to a control value of 0. Significant variations from controls marked with (*) and global IBR scores are colour-coded to the corresponding exposure condition.

Results:

- Genes with similar functions = similar responses to SCCPs in a given condition. Antioxidant, antitoxic stress + immunity loosely grouped together, same for endocrine genes (fig. 1).
- **♂ 16°C**: ↑ expression for MIH, HC, MnSOD = Possible short term inhibition of moulting, issues with oxygen transportation and increase in oxidative stress.
↓ expression of ProPO and NaK = smaller immune response and less osmoregulatory capability. Most effects only at 1000ng/L SCCP.
- **♀ 16°C**: ↓ expression of antioxidant + antitoxic stress genes, ProPO, NaK, and endocrine genes
Some function responses = ♂, but others opposite. EcR + MIH both lowest just before moult so do SCCPs encourage moult in females?
Most effects at 100 ng/L, some at 1000 ng/L. Females = more sensitive to SCCPs in the exposure conditions.
- **♂♀ 20°C**: males: unimpacted
females: ↑↑↑ antioxidant + antitoxic stress genes at 100ng/L
↓ Famet expression (enzyme which forms methylfarnesoate = growth hormone).
Higher stress for females + endocrine disruption caused by heat stress and toxicity?

Conclusions:

- SCCPs = **sublethal toxic** effects in *G. pulex* males + females after **7 days** at environmental concentrations: **potentially endocrine disruption, oxidative stress, general stress, ...**
- Cooler t° range: males over-express antioxidant defence + moult cycle endocrine genes. Females = opposite. Immunity + osmoregulation impacted similarly in both sexes.
=> **SEX DEPENDENT RESPONSE TO SCCP EXPOSURE**
- Warmer t° range: Female antioxidant genes highly overexpressed. Combined stress effect of t° and SCCPs?
Males seemingly unaffected. Effects hidden or compensated for by warm t° physiological response?
=> **TEMPERATURE DEPENDENT RESPONSE TO SCCP EXPOSURE**
- **Further research needed on SCCP effects on ecologically important but under-studied groups. What are the mechanisms of endocrine disruption and sublethal toxicity?**