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# Acute and sub-lethal response to mercury in Arctic and boreal calanoid copepods

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ABSTRACT

Acute lethal toxicity, expressed as $LC_{50}$ values, is a widely used parameter in risk assessment of chemicals, and has been proposed as a tool to assess differences in species sensitivities to chemicals between climatic regions. Arctic *Calanus glacialis* and boreal *Calanus finmarchicus* were exposed to mercury ($Hg^{2+}$) under natural environmental conditions including sea temperatures of 2° and 10 °C, respectively. Acute lethal toxicity (96 h $LC_{50}$) and sub-lethal molecular response (*GST* expression; in this article gene expression is used as a synonym of gene transcription, although it is acknowledged that gene expression is also regulated, e.g., at translation and protein stability level) were studied.

The acute lethal toxicity was monitored for 96 h using seven different Hg concentrations. The sub-lethal experiment was set up on the basis of nominal $LC_{50}$ values for each species using concentrations equivalent to 50, 5 and 0.5% of their 96 h $LC_{50}$ value. No significant differences were found in acute lethal toxicity between the two species.

The sub-lethal molecular response revealed large differences both in response time and the fold induction of *GST*, where the Arctic species responded both faster and with higher mRNA levels of *GST* after 48 h exposure. Under the natural exposure conditions applied in the present study, the Arctic species *C. glacialis* may potentially be more susceptible to mercury exposure on the sub-lethal level.



## 1. Introduction

Toxicity testing and risk assessment of chemicals have traditionally focused on temperate species, extrapolating toxicity thresholds to polar organisms (Chapman and Riddle, 2005b). This may indeed be applicable in some cases. However, abiotic factors influencing the life history of polar organisms are very different from those found in temperate areas, resulting in marked differences in ecological and physiological traits. For polar marine organisms, the most prominent abiotic factor is the large annual variation in daylight, resulting in a short period of massive primary production and growth during summer followed by a period of dormancy in the dark period. Ambient temperatures in polar oceans are low (−1.8–2 °C) throughout the year (Hop et al., 2002).

When measured at their normal temperature, polar ectothermic organisms tend to have a lower metabolic rate compared to species from warmer climatic regions (Peck, 2002). This results in Arctic species having longer life spans, extended developmental time and a tendency to grow larger than in temperate areas. These physiological differences between Arctic and temperate organisms influence their ability to grow and develop during contaminant exposure. Thus, it has been emphasized that toxicity testing should be focused on native polar species (Arctic or Antarctic) when evaluating risks of contaminants in these areas (Chapman and Riddle, 2005a).

Calanoid copepods of the *Calanus* genera constitute a key group of organisms linking primary producers and higher trophic levels in Atlantic and Arctic marine food webs (Falk-Petersen et al., 2009; Søreide et al., 2008). Due to their ability to accumulate large quantities of lipids, the *Calanus* species are regarded the most important energy source supporting large stocks of fish, birds

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and marine mammals in the Northern Atlantic and Arctic oceans. Three species of *Calanus* dominate the Northern Atlantic and Arctic oceans; *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus*. The two species examined in the present study, *C. glacialis* and *C. finmarchicus*, are similar in size and morphology, but have different temperature preferences determining their distribution. *C. glacialis* is considered a true Arctic species found mainly in cold polar waters in the northern Barents Sea and the Arctic Ocean. *C. finmarchicus* has a boreal distribution preferring sub-Arctic and Atlantic waters. *C. finmarchicus* is adapted to areas with a regular spring bloom of algae and has a longer productive season, completing its life cycle in one year (Falk-Petersen et al., 2009). *C. glacialis*, on the other hand, is better adapted to the large inter-annual variations in the onset and duration of the spring bloom in Arctic areas. Compared to temperate species, it takes a longer time for the Arctic copepodites to build up sufficient lipid stores to reach mature reproductive state, and they only complete the life cycle in one year if the conditions are highly favourable. On average *C. glacialis* has a two-year life cycle (Falk-Petersen et al., 2009). Considering the energy budget of an organism, defence against xenobiotic exposure and effects are causing an increased demand of energy otherwise available for growth and reproduction (Kooijman, 2001). This is particularly relevant for Arctic species with a limited time frame for growth and reproduction.

Mercury (Hg) occurs naturally in several forms, and the speciation determines its tendency of bioaccumulation and toxic effects. Methyl mercury (MeHg) is commonly known as neurotoxic, whereas inorganic mercury primarily perturbs the cellular redox balance (Stohs and Bagchi, 1995). Despite low emission of Hg in Arctic areas, high levels are documented in polar organisms (reviewed by Poissant et al., 2008). Although extensive regulations of global Hg emission have been implemented, temporal trend analyses show increasing Hg levels in Arctic biota (Braune et al., 2005). Mercury is deposited on snow and ice during the Arctic spring, and although some Hg is reemitted into the atmosphere, a net input of Hg to Arctic ecosystems has been reported (Poissant et al., 2008; Steffen et al., 2008). High levels of Hg have been documented in snow, where the bioavailable fraction is reaching up to 100% at the onset of snowmelt (Dommergue et al., 2010; Larose et al., 2011). Mercury concentrations in seawater increase significantly during snow melt (Dommergue et al., 2010), potentially leading to high exposure of zooplankton in Arctic areas. The relative proportion of MeHg in seawater is generally low compared to inorganic Hg (3–5%); however, the fraction may vary with season, region and water depth (Leermakers et al., 2001; Steffen et al., 2008). At the peak concentrations of total Hg (2.9 ng/L) during snowmelt in Kongsfjorden, the fraction of MeHg was approximately 5.6% (Dommergue et al., 2010).

Toxic effects of Hg occur at very low concentrations. Marine copepods appear to have a very species-dependent sensitivity to acute Hg exposure, with $LC_{50}$-values ranging between 10 and 600 μg/L (ECOTOX-database, 2013). Sub-lethal exposures to dissolved Hg are causing a variety of responses in marine copepods, some of which have direct ecological implications (e.g reduced egg production; Hook and Fisher, 2001, 2002; Valko et al., 2005). Both organic and inorganic mercury are known to deplete biological systems of free sulfhydryl groups (—SH) that are normally available for protection against oxidative stress (Valko et al., 2005). Inorganic $Hg^{2+}$ disrupts multiple steps in the metabolism of the endogenous antioxidant glutathione (GSH) (Stohs and Bagchi, 1995; Valko et al., 2005). Lipid peroxidation increases shortly after $Hg^{2+}$ exposure, mainly due to a combination of antioxidant depletion and enhanced production of reactive oxygen species (Lund et al., 1991, 1993; Monteiro et al., 2010; Valko et al., 2005).

Up-regulation of genes involved in xenobiotic defence is a useful tool to compare sub-lethal sensitivity in short term experiments. Glutathione-*S*-transferase (*GST*) is a group of enzymes with intrinsic xenobiotic defence properties, including the facilitation of conjugation reactions with GSH and a peroxidative activity attenuating lipid peroxidation (Hayes et al., 2005; Parkes et al., 1993; Vontas et al., 2001).

The purpose of the present study was to elucidate to which extent results from toxicity studies of boreal *C. finmarchicus* can be extrapolated to Arctic *C. glacialis* when they are exposed to Hg under their respective natural environmental conditions, including different sea temperatures. *C. finmarchicus* originating from the Trondheim fjord (63°N, 10°E) was exposed to Hg at a temperature of 10 °C in the laboratory in Trondheim. *C. glacialis* was collected in Kongsfjorden (79°N, 12°E) and exposed to Hg at a temperature of 2 °C in the Marine Laboratory of Ny-Ålesund. Two experiments were performed: first, we examined acute lethal toxicity ($LC_{50}$, 48 and 96 h) and Hg accumulation in the two species. Second, we performed a sub-lethal exposure experiment where we examined the relationships between exposure intensity (time and concentration) and molecular response in form of *GST* transcription in the two species.

## 2. Materials and methods

### 2.1. Experimental animals

Exposure experiments on the boreal model species *C. finmarchicus* were performed at SINTEF/NTNU Sealab in Trondheim. These animals, originating from the Trondheim fjord (63°N, 10°E), have been kept in the lab at 10 °C for several generations. Hansen et al. (2007) described details regarding the routine culturing. *C. glacialis* were collected in Kongsfjorden, Svalbard (79°N, 12°E) using a 1000 μm WP3 plankton net hauled at approximately 1 m/s (Hydro-Bios, Kiel, Germany). The net was fitted with a closed end container. Collected zooplankton was transferred to the Kings Bay Marine Laboratory, Ny-Ålesund, where the exposure experiments were performed. The developmental stage copepodite V (CV) of *C. glacialis* was individually identified using stereo microscopes. These individuals were kept in holding tanks at 2 °C for 2–3 days prior to the exposure experiment. All individuals used in the experiments were at developmental stage CV. The individuals from the culture were taken out at a time when the developmental stage CV was dominant, determined by close examination of a representative subsample. All individuals caught in Kongsfjorden were separated from species with an overlapping size distribution by close examination of the tail segments using a stereomicroscope. It is possible that a few individuals developed into the mature stage during the acclimation and exposure period; however, empty shells were not observed in the exposure chambers.

### 2.2. Acute toxicity testing ($LC_{50}$)

The acute toxicity test procedure applied was a modified version of the standard ISO 14669:1999. The test was performed in polypropylene containers (2L, NordicPack, Dilling, Norway), with a total volume of 0.5 L per bucket. A stock solution of 1000 mg/L $Hg^{2+}$ was prepared by dissolving $HgCl_2$ (Pro analysis 99.5%, Riedel-de-Häen Aktien Gesällschaft) in Milli-Q water, which was subsequently diluted in filtered seawater to the following concentrations: 7, 12, 20, 33, 54, 91 and 152 μg Hg/L. Triplicates of each concentration and six controls were applied. No lethality was observed in the controls. Each bucket contained seven individuals, and the two tests were performed at 10 °C and 2 °C for *C. finmarchicus* and *C. glacialis*, respectively. After 24, 48, 72 and 96 h of exposure the immobilised copepods in each container were counted. After 96 h, copepods from individual containers were sampled and rinsed