Ecotoxicological responses to chalcopyrite exposure in a proxy for deep-sea hydrothermal vent shrimp: Implications for seafloor massive sulphide mining

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Deep-sea mineral prospecting has raised concerns regarding potential ecotoxicological impacts of deep-sea mineral extraction. Although metal mineral phases are predicted to exhibit low bioavailability, few data explore the relative toxicity of mineral phases and dissolved constituent metals. Acute 96 h chalcopyrite (CuFeS2) (<250 µm grain size) exposures using the shallow-water ecophysiological model organism *Palaemon varians* as an ecotoxicological proxy for deep-sea hydrothermal vent shrimp revealed no effect in both lethal and sublethal assays up to 2.888 g l-1, suggesting that chalcopyrite is not bioavailable. Deep-sea species therefore appear at greater ecotoxicological risk from dissolved metals during seafloor massive sulphide (SMS) mining. Consequently, an approach combining modelling the release, and spatial and temporal dilution of dissolved metals during SMS mining, with data on sublethal effects of dissolved metals on shallow-water proxies, may best constrain the potential ecotoxicological impacts of SMS mining and deliver ecotoxicological threshold concentrations for active SMS extraction.

Keywords: aquatic toxicology; chalcopyrite; deep-sea mining; heavy metal; metal bioavailability

# Introduction

Exploration for deep-sea mineral resources is increasing, driven by increasing demand for metals and rare-earth elements and the challenges of achieving environmentally acceptable operations in the extraction, processing, and disposal of rock materials from low-grade terrestrial deposits [1]. Further, deep-sea mining technologies are sufficiently developed to allow extraction of hydrothermally-formed seafloor massive sulphides (SMS) at least as deep as 1600 m depth [2]. However, extraction and dewatering of deep-sea mineral deposits may have significant ecotoxicological impacts on the deep-sea fauna [3]. For example, SMS comprise high concentrations of mineral phases which are potentially toxic, such as chalcopyrite (CuFeS2) [4]. Consequently, accurately constraining the impacts of SMS extraction on the deep-sea fauna will require assessment of ecotoxicological effects [3].

Whilst metals may be released into solution by the extraction and dewatering of SMS deposits [5], there are few data available on the relative toxicity of sulphide mineralic phases and their dissolved constituent metals [6]. The limited data that are available suggest that metal sulphide phases are significantly less bioavailable than dissolved metals [6]. For example, Simpson and Spadaro [6] observed that the bioavailability of copper determined according to acute lethal toxicity thresholds is 4-12 times greater in dissolved copper-spiked sediments than in chalcopyrite-spiked sediments in juvenile *Melita plumulosa* (shallow-water amphipods) and *Spisula trigonella* (shallow-water bivalves). Although the dependence of marine metal toxicity on organismal physiology suggests that closely related species with similar physiologies can be expected to display similar tolerances to toxicants [7], tolerances to toxicants may differ significantly among closely related species which inhabit contrasting environments [8]. For example, physiological adaptations to the constant low temperature and high hydrostatic pressure environmental conditions of the deep-sea [9] may affect sensitivity to toxicants, potentially confounding the use of shallow-water species as ecotoxicological proxies [10]. Despite this, the urgency to exploit deep-sea mineral resources and the difficulties in experimentally assessing ecotoxicological impacts on deep-sea fauna have typically driven researchers to utilise shallow-water species as ecotoxicological proxies for deep-sea species without determining suitability.

The suitability of the shallow-water estuarine, salt marsh, and saline lagoon shrimp *Palaemon varians* as a shallow-water ecotoxicological proxy for the deep-sea hydrothermal vent shrimp *Rimicaris exoculata* has been assessed [8]. *P*. *varians* is an excellent comparator for deep-sea shrimp [8], with close phylogenetic relationship to both hydrothermal vent shrimp species and non-vent deep-sea species [11]. Furthermore, *P. varians* and hydrothermal vent shrimp inhabit similar temperature regimes and demonstrate similar acute temperature tolerance at native hydrostatic pressures [12-14]. Sublethal responses to dissolved copper in *P. varians* and *R*. *exoculata* at native hydrostatic pressure (0.1 MPa and 30.0 MPa respectively; 10.0 MPa ≈ 1000 m depth) and at a common temperature (10°C) appear consistent, suggesting that shallow-water species that are adapted to habitats with similar environmental variability may be suitable ecotoxicological proxies for deep-sea species [8]. Hydrothermal vent taxa, including shrimp, are considered to be at high risk from SMS mining [3]. Therefore, the aim of the present study was to determine the toxicity of mineral phase chalcopyrite, a major component of SMS deposits [3], to *P*. *varians* at native hydrostatic pressure (0.1 MPa) and ecologically relevant temperature (10°C), as a proxy for deep-sea hydrothermal vent shrimp species.

Although acute mortality provides an indication of survival in the short-term, it is the bioenergetic consequences of sublethal metal exposures that appear critical to the long-term persistence of individuals, populations, and species [15]. Increased basal metabolic demand by the cellular protective mechanisms that respond to metal exposure (e.g. antioxidant, metallothionein, glutathione, and molecular chaperone expression) is met by increased energy allocation to basal maintenance, which comprises key cellular processes and essential systemic activities such as ventilation and circulation [15]. Increased allocation of energy to maintenance during stress diminishes energy available for other functions, and consequently reduces scope for growth, activity and reproductive output, and therefore individual fitness is reduced as the additional homeostatic energy costs lead to energetic trade-offs [15]. Decreased sequestration of energy as reserves and/or reallocation of energy reserves to maintenance decreases capacity to buffer fluctuating food availability and ensure continuous metabolic energy supply [15]. This may crucially affect survival in the deep-sea, where energy constraints are typically high [16], and increasing with climate change [17]. Consequently, both lethal response and sublethal respiratory response to chalcopyrite exposure were assessed.

# Methods

Sampling and experimentation was based on adaptation of established protocols [8]. In brief, hand nets were used to collect adult specimens of *Palaemon varians* (4 to 5 cm in total length) from Lymington salt marshes (Hampshire, England: 50°45’N, 1°32’W). Shrimp were transported to the National Oceanography Centre Southampton (NOCS) in 10 litre buckets containing water from the sampling location, where the shrimp were transferred to a 185 litre flow-through holding aquarium (salinity ~32, temperature ~15°C, light:dark cycling following seasonal changes, fed to excess three times per week with Tetra Goldfish flakes). To minimise the potential for influences of seasonal temperature acclimation, shrimp were maintained for 4 to 8 weeks before experimental treatment: temperature acclimation predominantly occurs within 4 weeks in *P*. *varians* [18]. Seven days prior to experimental treatments animals were transferred to 10 litre PVC plastic tanks filled with continuously aerated artificial seawater (salinity 32) acclimated to 15°C using a water bath (controlled by a Haake EK20 chiller and a Haake DC10 heater) (12:12 photoperiod). Temperature was shifted to 10°C over ~5 hours. 50% water changes were conducted three times per week using artificial seawater with salinity 32 at 10°C. Potential for effects of differences in digestive state on sensitivity to toxicants were reduced by starving shrimp for 3 days prior to experimental treatments.

Static 96-h copper LC50 (concentration lethal to 50% of test individuals) was assessed with individuals exposed to artificial seawater or artificial seawater spiked with monomineralic chalcopyrite (CuFeS2). Chalcopyrite was presented as fine sand (<250 µm grain size) to simulate challenge anticipated during SMS mining. Exposure concentrations were selected based on lethal copper toxicity in *P*. *varians* at 10°C and 0.1 MPa (96-h LC50 = 26.9 mg l-1) [13]. Chalcopyrite additions were 0, 2.9, 28.9, 288.8, and 2887.8 mg l-1, representing additions of 0, 1, 10, 100, 1000 mg Cu l-1). Ten individuals were exposed in each treatment and treatments were performed in triplicate (total n per exposure concentration = 30). Mortality was assessed every 24 hours and no mortalities were observed in control treatments. Oxygen saturation was determined every 24 hours using an oxygen micro-optode connected to a PreSens Microx TX3 array and calibrated according to manufacturer’s instructions, and did not decrease below 70% in any treatment reducing the potential for influences of hypoxia [19].

The respiratory response to chalcopyrite was assessed by measuring oxygen consumption rates using an adaptation of established protocols [8]. In brief, 5 individuals exposed to 0, 2.9, 28.9, 288.8, and 2887.8 mg l-1 chalcopyrite additions for 96 hours as described previously, were subsequently transferred to 33 ml plastic vials filled with water from their incubation. To ensure the absence of air bubbles, vials were closed underwater. Each vial was then placed inside a temperature-acclimated hydrostatic pressure vessel filled with freshwater previously incubated at 10°C. To maintain experimental temperature, hydrostatic pressure vessels were placed within LMS model 230 series 2 cooled incubators. Animals were isolated for 45 minutes. Oxygen concentration within the vial did not fall below 70% oxygen saturation, reducing the potential for hypoxic metabolic influences in *P*. *varians* [19]. To control for microbial respiration within the seawater, 5 control vials containing only seawater from the 96-hour incubation were isolated for each treatment using an identical procedure.

Each vial was removed from the hydrostatic pressure vessel following the isolation period and inverted three times to ensure homogeneity of seawater oxygen within the vial. Oxygen saturation of the seawater was determined using an oxygen micro-optode connected to a PreSens Microx TX3 array, calibrated according to the manufacturer’s instructions, immediately after removal of the vial lid. Each animal was then removed from the vial, gently blotted on tissue paper, transferred to a 1.5 ml centrifuge tube, and flash frozen whole in liquid nitrogen. Samples were stored at -80°C for subsequent biomass analysis: shrimp total wet mass was 189.3 ± 25.3 mg (mean ± SD).

The difference between the oxygen saturation in the control vial and the oxygen saturation in the treatment vials was used to calculate molar oxygen consumption (MO2, µmol O2 mg-1 h-1), based on established methods for determining oxygen concentration in air-saturated seawater [20]. The respiratory response to chalcopyrite was analysed using one-way ANOVA; data were normal (Shapiro-Wilk test) and homoscedastic (Levene’s test) (p > 0.05).

# Results and Discussion

No exposure treatment resulted in mortality or significantly affected respiration rate in *Palaemon varians* (Figure 1) (F4,20 = 0.098, p = 0.982). Thus, exposure to 2887.8 mg l-1 chalcopyrite (<250 µm grain size) addition, equivalent to a copper concentration ~37 times greater than the acute lethal threshold for dissolved copper, did not result in mortality in *P*. *varians* [8]. Similarly, respiration rate in *P*. *varians* was not significantly affected by exposure to 2887.8 mg l-1 chalcopyrite (<250 µm grain size) addition, equivalent to a copper concentration 1000 times greater than that eliciting a respiratory response to dissolved copper [8]. These results are consistent with other available data on the relative toxicity of dissolved copper and mineralic metal phases, indicating greater bioavailability in dissolved form than in mineralic form, but the magnitude of the difference in bioavailability is unprecedented [6]. This disparity suggests that chalcopyrite may not be bioavailable to *P*. *varians*, and by extension to deep-sea shrimp species. However, the methodological approaches demand caution; the significant potential for bioavailability through dietary exposure [6] was not assessed. Nonetheless, the contrast in acute toxicity from exposure to ground chalcopyrite and from dissolved copper in a shallow-water ecotoxicological proxy for deep-sea hydrothermal vent shrimp has important implications for assessment of SMS mining risks.

Although exposure to greater chalcopyrite concentrations with smaller grain size than employed in this study may occur during SMS extraction and dewatering and may result in acute toxicity, deep-sea species appear at greater ecotoxicological risk from dissolved metals, which are significantly more bioavailable, despite slow oxidative dissolution of reduced sulphide mineralic phases that will be exposed during SMS mining [5]. Nonetheless, integrating modelling approaches exploring the concentrations of dissolved metals released during SMS mining, and spatial and temporal dilution of dissolved metals concentrations, with approaches exploring sublethal effects on shallow-water ecotoxicological proxies, may best constrain the potential ecotoxicological impacts of SMS mining. Such an approach may provide thresholds for environmentally acceptable concentrations of dissolved metals, which can be monitored during active SMS extraction.

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AB and CH conceived the study. AB collected and analysed the data. AB wrote the manuscript with input from CH. All authors approved the final article. The authors declare no competing interests.

# Disclosure Statement

The authors declare no competing interests.

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**Figure Legends**

**Figure 1.** Respiratory response (mean ± SD; n = 5) in *Palaemon varians* to acute (96 h) exposure to copper presented as chalcopyrite (<250 µm grain size).

**Word Count**

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