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Comparative sensitivity of aquatic invertebrate and vertebrate species to wastewater from an operational coal mine in central Queensland, Australia

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ABSTRACT

Coal excavation and refinement processes generate substantial volumes of contaminated effluent that may be detrimental to aquatic ecosystems. As such, understanding the impacts of coal mine water releases on aquatic animals and ecosystems is essential for effectively managing and protecting neighboring environments. Such information will ultimately be applied towards developing ongoing monitoring strategies that are protective of native wildlife. Despite intensive mining operations in Australia, few studies have documented toxicity associated with coal mine wastewater (CMW) on native species. To address existing knowledge gaps, we investigated acute toxicity (48–96 h) using eight native invertebrate species and sub-chronic effects (2 week) using three vertebrate species following exposure to wastewater from two dams (CMW1 and CMW2) located at an open-cut coal mine licensed to discharge into the Fitzroy catchment (Queensland, Australia). Wastewater from these sites is characterized by elevated conductivity, pH, sulfates as well as relatively high total and dissolved metal(loid)s (including As, Al, B, Cu, Mn, Ni, Se and Zn). Acute exposures revealed cladocerans (*Daphnia carinata*) and planarians (*Dugesia* sp.) to be the most sensitive species, exhibiting significant mortality after 48 and 96 h exposure to CMW2, respectively. Neither wastewater was found to elicit acute toxicity in vertebrates, but a range of sub-lethal morphological effects were observed following the sub-chronic exposures. The overall response pattern was characterized by decreased condition factor and hepatosomatic index in the fish *Hypseleotris compressa* and *Pseudomugil signifier,* and in *Limnodynastes peronii* tadpoles. Tadpoles were generally more sensitive compared to the two fish species. Differences in responses were observed amongst CMW1 and CMW2, which likely relates to differences in physico-chemical properties between sites. Our results have identified several candidate vertebrate and invertebrate species that show promise for ongoing monitoring of water quality and toxicity risk in Central Queensland, Australia.

1. Introduction

Coal mining is an important industry globally, and particularly so in Australia, where coal exportation represents a significant part of the country's economy (Höök et al., 2010; IEA, 2012). Black coal is mined predominantly in Queensland, with over 50 mines currently producing approximately 200 million tons of saleable coal annually (DNRM, 2013). However, despite the economic benefits, coal mining presents a number of major environmental issues including landscape alteration, air pollution and aquatic contamination (Bian et al., 2010). One of the foremost environmental concerns related to the daily operations of a coal mine is the production of substantial volumes of process-affected water that may be discharged into adjacent aquatic ecosystems. The extraction and processing of coal require a constant supply of water (Scott et al., 2010; Haibin and Zhenling, 2010; Thiruvenkatachari et al., 2011), and despite regulated releases of mine wastewater, uncontrolled releases are not uncommon during extreme rainfall events and flooding. These discharges can be highly saline and/or acidic, and may often contain high levels of dissolved solids, suspended solids, metal(loid)s (*e.g.*, Al, As, Cd, Cu, Mn, Ni, Fe, Se, Zn), hydrocarbons (*e.g.*, polycyclic aromatic hydrocarbons), and other

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compounds (Tiwary, 2001; Thiruvenkatachari et al., 2011), which have all been shown to have detrimental impacts on aquatic biota (Birge 1978; Eisler, 1987; Atchison et al., 1987; Nielsen et al., 2003; Muschal, 2006; Langdon et al., 2009; Hogsden and Harding, 2012; Cañedo-Argüelles et al., 2013; DeForest and Meyer, 2015). It is therefore important that both the volume and the quality of coal mine wastewater being discharged into the environment be effectively managed.

Rapid industrialization in Australia over the last century has put pressure on many of its limited freshwater systems, and has subsequently put native species at risk. This holds particularly true for the Fitzroy Catchment in Central Queensland, which coincides with Australia's largest black coal deposit in the Bowen Basin. Environmental research in this region has largely focused on monitoring water quality and have provided extensive data on flows, sediment loads, physical and chemical water quality characteristics, and assemblages of aquatic flora and fauna (*e.g.*, Noble et al., 1996a, 1996b; DEH and DNR, 1999; DERM, 2001; FAB, 2008, 2009; DERM, 2009, 2010, 2011a, 2011b). In recent years, there has been considerable focus directed towards understanding the risks associated with elevated salinity from coal mine water discharge on streams in the Fitzroy Catchment (Vink et al., 2009; Vink and Robbins, 2012). However, despite these research efforts, there have been few studies directed at understanding the potential for organism-level toxicological effects of coal mine discharge on locally relevant aquatic species (Hart et al., 2008). There have been almost no studies investigating chronic toxicological effects related to coal mine discharge on native aquatic vertebrates. A cumulative impact assessment released by the then Queensland Department of Environment and Resource Management recently established that mining discharge into the Fitzroy Basin catchment is currently not protective of the downstream environment, and that data is extremely limited and inadequate for quantifying cumulative impacts (DERM, 2009). Similar conclusions have been raised globally (Bernhardt and Palmer, 2011; US EPA, 2011; Bernhardt et al., 2012; Bharti and Banerjee, 2014), highlighting the need to explore the potential for adverse toxicological outcomes in relevant aquatic invertebrate and vertebrate species exposed to coal mine wastewater.

Whole effluent toxicity tests have been widely applied to assess the potential toxicity of complex effluents to aquatic organisms, and to monitor industrial and municipal water discharge (US EPA, 2000; Chapman, 2000; van Dam and Chapman, 2001). Given the significance of coal mining operations in Australia and the relatively limited whole organism toxicity information that exists for the Fitzroy River Basin, there is a clear need for such testing with a range of native aquatic organisms. This is not only necessary to fill basic knowledge gaps regarding the potential for adverse effects in exposed animals, but to facilitate effective ongoing monitoring of water quality in aquatic systems receiving mine water discharge, through the identification of comparatively sensitive species. We therefore investigated the impacts of wastewater from a Queensland open-cut coal mine on a range of native aquatic organisms. Acute toxicity tests were used to compare the sensitivity of eight native freshwater invertebrates and three aquatic vertebrate species to various dilutions of wastewater. Sub-lethal impacts were considered in tests with vertebrates, including effects on growth, development and hepatic condition of two species of fish and one species of larval amphibian. The study aims to provide basic acute and sub-lethal toxicity information for a range of relevant species, to determine the relative sensitivities of the different organisms and help develop an effective monitoring suite directly relevant for assessing toxicity associated with the release of coal mine wastewater in the Fitzroy Catchment.

2. Materials and methods

2.1. Animals

Broad sampling was performed at uncontaminated sites in Central Queensland, Australia, to collect various species of aquatic organisms from the local environment (Average water quality parameters at the time of sampling were: temperature: 24 ± 2.3 °C, conductivity: 1.0 ± 0.2 mS cm^{-1}, pH: 8.5 ± 0.4 and dissolved oxygen: 89 ± 19%). Species were selected for their relevance to the coal mining region within the Bowen Basin. For vertebrates, empire gudgeons (*Hypseleotris compressa*) and pacific blue-eyes (*Pseudomugil signifer*) fish (standard length ~2 cm), as well as a fertilized striped marsh frog (*Limnodynastes peronii*) egg mass were collected. A range of invertebrate species was also collected, and sorted by species and size classes in the laboratory. Sufficient numbers were obtained for replicated toxicity testing with eight invertebrate species, including *Paratya australiensis* (Decapoda: Atyidae, 10–14 mm), *Chironomus* sp. larvae (Diptera: Chironomidae, 7–10 mm), Leptophepiid nymphs (Ephemeroptera: Leptophlebiidae, 3–9 mm), Coenagrionid larvae (Odonata: Coenagrionidae, 12–19 mm), *Dugesia* sp. (Tricladida:Dugesiidae), Erpobdellid (Arhynchobdellida: Erpobdellidae), *Sphaerium* sp. (Bivalvia: Sphaeriidae, 2–5 mm) and *Daphnia carinata* (Cladocera: Daphniidae, <48 h old). *L. peronii* eggs were hatched in the laboratory in natural pond water, which was slowly replaced by filtered rainwater. All organisms were acclimatized to laboratory conditions in aerated glass aquaria filled with filtered rainwater (Invertebrates >48 h; Fish and tadpoles >2 weeks) prior to testing. During this time, fish and tadpoles were fed *ad libitum* daily with flake and pellet foods (Ocean Nutrition™) or Sera Micron® powered food, respectively. Tadpoles of Gosner developmental stage (Gs; (Gosner, 1960)) 27 were used for experiments. All aspects of experimentation and sampling were approved by the Animal Ethics Committee of Central Queensland University and in accordance with the guidelines of the Australian Code for the Care and Use of Animals for Scientific Purposes (CQUniversity Approval No. A13/05-301).

2.2. Wastewater sampling and water quality

Wastewater was collected from an open-cut coal mine located within the Bowen Basin in Central Queensland, Australia (approximately 250 km west of the coast), in August 2013. At the time of experimentation, the mine had two active release points authorized for discharge of mine-affected water, hereafter referred to as CMW1 and CMW2 dams. *In situ* measurements of temperature, electrical conductivity (EC), pH, dissolved oxygen (DO) and turbidity were taken at the time of sampling using a YSI multiparameter handheld sonde (Xylem Analytics, Hemnant, Australia). Wastewater was collected for experiments in acid washed 5 L plastic containers. Wastewater was transported on ice, stored in the refrigerator at 4 °C, and brought to ambient temperature prior to experimentation (~ 48 h post collection). Wastewater was filtered using small mesh to remove any large particles for invertebrate tests. Water quality parameters (temperature, EC, pH and dissolved oxygen) were measured for each treatment group using YSI EcoSence® probes at start and end of each acute test, and before and after each water renewal in the chronic exposures.

2.3. Acute toxicity in invertebrates, fish and tadpoles

For acute toxicity tests, all organisms were exposed to wastewater dilutions from the two holding dams (CMW1 and CMW2), ranging from 0% to 100% for 96 h, with the exception of *Daphnia carinata,* which was exposed for 48 h (based on ASTM (2002) and