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PRIMARY RESEARCH PAPER

Impact of mining and industrial pollution on stream macroinvertebrates: importance of taxonomic resolution, water geochemistry and EPT indices for impact detection

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Abstract This study investigated freshwater macroinvertebrate communities in waterways contaminated by active and abandoned mining and industrial activities in order to ascertain any impact on freshwater ecosystems. We compared macroinvertebrate communities at the species, family and order levels of taxonomic resolution. We also collected water samples to compare ionic composition and metal concentrations from waste-affected and reference (non-affected) sites. In addition to assessing ecological impairment, the study also sought to determine whether the degree of sensitivity in detecting any impairment varied according to the taxonomic level of identification used. We calculated the biotic indices of EPT richness and taxonomic richness at the species, family and order levels, and performed multivariate analyses to measure differences in community structure at all three levels. We found significant differences in both biotic indices and macroinvertebrate community structure at each taxonomic level, indicating ecological impairment at waste-affected sites. We also concluded that the most appropriate taxonomic level for evaluating macroinvertebrates depends on the information required. In this study, the family level provided the clearest assessment of ecological impairment at waterways affected by mining and/or industrial wastes, and order-level data provided only a marginally less sensitive measure of this impairment.

Keywords Water quality · Heavy metals · Reference sites · Ecosystem health · Taxonomic sufficiency

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Introduction

Human activities can have long-term adverse impacts on waterways and freshwater ecosystems. Mining and industrial activities are particularly problematic as they can generate environmentally hazardous waste materials during their operation, the impacts of which can persist long after the activities have ceased (Johnson, 2003; Younger, 2004). Mining and industrial activities are often located in close proximity because the extracted ore is an important industrial raw material. Assessment of any consequent disturbance to surrounding waterways is often achieved through the collection of freshwater macroinvertebrates, which provide a measure of the health of the river ecosystem (Winner et al., 1975; Norris et al., 1982; Rosenberg & Resh, 1993; Malmqvist & Hoffsten, 1999; Sloane & Norris, 2003; Wright & Burgin, 2009a, b).

When using macroinvertebrates to perform such assessments, it is important to determine the taxonomic level of identification that is adequately sensitive to detect any impacts from pollution. This topic has generated considerable debate in the literature (Bailey et al., 2001), and the term *taxonomic sufficiency* has been introduced by Ellis (1985). *Taxonomic sufficiency* refers to the level of identification needed to detect dissimilar community composition in polluted waters (Ellis, 1985). The use of higher taxonomic levels has attracted criticism, and many authors support species-level identification (Resh & Unzicker, 1975; Cranston, 1990). Cranston (1990) argued for the importance of species identification in biological monitoring but recognised that major impediments are that neither appropriate taxonomic expertise nor keys for species identification are always readily available. The consensus view is that species-level identification is regarded as the industry's ideal (Lenat & Resh, 2001), although in some cases, coarser taxonomic identification (family, order and phylum) has proven sufficiently sensitive to determine ecological impairment (e.g. Warwick, 1988; Wright et al., 1995; Hewlett, 2000).

A popular biotic index for assessment of fresh water pollution is the EPT index, which represents the relative abundance of the pollution-sensitive Ephemeroptera, Plecoptera and Trichoptera (EPT) orders (Rosenberg & Resh, 1993). Many investigations have calculated the relative abundance and/or taxonomic richness of the EPT orders in order to assess the impacts of waterway pollution (Rosenberg & Resh, 1993; Lenat & Penrose, 1996; Pond et al., 2008). A limitation, however, in the use of EPT indices in pollution assessments has been identified, as some species in these orders have reported tolerance to metal pollution (Gray & Harding, 2012). Species belonging to several families of Trichoptera, for example, have been collected in waterways with elevated concentrations of metals (Norris et al., 1982; Gower et al., 1994; Clements & Kiffney, 1995; Hickey & Clements, 1998; Winterbourn, 1998; Wright & Burgin, 2009a).

Some researchers have attempted to develop more robust biotic indices that account for the variable response of invertebrate taxa to water pollution from mining wastes. They developed their own biotic index, which reflected both the abundance and taxonomic richness of invertebrates (e.g. Gray & Delaney, 2010). A New Zealand investigation of the ecological impacts of coal mine wastes (Gray & Harding, 2012) also developed a similar, acid mine drainage (AMD) index quantifying EPT and total macroinvertebrate richness, which effectively provided a reasonable estimate of ecological impact. One study in West Virginia (USA) employed an extensive sampling area incorporating multiple waterways affected by mountaintop coal mining (Pond et al., 2008). The authors concluded that most biotic indices were sufficiently sensitive to ecological impairment to enable discrimination between waste-impacted and non-impacted sites (Pond et al., 2008).

One of the many difficulties faced when investigating the ecological impact of pollution when there are numerous individual sources and types of pollution is accounting for the multiple possibilities (Clements et al., 2000). This makes it difficult to control for the natural degree of variation in ecosystems and other factors particular to the environmental setting of each waste discharge and receiving waterway. Furthermore, variations in the significance and nature of these sources may represent a considerable challenge when accounting for the composition and strength of the pollutants themselves. This is a particular problem when a group of waste discharge sources are responsible (e.g. Pond et al., 2008). One way of addressing these issues is to sample from multiple reference sites, as exemplified by Fairweather (1990), along with multiple impacted sites (Bartram & Balance, 1996; Clements et al., 2000).

We investigated the relationship between macroinvertebrate communities at different levels of taxonomic resolution and a range of different sources of mining and industrial waste (MIW) within a defined region. Given that the choice of taxonomic resolution has major research and cost implications, this study focused on elucidating the taxonomic level and type of biotic index most appropriate to determine whether a waterway affected by waste discharge is adversely impacted.

Specifically, the questions we addressed in this study were

(i) Do freshwater macroinvertebrates respond differently to different types of MIW discharges within a region? (ii) What is the relative sensitivity of the biotic indices EPT and taxonomic richness to a variety of MIW discharges? (iii) How critical is the factor of taxonomic resolution when detecting ecological impairment at MIW-affected sites?

Methods

Study area and sampling sites

The study was conducted in the western Blue Mountains, in south eastern Australia (Fig. 1). The sampling area covers an area of approximately 2400 km^2 (33°20′–33°45′S, 149°56′–150°16′E) and is situated about 120–180 km west of the Central Business District of Sydney. This area straddles the Great Dividing Range, with the majority of the study area falling in the easterly flowing Hawkesbury-Nepean catchment, and a smaller proportion in waterways flowing to the north-west into Australia's largest catchment, the Murray-Darling river system.

There is a particular concern about water pollution in the largest river in the area (Coxs River) from mining and industrial activity in the Lithgow area as it is part of the water catchment for Sydney's main drinking water supply (Warragamba Dam—Lake Burragorang) (Birch et al., 2001). The pioneering study by Jolly & Chapman (1966) used macroinvertebrates as part of a biological investigation of water pollution in the study area (Lithgow, NSW) and examined the impact of industry, urban land and sewage effluent on water quality and freshwater ecosystems. Mining and industry have co-existed in the Lithgow area in the western Blue Mountains for nearly 150 years (Cremin, 1989).

Macroinvertebrates were collected from 16 sampling sites on 12 small to medium-sized upland waterways in the western Blue Mountains area (Fig. 1; Table 1) in the austral summer from January to March 2009. Catchments of the study area ranged from undisturbed and naturally vegetated areas to highly modified landscapes. Much of the area in the east of the study area is protected as Blue Mountains National Park, with the international significance of

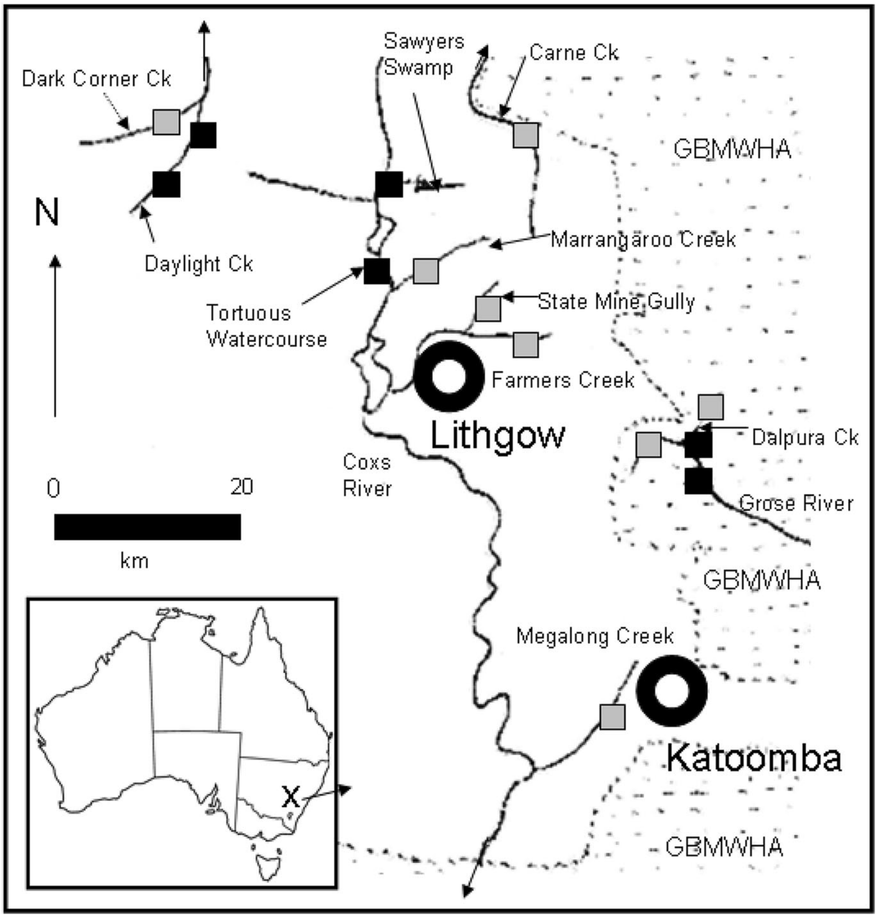

Fig. 1 Location of major waterways and study sites in the western Blue Mountains area. *Black rectangles* indicate sites below mining or industrial waste sources, and *unshaded rectangles* indicate reference localities. Major settlements in the area (Katoomba and Lithgow) are included. The Greater Blue Mountains World Heritage Area (GBMWHA) is shown. Two sampling sites on the Coxs River are obscured by the nearby site located on the Tortuous Watercourse. *Inset* indicates study area location in south eastern Australia

the area's natural environment recognised through its UNESCO listing as Greater Blue Mountains World Heritage Area (UNESCO, 2009). The sources of industrial and mining waste discharges are complex and reflect a wide spectrum of previous and current human land use activities. The individual waste discharges include the Sunny Corner Mining area. The derelict Sunny Corner mining area includes many abandoned gold mines, and associated smelting ruins from a heavy-metal mining and processing area that closed in the early 20th century (Napier, 1992). Another sampling site was a waterway receiving waste water from a coal-powered electricity power station (Wallerawang Power Station) (Graham & Wright, 2012). Two sampling sites were downstream of a waste discharge from a disused underground coal mine (Canyon Coal Mine) in an otherwise unpolluted catchment (Wright & Burgin, 2009a, b; Wright et al., 2011). The largest single waterway in the area, Coxs River, is subject to multiple waste impacts from current and historic coal mines and industrial sites (Birch et al., 2001).

The area has a cool, temperate climate with annual daily minimum and maximum temperatures ranging from 0.7 °C to 25.5 °C (BoM, 2014). Mean annual rainfall is heavier in the east (annual mean of 1400 mm at Katoomba) than in the west (annual mean of 858.5 mm at Lithgow) (BoM, 2014).

Macroinvertebrate sampling

Each site was sampled on a single occasion, with duplicate macroinvertebrate samples being collected from random locations within riffle zones. The riffle zone was chosen as it was a widely available habitat that was present at each waterway and sampling site.

Sampling was undertaken at least 1 week after any significant rainfall event (5 mm/day). Macroinvertebrate samples were collected according to the Australian National River Health Program protocols (DEST et al., 1994; Chessman, 1995). This was a rapid biological assessment method involving collection using a kick net with a 250 µm mesh and a square 30 × 30 cm net frame (Chessman, 1995). About 10 min was spent disturbing the benthos in a 15–20 m reach of waterway, along a riffle zone. The first of the duplicate samples was collected downstream of the other. The net was swept through the water column and sediment on the stream bed as kick sampling disturbed and dislodged rocks, sand and sediment in the riffle benthos. The mosaic of sub-habitats swept by the net was randomised by the collector to maximise the diversity of substrates, depths and current speed. Invertebrates and associated detritus (such as leaves, algae and sand) were emptied from the sampling net into a sorting tray and were live picked in the field for 30 min (as per methods described in DEST et al., 1994; Chessman, 1995). In the laboratory, animals were identified to species level, where possible, using reference specimens and a broad range of the latest Australian taxonomic keys (Hawking, 2000; MDFRC, 2013). Numbers of individuals in each species were recorded.

Water sampling

Water quality data were collected from each sampling site on the same day as macroinvertebrate sampling. Water samples were collected just before the macroinvertebrates to minimise possible disturbance due to kick sampling. At each site, sampling was conducted in the middle of a flowing stretch of stream using a field chemistry meter (Yeo-Kal 611 Meter, Warringah, Australia) to measure electrical conductivity, pH, dissolved oxygen, turbidity and water temperature. Water samples were also collected in clean 200-ml plastic bottles from the centre of the stream for later laboratory analysis. These samples were refrigerated in the field and analysed by a commercial laboratory using quality control procedures consistent with standard chemical analysis methods (APHA, 1998). Chemical analysis measured concentrations of heavy metals and major anions and cations (carbonate, bicarbonate, potassium, sodium, magnesium, calcium, chloride and sulphate). Samples were assessed for the metals commonly associated with acid mine drainage (AMD) including arsenic, cadmium, chromium, copper, lead, nickel and zinc (e.g. Norris et al., 1982; Mackey, 1988; Napier, 1992; Malmqvist & Hoffsten, 1999).

Biotic indices and data analysis

Student's *t* test was used to test for differences (for all water chemical attributes and macroinvertebrate biotic indices) between reference and MIW-affected sites. In the current study, two macroinvertebrate biotic indices were calculated for each macroinvertebrate sample at

Table 1 Summary information for each of the sampling sites used in this study including name of the waterway and sampling site, site latitude and longitude, width of the waterway, average depth of the waterway, altitude in m above sea level (ASL)

Site name	Latitude, longitude	Width	Average depth (m)	Altitude (m ASL)	Contamination
Dalpura Creek us/mine discharge	33° 32.5′S, 150° 18.3′E	1.0–2.0	0.45	860	Reference
Grose River above Jinki Creek	33° 32.6′S, 150° 18′E	2.0–4.0	0.65	605	Reference
Megalong Creek	33° 43.8′S, 150° 15.2′E	2.0–4.0	0.55	565	Reference
Carne Creek	33° 16.8′S, 150° 12.2′E	2.0–4.0	0.40	575	Reference
Marrangaroo Creek	33° 26.4′ S, 150° 6.8′E	2.0–4.0	0.50	905	Reference
Farmers Creek	33° 31.8′S, 150° 11.5′E	1.0–2.0	0.35	960	Reference
Dark Corner Creek	33° 18.7′S, 149° 56.1′E	1.0–2.0	0.45	870	Reference
State Mine Gully Creek	33° 27′S, 150° 10.5′E	1.0–2.0	0.30	955	Reference
Daylight Creek 1 km downstream mine	33° 21′S, 149° 54′E	1.0	0.45	980	Heavy-metal mining and smelting area from disused mines (Napier, 1992)
Daylight Creek 8 km downstream mine	33° 18.7′S, 149° 56.2′E	1.0–2.0	0.55	870	Heavy-metal mining and smelting area from disused mines (Napier, 1992)
Coxs River below Lake Wallace	33° 25.7′S, 150° 4.7′E	2.0–4.0	0.60	860	Coal mine drainage from disused and active mines, coal-ash drainage and power station cooling water (Graham & Wright, 2012).
Tortuous Watercourse	33° 25.3′S, 150° 4.9′E	1.0	0.40	865	Artificial water course containing power station cooling and waste water (Graham & Wright, 2012)
Coxs River below Tortuous power station cooling water	33° 25.5′S, 150° 4.8′E	2.0–4.0	0.70	855	Coal mine drainage from disused active mines, coal-ash drainage and power station cooling water (Graham & Wright, 2012).
Sawyers Swamp Creek	33° 22.7′S, 150° 5.3′E	1.0	0.35	870	Below coal-ash settlement dam and coal mine
Grose River below Dalpura	33° 32.9′S, 150° 18.2′E	2.0–4.0	0.70	585	Coal mine drainage from disused underground mine (Wright & Burgin, 2009a)
Dalpura Creek below mine discharge	33° 32.5′S, 150° 18.3′E	1.0	0.55	830	Coal mine drainage from disused underground mine (Wright & Burgin, 2009a)

The type of mining or industrial contamination in the catchment of each sampling site is provided. If no source of contamination is present, then the site is classified as ‘reference’

the species, family and order taxonomic level: EPT taxonomic richness (Lenat & Penrose, 1996) and overall taxonomic richness (Rosenberg & Resh, 1993).

Non-metric multidimensional scaling (NMDS) was performed on the similarity matrix, computed with macroinvertebrate taxon abundance data (4th root transformed), using the Bray–Curtis dissimilarity measure (Clarke, 1993; Warwick, 1993). Two-dimensional ordination plots represented the dissimilarity among samples. Ecological differences between the two groups were tested by one-way analysis of similarity (ANOSIM; Clarke, 1993) between reference and MIW-affected sites. ANOSIM analysis produced a ‘global R value’ which ranges from 1.0 signifying that communities have a completely dissimilar composition to 0, which indicates that communities have an identical composition

(Clarke, 1993). In the ordinations, the influence of particular taxa on dissimilarities between communities was quantified using the similarity percentage procedure (SIMPER). The BIOENV procedure (Clarke & Ainsworth, 1993) was used to evaluate which water quality variables were most highly correlated with the variation in the macroinvertebrate community data. These multivariate analyses were achieved using the software package PRIMER version 5 (Clarke, 1993).

Results

Water chemistry

The effects of mining and industrial waste (MIW) discharges on the water chemistry of streams in this study (Table 2) were clearly apparent, when compared with streams not receiving MIW (reference sites). Electrical conductivity was significantly higher at MIW waterways with median electrical conductivity

Table 2 Differences between mining and industrial waste (MIW)-affected sites and reference sites using macroinvertebrate and water chemistry data

	MIW affected			Reference			*t* value (*p*)
	Mean	SE	Range	Mean	SE	Range	
Macroinvertebrates							
Species Richness (whole community)	14.7	1.8	4–26	24.0	2.7	8–43	2.8 (*)
Species Richness (EPT)	3.9	0.9	1–13	11.9	1.7	3–26	4.1 (**)
Family Richness (whole community)	10.8	1.2	3–19	15.0	1.3	8–24	2.3 (*)
Family Richness (EPT)	3.0	0.6	1–8	6.7	0.8	2–13	3.6 (**)
Order Richness (whole community)	6.1	0.5	3–9	7.8	0.3	6–10	2.9 (*)
Order Richness (EPT)	1.8	0.2	1–3	2.9	0.1	2–3	4.9 (***)
Water chemistry							
pH (pH units)	6.6	0.5	3.7–8.0	6.1	0.2	4.9–6.7	0.78 (ns)
Electrical cond. (μS cm^{-1})	745	220	123–1710	57.6	14.6	17–127	5.1 (**)
Dissolved oxygen (% sat.)	64.5	5.1	39–81.3	66.2	8.0	8.9–110	0.03 (ns)
Turbidity (NTU)	18.4	2.0	13.1–29.6	16.5	0.9	13.3–21.6	0.71 (ns)
Bicarbonate (mg l^{-1})	143	64.9	1–535	13.9	7.2	bd–63	1.97 (*)
Sulphate (mg l^{-1})	189.9	68.7	29–579	8.1	4.7	1–40	5.97 (***)
Chloride (mg l^{-1})	18.5	6.6	4–52	6.6	1.8	2–18	1.82 (*)
Calcium (mg l^{-1})	18.8	5.3	4–44	3.9	1.5	bd–11	3.49 (**)
Magnesium (mg l^{-1})	12.2	2.8	3–26	1.5	0.6	bd–5	5.6 (***)
Sodium (mg l^{-1})	113.6	46.4	3–312	4.8	0.9	2–9	2.37 (*)
Potassium (mg l^{-1})	11.4	4.0	2–31	0.75	0.2	bd–2	4.75 (**)
Zinc (μg l^{-1})	4849	4020	bd–32,600	4.4	1.3	bd–12	3.06 (*)
Nickel (μg l^{-1})	69.9	35.9	4–273	1.1	0.3	bd–3	5.95 (**)
Copper (μg l^{-1})	200.3	174.6	bd–1420	0.6	0.1	bd–1	2.44 (*)
Arsenic (μg l^{-1})	3.0	1.1	bd–9	0.6	0.06	bd–1	2.71 (*)
Cadmium (μg l^{-1})	18.8	15.6	bd–1 26	–		bd	1.89 (ns)
Chromium (μg l^{-1})	0.7	0.1	bd–1	–		bd	2.05 (ns)
Lead (μg l^{-1})	174.4	170	bd–1370	–		bd	1.51 (ns)

Summary statistics: mean, standard error (SE) and range are also provided. *T* test results (*t* value and *P*) are given for differences between MIW and reference sites. Macroinvertebrate data were tested for the whole community (and also using EPT groups) at the species, family and order levels of taxonomic resolution

ns not significant, *bd* below detection

* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

more than 14 times higher at MIW streams (608.5 μS cm^{-1}) than reference streams (43.5 μS cm^{-1}; Table 2). The concentrations of all major anions and cations were also significantly more elevated at MIW-affected streams than at reference streams. The mean sulphate concentration at MIW streams (189.9 mg l^{-1}) was more than 23 times higher than that (8.1 mg l^{-1}) recorded at reference waterways. The concentrations of potassium and bicarbonate were much higher at MIW waterways, with the mean concentrations of potassium (11.4 mg l^{-1}) and bicarbonate (143.0 mg l^{-1}) more than 10 times higher at MIW sites than at reference sites (potassium: 0.75 mg l^{-1}; bicarbonate: 13.9 mg l^{-1}; Table 2).

Metal concentrations were also much higher at MIW-affected sites, but there was considerable variation in these values (Table 2). Metal concentrations at reference sites were often below detection limits. Nickel and zinc had significantly higher concentrations at MIW waterways compared to reference waterways. Nickel was the only metal found at detectable levels at all MIW sites (ranging from 4 to 273 μg l^{-1}), with zinc detected in all but one (ranging from 8 to 32.6 mg l^{-1}; Table 2). The most severe heavy-metal water pollution in this study was encountered at Daylight Creek, below the abandoned Sunny Corner Mining and smelting area. This waterway had the highest concentrations of cadmium (12.6 μg l^{-1}), copper (1.42 mg l^{-1}), lead (1.37 mg l^{-1}) and zinc (32.6 mg l^{-1}) and exceeded concentrations known to be hazardous for aquatic ecosystems at both sites sampled on Daylight Creek (ANZECC, 2000) often by hundreds of times (Table 2).

Macroinvertebrates

We collected and identified a total of 2051 aquatic macroinvertebrates from 212 species, representing 61 families and 18 orders. The majority were insects (201 species, 44 families and nine orders). The three EPT orders contributed 38 % ($n = 81$) of all species from 19 families. The most species-rich order was Diptera with 58 species, and the next most species-rich orders were Trichoptera with 47 species and Coleoptera with 28. The most species-rich family was Chironomidae with 39 species (Chironominae 19; Orthocladiinae with 14 and Tanypodinae with 6). The second most species-rich family was Leptophlebiidae (Ephemeroptera) with 16 species.

Taxonomic richness was significantly higher at reference sites than MIW sites at all levels of taxonomic resolution (Table 2). Mean taxonomic richness was always higher at reference sites with mean species richness 63 % higher than at MIW sites. Similarly, mean family richness was 38.9 % higher at reference sites. Mean order richness was 27.9 % higher at reference sites.

EPT taxonomic richness was also consistently higher at reference sites when considered at all three taxonomic levels (species, $P = 0.0002$; family, $P = 0.0006$; order, $P < 0.0001$; Table 2). EPT species richness was approximately three times higher at reference sites than MIW sites. In comparison, EPT family richness was twice as high at reference sites than MIW sites. EPT order richness was less than twice as high at reference sites than MIW sites.

Multivariate analysis of macroinvertebrate assemblage data revealed that the MIW sites were ecologically dissimilar to the reference sites (Fig. 2). Stress values ranged from 0.17 to 0.20 indicated that, in two dimensions, the NMDS ordinations were considered to be good to fair representations of the original multidimensional data (cf. Clarke, 1993). At all three taxonomic levels, reference sites were clustered separately from the MIW sites, although there was a small amount of overlap between reference and MIW samples at the species level and order level (Fig. 2). In both cases, the overlapping sites were from the Grose River downstream of a closed coal mine (Table 1). The ANOSIM results confirmed that the differences between the MIW and reference sites were consistent and significant at all three taxonomic levels. Global R values for the MIW versus reference assemblages were species (0.251, $P = 0.1$ %), family (0.440, $P = 0.1$ %) and order (0.402, $P = 0.1$ %).

Using SIMPER, data from the reference sites were compared with the MIW sites (Table 3) at the species, family and order level. Comparisons were made using the ten most influential individual taxonomic groups that contributed to the dissimilarity between the reference and MIW sites and repeated with data at each taxonomic level. The ten most influential species were all in the EPT orders and comprised five Ephemeroptera and five Trichoptera species with 8 of the 10 species having higher abundances at reference sites. At the family level, there were five families from the EPT orders that SIMPER analysis found to be important. At the order level,

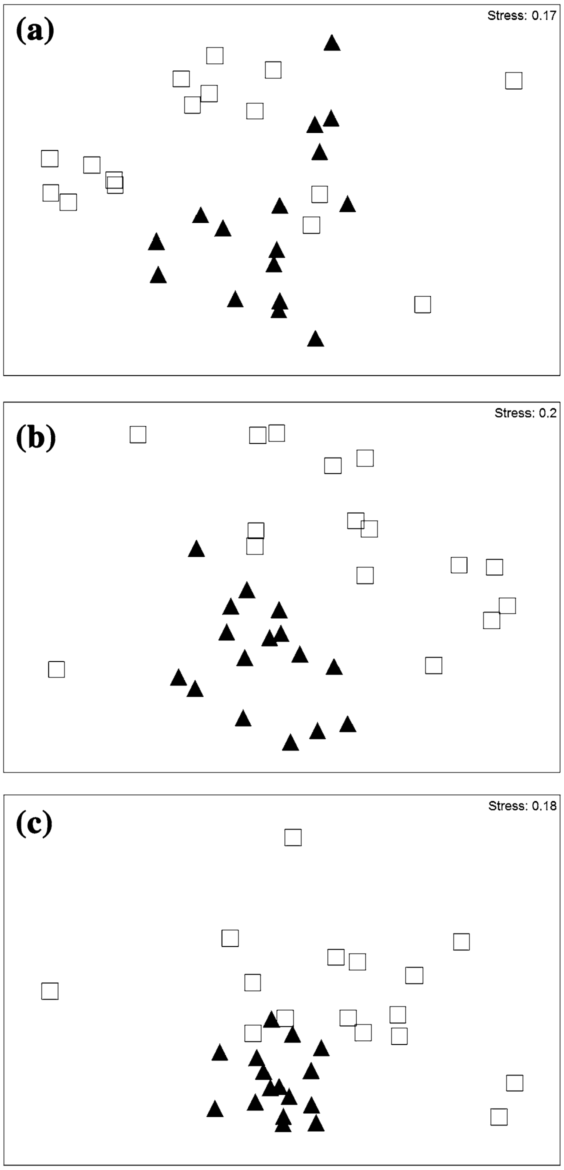

Fig. 2 NMDS ordination of macroinvertebrate data at the species (**a**), family (**b**) and order (**c**) taxonomic levels. Each *symbol* represents a macroinvertebrate sample. MIW-affected sites are denoted by *unshaded square symbols*, and reference sites are denoted by *black triangle symbols*. The stress value is provided on each ordination signifying whether the two-dimensional ordination is a reasonable representation (i.e. 0.20 or less) of the original multidimensional data

Ephemeroptera was most influential at splitting the communities between MIW and reference sites. Diptera was the second most influential order, Trichoptera third and Plecoptera sixth (Table 3).

BIOENV analysis revealed that the most influential water quality factors associated with variation in invertebrate assemblages (at the species level) was a combination of pH, electrical conductivity, cadmium and nickel concentrations (maximum P rank correlation = 0.428). Other influential attributes were anion and cation concentrations (sodium, chloride, calcium and magnesium). At the family level, BIOENV determined that the combination of dissolved oxygen, Mg, Na, K and Zn was most highly correlated (maximum P rank correlation = 0.558) with invertebrate assemblages. At the order level, BIOENV showed that a combination of Cd and Cr (maximum P rank correlation = 0.753) was most highly correlated.

Discussion

The results of this study demonstrate marked differences between macroinvertebrate communities at MIW-affected sites compared to reference sites. Specifically, both taxonomic richness and EPT richness were significantly lower, and macroinvertebrate community assemblages were clearly dissimilar to those observed at reference sites. These findings corroborate those of previous studies showing the adverse impacts of mining and metal pollution on freshwater stream ecosystems (Clements et al., 2000; Gray & Delaney, 2008; Pond et al., 2008; Gray & Harding, 2012).

In this study, analysis at each of the three taxonomic levels enabled detection of ecological impairment with broadly similar success, with both family-level and order-level data most clearly revealing ecological degradation linked to MIW and associated water quality impairment. In particular, multivariate analysis of macroinvertebrate assemblages at the family level provided the greatest insight, generating both the highest Global R value and the clearest discrimination between MIW-affected and reference streams in the NMDS plot. Order-level data were also meaningful, generating a similar Global R value to that of family data. A clear discrimination between MIW-affected streams and reference sites at all three levels of taxonomic resolution was similarly achieved in a previous study, which analysed freshwater sites impacted by a single point source sewage discharge together with multiple reference streams in the Blue

Table 3 Results of SIMPER breakdowns for square-root transformed data at the species, family and order level

Taxon	MIW sites	Reference sites	Contribution (%)	Cumulative (%)
Species data				
Koornonga sp. 1	0.00	0.94	6.20	6.20
Austrophlebiodes pusillus	0.00	1.56	6.13	12.33
Nousia sp. 1	0.00	1.03	6.08	18.41
Cheumatopsyche sp. 2	0.94	0.00	5.18	23.59
Taschorema evansi	0.09	0.50	3.83	27.41
Edmundsiops hickmani	0.00	0.57	3.52	30.93
Austropsyche sp.1	0.00	0.30	3.46	34.39
Tasmanocaenis rieki/tillyardi	0.57	0.09	3.05	37.44
Notalina fulva	0.00	0.70	2.96	40.40
Notalina ordina	0.13	0.69	2.94	43.33
Family data				
Leptophlebiidae	0.06	4.24	11.64	11.64
Simuliidae	1.55	1.16	5.28	16.92
Chironominae	2.08	0.96	5.25	22.17
Leptoceridae	1.36	2.44	5.08	27.25
Hydropsychidae	1.57	0.73	4.33	31.58
Veliidae	1.41	1.03	4.05	35.62
Elmidae	0.32	1.54	3.83	39.46
Orthocladiinae	1.38	1.25	3.69	43.15
Gripopterygidae	0.36	1.17	2.99	46.15
Hydrobiosidae	0.14	1.18	2.96	49.11
Order data				
Ephemeroptera	0.76	4.68	21.97	21.97
Diptera	4.04	3.11	13.23	35.20
Trichoptera	2.75	3.94	11.75	46.95
Hemiptera	2.20	1.28	9.75	56.70
Coleoptera	1.27	2.45	8.79	65.49
Plecoptera	0.87	1.56	8.50	73.98
Odonata	0.54	1.13	5.84	78.82
Oligochaeta	0.13	1.13	5.79	85.61
Tricladida	0.75	0.09	3.89	89.50
Megaloptera	0.35	0.45	3.29	92.79

The ten taxa (at each taxonomic level) contributing most to the average dissimilarity between the MIW sites and reference sites are listed in order of influence

Mountains (Wright et al., 1995). Detection of the ecological impact of sewage effluent was achieved at the species, family and order levels. These findings together support the examination of freshwater macroinvertebrates identified at higher levels of taxonomic resolution when investigating the ecological impacts of pollution (Warwick, 1988; Ferraro & Cole, 1990; Gray et al., 1990; Wright et al., 1995; Hewlett, 2000; Buss & Vitorino, 2010; Wright-Stow & Winterbourn, 2010).

The current study makes a useful contribution to the literature on taxonomic sufficiency and the use of macroinvertebrates in the ecological assessment of heavy-metal pollution in freshwater streams. It is also the first study of these themes to be conducted in Australia. Our findings are very similar to those obtained in a study of the impact of metal pollution in Colorado (USA) streams by Clements et al. (2000), who concluded that it may be most appropriate to adopt family-level identification when pollution studies are conducted over large spatial scales. Perhaps this explains why the impact of MIW pollution in the current study, also conducted over a large spatial scale, was more clearly demonstrated at the family level than at the species level. Macroinvertebrate community assemblages identified at reference sites in this study were found to be variable, which reflected different degrees of scattering observed within clusters in the NMDS ordinations. Tighter clusters were found at the order level, whereas species-level clusters were more widely dispersed. In identifying family-level data as most useful for the detection of ecological impairment in this study, our findings differ from those of other studies, which conclude that macroinvertebrate taxonomic resolution below the family level is required for the effective detection of the impacts of mining on freshwater ecosystems.

For example, Pond et al. (2008) concluded that identification at the genus level enabled the more effective detection of ecosystems impacted by coal mine pollution in a study of 37 sites, consisting of ten reference and 27 coal mine-affected, located in Appalachian Mountains. In agreement with the findings of our study, Wright-Stow & Winterbourn (2010) concluded that low level identification at order, class and phylum levels was as effective as higher, predominantly genus-level identification when evaluating organic stream pollution using New Zealand's macroinvertebrate community index. The authors suggested that the relatively low natural diversity of the fauna, as is the also case in the streams of Australia and the United Kingdom, may have contributed to this conclusion.

Unlike other macroinvertebrate studies, such as those conducted by Gray & Delaney (2008) and Wright et al. (1995), both of which investigated a single point source of pollution, MIW in the current study was a consequence of several different mining and industrial activities. This factor, together with the different catchment conditions and histories of the multiple MIW sites gave rise to highly variable combinations and concentrations of polluting toxicants. The aim of this study was to draw general conclusions regarding the ecological condition of regional waterways affected by MIW. To best achieve this, the sampling of multiple impacted streams downstream of the mine/industry alongside multiple unaffected reference streams was deemed more appropriate than the upstream versus downstream approach, which has some limitations (Dixon & Chiswell, 1996). One of the most significant of these is that it is often impossible to sample upstream of a waste discharge source. Our inclusion of multiple reference sites, as exemplified by Fairweather (1990), was in the interest of rigour, as this introduced considerable variability in terms of the range of water chemistry and macroinvertebrate attributes found.

It is worth noting that whilst the EPT index is commonly employed as an assessment tool, it is sometimes not specified which of its various aspects, for example, taxonomic richness or relative abundance, is chosen to be measured. To ensure clarity in the current study, we have described our choices precisely. One of our major findings was that both taxonomic richness and EPT richness considered at three taxonomic levels successfully discriminated between MIW-affected and reference sites. The calculation of these biotic indices is a simple process, which supports their use in the assessment of mining and industrial pollution of streams. Nevertheless, other studies have reported mixed success. For example, Gray & Delaney (2008, 2010) measured a wide range of biotic and diversity indices, including the EPT abundance index, in the River Avoca in South East Ireland, which receives waste from an abandoned copper and sulphur mine. They concluded that EPT abundance represented a less sensitive biotic index than a number of alternatives, but this may have been because the river had a single point source of pollution. An investigation of benthic communities in waterways affected by MIW in Colorado also reported that the EPT index was inadequate to detect ecological impairment at low levels of metal contamination (Clements & Kiffney, 1995).

The current study revealed that some EPT species and families were tolerant of MIW contamination, as demonstrated by the frequent collection of Plecoptera and Trichoptera at MIW-contaminated sites. Similar observations have been made in other Australian and international studies of streams and rivers affected by metal pollution. Clements et al. (2000) noted that measures of EPT taxonomic richness were complicated by the metal tolerance of some Trichoptera taxa, and in the current study, Hydropsychidae was one of the families most frequently collected at sites contaminated by metal and other inorganic pollution. Hickey & Clements (1998) also found Hydropsychidae were numerous at sites affected by metal pollution, as have several studies in the UK (Armitage, 1980; Gower et al., 1994) and the USA (Short et al., 1990; Clements et al., 2000; Pond et al., 2008). Tolerance to metal pollution by Hydropsychidae has previously been reported in two of the waterways included in the current study: Daylight Creek (Napier, 1992) and Grose River (Wright & Burgin, 2009a). Results of analyses of the two samples taken from the Grose River site, located below a coal mine waste discharge, overlapped with those from reference sites in the NMDS plots (Fig. 2c), due to a relatively high Trichoptera species richness in the families Leptoceridae and Hydroptilidae, along with a higher abundance of Hydropsychidae. This family and species response, however, could be misleading as not all Hydropsychidae species were found to be tolerant to metal pollution in the current study. Whilst three undescribed species (*Asmicridea* sp. 1, *Cheumatopsyche* sp. 2 and *Diplectrona* sp. 3) demonstrated tolerance to MIW pollution, the identification of another undescribed species only at reference sites was suggestive of pollution intolerance (*Austropsyche* sp. 1).

Of the insects, the ephemeropterans, especially Leptophlebiidae, were most sensitive to metal pollution. Only one mayfly, an early *Austrophlebioides* nymph, was found at a site affected by metal pollution and Wright & Burgin (2009a) reported to find no Leptophlebiidae at sites below either coal mine or sewage waste discharge in the Grose River. This family has also been reported to be intolerant of metal pollution in Queensland (Mackey, 1988), Kentucky (Short et al., 1990) and Portugal (Gerhardt et al., 2004). Our results therefore reinforce the belief that Leptophlebiidae is one of the most pollution-sensitive macroinvertebrate families worldwide. Mayflies have also been shown to be intolerant of coal mine pollution in Appalachian streams (Pond et al., 2008), zinc contamination in high elevation Colorado streams (Clements & Kiffney, 1995) and to zinc, lead, copper and cadmium pollution in New Zealand (Hickey & Clements, 1998).

Conclusion

Our findings demonstrate that mining and industrial activities can result in water quality and ecological impairment of a variable nature in a relatively small region. The water chemistry and macroinvertebrate assemblages found in rivers and streams affected by mining and industrial wastes had all suffered impairment, the level and particulars of which reflected the severity and type of water pollution. Our study provides evidence in support of identifying macroinvertebrates at the higher taxonomic levels whilst detecting the ecological impacts of waste discharges such as mining and industrial water pollution. We found that identification at the species level offered no advantage when detecting these impacts. On the contrary, family-level data enabled the clearest assessment and detection of ecological impairment by mining/industrial waste discharges in this study, with order-level data providing only a marginally less sensitive measure of this impairment. We share the opinion of Bailey et al. (2001) that the question being asked in each bioassessment study should be central to the choice of which level of identification is required. Our findings also support the use of the biotic indices taxonomic richness and EPT richness for the detection of ecological impairment. Finally, whilst performing studies of water pollution from mining and industrial waste, we advocate the collection of water geochemical data including electrical conductivity, major ions and a suite of metals that may be ecologically hazardous to freshwater ecosystems.

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