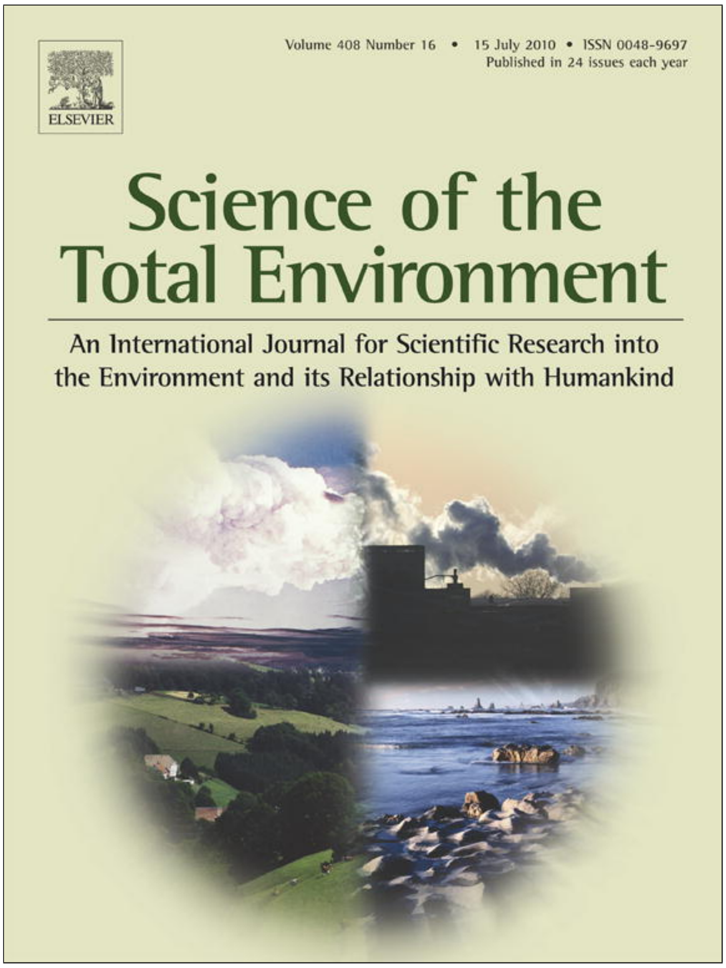
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Heavy metals: confounding factors in the response of New Zealand freshwater fish assemblages to natural and anthropogenic acidity

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ABSTRACT

Acidification of freshwaters is a global phenomenon, occurring both through natural leaching of organic acids and through human activities from industrial emissions and mining. The West Coast of the South Island, New Zealand, has both naturally acidic and acid mine drainage (AMD) streams enabling us to investigate the response of fish communities to a gradient of acidity in the presence and absence of additional stressors such as elevated concentrations of heavy metals. We surveyed a total of 42 streams ranging from highly acidic (pH 3.1) and high in heavy metals (10 mg L^{-1} Fe; 38 mg L^{-1} Al) to circum-neutral (pH 8.1) and low in metals (0.02 mg L^{-1} Fe; 0.05 mg L^{-1} Al). Marked differences in pH and metal tolerances were observed among the 15 species that we recorded. Five *Galaxias* species, *Anguilla dieffenbachii* and *Anguilla australis* were found in more acidic waters (pH<5), while bluegill bullies (*Gobiomorphus hubbsi*) and torrentfish (*Cheimarrichthys fosteri*) were least tolerant of low pH (minimum pH 6.2 and 5.5, respectively). Surprisingly, the strongest physicochemical predictor of fish diversity, density and biomass was dissolved metal concentrations (Fe, Al, Zn, Mn and Ni) rather than pH. No fish were detected in streams with dissolved metal concentrations >2.7 mg L^{-1} and nine taxa were only found in streams with metal concentrations <1 mg L^{-1}. The importance of heavy metals as critical drivers of fish communities has not been previously reported in New Zealand, although the mechanism of the metal effects warrants further study. Our findings indicate that any remediation of AMD streams which seeks to enable fish recolonisation should aim to improve water quality by raising pH above ≈4.5 and reducing concentrations of dissolved Al and Fe to <1.0 mg L^{-1}.

1. Introduction

Anthropogenic acidification is a widespread phenomenon and often has severe impacts on community structure and function in freshwaters (Schindler, 1988; Stoddard et al., 1999; Monteith et al., 2005), but many landscapes also include waterways that are naturally acidic from leaching of organic acids from soils with high organic matter content (Collier et al., 1990; Herrmann et al., 1993; Petrin et al., 2007). Unlike anthropogenically acidified systems these naturally acidic streams often support diverse aquatic communities (Dangles et al., 2004). Therefore, comparing the effects of contrasting sources of acidity on biotic communities may reveal the mechanisms behind the negative effects of anthropogenic acidity on freshwater biota (Collier et al., 1990; Petrin et al., 2008a).

Recent work has indicated anthropogenic sources of acidity can have greater effects on freshwater communities than natural acidity, potentially because of two non-mutually exclusive mechanisms (Petrin et al., 2008a,b). First, anthropogenic acidification is often associated with stressors other than reduced pH, such as the elevated concentration of bioavailable forms of toxic metals (Campbell and Stokes, 1985; Nelson and Campbell, 1991). This is especially the case for acidification through mine drainage, where acidic water (pH often <3) also contains elevated concentrations of metals (e.g., Fe, Al, Zn) and other ions (e.g., sulphate). In addition, where acid mine drainage (AMD) enters natural streams, some metals, especially Fe and Al, can form insoluble metal hydroxides that adhere to benthic substrata (McKnight and Feder, 1984; Evangelou and Zhang, 1995; Banks et al., 1997). These interactive stressors are largely absent from naturally acidic streams because metals are either at low concentrations or their bioavailability is mediated by the high concentration of organic acids (Driscoll et al., 1980; Kullberg et al., 1993; Winterbourn and McDiffett, 1996; Petrin et al., 2008a).

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The second hypothesis for weak effects of natural acidity on stream communities is that exposure to naturally low pH over evolutionary time selects for adaptations to low pH in the regional species pool, whereas anthropogenic acidification that has occurred within the last few hundred years is too recent for these traits to become established (but see Rasanen et al., 2003). This suggests that biota inhabiting landscapes with naturally acidic freshwaters may be more resilient to anthropogenic acidification, or able to adapt to novel stressors more easily than species in landscapes without a history of natural acidity (Petrin et al., 2008a). There is considerable evidence that populations of some species of freshwater invertebrates, amphibians, and fish that occur in acidic waters are more tolerant of acidification than conspecifics from circum-neutral waters (reviewed by Collier et al. 1990; O'Halloran et al., 2008; Petrin et al. 2008a). However, tolerance to natural acidity may not be sufficient to buffer regional pools of species to anthropogenic acidity if pH is reduced markedly below natural ranges, or if there are additional stressors such as elevated metal concentrations.

The West Coast region on the South Island of New Zealand provides an ideal landscape to test these alternative hypotheses as there are numerous streams influenced by both natural and anthropogenic acidity. Naturally acidic, brown-water streams are common on the West Coast and are formed by leaching of fulvic and humic acids from slow draining organic soils (Collier et al., 1990). The water chemistry of these brown-water streams is characterised by low pH (4–6), high concentrations of dissolved organic carbon (>10 mg L^{-1}) and low concentrations of bioavailable toxic metals (Collier and Winterbourn, 1987; Winterbourn and Collier, 1987). Active and abandoned coal mines are also widespread on the West Coast, and those associated with Brunner coal measures have high sulphur content and produce chemical conditions typical of AMD (Winterbourn and McDiffett, 1996; Harding et al., 2000; Harding and Boothroyd, 2004). The most severely contaminated sites are highly acidic (pH<3) and have concentrations of dissolved Al up to 35 mg L^{-1} and Fe up to 8 mg L^{-1} (Winterbourn and McDiffett, 1996).

Much of the research comparing naturally acidic streams with those influenced by mining in New Zealand has focussed on the invertebrate fauna. Many New Zealand stream invertebrates are remarkably tolerant of low pH and form diverse communities in naturally acidic streams (Winterbourn and Collier, 1987; Winterbourn and McDiffett, 1996). Furthermore, ecotoxicological experiments with the ubiquitous New Zealand mayfly, *Deleatidium* sp., indicate that populations from naturally acidic streams are locally adapted to tolerate low pH (O'Halloran et al., 2008). Despite these apparent adaptations, mining-affected streams consistently have much lower macroinvertebrate diversity and abundance than unaffected streams (reviewed by Harding et al., 2000; Harding and Boothroyd, 2004). However, some macroinvertebrates are surprisingly tolerant to metal contaminants (Hickey and Clements, 1998; Harding, 2005) and regularly occur in systems with reduced pH and elevated concentrations of metals (e.g., the caddisfly *Psilochorema*, and the stonefly *Spaniocerca*) (Winterbourn and McDiffett, 1996; Winterbourn, 1998).

In contrast to invertebrates, very little is known of how New Zealand fish species respond to both natural acidity and acid mine drainage, despite their high endemism and recreational and economic value. Fish communities may be more severely impacted by both acidity and metal toxicity than invertebrates, due to the vulnerability of ion exchange and respiration across the gill membranes of fish (Wood, 1989; Rosseland and Staurnes, 1994). Indeed, a number of international studies have observed marked declines in fish diversity and abundance in naturally acidic streams and those impacted by acid mine drainage (Somers and Harvey, 1984; Carline et al., 1992; Baldigo and Lawrence, 2000; Diamond et al., 2002). New Zealand native fishes appear to be tolerant of very low pH in naturally acidic streams. For example, up to eight species, including all five migratory galaxiids, have been observed in streams with pH<4.5 on the West Coast of the South Island (Collier et al., 1990; Olsson et al., 2006). In contrast, bluegill bullies (*Gobiomorphus hubbsi*), torrentfish (*Cheimarrichthys fosteri*) and brown trout (*Salmo trutta*) appear to avoid waters with pH<5 (Collier et al., 1990; Olsson et al., 2006). However, the influence of natural acidity in structuring fish communities is largely unknown because few studies have separated the effects of pH from other confounding habitat variables such as distance from the sea, riparian conditions, in-stream physical habitat, and biotic interactions (reviewed by Jowett et al., 1996; McIntosh and McDowall, 2004). Moreover, despite apparent differences in pH tolerance between fishes, many New Zealand taxa do not show strong preferences for particular pH ranges in experimental trials (West et al., 1997). Similarly, very little is known of the responses of New Zealand fish communities to the combination of chemical and physical stressors associated with acid mine drainage (Harding, 2005), although anecdotal evidence from the whitebait fishery indicates declines in abundance in catchments affected by mining (Harding and Boothroyd, 2004). This observation was supported by the absence of fish in mining-affected streams with pH<5.2 in a survey of two North Westland catchments (Harding et al., 2005).

The aim of this study was to conduct a spatially extensive survey to compare the effects of natural and mining-induced acidity on fish communities on the West Coast of New Zealand, and use this data to develop field-based estimates of pH and metal tolerance thresholds for common New Zealand fishes. We also used a variance partitioning and model selection technique to assess the effects of natural and anthropogenic acidity relative to other potential influences on freshwater fish community structure.

2. Materials and methods

2.1. Site selection

In order to generate presence/absence fish data across natural and mining-induced pH gradients, sampling focused around the three main coal mining regions on the West Coast, South Island, New Zealand: the Stockton and Denniston Plateaus (north of Westport), Reefton, and the Paparoa Ranges (north of Greymouth) (Fig. 1). Streams were selected based on three criteria: (1) those draining catchments with active or abandoned coal mines and are thereby influenced by reduced pH and elevated dissolved metal concentrations, (2) naturally acidic streams, and (3) naturally circum-neutral streams. Naturally acidic streams typically drained pakahi wetlands or low gradient, podocarp-dominated forest. Sampling was also stratified by geographic location, so where possible, circum-neural and naturally acidic sites were selected near to those impacted by mining to provide comparative reference sites. Sites with fish barriers (culverts, waterfalls or steep cascades) were avoided where possible, although two mining-affected and two reference streams did have barriers that were likely to constrain the number of potential fish taxa.

2.2. Fish survey

Fish communities in 42 streams were surveyed from February to May 2009 (austral summer and autumn). Fish were sampled with an intensive single pass with a Kainga EFM 300 backpack electrofishing machine (NIWA Instrument Systems, Christchurch, New Zealand) over a 40 m reach that encompassed the range of habitat types present in the stream segment. Fish were captured in a downstream push net or in a fixed downstream stop net, which was

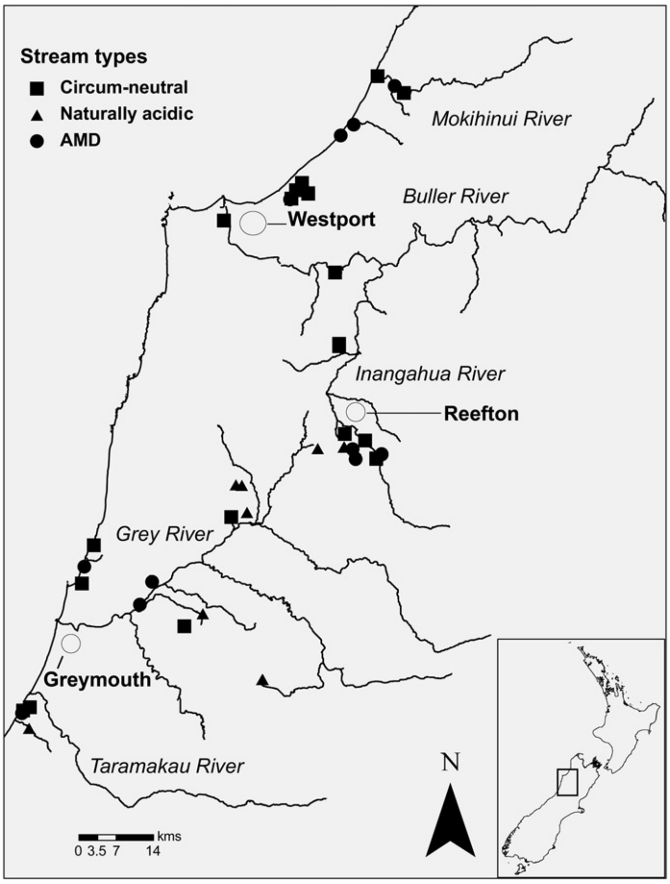

Fig. 1. The distribution of study sites on the West Coast, South Island, New Zealand. Streams were classified into those affected by acid mine drainage (AMD) from active and abandoned coal mines, naturally acidic streams (pH ≤ 6.0), and naturally circum-neutral streams (pH 6.1–7.6).

used in all but the smallest or most inaccessible sites. The fork length of captured fish was measured in the field, and wet weights were either measured with a portable balance, or determined from length–weight regressions (PG Jellyman, DJ Jellyman and ML Bonnett unpublished data). Up to 100 m of additional habitat was spot-electrofished to confirm the absence of taxa not observed in the 40 m single pass. Additionally, streams in the Westport and Greymouth areas were surveyed at night with spotlights to assess the presence/absence of migratory galaxiids, which can be difficult to capture by electric fishing. A subset of the Reefton streams were also spotlighted, but in all cases no additional species were found. Reefton is approximately 75 km from the sea (via the Buller River) and was unlikely to have migratory galaxiids (except koaro). Overall, the sampling programme was designed to yield a rigorous assessment of species presence/absence and also provide estimates of the density (no. m^{-2}) and biomass (g m^{-2}) of fish species.

2.3. Physicochemical assessment of environmental conditions

Dissolved oxygen, specific conductivity and water temperature were measured in the field with YSI 550 and YSI 63 meters, respectively. Stream pH was measured from grab water samples collected from pools and chilled above freezing (<4 °C) until analysis. pH was determined to 0.1 units with a laboratory meter within 48 h of sample collection (Hill Laboratories, Christchurch, New Zealand). The dissolved concentrations of five metals (Fe, Al, Zn, Mn and Ni) were measured from water samples filtered in the field and preserved in nitric acid. Samples were analysed with ICP-MS by Hill Laboratories, Hamilton, New Zealand.

A number of physical habitat parameters were quantified at each site over the 40 m reach, focusing on variables that had demonstrated effects on stream fish communities in New Zealand (reviewed by Harding et al., 2009). Stream cross-sectional area (m^2) was calculated from measurements of wetted width and three depths across five transects. The proportion of pools, riffles, runs and rapids were measured along the reach following Protocol 2 of Harding et al. (2009). Substrate size at each site was determined from measurements of 30 randomly selected particles, equally split across a representative pool, riffle and run where possible. These were used to calculate the percentage of boulder (>265 mm), percentage of silt and sand (<1 mm), and the substrate index of Jowett et al. (1991). In-stream habitat (percentage cover of macrophytes, wood/leaf packs, filamentous algae, obstructions to the flow, and bank cover) was visually assessed in a representative pool, riffle and run following Protocol 2 in Harding et al. (2009). The frequency and severity of flow disturbance was estimated with the channel stability index (Pfankuch, 1975) including upper banks, lower banks, and in-stream components. The condition of the riparian zone was assessed with Protocol 2 of Harding et al. (2009), which involved categorical (1–5) classification of riparian characteristics of each bank. A riparian index relevant to fish cover was generated from this assessment by taking the sum of the scores for both stream banks for shading, buffer width, buffer intactness, vegetation composition, livestock access and groundcover. Catchment variables were quantified using Freshwater Environments of New Zealand (FWENZ) database information provided by Harding et al. (2009), and included the percentage of the upstream catchment in native vegetation, and the percentage in agriculture, as well as distance from the sea. Altitude at each site was estimated from topographical maps (1:50000 NZ260 series).

2.4. Statistical analysis

Differences in water quality between naturally acidic streams, circum-neutral streams and those downstream of coal mines (mining streams) were assessed with principle components analysis (PCA) of nine key variables. Prior to analysis, the concentration of each of five dissolved metals (Fe, Al, Zn, Mn and Ni) and specific conductance were ln-transformed to meet the assumptions of normality and to produce scales comparable to that of pH. Dissolved oxygen (mg L^{-1}), pH and spot water temperature (°C) were not transformed. Separation among mining, naturally acidic and circum-neutral streams along axes one and two of the PCA were assessed with multivariate analysis of variance (MANOVA) followed by univariate analysis of variance (ANOVA) on specific water chemistry variables.

Relationships between environmental variables and fish community descriptors (composition, diversity, biomass and density) were assessed with general linear models (GLMs) and ordination. Prior to analysis, PCA was used as a variable reduction technique to combine highly intercorrelated variables into independent predictors. PCA of the concentration of each of the five metals and specific conductance produced a single axis explaining 71% of the variation among sites in conductivity and the dissolved metal concentrations. Conductivity and each of the metals were highly correlated with this axis (Pearson's r 0.76–0.95). Because we were interested in separating the effects of pH and metal concentration, stream pH was retained as a separate predictor variable and was moderately correlated with the metal index ($r = 0.61$). Dissolved oxygen

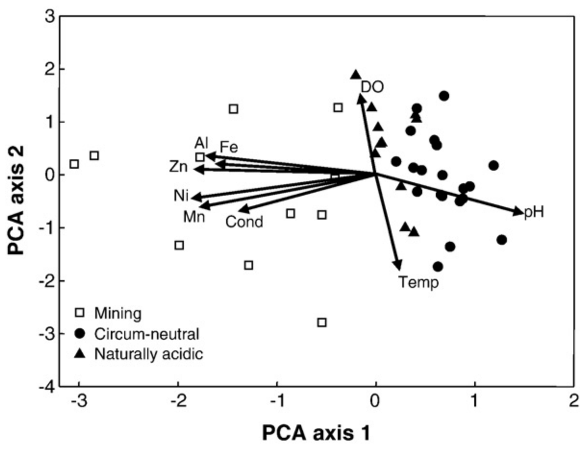

Fig. 2. Principle components analysis of nine water quality parameters in streams impacted by mining, and naturally acidic and circum-neutral reference streams. Arrows indicate correlation of variables with axes, and are scaled to twice the correlation coefficient (r). Mining, naturally acidic and circum-neutral streams were significantly differentiated across Axis 1 but not Axis 2 (MANOVA: Wilks $\lambda_{4,76}=0.22$, $P<0.0001$. Univariate ANOVAs: Axis 1 $F_{2,39}=53.9$, $P<0.0001$; Axis 2 $F_{2,39}=2.2$, $P=0.12$).

concentration and spot water temperature were not included in further analyses.

The substrate index and altitude were highly intercorrelated with other physical variables and were removed prior to further variable reduction. The substrate index was moderately correlated with % silt and sand ($r=0.74$) and % boulder ($r=0.66$), and altitude was highly correlated with distance inland ($r=0.91$). The remaining nine variables (riparian index, channel stability index, % boulder, % silt and sand, % in-stream cover, % pool in the reach, stream cross-section area, the proportion of the catchment in native vegetation and the proportion of the catchment modified for agriculture) were reduced into three independent axes using PCA (see Results). Prior to analysis, the riparian index and cross-sectional area were ln-transformed and all proportions were arcsine transformed.

Best subsets GLMs followed by hierarchical partitioning analysis were used to assess relationships between fish community metrics (fish species richness, biomass and density) and six environmental variables: pH, the metal index derived from PCA, distance from the sea (ln-transformed) and three principle components describing physical habitat parameters. Fish density was square root-transformed and fish biomass was ln +1-transformed (as several streams were fishless) prior to analysis. In each of the best subset regressions, models with all possible combinations of variables were fitted, and the model with the highest explanatory power weighted to the number of predictor variables (the 'best' model) was selected using Akaike's information criterion corrected for small sample sizes (AIC*c*) (Burnham and Anderson 1998). Wald's statistic was used to determine the significance of each predictor in the final model. Using hierarchical partitioning analysis, we calculated the independent and shared contribution (R^2) of intercorrelated predictors to the total explained variation in response variables (Mac Nally, 2000; Quinn and Keough, 2002), in order to assess the relative importance of each variable in explaining variation in fish community metrics. The significance of independent contributions of each variable was tested with Z-scores after 1000 randomizations. Hierarchical partitioning was conducted with "hier.part" and "rand.hp" functions (Mac Nally and Walsh, 2004) in R (R Development Core Team 2006), and best subsets regressions were performed in Statistica 8.

Predictors of fish community composition in streams that contained fish were investigated with redundancy analysis (RDA) using the same six environmental variables used in the GLMs. Fish communities were described by a species presence–absence matrix, with species lists compiled from electrofishing and spotlighting observations for each stream. Preliminary detrended correspondence analysis indicated the fish community matrix exhibited a short gradient that was most appropriate for RDA (Leps and Smilauer, 2003). Significant environmental predictors of fish community composition in the RDA ordination were extracted after 999 Monte-Carlo permutations with the forward stepwise procedure in Canoco 4.0.

3. Results

3.1. Physicochemical parameters

Eleven of the 42 sites sampled drained catchments that were affected by mining. Seven streams were associated with abandoned mines, and four streams had currently active or both active and abandoned mines. PCA indicated the water chemistry of streams draining catchments with coal mines was markedly different from mining-unaffected streams (Fig. 2), but streams with active mines were not significantly different from streams with abandoned mines (MANOVA on PCA axes 1 and 2: Wilks $\lambda_{2,8}=0.60$, $P=0.13$). Mining streams were significantly separated from naturally acidic and circum-neutral streams along Axis 1 (Scheffe's comparison: $P<0.0001$), which was strongly negatively correlated with the concentration of each of the five dissolved metals and specific conductance, and positively correlated with pH (Fig. 2). However, the three stream categories were not differentiated along Axis 2, which was positively correlated with dissolved oxygen and negatively correlated with stream temperature (Fig. 2). Analysis of individual water chemistry parameters supported the multivariate separation of sites along Axis 1. Circum-neutral sites had significantly higher pH than naturally acidic and mining streams, which

Table 1
Water quality parameters in streams affected by coal mines, naturally acidic streams (pH<6) and circum-neutral streams (pH 6–8). Different letters indicate significant differences ($P<0.05$) in parameters in Scheffe's comparisons across stream categories.

Stream type		pH	Conductivity (μS_{25} cm^{-1})	Fe (mg L^{-1})	Al (mg L^{-1})	Zn (mg L^{-1})	Ni (mg L^{-1})	Mn (mg L^{-1})	DO (mg L^{-1})	Temperature (°C)
Mining	Mean ± se	5.17 ± 0.52^a	446 ± 110^a	2.23 ± 0.95^a	7.29 ± 3.92^a	0.17 ± 0.067^a	0.035 ± 0.013^a	0.58 ± 0.24^a	10.78 ± 0.22^a	8.56 ± 0.46^a
($n=11$)	Range	3.1–8.1	62–1160	0.02–10.0	0.02–38.0	0.002–0.59	0.0005–0.120	0.0005–2.6	9.12–11.78	6.7–11.6
Naturally acidic	Mean ± se	5.17 ± 0.19^a	31.7 ± 2.2^b	0.29 ± 0.04^b	0.52 ± 0.08^a	0.012 ± 0.006^b	0.0006 ± 0.0001^b	0.007 ± 0.001^b	10.52 ± 0.23^a	8.09 ± 0.43^a
($n=11$)	Range	4.3–6.0	21.7–45.4	0.082–0.5	0.26–0.95	0.004–0.067	0.0005–0.0014	0.0009–0.012	9.11–11.67	6.0–9.9
Circum-neutral	Mean ± se	7.02 ± 0.09^b	73.9 ± 9.2^c	0.15 ± 0.02^c	0.11 ± 0.02^b	0.0056 ± 0.001^b	0.0006 ± 0.0001^b	0.0049 ± 0.0011^b	10.89 ± 0.13^a	8.89 ± 0.34^a
($n=20$)	Range	6.2–7.6	31.1–188	0.02–0.36	0.02–0.34	0.0021–0.027	0.0005–0.0013	0.0005–0.02	9.65–12.15	6.2–11.9

themselves were not significantly different (Table 1). Conductivity and concentrations of each of the five dissolved metals were one to two orders of magnitude higher in mining streams compared to naturally acidic and circum-neutral streams, although Al concentrations in mining streams were highly variable (Table 1). Naturally acidic streams had significantly higher concentrations of Al and Fe than circum-neutral streams (Table 1). The concentrations of Mn and Ni were highly variable across mining streams, and close to minimum detection concentrations in both the naturally acidic and circum-neutral streams (Table 1). Dissolved oxygen and spot water temperature did not differ significantly between the three stream types (Table 1).

The streams surveyed were 1.2–11 m wide, 9–47 cm deep, and were moderately stable to highly disturbed (Pfankuch index 67–128). Stream reaches were generally highly shaded (50–80%), had intact riparian zones, and drained catchments with a high proportion of native vegetation (mean $\pm$ se: 60% $\pm$ 0.05) and little agriculture (0.05% $\pm$ 0.02). PCA of the nine physical variables produced three significant principle components which explained a total of 61% of the variation in physical habitat variables among sites (Table 2). The first component was associated with stream morphology and disturbance, being positively correlated with % in-stream cover and % pool habitat, and negatively correlated with the channel stability index, % boulder substrate, and stream cross-sectional area (Table 2). The second component described riparian conditions, and was positively correlated with the riparian index and the proportion of catchment in native vegetation, and negatively correlated with the proportion of catchment in agriculture. Finally, the third principle component was positively correlated with % silt and sand, and therefore reflected the degree of stream sedimentation (Table 2). Streams with and without coal mines did not differ in physical habitat properties, as summarised by three habitat principle components (MANOVA Wilk's $\lambda_{3,38} = 0.87$, $P = 0.14$). Nevertheless, there was a trend towards mining streams being larger and more disturbed than streams unaffected by mining (Univariate ANOVA on Habitat PC1 $F_{1,40} = 4.1$, $P = 0.05$).

3.2. Environmental correlates of fish communities

Fifteen fish taxa were observed across the 42 streams. Longfin eels (*Anguilla dieffenbachii*) were the most common taxon across sites and were present in 64% of the streams. Brown trout (*S. trutta*) were also widely distributed, being found in 17 of the 42 streams.

Table 2
Results of variable reduction of nine descriptors of physical stream habitat using principle components analysis. Three axes with Eigen values >1 were retained for use as predictor variables in further analysis (Habitat PC1-3).

Component	Habitat PC1	Habitat PC2	Habitat PC3
Eigen value	2.28	1.93	1.27
% variation explained	25.3	21.4	14.1
Positive correlations	% in-stream cover[a] % pool	Riparian index[b] % catchment in native vegetation	% silt and sand
Negative correlations	Channel stability index[c] % boulder Stream cross-section area	% catchment in agriculture	

[a] Includes submerged logs, macrophytes and overhanging banks.
[b] Subjective assessment of the size, intactness and composition of the riparian zone.
[c] Subjective index of flow disturbance (Pfankuch, 1975).

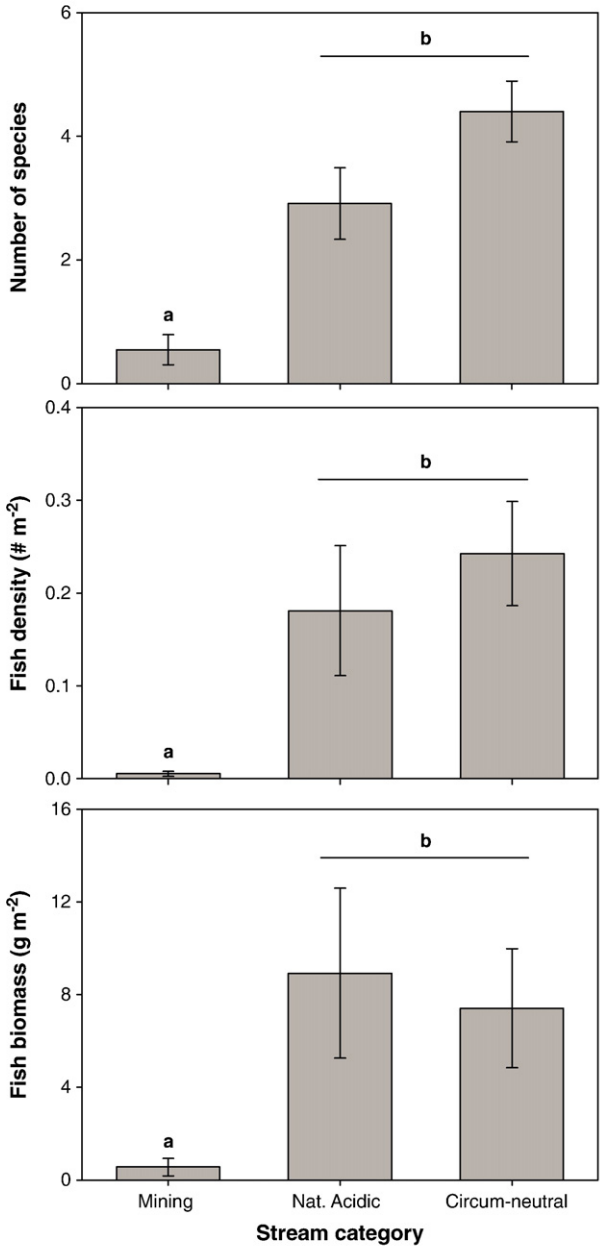

Fig. 3. Mean fish species richness and abundance ($\pm$se) across three categories of streams sampled on the West Coast, South Island, New Zealand. Mining streams drained catchments with active or historical coal mines (pH range 3.1–8.1, with elevated dissolved metal concentrations). Reference streams (no mines in catchment) were divided into naturally acidic streams (pH $\leq$ 6), and circum-neutral streams (pH 6.1–7.5). Categories with different letters were significantly different (P<0.05) in Scheffe's comparisons following significant one-way ANOVAs.

Rare species included upland bullies (*Gobiomorphus breviceps*) and lamprey (*Geotria australis*), which were found in only 3 and 2 streams, respectively. Dwarf galaxias (*Galaxias divergens*), inanga (*Galaxias maculatus*) and shortfin eels (*Anguilla australis*) were also rarely encountered (4 streams each). The mean number of fish species observed per stream was 3.0 ($\pm$0.4 se), and the most diverse stream had 9 species. Eight of the 42 streams were fishless, and seven of these drained mining-affected catchments. No fish were observed in streams with pH<4.4 and concentrations of dissolved metals >2.7 mg L^{-1}. Several mining streams did contain fish, but these were depauperate communities with much lower fish diversity, density and biomass than reference streams (Fig. 3). There was no difference in biomass and density between naturally acidic and circum-neutral reference streams, although there was a trend towards fewer taxa in naturally acidic streams (Scheffe's posthoc comparison, $P = 0.11$; Fig. 3).

The concentration of dissolved metals as summarised by the metal index, had consistently strong negative effects on fish communities, whereas pH generally had little influence. Best subsets regression (Table 3) indicated both the metal index and distance from the sea together were the best predictors of species richness, each having a significant negative effect. However, the metal index explained four times the variation in species richness than did distance from the sea. Similarly, the metal index was negatively associated with fish density and explained more variation in density than the other significant predictors (pH, distance from the sea, and each of the three physical habitat variables). Fish biomass was best predicted by a model that included the metal index and each of the three physical habitat components. The negative effect of sedimentation (Habitat PC3) explained the most variation in biomass. Metal concentrations and the intactness of native vegetation in the catchment and riparian zone (Habitat PC2) were also negatively associated with fish biomass. Small stable streams with high in-stream cover (Habitat PC1) supported higher fish biomass than large, disturbed streams with sparse in-stream cover.

When only naturally acidic and circum-neutral sites were included in regression analyses (Table 3), habitat variables and distance from the sea were consistently selected in the best models explaining fish community variables, and water chemistry variables had little or no influence. Species richness in reference streams decreased with distance from the sea, but increased with stream size, disturbance and % boulder content of the river bed (Habitat PC1). Riparian conditions (Habitat PC2), sedimentation (Habitat PC3), pH and metal concentrations had little influence on species richness in reference

Table 3
Best subsets regression and hierarchical partitioning of environmental predictors of fish community parameters in West Coast streams. Variables in bold were selected in 'best' models based on AICc selection criteria. Asterisks indicate significance of effects: *$P = 0.01$–0.05; **$P = 0.001$–0.01; and ***$P < 0.001$. Habitat PC1-3 are principle components describing habitat morphology, riparian condition and sedimentation, respectively. Metal index is a principle component positively correlated with the concentration of dissolved Fe, Al, Zn, Mn and Ni.

Variable	Slope	Independent R^2	Total R^2
a) All streams			
Species richness			0.51
pH	0.17	0.10*	0.15
Metal index	**−1.70*****	0.28***	0.33
Distance	**−0.59*****	0.07*	0.06
Habitat PC1	−0.35	0.02	0.01
Habitat PC2	−0.37	0.04	0.05
Habitat PC3	−0.39	0.06	0.07
Density			0.69
pH	**0.05***	0.09**	0.11
Metal index	**−0.12*****	0.22***	0.29
Distance	**−0.06*****	0.14***	0.12
Habitat PC1	**0.07****	0.05*	0.04
Habitat PC2	**−0.07****	0.13***	0.17
Habitat PC3	**−0.05***	0.06	0.08
Biomass			0.47
pH	0.21	0.05	0.05
Metal index	**−0.42*****	0.13***	0.20
Distance	−0.07	0.02	0.01
Habitat PC1	**0.29***	0.11**	0.11
Habitat PC2	**−0.32****	0.06	0.06
Habitat PC3	**−0.38****	0.14***	0.15
b) Reference streams[a]			
Species richness			0.47
pH	−0.06	0.02	0.05
Metal index	−0.79	0.01	0.01
Distance	**−0.69*****	0.20***	0.33
Habitat PC1	**−0.92*****	0.16***	0.20
Habitat PC2	−0.41	0.07	0.13
Habitat PC3	−0.40	0.06	0.09
Density			0.63
pH	0.04	0.01	0.01
Metal index	0.14	0.02	0.02
Distance	**−0.08*****	0.34***	0.55
Habitat PC1	0.03	0.01	0.01
Habitat PC2	**−0.09*****	0.26***	0.46
Habitat PC3	−0.04	0.05	0.08
Biomass			0.33
pH	0.20	0.01	0.01
Metal index	**0.90**	0.06	0.07
Distance	−0.07	0.06	0.13
Habitat PC1	**0.18**	0.04	0.05
Habitat PC2	−0.23	0.09	0.16
Habitat PC3	**−0.51*****	0.20***	0.21

[a] Reference streams included both naturally acidic and circum-neutral streams.

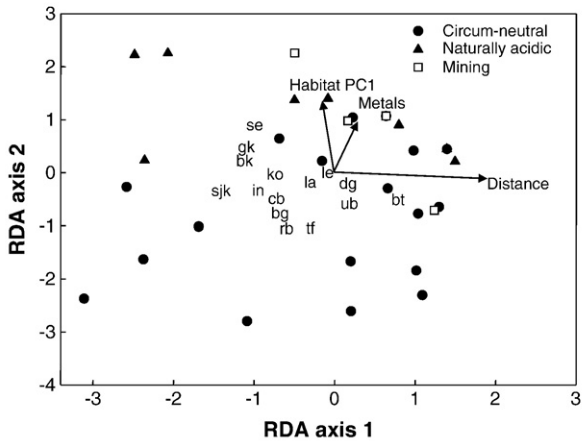

Fig. 4. Redundancy analysis (RDA) ordination of fish presence/absence in naturally acidic and circum-neutral reference streams and streams affected by coal mine drainage. Vectors show the correlation of significant environmental predictors (selected by forward stepwise regression) with each axis. Letters are fish names: sjk = shortjaw kokopu, bk = banded kokopu, gk = giant kokopu, ko = koaro, in = inanga, dg = dwarf galaxias, cb = common bully, rb = redfin bully, bg = bluegill bully, ub = upland bully, bt = brown trout, le = longfin eels, se = shortfin eels, la = lamprey, and tf = torrentfish. Environmental vectors and species scores are multiplied by two for clarity. Axes 1 and 2 explained 17% and 11% of the variation in fish communities, respectively.

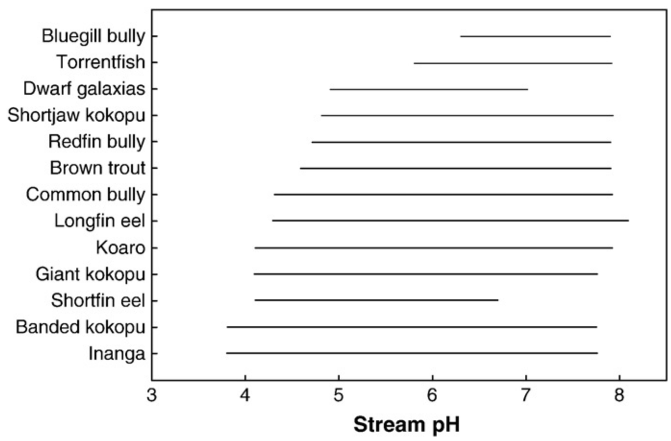

Fig. 5. Upper and lower pH limits for 12 New Zealand freshwater fish species on the West Coast, South Island, New Zealand. Data were compiled from surveys using qualitative and quantitative electric fishing, night spotlighting, traps and nets. Main et al., 1985; Taylor and Main, 1987; Harding et al., 2002, 2005; Olsson et al., 2006; this paper.

sites. The density of fish in reference sites decreased with distance from the sea and also with increasing riparian cover and proportion of native forest in the catchment (Habitat PC2). In contrast, fish biomass in reference streams was best predicted by a combination of the metal index, Habitat PC1 and Habitat PC3. However, of these three variables, Habitat PC3 was the only variable with a significant regression coefficient, which reflected a decrease in fish biomass with increasing sedimentation.

RDA ordination revealed patterns consistent with the univariate analyses (Fig. 4). When all sites containing fish were considered, communities were most strongly influenced by distance from the sea, but Habitat PC1 and metals were also important. Fish communities close to the coast were dominated by the three kokopu species (*Galaxias argenteus*, *G. fasciatus*, *G. postvectis*), inanga and shortfin eels, whereas brown trout were positively correlated with distance inland. Redfin bullies (*Gobiomorphus huttoni*), bluegill bullies (*G. hubbsi*) and torrentfish (*C. fosteri*) were positively associated with large, disturbed streams (Axis 2), and the kokopu species and shortfin eels were associated with small, stable streams with abundant in-stream cover. Community composition was variable among the four mining streams that supported fish (Fig. 4): shortfin eels and giant kokopu were present in Pages Stream, Ford Creek contained brown trout and torrentfish, and Devils Creek and Waimangaroa River had longfin eels and koaro, respectively. Subsequently, these six taxa (shortfin and longfin eels, giant kokopu, koaro, torrentfish and brown trout) appeared to have the highest tolerance to dissolved metals of the 15 species observed (Table 3, Appendix A), whereas the other nine taxa were only found in sites with individual dissolved metal concentrations <1 mg L^{-1}.

We combined our data with several previous studies to examine pH distributions for New Zealand fishes. The frequency of occurrence of 11 species across a pH gradient was collated from a 124 site dataset compiled from our data and other recent New Zealand studies (Fig. 6) (Harding et al., 2002, 2005; Olsson et al., 2006). These data, plus pH ranges reported in Main et al. (1985) and Taylor and Main (1987) were used to determine maximum and minimum pH thresholds for 13 commonly observed taxa. Although pH was a weak predictor of fish community composition when compared to

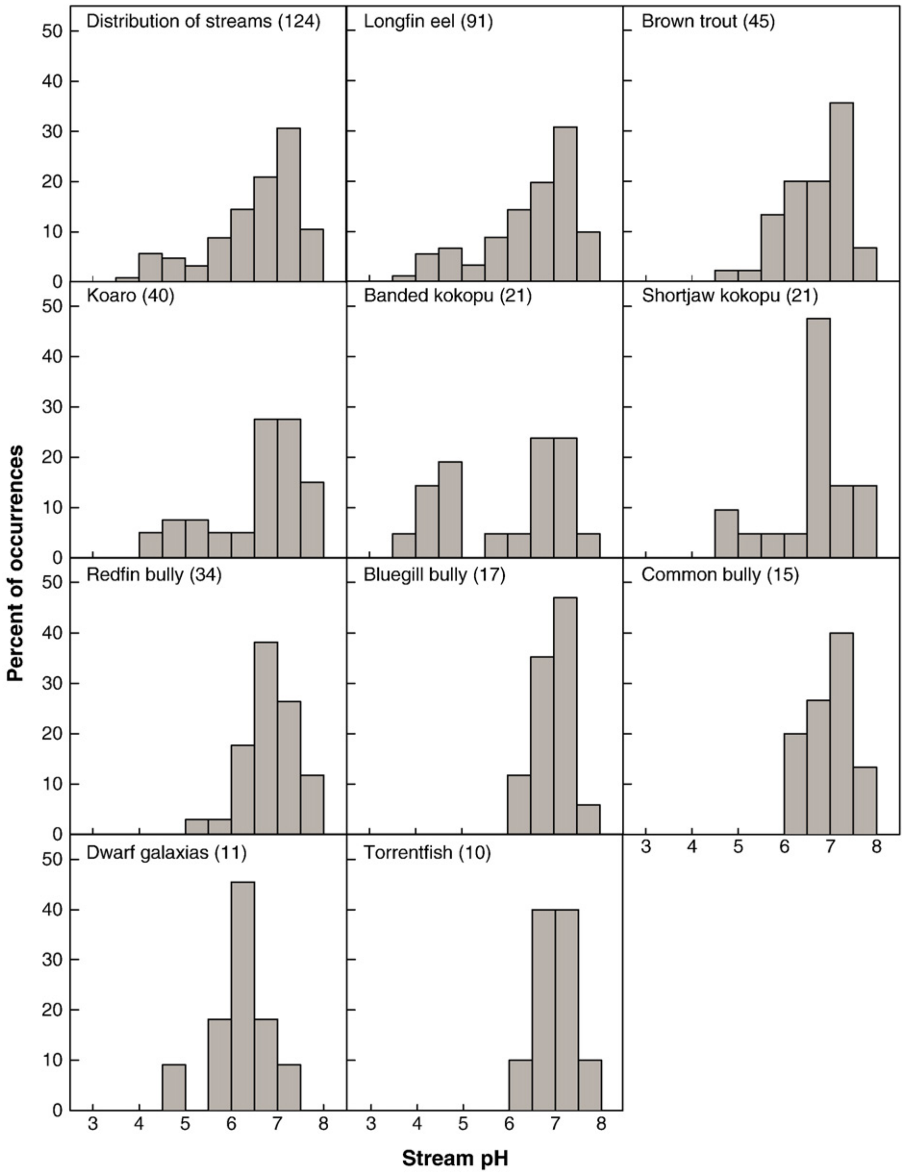

Fig. 6. The distribution of common freshwater fishes in 124 streams that were unaffected by mining. Data were compiled from published quantitative or semi-quantitative electric fishing surveys. Taxa shown were present in ≥10 streams with number of occurrences for each species presented in parenthesis. In some cases streams were sampled twice or more, but these were usually different reaches and across different years. Harding et al., 2002, 2005; Olsson et al., 2006; this study.

other environmental variables, several species were not found in naturally acidic streams, including bluegill bullies, torrentfish and lamprey (Figs. 5 and 6 and Appendix A). In contrast, 11 other species have been observed in highly acidic streams (pH<5; Fig. 5, Appendix A), although pH distributions indicate common bullies, redfin bullies and brown trout were rarely observed below pH 6 (Fig. 6). Over 40% of the banded kokopu occurrences were in streams with pH<5, but this species was also common in circum-neutral streams (Fig. 6).

4. Discussion

Recent syntheses have indicated that natural acidity from organic acids can impose stress on aquatic biota, but it has much less influence on communities than acidity generated by human impacts (Petrin et al., 2008a). Several reasons account for this difference (Collier et al., 1990; Petrin et al., 2008a,b). Anthropogenic acidity can result in more extreme pH (<3.5) in some cases, compared to natural acidity from organic acids (pH usually >4). Furthermore, anthropogenic acidity is often associated with additional stress from elevated concentrations of bioavailable metal ions. Finally, anthropogenic impacts have occurred over a time scale too short to enable local adaptation. We observed strong evidence for negative effects of mining-induced acidity on fish communities in West Coast streams of New Zealand. Seven of the eleven mining-affected streams were fishless, and those that did contain fish supported communities with markedly lower diversity and abundance than nearby reference streams. However, in catchments unaffected by mining, fish communities in naturally acidic streams were not significantly different from those in circum-neutral streams, and most fish species exhibited a very broad pH tolerance.

4.1. The effects of mine drainage on fish communities

The water chemistry of streams affected by mining was characterised by elevated concentrations of dissolved Fe, Al, Zn, Ni (and sometimes Mn), and generally reduced pH, although four streams were mildly acidic to circum-neutral. However, mining-affected streams were less acidic and had lower concentrations of dissolved metals than extremes observed in New Zealand and elsewhere (e.g., pH<3, Zn>1 mg L^{-1}, Fe and Al>35 mg L^{-1}; Winterbourn and McDiffett, 1996; Niyogi et al., 2002), largely because the small headwater streams where AMD is most concentrated were not sampled because of barriers to fish dispersal. Streams with active mining were very similar in water chemistry to those with abandoned mines, although we did not measure suspended sediment which can become elevated during active mining and remediation (Kelly, 1988). In our dataset on all streams, the concentration of metals, rather than pH, was most closely related to negative effects on fish community composition, richness, biomass and density. The metal index, which summarised the combined dissolved concentrations of Fe, Al, Zn, Mn and Ni, consistently had the strongest negative effects on fish communities of the six predictor variables, and explained the most variation in species richness and biomass independent of other predictors (Table 3). Although some streams were very acidic (pH<3), the pH of mining streams was not significantly different from naturally acidic streams and pH overall was a poor predictor of fish community metrics. When all sites were considered, there was no significant effect of pH on species richness and biomass after accounting for effects of dissolved metals, although there was a significant but minor effect on fish density when compared to the effects of other environmental variables.

Several interactive mechanisms could explain the strong negative relationship between the concentration of metals and fish community metrics. First, dissolved metals may be present in sufficient concentrations to cause acute or chronic toxicity. The mechanisms of toxicity generally involve electrolyte loss from the inhibition of ion exchange, and adherence of metal hydroxides to gill membranes leading to respiratory stress (Baker and Schofield, 1982 and references therein). Published data on the toxicity of dissolved metals to New Zealand fishes are limited to short term behavioural trials (Hickey, 2000), but comparisons with international studies suggest that several streams contained concentrations of dissolved metals sufficient to cause acute mortality. Inorganic monomeric aluminium is acutely toxic to fish at low concentrations (0.1–0.2 mg L^{-1}) (Baker and Schofield, 1982) and Fe can cause acute mortality in brown trout at 2 mg L^{-1} (Peuranen et al., 1994). These observations suggest total dissolved Al and Fe concentrations in the two most heavily impacted streams (Al: 28–38 mg L^{-1}; Fe: 6.2–10 mg L^{-1}) were very likely to cause acute toxicity. Similarly, Zn acute LC_{50} values for rainbow trout (0.29 mg L^{-1}) (Besser et al., 2007) were exceeded in three mining-affected streams, although behavioural trials with New Zealand species indicated rainbow trout are more sensitive to Zn than both eel species and common bullies (Hickey, 2000). Ni and Mn were unlikely to have caused fish mortality, as even the most contaminated streams contained concentrations of Ni and Mn three orders of magnitude lower than concentrations required for acute toxicity in several fish taxa (Krishnani et al., 2003; Pane et al., 2004b).

Chronic exposure to metal concentrations below those required for acute mortality can cause mortality or non-lethal effects such as impaired predator avoidance, feeding, migration and fecundity which affect population persistence (Pane et al., 2004a,b). For example, chronic lethal effects of Ni on rainbow trout occurred at 2.03 mg L^{-1}, but non-lethal chronic effects of Ni on exercise physiology were observed at 0.39 mg L^{-1} (Pane et al., 2004a,b). Similarly, Zn chronic toxicity values for rainbow trout and mottled sculpins (0.219 and 0.117 mg L^{-1}, respectively) were 70–75% of acute LD_{50} levels (Besser et al., 2007). The concentrations of metals in moderately impacted mining streams in our study may be sufficient to induce chronic toxicity, and contribute to the negative effect of metals on fish communities.

The precipitation of metals, especially iron can impose direct lethal and non-lethal effects on fish, or indirect effects through altering habitat and food resources (Vuori, 1995; Niyogi et al., 2002). Iron hydroxide deposition is particularly prevalent in West Coast, New Zealand streams affected by coal mining (Barnden and Harding, 2005; Bray et al., 2008). Moreover, iron flocculation and sedimentation from active mines can produce elevated suspended sediment loads (>25 NTU) sufficient to deter migrating galaxiids and impair feeding (Rowe and Dean, 1998; Richardson et al., 2001) but not cause direct mortality (Rowe et al., 2009). Although we did not directly measure suspended sediment, benthic sedimentation (Habitat PC3) had strong negative effects on fish biomass in analyses both with and without mining streams (Table 3). Fish may also actively avoid high metal concentrations in mining streams during migration, or evade pulses of contaminated water following settlement (Atland and Barlaup, 1996; Atland, 1998). It is also worth noting that although pH was not selected as a strong predictor of fish community metrics in our analyses, acidity is likely to have contributed to the absence of fish in the most highly acidic sites (pH<3.3) where pH was well below the most extreme naturally acidic streams (pH 3.8; Main et al., 1985). Moreover, physiological stress from low pH and dissolved metals and their precipitates is likely to be synergistic and lead to more pronounced impacts than each of these stressors in isolation.

4.2. Influences on fish communities in reference streams

Naturally acidic streams often supported diverse fish communities with a biomass and density similar to circum-neutral streams. Moreover, the integration of our results with six other studies of pH distributions of West Coast freshwater fishes indicate that the vast majority of taxa are not limited by low pH observed in naturally acidic streams. Eleven species have been found in streams with pH<5, including the exceptional observations of inanga and banded kokopu occurring in streams of pH 3.8 (Main et al., 1985; Harding et al., 2002). Some species, especially kokopu and koaro, may utilize these naturally acidic streams as refugia from exotic salmonid predation (Main, 1988; Olsson et al., 2006). Analyses of distributions across 124 streams indicated banded kokopu were overrepresented in naturally acidic streams, providing support for positive selection for low pH. However, the pH distributions of shortjaw kokopu and koaro were consistent with the pH distribution of streams. In contrast, bluegill bullies and torrentfish have never been observed below pH 6.1 and 5.6, respectively, across the six studies (Fig. 4), and although common bullies, redfin bullies, and brown trout can tolerate low pH, these species were underrepresented in naturally acidic streams.

As in other studies (e.g., Winterbourn and Collier, 1987), a number of naturally acidic brown-water streams in our survey exhibited elevated concentrations of heavy metals. Five of the 31 streams that were unaffected by mining had concentrations of Al>0.5 mg L^{-1} and Fe>0.25 mg L^{-1}, yet these streams supported communities of 2–6 fish species. Acidic brown-water streams on the West Coast typically contain high concentrations of DOC (>10 mg L^{-1}), which promotes the complexation of aluminium with organic ligands (Collier and Winterbourn, 1987; Winterbourn and Collier, 1987; Collier et al., 1990). Organic monomers of Al are less bioavailable, and therefore considerably less toxic to fish than inorganic monomeric forms of Al (Driscoll et al., 1980; Campbell, 1995). Binding to organic ligands associated with DOC also decreases the toxicity of other metals, such as Zn (Bringolf et al., 2006). Thus, elevated concentrations of metals in naturally acidic streams are unlikely to impact fish communities.

Despite apparent differences in the pH preferences of fish, and elevated concentrations of metals in naturally acidic streams, water chemistry variables were not significant predictors of fish community composition, richness or abundance in streams unaffected by mining. Rather, physical habitat conditions and distance inland were consistently the most significant predictors. Distance from the sea had moderate negative effects on species richness and density, which is consistent with other studies (McDowall, 1996, 1998; Joy et al., 2000; Jowett and Richardson, 2003) and occurred through the decline in occurrence and abundance of diadromous species inland. This effect also likely encompassed the influence of altitude as distance and altitude were highly correlated. Species richness also decreased with Habitat PC1, which appears to be driven by the dominance of one or two large bodied galaxiid species in the streams with high amounts of in-stream cover, while larger more disturbed streams supported communities with multiple galaxiid and bully species, torrentfish and eels (see also Fig. 3). Riparian conditions as summarised by Habitat PC2 had little effect on species richness and fish biomass, probably because the majority of streams sampled had intact or mostly intact riparian zones in native forest catchments. Nevertheless, Habitat PC2 explained 26% of the variation in fish density independent of all other predictors and the highest densities of fish were observed in streams with agricultural catchments and sparse riparian cover. This pattern, which is similar to observations in the North Island (Rowe et al., 1999), was driven by the very high abundance of agriculture-tolerant species such as longfin eels and common bullies, possibly due to increased productivity as a result of agricultural inputs and reduced shading.

4.3. Local adaptation and vulnerability to anthropogenic acidification

Anthropogenic impacts act upon the template of habitat conditions present within a landscape. In New Zealand, the close spatial proximity of naturally acidic and circum-neutral waterways in New Zealand (Collier et al., 1990) is likely to have contributed to the evolution of the wide pH tolerance of fishes observed in this study. Moreover, the prevalence of diadromous life histories in the New Zealand fish fauna may reinforce selection for these generalist traits, as gene flow from the recruitment of marine dispersing larvae to non-natal streams appears to have homogenised genotypes (Allibone and Wallis, 1993; McIntosh and McDowall, 2004). While these adaptations may buffer assemblages to altered physicochemical conditions, our results indicate that stressors that are elevated outside the historical natural range, such as high concentrations of bioavailable metals, can have pronounced negative impacts on stream fauna.

4.4. Conclusions and management recommendations

Our findings suggest that the stressors associated with elevated concentrations of dissolved metals may be more important than acidity in affecting the diversity, density and biomass of fish assemblages. This is consistent with the hypothesis that additional stressors from anthropogenic sources drive the disparity between the effects of natural and anthropogenic acidification on freshwater communities (Collier et al., 1990; Petrin et al., 2008a,b). However, the paucity of data on mortality levels of dissolved metals for New Zealand fish taxa, combined with potentially interactive stressors of dissolved metals, metal hydroxide precipitates and low pH make it difficult to ascribe the effects of mine drainage observed in this study to one particular mechanism, or to determine robust chemical thresholds. Nevertheless, our results provide indicative water chemistry thresholds for mining companies and regulatory agencies seeking improved water quality limits for the maintenance of fish species. Although in extreme cases several species may occur in waters with pH<4, our results indicate that pH>4.5 would be a suitable target. Most importantly, mining companies and regulators should generally strive for the combined concentrations of dissolved metals <2.5 mg L^{-1} and individual concentrations of Al and Fe <1 mg L^{-1} and Zn <0.1 mg L^{-1} to support fish species. In order to better understand how to manage and remediate anthropogenic acidification in freshwaters, future research should focus on the interactive effects of multiple heavy metals and reduced pH, and how these effects may propagate directly and indirectly though food webs.

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References

Allibone RM, Wallis GP. Genetic variation and diadromy in some native New Zealand galaxiids (Teleostei, Galaxiidae). Biol J Linn Soc 1993;50:19–33.

Atland A. Behavioural responses of brown trout, *Salmo trutta*, juveniles in concentration gradients of pH and Al: a laboratory study. Environ Biol Fish 1998;53:331–45.

Atland A, Barlaup BT. Avoidance behaviour of Atlantic salmon (*Salmo salar* L.) fry in waters of low pH and elevated aluminum concentration: laboratory experiments. Can J Fish Aquat Sci 1996;53:1827–34.

Baker JP, Schofield CL. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut 1982;18:289–309.

Baldigo BP, Lawrence GB. Composition of fish communities in relation to stream acidification and habitat in the Neversink River, New York. Trans Am Fish Soc 2000;129:60–76.

Appendix A

Fish species and the range of pH and dissolved metal concentrations in 42 streams sampled on the West Coast of the South Island.

Fish species	n	pH range	Mean ± se	Al (mg L^{-1})		Fe (mg L^{-1})		Mn (mg L^{-1})		Ni (mg L^{-1})		Zn (mg L^{-1})	
				Max	Mean ± se	Max	Mean ± se	Max	Mean ± se	Max	Mean ± se	Max	Mean ± se
Longfin eel	29	4.4–8.1	6.5 ± 0.18	0.95	0.25 ± 0.05	1.50	0.21 ± 0.02	1.50	0.057 ± 0.052	0.029	0.0016 ± 0.0009	0.067	0.008 ± 0.002
Brown trout	17	5.3–7.6	6.7 ± 0.16	0.41	0.17 ± 0.03	1.00	0.21 ± 0.050	0.06	0.008 ± 0.003	0.0042	0.0009 ± 0.0002	0.067	0.012 ± 0.004
Redfin bully	11	5.9–7.5	6.8 ± 0.14	0.56	0.16 ± 0.04	0.25	0.15 ± 0.02	0.013	0.004 ± 0.0009	0.0013	0.0006 ± 0.00007	0.027	0.0072 ± 0.0021
Banded kokopu	10	4.4–7.4	5.9 ± 0.34	0.89	0.38 ± 0.10	0.50	0.26 ± 0.049	0.012	0.0056 ± 0.001	0.0006	0.0005 ± 0.00001	0.011	0.005 ± 0.001
Shortjaw kokopu	9	4.8–7.4	6.3 ± 0.31	0.67	0.25 ± 0.08	0.38	0.18 ± 0.038	0.008	0.0042 ± 0.0009	0.0006	0.0005 ± 0.00001	0.011	0.006 ± 0.001
Koaro	9	4.8–7.4	6.2 ± 0.33	0.71	0.32 ± 0.09	0.50	0.22 ± 0.053	0.024	0.0070 ± 0.0024	0.0039	0.0009 ± 0.0004	0.033	0.008 ± 0.003
Torrentfish	7	6.5–7.5	7.0 ± 0.13	0.12	0.08 ± 0.02	1.00	0.25 ± 0.163	0.06	0.012 ± 0.008	0.0042	0.0012 ± 0.0005	0.030	0.008 ± 0.004
Giant kokopu	6	4.7–7.4	5.7 ± 0.47	2.70	0.76 ± 0.40	0.96	0.33 ± 0.13	0.13	0.026 ± 0.020	0.0064	0.0015 ± 0.0009	0.057	0.016 ± 0.008
Bluegill bully	6	6.5–7.4	6.8 ± 0.13	0.22	0.12 ± 0.03	0.23	0.15 ± 0.03	0.006	0.003 ± 0.0005	0.0006	0.0005 ± 0.00005	0.007	0.0036 ± 0.0007
Common bully	5	6.2–7.5	6.8 ± 0.22	0.30	0.15 ± 0.04	0.26	0.17 ± 0.04	0.02	0.009 ± 0.0033	0.0013	0.0006 ± 0.0002	0.011	0.0064 ± 0.0015
Shortfin eel	4	4.7–6.7	5.3 ± 0.48	2.70	1.00 ± 0.57	0.96	0.46 ± 0.17	0.13	0.038 ± 0.031	0.0064	0.0020 ± 0.0015	0.057	0.020 ± 0.012
Inanga	4	6.2–7.5	6.9 ± 0.29	0.30	0.14 ± 0.05	0.26	0.19 ± 0.038	0.02	0.011 ± 0.0037	0.0013	0.0007 ± 0.0002	0.0085	0.0005 ± 0.001
Dwarf galaxias	4	4.9–7.0	6.0 ± 0.46	0.95	0.41 ± 0.18	0.36	0.19 ± 0.060	0.009	0.005 ± 0.001	0.0005	0.0005 ± 0.00001	0.011	0.0064 ± 0.002
Upland bully	3	6.5–7.0	6.8 ± 0.17	0.22	0.17 ± 0.03	0.16	0.13 ± 0.026	0.005	0.003 ± 0.0007	0.001	0.0007 ± 0.0002	0.011	0.0052 ± 0.0029
Lamprey	2	7.0–7.5	7.3 ± 0.25	0.22	0.13 ± 0.09	0.26	0.21 ± 0.05	0.02	0.012 ± 0.007	0.0005	0.0005	0.0038	0.0030 ± 0.0008

Banks D, Younger PL, Arnesen RT, Iversen ER, Banks SB. Mine-water chemistry: the good, the bad and the ugly. Environ Geol 1997;32:157–74.

Barnden AR, Harding JS. Shredders and leaf breakdown in streams polluted by coal mining in the South Island, New Zealand. N Z Nat Sci 2005;30:35–48.

Besser JM, Mebane CA, Mount DR, Ivey CD, Kunz JL, Greer IE, et al. Sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Onchorhynchus mykiss*) to acute and chronic toxicity of cadmium, copper, and zinc. Environ Toxicol Chem 2007;26:1657–65.

Bray JP, Broady PA, Niyogi DK, Harding JS. Periphyton communities in New Zealand streams impacted by acid mine drainage. Mar Freshw Res 2008;59:1084–91.

Bringolf RB, Morris BA, Boese CJ, Santore RC, Allen HE, Meyer JS. Influence of dissolved organic matter on acute toxicity of zinc to larval fathead minnows (*Pimephales promelas*). Arch Environ Contam Toxicol 2006;51:438–44.

Burnham KP, Anderson DR. Model selection and inference: a practical information-theoretic approach. New York, USA: Springer; 1998. 353 pp.

Campbell PGC. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier A, Turner DR, editors. Metal speciation and bioavailability in aquatic systems. London: John Wiley and Sons; 1995. p. 45-102.

Campbell PGC, Stokes PM. Acidification and toxicity of metals to aquatic biota. Can J Fish Aquat Sci 1985;42:2034–49.

Carline RF, Sharpe WE, Gagen CJ. Changes in fish communities and trout management in response to acidification of streams in Pennsylvania. Fisheries 1992;17:33–8.

Collier KJ, Winterbourn MJ. Faunal and chemical dynamics of some acid and alkaline New Zealand streams. Freshw Biol 1987;18:227–40.

Collier KJ, Ball OJ, Graesser AK, Main MR, Winterbourn MJ. Do organic and anthropogenic acidity have similar effects on aquatic fauna. Oikos 1990;59:33–8.

Dangles O, Malmqvist B, Laudon H. Naturally acid freshwater ecosystems are diverse and functional: evidence from boreal streams. Oikos 2004;104:149–55.

Diamond JM, Bressler DW, Serveiss VB. Assessing relationships between human land uses and the decline of native mussels, fish, and macroinvertebrates in the Clinch and Powell River watershed, USA. Environ Toxicol Chem 2002;21:1147–55.

Driscoll CT, Baker JP, Bisogni JJ, Schofield CL. Effect of aluminum speciation on fish in dilute acidified waters. Nature 1980;284:161–4.

Evangelou VP, Zhang YL. A review: pyrite oxidation mechanisms and acid-mine drainage prevention. Crit Rev Environ Sci Technol 1995;25:141–99.

Harding JS. Impacts of metals and mining on stream communities. In: Moore TA, A.B., Centeno AA, editors. Metal contaminants in New Zealand. Christchurch: Caxton Press; 2005. p. 343–57.

Harding JS, Boothroyd IKG. Impacts of mining. In: Harding JS, Mosley MP, Pearson CP, Sorrell BK, editors. Freshwaters of New Zealand. Christchurch: New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc; 2004. p. 36.1-36.10.

Harding JS, Quinn GP, Hickey CW. Effects of mining and production forestry. In: Collier KJ, Winterbourn MJ, editors. New Zealand stream invertebrates: ecology and implications for management. Christchurch: New Zealand Limnological Society; 2000. p. 230–59.

Harding JS, McIntosh AR, Eikaas HS. An ecological assessment of river environment classification in West Coast beech forest streams. Christchurch, New Zealand: University of Canterbury; 2002. Available from http://www.biol.canterbury.ac.nz/ferg/pubs.shtml.

Harding JS, McIntosh AR, Gray D, Jellyman PG, Eikaas HS. Distribution of native fish in the Ngakawau and Waimangaroa Rivers. Christchurch, New Zealand: University of Canterbury; 2005. Available from http://www.biol.canterbury.ac.nz/ferg/pubs.shtml.

Harding JS, Clapcott JE, Quinn JM, Hayes JW, Joy MK, Storey RG, et al. Stream habitat assessment protocols for wadable rivers and streams of New Zealand. Christchurch, New Zealand: School of Biological Sciences, University of Canterbury; 2009. 133 pp.

Herrmann J, Degerman E, Gerhardt A, Johansson C, Lingdell PE, Muniz IP. Acid-stress effects on stream biology. Ambio 1993;22:298–307.

Hickey CW. Ecotoxicology: laboratory and field approaches. In: Collier KJ, Winterbourn MJ, editors. New Zealand stream invertebrates: ecology and implications for management. Christchurch: New Zealand Limnological Society; 2000. p. 313–43.

Hickey CW, Clements WH. Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. Environ Toxicol Chem 1998;17:2338–46.

Jowett IG, Richardson J. Fish communities in New Zealand rivers and their relationship to environmental variables. NZ J Mar Freshwat Res 2003;37:347–66.

Jowett IG, Richardson J, Biggs BJF, Hickey CW, Quinn JM. Microhabitat preferences of benthic invertebrates and the development of generalized *Deleatidium* spp. habitat suitability curves, applied to four New Zealand rivers. NZ J Mar Freshwat Res 1991;25:187–99.

Jowett IG, Richardson J, McDowall RM. Relative effects of in-stream habitat and land use on fish distribution and abundance in tributaries of the Grey River, New Zealand. NZ J Mar Freshwat Res 1996;30:463–75.

Joy MK, Henderson IM, Death RG. Diadromy and longitudinal patterns of upstream penetration of freshwater fish in Taranaki, New Zealand. NZ J Mar Freshwat Res 2000;34:531–43.

Kelly M. Mining and the freshwater environment. London: Elsevier Applied Science; 1988.

Krishnani KK, Azad IS, Kailasam M, Thirunavukkarasu AR, Gupta BP, Joseph KO, et al. Acute toxicity of some heavy metals to *Lates calcarifer* fry with a note on its histopathological manifestations. J Environ Sci Health Part A—Toxic/Hazard Subst Environ Eng 2003;38:645–55.

Kullberg A, Bishop KH, Hargeby A, Jansson M, Petersen RC. The ecological significance of dissolved organic-carbon in acidified waters. Ambio 1993;22:331–7.

Leps J, Smilauer P. Multivariate analysis of ecological data using CANOCO. Cambridge, USA: Cambridge University Press; 2003. 269 pp.

Mac Nally R. Regression and model-building in conservation biology, biogeography and ecology: the distinction between and reconciliation of 'predictive' and 'explanatory' models. Biodivers Conserv 2000;9:655–71.

Mac Nally R, Walsh CJ. Hierarchical partitioning public-domain software. Biodivers Conserv 2004;13:659–60.

Main MR. Factors influencing the distribution of kokopu and koaro (Pisces: Galaxiidae). Unpublished MSc thesis. University of Canterbury, 1988.

Main MR, Nicoll GJ, Eldon GA. Distribution and biology of freshwater fishes in the Cook River to Paringa River area, South Westland. Fisheries Environment Report 60. Fisheries Research Division, NZ Ministry of Agriculture and Fisheries; 1985.

McDowall RM. Diadromy and the assembly and restoration of riverine fish communities: a downstream view. Can J Fish Aquat Sci 1996;53(Supp 1):219–36.

McDowall RM. Fighting the flow: downstream–upstream linkages in the ecology of diadromous fish faunas in West Coast South Island rivers. Freshw Biol 1998;40:111–22.

McIntosh AR, McDowall RM. Fish communities in rivers and streams. In: Harding JS, Mosley MP, Pearson CP, editors. Freshwaters of New Zealand. Christchurch, New Zealand: New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc; 2004. p. 17.1-17.20.

McKnight DM, Feder GL. The ecological effect of acid conditions and precipitation of hydrous metal-oxides in a Rocky-Mountain stream. Hydrobiologia 1984;119:129–38.

Monteith DT, Hildrew AG, Flower RJ, Raven PJ, Beaumont WRB, Collen P, et al. Biological responses to the chemical recovery of acidified fresh waters in the UK. Environ Pollut 2005;137:83-101.

Nelson WO, Campbell PGC. The effects of acidification on the geochemistry of Al, Cd, Pb and Hg in fresh-water environments: a literature review. Environ Pollut 1991;71:91-130.

Niyogi DK, Lewis WM, McKnight DM. Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. Ecosystems 2002;5:554–67.

O'Halloran K, Cavanagh JA, Harding JS. Response of a New Zealand mayfly (*Deleatidium* spp.) to acid mine drainage: implications for mine remediation. Environ Toxicol Chem 2008;27:1135–40.

Olsson K, Stenroth P, Nystrom P, Holmqvist N, McIntosh AR, Winterbourn MJ. Does natural acidity mediate interactions between introduced brown trout, native fish, crayfish and other invertebrates in West Coast New Zealand streams? Biol Conserv 2006;130:255–67.

Pane EF, Haque A, Wood CA. Mechanistic analysis of acute, Ni-induced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): an exclusively branchial phenomenon. Aquat Toxicol 2004a;69:11–24.

Pane EF, Haque A, Goss GG, Wood CM. The physiological consequences of exposure to chronic, sublethal waterborne nickel in rainbow trout (*Oncorhynchus mykiss*): exercise vs. resting physiology. J Exp Biol 2004b;207:1249–61.

Petrin Z, Laudon H, Malmqvist B. Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? Freshw Biol 2007;52:2172–83.

Petrin Z, Englund G, Malmqvist B. Contrasting effects of anthropogenic and natural acidity in streams: a meta-analysis. Proc R Soc B—Biol Sci 2008a;275:1143–8.

Petrin Z, Laudon H, Malmquist B. Diverging effects of anthropogenic acidification and natural acidity on community structure in Swedish streams. Sci Total Environ 2008b;394:321–30.

Peuranen S, Vuorinen PJ, Vuorinen M, Hollender A. The effects of iron, humic acids and low pH on the gills and physiology of brown trout (*Salmo trutta*). Annales Zoologici Fennici 1994;31:389–96.

Pfankuch DJ. Stream reach inventory and channel stability evaluation. Missoula, Montana, USA: U.S. Department of Agriculture & Forest Service; 1975.

Quinn GP, Keough MJ. Experimental design and data analysis for biologists. Cambridge, UK: Cambridge University Press; 2002.

Rasanen K, Laurila A, Merila J. Geographic variation in acid stress tolerance of the moor frog , *Rana arvalis*. I. Local adaptation. Evolution 2003;57:352–62.

Richardson J, Rowe DK, Smith JP. Effects of turbidity on the migration of juvenile banded kokopu (*Galaxias fasciatus*) in a natural stream. NZ J Mar Freshwat Res 2001;35:191–6.

Rosseland BO, Staurnes M. Physiological mechanisms for toxic effects and resistance to acidic water: an ecophysiological and ecotoxicological approach. In: Steinberg CEW, Wright RF, editors. Acidification of freshwater ecosystems: implications for the future. New York: John Wiley & Sons; 1994. p. 227–46.

Rowe DK, Dean TL. Effects of turbidity on the feeding ability of the juvenile migrant stage of six New Zealand freshwater fish species. NZ J Mar Freshwat Res 1998;32:21–9.

Rowe DK, Chisnall BL, Dean TL, Richardson J. Effects of land use on native fish communities in east coast streams of the North Island of New Zealand. NZ J Mar Freshwat Res 1999;33:141–51.

Rowe DK, Hicks M, Smith JP, Williams E. Lethal concentrations of suspended solids for common native fish species that are rare in New Zealand rivers with high sediment loads. NZ J Mar Freshwat Res 2009;43:1029–38.

Schindler DW. Effects of acid rain on fresh water ecosystems. Science 1988;239:149–57.

Somers KM, Harvey HH. Alteration of fish communities in lakes stressed by acid deposition and heavy metals near Wawa, Ontario. Can J Fish Aquat Sci 1984;41:20–9.

Stoddard JL, Jeffries DS, Lukewille A, Clair TA, Dillon PJ, Driscoll CT, et al. Regional trends in aquatic recovery from acidification in North America and Europe. Nature 1999;401:575–8.

Taylor MJ, Main MR. Distribution of freshwater fishes in the Whakapohai River to Waita River area, South Westland. Fisheries Environment Report No. 77. Christchurch: Fisheries Research Division, NZ Ministry of Agriculture and Fisheries; 1987. Available from http://www.biol.canterbury.ac.nz/ferg/pubs.shtml.

Vuori KM. Direct and indirect effects of iron on river ecosystems. Annales Zoologici Fennici 1995;32:317–29.

West DW, Boubee JAT, Barrier RFG. Responses to pH of nine fishes and one shrimp native to New Zealand freshwaters. NZ J Mar Freshwat Res 1997;31:461–8.

Winterbourn MJ. Insect faunas of acidic coal mine drainages in Westland, New Zealand. N Z Entomol 1998;21:65–72.

Winterbourn MJ, Collier KJ. Distribution of benthic invertebrates in acid, brown water streams in the South Island of New Zealand. Hydrobiologia 1987;153:277–86.

Winterbourn MJ, McDiffett WF. Benthic faunas of streams of low pH but contrasting water chemistry in New Zealand. Hydrobiologia 1996;341:101–11.

Wood CM. The physiological problems of fish in acid waters. In: Morris R, Taylor EW, Brown DJA, Brown JA, editors. Acid toxicity and aquatic animals. Cambridge: Cambridge University Press; 1989. p. 125–52.