Evaluation of the likely ecological impacts of aluminium, copper and zinc in Southland rivers

Envirolink Medium Advice Grant 2532-ESRC306

Report prepared for Environment Southland by

Christoph D. Matthaei [1]* & Grant L. Northcott [2]

[1] University of Otago, Department of Zoology, Dunedin

[2] Northcott Research Consultants Limited, Hamilton

*Corresponding author: christoph.matthaei@otago.ac.nz

20 April 2026

Executive Summary

This report comprises a preamble, three main sections, and a concluding section outlining future research needs. **Section 1** summarises the survey-based work on metal concentrations in Southland rivers carried out by Environment Southland in 2023 and 2024. It details the metals detected in the two river surveys, the metal concentration ranges under wet and dry weather conditions, and whether or not their concentrations exceeded any of the guidelines considered. The latter was the case for aluminium, copper and zinc, but not for lead, nickel, arsenic and cadmium. Consequently, the next section of this report focuses on aluminium, copper and zinc.

Section 2 provides an overview of the known ecotoxicological effects on freshwater organisms for aluminium, copper and zinc, focusing on these metals' effects on ecologically relevant responses of freshwater fish, invertebrates and algae or (in a few cases) other plants. This overview is based on a review of the ecotoxicological literature on this topic. Most of the studies included in this section are from overseas; where possible, these are complemented by studies completed in New Zealand. For each metal, the key effects on the tested organisms are discussed in the context of the river water sampling conducted by Environment Southland in 2023 and 2024. In brief, the combined evidence presented in Section 2 indicates that the aluminium, copper and zinc concentrations detected in the dry and wet weather surveys of Southland rivers are all high enough to raise concerns regarding potential detrimental effects on the freshwater biota in these rivers – with a few cautionary notes regarding this overall assessment.

Aluminium: No relevant ecotoxicological studies for this metal exist from New Zealand. For seven freshwater species investigated in overseas studies, clear detrimental effects of aluminium exposure were found at experimental concentrations within the range detected in Southland rivers during the 2023 dry weather survey. For another five overseas freshwater species, clear negative effects of aluminium exposure were reported during acute 96-h experiments at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (when aluminium concentrations were generally higher than during the dry weather survey).

Copper: In a 48-h exposure study on the NZ freshwater mussel *Echyridella menziesii* (kākahi or kāeo), a taonga species for Māori, survival of mussel glochidia was reduced at dissolved Cu concentrations well within the range detected in the 2024 wet weather Southland river survey. For seven further freshwater taxa investigated in overseas studies, clear detrimental effects of copper exposure were found at experimental concentrations within the range detected in Southland rivers during the 2023 dry weather survey. For another six overseas freshwater species, clear negative effects of copper exposure were found in acute-exposure experiments at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (when copper concentrations were generally higher than during the dry weather survey).

Zinc: In one of three relevant studies from NZ, two freshwater species were affected by zinc exposure at concentrations within the range detected in Southland rivers (which was near-identical in the dry and wet weather surveys). For an additional nine overseas freshwater taxa (and in one

community-level, overseas mesocosm experiment), detrimental effects of zinc exposure were found at experimental concentrations within the range detected in Southland rivers.

Section 3 outlines the chemistry of aluminium in natural waters, the speciated forms that co-exist in response to pH, and importantly, identifies the individual labile inorganic species that collectively constitute the more highly toxic forms of aluminium (Al_{Mono}). While this section primarily focuses on aluminium, the methods and techniques discussed are similarly applicable to copper, zinc and other heavy metals whose toxicity similarly derives from labile inorganic mononuclear cationic molecules.

The instrumental methods applied to investigate and quantify aluminium in natural freshwaters are discussed, ranging from earlier wet chemistry techniques to specialist instrumental analysis methods, some of which are restricted to advanced research applications. The methods that are economically feasible to be adopted into typical research and commercial laboratory operations are identified. Previous methods developed to quantify the highly toxic fraction of aluminium by reactive complexing followed by colorimetric analysis are summarised together with the accepted and widely applied cationic exchange technique(s) that selectively isolate Al_{Mono} from natural water samples. These combined methods enable Al_{Mono} to be differentiated from other aluminium species in natural waters that are included within measurements of total and total dissolved aluminium, but they do not contribute to its toxicity to aquatic organisms. The application of diffusion in thin gels (DGT) passive sampling devices (PSDs) as a viable approach to monitoring aluminium and other heavy metals and metalloids in freshwaters is summarised and discussed. Importantly, DGT-PSDs have been previously shown to selectively accumulate the toxic Al_{Mono} species that are toxic to brown trout.

The last part of Section 3 discusses approaches that could be applied to better characterise the natural background concentrations of metals in rivers and streams of Southland and assist the identification of natural sources and those arising from anthropogenic activities. The experimental approaches discussed focus on characterising the particulate matter loading that is mobilised during wet weather high flow events within Southlands rivers and streams. Section 3 concludes with a summary of points to consider in the design of a future water quality monitoring program for metals and metalloids in Southland rivers and streams, including methods that selectively sample and enable the quantification of the bioavailable toxic fraction.

The final section of the entire report points out several key knowledge gaps and future research needs on the topic.

Table of Contents

Preamble

This report comprises a preamble, three main sections, and a concluding section outlining future research needs. **Section 1** contains a summary of the survey-based work on metal concentrations in Southland rivers that was carried out by Environment Southland staff in 2023 and 2024 (Blakemore 2023, 2024). This section details the metals detected in the two regional river surveys, the concentration ranges of each metal under wet and dry weather conditions, and whether their concentrations exceeded any of the guidelines considered or calculated by Environment Southland. The latter was the case for aluminium, copper and zinc, but not for lead, nickel, arsenic, and cadmium. Consequently, the remaining report focuses on the three priority metals **aluminium**, **copper** and **zinc**.

Notably, the same three metals were identified as the top three metals of concern in a review study conducted in the UK (Donnachie et al. 2014) that used risk-ranking of 12 metals (based on comparing information on ecotoxicological thresholds with measured concentrations in rivers, the sane approach as in the present report) to identify which metals posed the greatest threat to freshwater organisms in the UK.

Section 2 provides an overview of the known ecotoxicological effects on freshwater organisms for aluminium, copper and zinc, focusing on these metals' effects on ecologically relevant responses of freshwater fish, invertebrates and algae or (in a few cases) other freshwater plants. This overview is based on a review of the ecotoxicological literature on this topic. Most of the studies included in this section are from overseas and where available these are complemented by specific studies completed on New Zealand species and/or water quality parameters .

For each metal, the key effects on the tested organisms are discussed in the context of the river water sampling conducted by Environment Southland in 2023 and 2024. When doing so, the metal concentrations used in chronic-exposure laboratory experiments involving aluminium or copper are compared directly with the concentrations found in the Southland river water samples collected under dry weather conditions (which are likely to represent "baseline" contaminant levels). By contrast, the concentrations used in acute-exposure laboratory experiments involving aluminium or copper are compared directly with the concentrations in the river water samples collected under wet weather conditions (which were likely to include contaminant pulse peaks due to surface runoff). For zinc, this distinction between dry weather and wet weather conditions was not necessary because the zinc concentration ranges detected in the two Southland river surveys were nearly identical (see Section 1.3).

Section 3 summarises methods to analyse aluminium and heavy metals in natural freshwaters with an emphasis on aluminium which exceeded ANZEEC water quality guideline values in rivers in Southland under both dry and wet weather conditions.

Because of its complexity and relevance to its toxicity, the chemistry of aluminium in fresh water is described, and the highly toxic speciated forms of aluminium are highlighted. Analytical methods used to specifically quantify the highly toxic forms of aluminium in water samples are summarised along with complimentary methods that are applied to fractionate highly toxic forms of aluminium (Al_{Mono}) from other speciated forms that contribute to measurements of total and dissolved concentrations of aluminium.

The relevance and applicability of these methods to the analysis of toxic forms of copper and zinc is considered so a common methodological approach could be applied in future studies of heavy metals in Southland rivers and streams.

The application of the diffusion in thin gel (DGT) passive sampling device (PSD) to sample heavy metals in freshwaters within water quality monitoring studies is summarised and the application of this method of sampling to quantify the bioavailable fraction of heavy metals in freshwater ecosystems, including the highly toxic Al_{Mono} species, is highlighted.

Important considerations to consider in the design of a water quality monitoring program to assess and monitor natural background concentrations of heavy metals in Southland rivers and streams are outlined. This includes a more comprehensive analysis of the properties of particulate matter mobilised in Southland rivers during wet weather high flow events including organic chemical markers that could be used to differentiate and identify sources of particulate matter in river and streams in Southland.

The final section of the entire report identifies the main knowledge gaps and future research needs on this topic.

1. Summary of the metal river surveys completed by Environment Southland

Metals concentrations in the 22 rivers sampled by Environment Southland in June 2023 (during dry weather conditions; Blakemore 2023) and in 20 of the same 22 rivers sampled in 2024 (on 12 April or 30 July, during wet weather conditions; Blakemore 2024) varied spatially and in relation to flow conditions. These 22 sites had been selected to cover the main stems of major rivers in Southland and the key tributaries of the Mataura River (which was also sampled monthly for total and dissolved aluminium in 2023/24), as well as sites that were assessed as having a higher risk of metals contamination due to current and historic land-use activities.

Metal concentrations were generally higher under wet than under dry conditions at the 20 sites sampled under both weather conditions. Consequently, the wet weather concentrations are presented first in this summary.

1.1. Aluminium

Total aluminium concentrations ranged from 0.529 - 3.52 g/m^3 (= 529 - 3520 µg L^{-1}) during the wet weather sampling in 2024 (Blakemore 2024). These concentrations exceeded the relevant ANZECC (2000) guideline (0.055 g/m^3 = 55 µg L^{-1}, at pH > 6.5) at all 20 river sites.

The same was the case for dissolved aluminium concentrations, which ranged from 0.0963 - 0.974 g/m^3 (96.3 - 974 µg L^{-1}). *(Note: the same "trigger values for freshwater" in the ANZECC guideline are commonly applied to both total metal and dissolved metal concentrations, and this approach is similarly applied throughout this report – see also Section 2).*

Moreover, all 20 sites also exceeded the site-specific Canadian Federal Environment Quality Guideline (FEQG) for total aluminium. This chronic toxicity guideline (which is adjusted for pH, water hardness and dissolved organic carbon concentrations) was calculated for each individual site and ranged from 0.107 - 0.795 g/m^3 (107 - 795 µg L^{-1}).

During the dry weather sampling in 2023 (Blakemore 2023), total aluminium concentrations ranged from 0.022 - 0.460 g/m^3 (22 - 460 µg L^{-1}). The relevant ANZECC (2000) guideline value (0.055 g/m^3 = 55.0 µg L^{-1} at pH > 6.5, a condition met at all sites) was exceeded at 19 of the 22 sites.

During dry weather conditions, the site-specific Canadian FEQG for total aluminium ranged from 0.216 - 1.021 g/m^3 (217 – 1021 µg L^{-1}). This site-specific guideline was not exceeded at any of the 22 sites.

During dry weather conditions, dissolved aluminium concentrations generally ranged from 0.010 to 0.048 g/m^3 (10 - 48 µg L^{-1}), and with the exception of Otepuni Stream at Nith Street (270 µg L^{-1}) the ANZECC guideline for aluminium was not exceeded.

1.2. Copper

During wet weather conditions, total copper concentrations ranged from 0.00141 - 0.00866 g/m^3 (1.41 – 8.66 µg L^{-1}) and the ANZECC guideline value of 0.0014 g/m^3 (= 1.4 µg L^{-1}) was exceeded at all 20 sites. In comparison, dissolved copper concentrations ranged from <0.0005 g/m^3 to 0.00321 g/m^3 (<0.50 - 3.21 µg L^{-1}) and exceeded the ANZECC guideline value for copper at 11 sites.

During dry weather conditions, total copper concentrations ranged from <0.00053 - 0.0026 g/m^3 (0.53 - 2.6 µg L^{-1}) and the ANZECC guideline value was exceeded at three of the 22 sites. Dissolved copper concentrations ranged from <0.0005 g/m^3 to 0.0012 g/m^3 (<0.5 - 1.2 µg L^{-1}), remaining below the ANZECC guideline value at all sites.

1.3. Zinc

During wet weather conditions, total zinc concentrations ranged from 0.00150 - 0.0271 g/m^3 (1.50 – 27.1 µg L^{-1}) and the ANZECC guideline value (0.008 g/m^3 = 8.0 µg L^{-1}) was exceeded at 10 sites. Dissolved zinc concentrations ranged from <0.0010 - 0.0233 g/m^3 (<1.0 - 23.3 µg L^{-1}) and only exceeded the ANZECC guideline value at a single site (Otepuni Creek at Nith Street).

During dry weather conditions, total and dissolved zinc concentrations ranged from <0.0011 - 0.030 g/m^3 (<1.1 – 30.0 µg L^{-1}) and <0.0010 - 0.030 g/m^3 (<1.0 – 30.0 µg L^{-1}), and the ANZECC guideline value was exceeded at a single site site (Otepuni Creek at Nith Street).

1.4. Lead, nickel, arsenic, and cadmium

During both wet and dry weather conditions, total and dissolved concentrations of nickel, arsenic, lead and cadmium were clearly below the relevant ANZECC guidelines at all sampled sites, with only total lead concentrations under wet conditions approaching the relevant trigger value.

During wet conditions, total lead ranged from 0.000219 - 0.00252 g/m^3 (0.219 - 2.52 µg L^{-1}) and dissolved lead from <0.00010 - 0.000707 g/m^3 (guideline: 0.0034 g/m^3 = 3.4 µg L^{-1}). During dry conditions, lead concentrations were generally lower. Total lead ranged from <0.00011 - 0.00033 g/m^3 and dissolved lead from <0.00010 - 0.000110 g/m^3.

During wet conditions, total nickel ranged from <0.00053 - 0.00435g/m^3 (0.53 - 4.35 µg L^{-1}) and dissolved nickel from <0.0005 - 0.00224 g/m^3 (guideline: 0.011 g/m^3 = 11.0 µg L^{-1}). During dry conditions, nickel concentrations were similar, with total nickel ranging from <0.00053 - 0.00410 g/m^3 (0.53 - 4.10 µg L^{-1}) and dissolved nickel from <0.0005 - 0.0038 g/m^3.

During wet conditions, total arsenic (As III) ranged from <0.0011 - 0.00132 g/m^3 (<1.1 - 1.32 µg L^{-1}), whereas dissolved arsenic was <0.0010 g/m^3 at all 20 sampled sites (guideline: 0.024 g/m^3 = 24.0 µg L^{-1}). During dry conditions, both total and dissolved arsenic were <0.0010 g/m^3 at all 22 sites.

During wet conditions, total cadmium ranged from <0.000053 - 0.000062 g/m^3 (<0.053 - 0.062 µg L^{-1}) while dissolved cadmium was <0.00005 g/m^3 at all sites (guideline: 0.0002 g/m^3 *[note: not 0.200]* = 0.20 µg L^{-1}). During dry conditions, cadmium concentrations were similar. Total cadmium ranged from <0.000053 - 0.000064 g/m^3 (<0.053 - 0.064 µg L^{-1}), while dissolved cadmium was <0.00005 g/m^3 at all sites.

2. Ecotoxicological effects of Aluminium, Copper and Zinc on freshwater organisms

This literature review focuses on ecologically informative response variables such as survival rates, growth rates or reproduction. Biomarker studies were not included, mostly because of fundamental limitations of the existing biomarker-based evidence. While biomarkers are widely used in toxicology studies, more research is needed to ground-truth biomarker responses against ecologically relevant responses of freshwater organisms, and to determine whether biomarkers can effectively predict changes in ecosystem resilience (Forbes et al. 2006). Although a wealth of biomarker information exists in the literature, this information still requires systematisation and streamlining to identify which biological pathways are most useful and relevant for evaluating the multitude of existing environmental stressors (Ebner 2021).

Literature review - Methods

In several consecutive Web of Science searches (with all databases), the following keywords were used:

- metal* and ((freshwater invertebrate*) or (freshwater fish*) or (freshwater alga*))
- alumin* and ((freshwater invertebrate*) or (freshwater fish*) or (freshwater alga*)) and Research area: first Toxicology, then Environmental Sciences or Marine Freshwater Biology or Toxicology
- (copper or zinc) and ((freshwater invertebrate*) or (freshwater fish*) or (freshwater alga*)) & Research area: Toxicology
- metal* and ((freshwater invertebrate*) or (freshwater fish*) or (freshwater alga*)) and (New Zealand) & Research area: Toxicology, then Research area: Environmental Sciences or Marine Freshwater Biology

As a result of these searches, the titles and abstracts of more than 3000 articles were read, focusing on articles published between 2025 and 1998 (complemented by a few older articles on species particularly relevant for New Zealand).

The goal of this literature review was to provide an overview of the known ecotoxicological effects on freshwater organisms for aluminium, copper and zinc, focusing on the effects these metals exert on ecologically relevant responses of freshwater fish, invertebrates and algae (or, in a few cases, aquatic plants).

With this goal in mind, the following criteria had to be met by the studies summarised in Tables 1-3 below: they had to be single-exposure laboratory experiments or mesocosm experiments (with the latter being extremely rare), preferably with gradient (dose-response) designs. These designs enabled the determination of acute effect LC50s (the concentration expected to kill 50% of a group of test animals when administered as a single exposure) and/or chronic effect EC50s (the 50% effect concentration, i.e. the concentration that causes half of the maximum possible effect) of ecologically relevant responses (e.g. 50% reduction in growth rate compared to control performance). Further, achieved (measured) concentrations of the metal contaminants had to be presented in addition to the nominal (target) concentrations. Finally, to allow comparisons across multiple studies, exposure concentrations had to be presented in mg/L or µg/L (the units used in the vast majority of studies) or in g/m^3, not in mM/L or µM/L.

In keeping with these criteria, the following types of studies were excluded:

- laboratory experiments where only nominal concentrations were presented, or achieved concentrations were below the detection limit (e.g. in Santos et al. 2021 for copper);
- laboratory experiments (or experimental treatments) with exposures to contaminant mixtures;
- studies that presented their exposure concentrations only in mM/L or µM/L (e.g. Wan et al. 2021 for copper);
- all field-based surveys/observational studies.

Applying these selection criteria to the assessed literature yielded 78 studies in total, as follows:

- 11 ecotoxicological studies on 28 test organisms (mostly single species, sometimes single genera) for aluminium;
- 44 studies on 66 test organisms (plus 1 mesocosm experiment on stream macroinvertebrate communities) for copper; and
- 24 studies on 41 test organisms (plus 1 mesocosm experiment on lake zooplankton, phytoplankton and periphyton communities) for zinc.

Literature Review - Results & Discussion

For all three focal metals, the key effects on the tested organisms are discussed below in the context of the river water sampling conducted by Environment Southland. When doing so for aluminium and copper, the metal concentrations used in chronic-exposure laboratory experiments are compared directly with the measured concentrations fond in the Southland river water samples collected under dry weather conditions (which are likely to represent "baseline" contaminant levels). By contrast, the concentrations used in acute-exposure laboratory experiments are compared directly with the concentrations in the river water samples collected under wet weather conditions (which were likely to include contaminant pulse peaks due to surface runoff). For zinc, the concentration ranges found in both Southland river surveys were very similar, therefore this distinction was not necessary (see Section 2.3).

Moreover, as the same "trigger values for freshwater" in the ANZECC guideline are commonly applied to both total metal and dissolved metal concentrations (as discussed with K. Blakemore from Environment Southland on 9 July 2025), the total metal concentrations found in Southland rivers are compared with the dissolved metal concentrations presented in most of the ecotoxicological studies. This cautionary approach was used to avoid underestimating the potential toxicity of aluminium, copper or zinc. (The few exceptions where total metal concentrations were provided in ecotoxicological studies have been identified in Tables 1-3.)

2.1. Aluminium

As discussed in depth in Cardwell et al. (2018), the toxicity of aluminium in freshwater ecosystems is influenced by several water quality parameters, principally pH, hardness and dissolved organic carbon. The single-most important water chemistry parameter affecting Al toxicity is pH because the speciation and solubility of Al are strongly correlated with pH (Cardwell et al, 2018). Due to concerns regarding impacts of acid rain on freshwater ecosystems, much of the Al toxicity data prior to the mid-1990s were derived under acidic conditions (pH < 5), under which Al speciation is dominated by the Al^{3+} species. In contrast, under circumneutral conditions (pH 6–8) Al speciation is dominated by several different dissolved species. Thus, Al^{3+} dominates at pH < 5, $Al(OH)^{+}{}_{2}$ at pH 5-6, and the anion $Al(OH)^{-}{}_{4}$ at pH > 7. The target water pH in the studies reported in Cardwell et al. (2018), pH 6, represented the lower pH range of most natural waters found in the United States and Europe. It was chosen by these authors because it represented a "reasonable worst-case scenario for Al toxicity at an environmentally relevant pH".

Given this, pH clearly needs to be considered when assessing the potential ecotoxicity of aluminium in Southland's rivers. In Blakemore (2023, dry weather conditions), all 22 sampled river sites had a pH of > 6.5. In Blakemore 2024 (wet weather conditions), six of 20 sites had a pH between 6.0 and 6.4, while the remaining 14 sites had a pH > 6.5. Further, multi-year data on pH values determined at 60 Southland river sites (Stauber et al, 2023) obtained approximately monthly over 5 years (Feb 2015–Feb 2020, supplied by Roger Hodson, Environment Southland) demonstrated a median pH of 7.5 and a range from pH 7.2 to 7.7. These pH data specific to Southland rivers are taken into account when interpreting the ecotoxicological studies below.

2.1.1. New Zealand studies

No studies from New Zealand investigating aluminium toxicity on freshwater biota matched the selection criteria of single-exposure experiments with dose-response designs.

2.1.2. Overseas studies

Comparison to dry-weather field data from Southland

For seven overseas freshwater species (listed below in this section), clear detrimental effects of aluminium exposure were found at experimental concentrations within the range detected in Southland rivers during the 2023 dry weather survey (total Al 22 - 460 µg L^{-1}; dissolved Al 10 - 270 µg L^{-1}) (Table 1, study results highlighted in yellow).

These seven overseas species include two of the most widely used species in ecotoxicology (*Daphnia magna* and *Danio rerio*), one fish species and six invertebrate species belonging to a broad range of taxonomic groups (a microcrustacean, an amphipod, a mussel, a snail, and two rotifers), and five species with natural geographical distributions that include temperate (rather than tropical) climates roughly comparable to that of New Zealand. The majority of these studies used chronic exposures of 14-42 days duration; therefore, the "baseline" Al contamination concentrations found in the 2023 dry weather survey of Southland rivers are directly relevant for these studies. Moreover, most of these laboratory experiments were conducted under circumneutral pH conditions similar to the median pH range observed in the 2023 Southland river survey. Consequently, the evidence presented in more detail below and in Table 1 indicates the "baseline" aluminium concentrations measured in the 2023 dry weather survey of Southland rivers are high enough to raise concerns regarding potential detrimental effects on the freshwater biota in these rivers – despite the limitation that no specific ecotoxicological studies exist from New Zealand.

For *Daphnia magna*, a freshwater microcrustacean widespread in the Northern Hemisphere and arguably the most-used invertebrate model species in ecotoxicology worldwide, both reproduction (number of neonates per female) and growth (body length increase) of a lab clone were significantly reduced during 21 days compared to controls following exposure to 50 µg/L of dissolved Al (Rodrigues et al. 2020, Study 11 in Table 1, pH 7.0-8.0). Furthermore, in acute 72-h and 6-d tests conducted in the same study, survival of *D. magna* was reduced to zero (LC100 = 100% lethality) at 150 µg/L Al after 72 h and at 100 µg/L after 6 days.

For *Lecane quadridentate*, a cosmopolitan freshwater rotifer, the LC50 for the survival of wild-caught rotifers from a Mexican lake exposed to aluminium for 96 h was 157.2 µg/L of dissolved Al, with a corresponding LOEC (lowest-observed-effect concentration) of 10 µg/L (Rodrigues et al. 2020, Study 11 in Table 1, pH 7.0-8.0).

For the zebrafish (*Danio rerio*), a tropical and subtropical species that is probably the most widely used fish model in ecotoxicology, the growth of juveniles (biomass increase) was reduced (EC10) upon exposure to 98.2 µg/L of dissolved Al for 33 days (Cardwell et al. 2018, Study 3 in Table 1, pH 6.0). In the same study, reproduction of *Hyalella azteca* (a North American amphipod) was reduced during 42 days of Al exposure (EC10, 170.6 µg/L; EC50, 264.8 µg/L), and population growth of *Brachionus calyciflorus* (another cosmopolitan rotifer) was reduced following 48 h of Al exposure (EC10, 303.7 µg/L).

For two further North American species, the fatmucket mussel *Lampsilis siliquoidea* and the amphipod *Hyalella azteca*, Wang et al. (2018, Study 8 in Table 1, pH 5.0-6.1) reported that two growth measures (dry weight per individual, combined survivor biomass) were reduced after 28 days of exposure to aluminium (total Al, EC20 163 µg/L and 169 µg/L, respectively, for the mussel, 409 µg/L and 425 µg/L for the amphipod).

For a tropical freshwater snail from the Philippines, *Radix quadrasi*, Factor & de Chavez (2012, Study 10 in Table 1, pH 7.8) exposed embryos to aluminium for 14 days. They reported several developmental abnormalities (growth retardation, edema, thinning of the shell) at a LOEC of 43.0 µg/L of dissolved Al. In the same study, snail embryos exposed to aluminium for a shorter, acute period (96 h) from 2 days after oviposition showed an LC50 (survival) of 1,879 µg/L Al – an experiment that is also relevant for the wet-weather comparison below.

Comparison to wet-weather field data from Southland

For another five overseas freshwater species, all mainly tropical in their natural geographical distributions, clear negative effects of aluminium exposure were found during 96-h experiments at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (total Al 529 - 3520 µg L^{-1}; dissolved Al 96.3 - 974.0 µg L^{-1}) (Table 1, study results highlighted in blue font).

The findings of these 96-h acute exposure studies add some further weight to the interpretation of the mostly chronic-exposure studies discussed above in the comparison with the dry-weather field data from Southland. The pH range in these 96-h-exposure studies is once again circumneutral and thus comparable to the pH at the majority of Southland river sites sampled under wet weather conditions in 2024. However, the fact that all five model species are mostly tropical in their geographical distributions (as opposed to the five species with temperate geographical distributions discussed above) means that more caution should be applied when trying to extrapolate the findings of the 96-h acute exposure studies summarised in the next two paragraphs to the freshwater biota in Southland's rivers.

Rasbora sumatrana, a ray-finned fish found in South-east Asia, showed reduced survival after 96-h exposure to aluminium (LC50 1,530 µg/L) (Shuhaimi-Othman et al. 2015, Study 5 in Table 1, pH 6.5).

In a related 96-h-exposure study, Shuhaimi-Othman et al. (2013, Study 6 in Table 1, pH 6.7), tadpoles of the Asian common toad *Duttaphrynus melanostictus* had an LC50 of 1,870 µg/L of dissolved Al. The corresponding values for the Asian glass shrimp *Macrobrachium lanchesteri* were 2,900 µg/L, 3,100 µg/L for the freshwater ostracod *Stenocypris major*, and (1,430 µg/L) for the midge larva *Chironomus javanus*.

Table 1. Summary of single-exposure experiments with dose-response designs on the effects of ***aluminium*** exposure on freshwater organisms. Clear, ecologically relevant effects found in acute-exposure studies at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (total Al 529 - 3520 µg L^{-1}; dissolved Al 96.3 - 974.0 µg L^{-1}) are highlighted in blue font. Moreover, such clear effects found in chronic-exposure studies at concentrations within the range detected in Southland rivers during the 2023 dry weather survey (total Al 22 - 460 µg L^{-1}; dissolved Al 10 - 270 µg L^{-1}) are highlighted in yellow.

LC50 = concentration expected to kill 50% of a group of test animals when administered as a single exposure; EC50 = 50% effect concentration (e.g. reduction in growth compared to control performance); EC20 = 20% effect concentration; EC10 = 10% effect concentration; LOEC = lowest-observed-effect concentration (compared to control treatment). In Study 3, the EC results for five taxa where the LOEC was > ECs should be interpreted with somewhat reduced confidence.

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
1	*Cyprinus carpio* Common carp (Cyprinidae)	3-month old juveniles	Acute - 96 h 210 - 400 mg/L $Al_2(SO4)_3$ pH 7.8 Hardness 125	LC50 (survival)	LC50 = 224.2 mg/L (224,200 µg/L)	Yes	Yes	Das & Ray (2022) India
2	*Danio rerio* Zebrafish (Danionidae)	Embryos	Acute – 96 h 0.01 – 8.0 mg/L $Al\ Cl_3$ pH 7.2-7.6 Hardness not given	LC50 (survival) EC50 (teratogenic effects)	LC50 = 5.0 mg/L (5,000 µg/L) EC50 = 3.58 mg/L (3,580 µg/L)	Yes Yes	Yes Yes	Sanchez-Aceves et al. (2021) Mexico
3	*Pimephales promelas* Fathead minnow (Leuciscidae)	Fertilised eggs & juveniles	Chronic – 33 d 0 - 1200 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0 Hardness 96	Fry survival (most sensitive response) Growth (33-day mean dry biomass)	LOEC 558.1 µg/L But: EC10 & EC20 "undeterminable" LOEC 1104.6 µg/L EC10 417.4 µg/L EC50 719.0 µg/L	Yes Yes	No Mostly *(LOEC>ECs)*	Cardwell et al. (2018) USA
	Danio rerio Zebrafish (Danionidae)	Fertilised eggs & juveniles	Chronic - 33 d 0 - 600 µg/L $Al(NO_3)_3 \times 9H_2O$	Growth (33-day mean dry biomass) (most sensitive response)	LOEC 139.4 µg/L EC10 98.2 µg/L EC50 1318.7 µg/L	Yes	Yes	

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
			pH 6.0 Hardness 83					
	Hyalella azteca (an amphipod) (Hyalellidae)	From 7-9 days old	Chronic - 42 d 0 - 450 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0 Hardness 95	Reproduction (in 42 days) (most sensitive response)	LOEC 453.8 µg/L EC10 170.6 µg/L EC50 264.8 µg/L	Yes	Mostly *(LOEC>ECs)*	
	Lymnaea stagnalis Great pond snail (Lymnaeidae)	From < 1 day old	Chronic - 30 d 0 - 2000 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0 Hardness 117	Growth in 30 days (wet weight) (most sensitive response)	LOEC 2099.2 µg/L EC10 860.7 µg/L EC50 2036.0 µg/L	Yes	Mostly *(LOEC>ECs)*	
	Chironomus riparius (a midge) (Chironomidae)	From 3 days old	Chronic - 28 d 0 - 5000 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0 Hardness 91	Reproduction (number of eggs/case/female)	LOEC >4281.8 µg/L EC10 1271.5 µg/L EC50 23,667 µg/L (extrapolated)	Yes	No	
	Aelosoma sp. (an oligochaete) (Aeolosomatidae)	From < 1 day old	Chronic - 17 d 0 - 4000 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0 Hardness 54	Population growth (most sensitive response)	LOEC 2156.9 µg/L EC10 987.9 µg/L EC50 1923.9 µg/L	Yes	Mostly *(LOEC>ECs)*	
	Brachionus calyciflorus (a rotifer) (Brachionidae)	From < 2 hours old	Chronic - 48 h 0 - 1600 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0	Population growth (most sensitive response)	LOEC 820 µg/L EC10 303.7 µg/L EC50 863.5 µg/L	Yes	Mostly *(LOEC >EC10)*	

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
			Hardness 100					
	Lemna minor (a duckweed) (Araceae)	10-day old plants	Chronic - 7 d 0 – 10,000 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.0 Hardness not given	Plant growth (total dry weight)	LOEC 5318.8 µg/L EC10 2175.0 µg/L EC50 15,966 µg/L (extrapolated)	Yes	No	
4	*Labeo rohita* Rohu (Cyprinidae)	90-150-day old fish	Acute - 96 h 5 – 135 mg/L $Al(NO_3)_3 \times 9H_2O$ pH 7.5, Hardness 300	LC50 (survival of 90-day old fish – most sensitive response)	LC50 = 57.77 mg/L (57,770 µg/L)	Yes	Yes	Kousar et al. (2016) Pakistan
	Cirrhina mrigala Mrigal carp (Cyprinidae)		Acute - 96 h Other details also as above		LC50 = 48.06 mg/L (48,060 µg/L)	Yes	Yes	
	Catla catla Catla (Cyprinidae)		Acute - 96 h Other details also as above		LC50 = 38.01 mg/L (38,010 µg/L)	Yes	Yes	
	Ctenopharyngodon idella Grass carp (Cyprinidae)		Acute - 96 h Other details also as above		LC50 = 37.85 mg/L (37,850 µg/L)	Yes	Yes	
5	*Rasbora sumatrana* (a ray-finned fish) (Cyprinidae)	Adults (4-5 cm long)	Acute - 96 h 0.1 – 18 mg/L $Al_2(SO_4)_3$ x 18H2O	LC50 (survival)	LC50 = 1.53 mg/L (1,530 µg/L)	Yes	Yes	Shuhaimi-Othman et al. (2015)

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
			pH 6.5, Hardness 20					Malaysia
	Poecilia reticulata Guppy (Poeciliidae)	Adults (2-3.5 cm long)	Acute - 96 h 3.2 – 56 mg/L $Al_2(SO_4)_3$ x 18H2O pH 6.5, Hardness 20	LC50 (survival)	LC50 = 6.76 mg/L (6,760 µg/L)	Yes	Yes	
6	*Duttaphrynus melanostictus* Asian common toad (Bufonidae)	Tadpole	Acute - 96 h Conc. ranges given in 5 earlier papers $Al_2(SO_4)_3$ x 18H2O Mean pH 6.7, Hardness 19	LC50 (survival)	LC50 = 1.87 mg/L (1,870 µg/L)	Yes	Yes	Shuhaimi-Othman et al. (2013) Malaysia
	Macrobrachium lanchesteri Asian glass shrimp (Palaemonidae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 2.90 mg/L (2,900 µg/L)	Yes	Yes	
	Stenocypris major A freshwater ostracod (Cyprididae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 3.10 mg/L (3,100 µg/L)	Yes	Yes	
	Melanoides tuberculata A snail	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 68.23 mg/L (68,230 µg/L)	Yes	Yes	

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
	(Thiaridae)							
	Chironomus javanus A midge larvae (Chironomidae)	Larvae (4th instars)	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 1.43 mg/L (1,430 µg/L)	Yes	Yes	
	Nais elinguis An aquatic oligochaete (Naididae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 3.90 mg/L (3,900 µg/L)	Yes	Yes	
7	*Margaritifera margaritifera* Freshwater pearl mussel (Margaritiferidae)	3-day old juveniles	Acute - 96 h 10 - 693 µg/L Al Cl_3 Hardness 40-48	LC50 (survival) LC10 (survival)	LC50 >693 µg/L LC10 >693 µg/L	Yes Yes	No No	Belamy et al. (2022) France
8	*Lampsilis siliquoidea* (Fatmucket mussel) (Unionidae)	7-8 day-old juveniles	Acute 96-h 2.5 – 6680 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.1 Hardness 108	LC50 (survival)	LC50 >6200 µg/L (Total Al)	Yes	No	Wang et al. (2018) USA
		6-week-old juveniles	Chronic – 28 d 3.7 – 1103 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 5.0-6.1 Hardness 105-106	EC10, EC20 (dry weight) EC10, EC20 (survivor biomass per replicate)	EC10 = 109 µg/L EC20 = 163 µg/L (Total Al) EC10 = 116 µg/L EC20 = 169 µg/L (Total Al)	Yes Yes	Yes Yes	

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
	Hyalella azteca (an amphipod) (Hyalellidae)	7 day-old juveniles	Acute 96-h 0 – 5997 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 6.1-6.2 Hardness 108	LC50 (survival)	LC50 >6200 µg/L (Total Al)	Yes	No	
		7 day-old juveniles	Chronic – 28 d 0 - 1004 µg/L $Al(NO_3)_3 \times 9H_2O$ pH 5.0-6.1 Hardness 105-107	EC10, EC20 (dry weight)	EC10 = 272 µg/L EC20 = 409 µg/L (Total Al)	Yes	Yes	
				EC10, EC20 (survivor biomass per replicate)	EC10 = 282 µg/L EC20 = 425 µg/L (Total Al)	Yes	Yes	
9	*Lecane quadridentate* (a freshwater rotifer) (Lecanidae)	Wild-caught rotifers from a lake	Acute - 96 h 0.8 – 500 µg/L $Al\ Cl_3$ pH 7.4-7.8 Hardness 80-100	LC50 (survival)	LOEC 0.01 mg/L = 10 µg/L LC50 0.1572 mg/L = 157.2 µg/L	Yes	Yes	Torres Guzmán et al. (2010) Mexico
10	*Radix quadrasi* (a freshwater snail) (Lymnaeidae)	Embryos (from 2 days after oviposition)	Acute – 96 h Range not given $Al_2(SO_4)_3 \times 18H_20$ pH 7.8 Hardness 147	LC50 (survival)	LC50 = 1.879 mg/L (1,879 µg/L)	Yes	Yes	Factor & de Chavez (2012) Philippines
			Chronic – 14 d 0 – 45 µg/L Al2(SO4)3 x 18H20	Developmental abnormality (growth retardation, edema or thinning of the shell)	LOEC = 0.0430 mg/L (43.0 µg/L)	Yes	Yes	

No.	Species	Developmental stage	Duration & Concentration (dissolved unless stated otherwise) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country of study
			pH 7.8 Hardness 147					
11	*Daphnia magna* (a freshwater crustacean) (Daphniidae)	Female neonates < 24 h old (from laboratory clones)	Acute – 72h+6d Chronic – 21 d 25 – 200 µg/L Al Cl_3 pH 7.0-8.0 Hardness 175-225	LC100 (survival) = 100% lethality	LC100 (72 h) = 0.15 mg/L (150 µg/L) LC100 (6 d) = 0.10 mg/L (100 µg/L)	Yes	Yes	Rodrigues et al. (2020) Brazil
				Reproduction (number of neonates per female in 21 days)	0.05 mg/L (50 µg/L): significant reduction vs control	Yes	Yes	
				Growth in 21 days (body length)	0.05 mg/L (50 µg/L): significant reduction vs control	Yes	Yes	

2.2. Copper

While the toxicity of copper to freshwater organisms is affected by several water quality parameters, including pH, hardness and dissolved organic carbon (DOC), the combined literature summarized in Table 2 suggests that the influence of these modifying factors may not be as strong as for aluminium (see Section 2.1) or zinc (see Section 2.3) – see e.g. Cusimano et al. (1986, Study 9 in Table 2) for rainbow trout or the mesocosm experiment on benthic stream invertebrate communities (Iwasaki et al. 2022, Study 7 in Table 2).

For Southland rivers, the abovementioned study by Stauber et al. (2023; see Section 2.1) includes multi-year data on pH values, hardness and DOC at 60 Southland stream and river sites. These data (supplied by Roger Hodson, Environment Southland) are median values (and 10^{th} - 90^{th} percentile range) from 5 years (Feb 2015–Feb 2020) of approximately monthly monitoring at 60 sites. Median pH was 7.5 (range 7.2–7.7), median hardness 36 (range 16-68), and median DOC 3.3 (range 1.5-5.9). These water quality data from Southland need to be kept in mind when interpreting the ecotoxicological studies below, and the findings from studies conducted under strongly different water quality conditions should only be extrapolated with caution to Southland rivers.

2.2.1. New Zealand studies

Two studies investigating copper toxicity on freshwater biota in New Zealand matched the selection criteria for inclusion in this review. In the first, Clearwater et al. (2014, Study 2 in Table 2), larvae (glochidia) of the NZ freshwater mussel *Echyridella menziesii* (kākahi or kāeo) were exposed to dissolved copper for 48 h. Even during this short exposure period, survival of the glochidia was reduced at dissolved Cu concentrations (EC50 = 1.7-3.4 µg/L, NOEC = 1.1-2.6 µg/L) that were within the ranges detected in both Southland river surveys, and clearly so within the range of the wet weather survey (which is arguably most relevant for a 48-h exposure study; total Cu 1.41-8.66 µg L^{-1}, dissolved Cu <0.50-3.21 µg L^{-1}). Moreover, during this mussel exposure experiment, water hardness (30) and pH (7.8) were either within the range or very close to the range of these water quality variables at the 60 Southland river sites in Stauber et al. (2023; see previous paragraph). Consequently, the findings of Clearwater et al. (2014) indicate that the concentrations detected in Southland rivers during the dry weather survey and especially during the wet weather survey are probably high enough to be detrimental for this native mussel, which is considered a taonga (treasured) species by Māori (Clearwater et al. 2014).

In the only other suitable New Zealand study (Glover et al. 2016, Study 1 in Table 2), survival of the native freshwater fish *Galaxias maculatus* (inanga) was not affected by copper exposure at the concentrations measured in Southland rivers during the two surveys.

For copper (and zinc), Hickey & Golding (2002) conducted the only existing stream mesocosm study from New Zealand, in which they investigated effects on benthic stream invertebrate communities. However, they only added zinc and copper together as a mixture (in 12 channels with 3 replicates per treatment) at quite high concentrations; therefore, this study had to be excluded from the present review.

2.2.2. Overseas studies

Comparison to dry-weather field data from Southland

For seven freshwater taxa investigated in overseas studies, mostly with chronic exposure durations (relative to the generation times of the organisms studied), clear detrimental effects of copper exposure were found at experimental concentrations within the range detected in Southland rivers during the 2023 dry weather survey (total Cu 0.53 - 2.6 µg L^{-1}; dissolved Cu <0.5 - 1.2 µg L^{-1}) (Table 2, study results highlighted in yellow). These seven overseas taxa include five animal and two plant species belonging to a broad range of taxonomic groups (a fish, an amphipod, a mussel, a cladoceran, a hydra, a green algae [which was investigated in 4 different studies] and a cyanobacterium). The studies on the fish and the amphipod, with exposure durations of 36 days and 10 weeks, revealed strikingly strong negative effects of dissolved copper at very low concentrations (0.56 µg/L and 1.4 µg/L), highlighting the powerful nature and importance of such truly long-term exposure experiments (which are rare in ecotoxicology worldwide). As a potential caveat, only two of the seven species have natural geographical distributions that include temperate climates roughly comparable to that of Southland (the amphipod and the cyanobacterium, which has a cosmopolitan distribution). The remaining five species are tropical or subtropical.

While the toxicity of copper to freshwater organisms is affected by several water quality parameters, including pH, hardness and dissolved organic carbon, the combined literature in Table 2 suggests that the influence of these modifying factors may not be as strong as for aluminium or zinc, as discussed at the start of Section 2.2. Moreover, most of the experiments on the seven taxa listed above were conducted under water quality conditions reasonably similar to those in the two Southland river surveys and/or at the 60 Southland river sites in Stauber et al. (2023). Consequently, the evidence presented in more detail below and in Table 2 complements the study on the NZ freshwater mussel (Clearwater et al. 2014) discussed in Section 2.2.1 and indicates that the copper contamination concentrations found in the 2023 and 2024 surveys of Southland rivers are high enough to raise concerns regarding potential detrimental effects on the freshwater biota in these rivers. This overall assessment of the relevant overseas studies comes with two cautionary notes: i. the specific water quality conditions should also be considered when evaluating to which extent the findings of a given overseas experiment might be extrapolated to the field conditions in Southland rivers, and ii. more ecotoxicological research on model species with temperate geographical distributions would lend more weight to the evidence from the seven overseas species below, five of which are subtropical or tropical.

In the first chronic study highlighted in the summary of this section above, adult *Labeo rohita* (Rohu), a cyprinid fish found in subtropical and tropical South Asia, were exposed for 36 days to dissolved copper at 0.00, 0.28, 0.42 and 0.56 µg/L (Naz et al. 2023, Study 17 in Table 2, pH or hardness not given). At 0.56 µg/L, significant body weight loss occurred compared to controls, together with severe symptoms of behavioural stress responses and clinical signs of stress (e.g. loss of equilibrium, air gulping, coordination loss, erratic swimming, mucus secretion from mouth and gills, rapid operculum movement, bulging eyes). Moreover, a number of different histopathological lesions occurred in multiple fish tissues (e.g. brain, gills, liver, kidneys) in 100% of exposed fish. In the second chronic study highlighted above, wild-caught adults of the freshwater amphipod *Gammarus fossarum* in France were exposed to 0.0 vs 1.4 µg/L of dissolved Cu for 10 weeks (Lebrun & Gismondi 2020, Study 21 in Table 2; pH 7.0, hardness 11.5). Amphipod survival and three sublethal responses (respiration, locomotory activity, feeding rate) were all reduced significantly at 1.4 µg/L Cu compared to the controls.

For the tropical green microalga *Chlorella* spp., Shakya et al. (2022) investigated growth of algae from a stock culture (isolated initially from a lake in Papua New Guinea) during 72-h exposure to dissolved copper, which resulted in reduced growth (EC50 2.0 µg/L, EC10 1.0 µg/L) (Study 35 in Table 2, pH 7.3, hardness 80-90). In another study on the same species conducted at two different levels of DOM (none added vs 9.9 mg C/L added), Macoustra et al. (2020, Study 36 in Table 2) reported a near-identical EC50 of 1.9 µg/L Cu for 72-h growth without added DOM, but a much higher EC50 of 63.0 µg/L when DOM was added. In a third 72-h exposure study on the same species, McKnight et al. (2023, Study 39 in Table 2, pH 7.8-8.1, hardness 80-90) reported an EC50 of 2.8 µg/L for growth. Similarly, *Monoraphidium arcuatum*, another tropical green microalga, had an EC50 of 1.1 µg/L in the same study.

McKnight et al. (2023) also conducted a multispecies 72-h Cu exposure experiment with *Monoraphidium arcuatum*, *Pediastrum duplex* (another tropical green microalga) and *Nannochloropsis* sp. (a tropical golden microalga) together. In this, the EC50s for 72-h growth were 3.7 µg/L for *Monoraphidium* and *Pediastrum*, and 15.0 µg/L for *Nannochloropsis*.

For stock-culture-derived organisms of the fast-growing cosmopolitan cyanobacterium *Synechococcus elongatus*, both growth rate (after 3 d, EC50 2.7 µg/L) and total cell yield (after 7 d, EC50 2.6 µg/L) were reduced under exposure to dissolved Cu (Fettweiss et al. 2023, Study 38 in Table 2, pH 7.5, hardness 60).

In a study on seven tropical freshwater species from a wide range of taxonomic groups that are all local to Kakadu National Park, Australia, Trenfield et al. (2022, Study 44 in Table 2, pH 6.1-6.8, hardness 1.9-3.3) investigated the effects of exposure to dissolved copper. The mussel *Velesunio* sp. (glochidia exposed to Cu for 24 h, LC10 for survival 1.7 µg/L), the cladoceran *Moinodaphnia macleaya* (6-d reproduction, EC10 1.0 µg/L), the green hydra *Hydra viridissima* (96-h reproduction, EC10 2.5 µg/L), the green alga Chlorella sp. (72-h growth, EC10 1.6 µg/L) were all negatively affected at Cu concentrations within the range measured during the dry weather survey of Southland rivers. Moreover, the snail *Amerianna cumingi* (96-h reproduction, EC50 8.0 µg/L, EC10 5.7 µg/L) and the duckweed *Lemna aequinoctialis* (96-h growth, EC10 6.8

μg/L) were negatively affected at Cu concentrations within the range measured during the wet weather survey of Southland rivers.

Comparison to wet-weather field data from Southland

For another six freshwater species (three fish species, an oligochaete, a snail and a cyanobacterium), clear negative effects of copper exposure were found in acute-exposure experiments (mostly 96 h, but only 3 h in one case) at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (total Cu 1.41 – 8.66 μg L^{-1}); dissolved Cu <0.50 - 3.21 μg L^{-1}) (Table 2, study results highlighted in blue font). Two of these six species have tropical or subtropical distributions; the remaining four are temperate or cosmopolitan species.

The findings of these acute exposure studies add further weight to the interpretation of the NZ mussel study and the mostly chronic-exposure studies on overseas species discussed above (in the comparison with the dry-weather field data from Southland) – also given that four of the six species used in the acute-exposure studies are not restricted to tropical or subtropical regions. Moreover, the pH range in these studies was mostly circumneutral and thus comparable to the pH at the majority of Southland river sites sampled under wet weather conditions in 2024.

Juvenile rainbow trout (*Oncorhynchus mykiss)*, a salmonid species native to North America and Asia that has been introduced to NZ where it has become widespread, showed reduced survival after 96-h or 7-day exposure to dissolved copper, with LC50s being slightly lower at pH 7.0 (96-h 2.8 μg/L, 7-d 2.3 μg/L) than at pH 5.7 (96-h 4.2 μg/L, 7-d 3.1 μg/L) (Cusimano et al. 1986, Study 9 in Table 2, hardness 9.2).

In an experiment focusing on sublethal, behavioural responses (Gosavi et al. 2020, Study 13 in Table 2, pH or hardness not given), individuals of the common spiny loach *Lepidocephalichthys thermalis*, a fish found in subtropical/tropical India and Sri Lanka, were exposed for three hours to 5 μg/L of dissolved copper. Even this short-term exposure reduced predator recognition (causing reduced swimming activity) in the loach significantly compared to controls, likely due to olfactory dysfunction according to the authors of the study, and loach survival in predation trials was significantly reduced, as well.

Survival of adult *Rasbora sumatrana*, a ray-finned fish found in subtropical/tropical south-east Asia, was reduced after 96-h exposure to dissolved copper (LC50 6.0 μg/L) (Shuhaimi-Othman et al. 2015, Study 15 in Table 2, pH 6.5, hardness 20). Similarly, *Nais elinguis*, an aquatic oligochaete with a cosmopolitan distribution, showed lower survival after 96-h exposure to dissolved Cu (LC50 7.0 μg/L) (Shuhaimi-Othman et al. (2013, Study 20 in Table 2, pH 6.7, hardness 19). Likewise, survival of juveniles of the North American freshwater snail *Planorbella pilsbryi* was reduced after 96-h exposure to dissolved Cu (LC50 8.0-10.0 μg/L) (Osborne et al. 2023, Study 31 in Table 2, pH 7.8-8.8, hardness 466).

For stock-culture-derived organisms of the cosmopolitan freshwater cyanobacterium *Microcystis aeruginosa*, growth rate after 96 h was reduced under exposure to dissolved Cu (EC50 4.8-5.0 µg/L) (Zang et al. 2024, Study 40 in Table 2, pH 8.0, hardness 7.8).

Mesocosm experiment with copper exposure

In the only community-level experiment investigating copper effects on freshwater biota that met the criteria of this literature review (Study 7 in Table 2), Iwasaki et al. (2022) exposed stream macroinvertebrate communities in 18 closed-system mesocosms located in a greenhouse in Colorada, USA for 10 days to two categorical concentrations of dissolved copper (3.2 and 24.7-29.1 µg/L) at five different water hardnesses (29-253, pH 7.6-7.9). In this experiment, the macroinvertebrate communities were strongly detrimentally affected (significant reductions in total invertebrate abundance, mayfly abundance, mayfly taxon richness and abundances of 4 of the 9 most common taxa) at the higher Cu concentration (24.7-29.1 µg/L) in all 5 water hardness treatments.

Although this Cu concentration is somewhat above the range detected in the two Southland river surveys, it is worth noting that a 10-day exposure is quite short for a mesocosm experiment where manipulative periods of 3-5 weeks are common and toxicant exposures can sometimes be as long as 98 days – see e.g. Hoang et al. 2021 on zinc (Study 23 in Table 3). Moreover, the relatively small number of experimental units in this experiment (18) allowed only using two metal concentrations (3.2 1 µg/L and the much higher 24.7-29.1 µg/L) in a categorical design (rather than a gradient design with multiple metal concentrations). Given these design limitations, and the fact that toxic effects of contaminants generally become more severe with increasing exposure time, it is possible that a Cu concentration within the range detected in Southland rivers during dry weather conditions might have been high enough to cause detrimental effects on the stream invertebrate communities in Iwasaki et al.'s study if their exposure period had been 3-5 weeks or longer.

Table 2. Summary of single-exposure experiments with dose-response designs on the effects of ***copper*** exposure on freshwater organisms. Clear, ecologically relevant effects found in acute-exposure studies at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (total Cu 1.41 – 8.66 µg L^{-1}); dissolved Cu <0.50 - 3.21 µg L^{-1}) are highlighted in blue font. Moreover, such clear effects found in chronic-exposure studies at concentrations within the range detected in Southland rivers during the 2023 dry weather survey (total Cu 0.53 - 2.6 µg L^{-1}; dissolved Cu <0.5 - 1.2 µg L^{-1}) are highlighted in yellow.

LC50 = concentration expected to kill 50% of a group of test animals when administered as a single exposure; EC50 = 50% effect concentration (e.g. reduction in growth compared to control performance); EC20 = 20% effect concentration; EC10 = 10% effect concentration; LOEC = lowest-observed-effect concentration (compared to control treatment).

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
1	*Galaxias maculatus* Inanga (Galaxiidae)	Juveniles	Acute - 48 h 0 - 137 µg/L $CuSO_4 \times 5H_2O$ pH 7.1 Hardness 0.7 mmol/L	Survival (most ecologically relevant response) in freshwater treatment	25 µg/L: 82% survival 55 & 137 µg/L: 45% survival	Yes Yes	Yes Yes	Glover et al. (2016) New Zealand
2	*Echyridella menziesii* (Kākahi or kāeo) NZ Freshwater Mussel (Hyriidae)	Larvae (glochidia)	Acute - 48 h 0 – 9.4 µg/L $Cu(NO_3)_2 \times 3H_2O$ pH 7.8 Hardness 30	EC50 (survival) NOEC (survival)	EC50 = 1.7-3.4 µg/L NOEC = 1.1-2.6 µg/L	Yes Yes	Yes Yes	Clearwater et al. (2014) New Zealand
3	*Margaritifera margaritifera* Freshwater pearl mussel (Margaritiferidae)	3-day old juveniles	Acute – 96 h 0.6 - 110 µg/L Cu Cl_2 Soft water Hardness 40-48	LC50 (survival) LC10 (survival)	LC50 = 33 µg/L LC10 = 21 µg/L	Yes Yes	Yes Yes	Belamy et al. (2022) France
4	*Villosa iris* Rainbow mussel (Unionidae)	Glochidia (no age given)	Acute – 48 h 0 - 50 µg/L (nominal) $CuSO_4 \times 5H_2O$	LC50 (glochidia viability = survival)	LC50 = 14.2 µg/L	Yes	Yes	Salerno et al. (2020) Canada

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			pH 7.7-8.2 Hardness 95					
5	*Villosa iris* Rainbow mussel (Unionidae)	Juveniles (age ca. 150 d)	Chronic – 42 d 0.6 & 8.0 µg/L Cu (no formula given) pH 8.4 Hardness 155 (as in a river with alkaline mine drainage)	Experiment 2 only (more informative)				Timpano et al. (2022)
				Length increase (growth)	61% reduced at 8.0 µg/L Cu	Yes	Yes	USA
				Dry weight increase (growth)	73% reduced at 8.0 µg/L Cu	Yes	Yes	
6	*Pseudunio auricularius* Spengler's freshwater mussel (Margaritiferidae)	Newly transformed juveniles (24 h old)	Acute – 96 h 0 - 120 µg/L (nominal) $CuSO_4$ x $5H_2O$ pH 7.5 – 8.27 Hardness 160-180	LC50 (survival)	LC50 = 58.6 µg/L (hardness 160-180) Estimated for soft water (hardness 42) using a biotic ligand model: LC50 = 24.0 µg/L	Yes	Yes	Nakamura et al. (2021) Spain/Portugal
				LC10 (survival)	LC10 = 43.8 µg/L (hardness 160-180)	Yes	Yes	
7	Stream macroinvertebrate communities	Stream macroinvertebrate communities collected after 30 days of colonisation	10-d mesocosm experiment (18 units, 76 x 46 x 14 cm) in a greenhouse	Total invertebrate abundance Mayfly abundance Mayfly taxon richness	All reduced significantly in Cu-only treatment (24.7 µg/L)	Yes	Yes	Iwasaki et al. (2022) USA

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
		of plastic trays (10 x 10 x 6 cm) in an unpolluted stream	3.2 & 24.7-29.1 µg/L (Cu alone & combined with different levels of water hardness) $CuSO_4 \times 5H_2O$ Hardness 29 - 253 (5 levels, via adding $CaCO_3$) pH 7.6-7.9	Four of the 9 most common taxa: *Baetis*, *Epeorus*, *Utacapnia*, Chironomidae)	$CaCO_3$ addition: no significant effects on any macroinvertebrate responses → no protective effect of Ca on Cu toxicity (as assumed in Biotic Ligand Models – see Cu-Study 6 by Nakamura et al. 2021)			
8	*Oncorhynchus mykiss* Rainbow trout (Salmonidae)	From eyed embryos to swim-up fry stage	Chronic – 31 d 0 - 104 µg/L Cu SO_4 pH 8.11 Hardness 87	LC25 (survival) EC10 (reduced body weight) LOEC (reduced yolk sac resorption) LOEC (reduced coelomic fat)	LC25 = 46.3µg/L EC10 = 55.0 µg/L LOEC = 70 µg/L LOEC = 47 µg/L LOEC = 47 µg/L	Yes Yes Yes	Yes Yes Yes	McKay et al. (2024) Canada/USA
9	*Oncorhynchus mykiss* Rainbow trout (Salmonidae)	Juveniles (fry) (laboratory-reared; 6.7-7.0 cm long)	Acute - 96 h Subchronic – 7 d 0 – not specified Cu $Cl_2 \times 2H_2O$ pH 4.7, 5.7 & 7.0 Hardness 9.2 (very soft)	LC50 (survival)	96h - pH 4.7: LC50 = 66.0 µg/L 96h - pH 5.7: LC50 = 4.2 µg/L 96h- pH 7.0: LC50 = 2.8 µg/L	Yes Yes Yes	Yes Yes Yes	Cusimano et al. (1986) USA

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
					7d - pH 4.7: LC50 = 36.7 µg/L	Yes	Yes	
					7d - pH 5.7: LC50 = 3.1 µg/L	Yes	Yes	
					7d - pH 7.0: LC50 = 2.3 µg/L	Yes	Yes	
10	*Danio rerio* (Zebrafish) (Danionidae)	Embryos from ca. 2h post fertilisation	Acute - 96 h 0.1 – 0.8 mg/L $CuSO_4$ pH 5.0, 6.0 & 7.0 Hardness not given	LC50 (survival)	pH 5.0: LC50 = 360.0 µg/L	Yes	Yes	Boyle et al. (2020) United Kingdom
					pH 6.0: LC50 = 220 µg/L	Yes	Yes	
					pH 7.0: LC50 = 270 µg/L	Yes	Yes	
11	*Danio rerio* (Zebrafish) (Danionidae)	Embryos from 3-6h post fertilisation	Acute - 96 h 0.025 – 1.0 mg/L $CuSO_4$ pH 7.3 Hardness not given	LC50 (survival)	LC50 = 303.1 µg/L	Yes	Yes	Pereira et al. (2023) United Kingdom
				EC50 (hatching success = embryos able to break chorion)	EC50 = 75.5 µg/L	Yes	Yes	
12	*Danio rerio* (Zebrafish) (Danionidae)	Embryos from 2h post fertilisation	Acute - 96 h 19.8, 52.0 and 136.4 µg/L $CuSO_4 \times 5H_2O$ pH 7.5-8	Survival	Lower than in Control at 52 and 136 µg/L	Yes	Yes	Santos et al. (2020) Portugal/Spain

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			Hardness not given	Hatching rate	Lower than in Control at >= 20 µg/L	Yes	Yes	
				Body length and eye size at 96h	Lower than in Control at 136 µg/L	Yes	Yes	
				Larval behaviour at 96h: avoidance of an adverse visual stimulus (most ecologically informative response)	Lower than in Control at 52 and 136 µg/L	Yes	Yes	
13	*Lepidocephalichthys thermalis* Common spiny loach (Cobitidae)		Acute – 3 hours 5 µg/L $CuSO_4$ pH not given Hardness not given	Predator recognition (kairomones of two predatory fish species)	5 µg/L reduced predator recognition (causing reduced swimming activity) in loach significantly; probably due to olfactory dysfunction	Yes	Yes	Gosavi et al. (2020) India
				Survival in predation trials with one non-native predatory fish (Tilapia, *Oreochromis mossambicus*)	5 µg/L reduced loach survival in predation trials significantly	Yes	Yes	
14	*Micropterus salmoides* Largemouth bass (Centrarchidae)	Juveniles (ca. 2.58-2.69 g)	Acute – 96 h 0 – 27.2 mg/L $CuSO_4 \times 5H_2O$	LC50 (survival)	LC50 = 12.8 mg/L (12,800 µg/L)	Yes	Yes	Wang et al. (2023)

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			pH not given Hardness not given					China
			Chronic – 28 d 0.03 - 5.142 mg/L $CuSO_4$ x $5H_2O$ pH 8.2 Hardness 166	Survival (significant reduction compared to control)	Reduced at 1,630 or 5,142 µg/L	Yes	Yes	
				Weight gain (significant reduction)	Reduced at 1,630 or 5,142 µg/L	Yes	Yes	
				Food intake & feeding efficiency (significant reductions)	Reduced at 1,630 or 5,142 µg/L	Yes	Yes	
15	*Rasbora sumatrana* (a ray-finned fish) (Cyprinidae)	Adults (4-5 cm long)	Acute - 96 h 0.0056 – 0.087 mg/L $CuSO_4$ x $5H_2O$ pH 6.5 Hardness 20	LC50 (survival)	LC50 = 0.006 mg/L (6.0 µg/L)	Yes	Yes	Shuhaimi-Othman et al. (2015) Malaysia
	Poecilia reticulata Guppy (Poeciliidae)	Adults (2-3.5 cm long)	Acute - 96 h 0.01 – 0.75 mg/L $CuSO_4$ x 5H2O pH 6.5 Hardness 20	LC50 (survival)	LC50 = 0.038 mg/L (38.0 µg/L)	Yes	Yes	
16	*Acrossocheilus fasciatus* Freshwater grouper (Cyprinidae)	Juveniles (initial weight 1.56 g)	Chronic – 30 d 0.00, 0.0097, 0.037 mg/L (3 levels) $CuSO_4$ x 5H2O	Survival (significant reduction compared to control)	0.037 mg/L (37 µg/L)	Yes	Yes	Wang et al. (2023) China
				Weight gain (significant reduction)	37 µg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			pH 7.97 Hardness not given	Food intake (significant reduction)	37 µg/L	Yes	Yes	
17	*Labeo rohita* Rohu (Cyprinidae)	Adult hatchery fish (200-215 g)	Chronic – 36 d 0.00, 0.28, 0.42, 0.56 µg/L (4 levels) $CuSO_4$ pH not given Hardness not given	Body weight (significant loss compared to control)	0.56 µg/L	Yes	Yes	Naz et al. (2023) Pakistan
				Behavioural responses & clinical signs of stress (loss of equilibrium, air gulping, increased swimming, coordination loss, erratic swimming, swimming in isolation, mucus secretion from mouth and gills, rapid operculum movement, bulging eyes)	0.28 µg/L: mild symptoms (4 categories: no ailments, mild, moderate, severe) 0.42 µg/L: moderate symptoms 0.56 µg/L: severe symptoms	Yes	Yes	
				Also a number of different histopathological lesions in multiple fish tissues (e.g. brain, gills, liver, kidneys)	0.56 µg/L: very severe (= 100% of fish had lesions)	Yes	Yes	
18	*Brachymystax lenok* Sharp-snouted lenok (Salmonidae)	Juveniles (ca. 45 days post-hatching, mean length 2.3 cm)	Acute - 96 h 0 – 630 µg/L $Cu(NO_3)_2$ pH 7.29 Hardness 106	LC50 (survival)	LC50 = 134 µg/L	Yes	Yes	Zheng et al. (2023) China

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
19	*Clarias batrachus* Walking catfish (Clariidae)	Adults (16-21 cm, 50-55 g)	Acute - 96 h 0 – 30 mg/L $CuSO_4$ pH not given Hardness not given	LC50 (survival) Behavioural changes: hyperactivity, loss of equilibrium, rapid swimming, disturbed opercular movements, increased surface activity	LC50 = 15.02 mg/L From 12 mg/L: moderate changes From 24 mg/L: strong changes	Yes Yes	Yes Yes	Kumar & Srivastava (2021) India
20	*Duttaphrynus melanostictus* Asian common toad (Bufonidae)	Tadpole	Acute - 96 h Conc. ranges given in 5 other papers $CuSO_4 \times 5H_2O$ Mean pH 6.7, Hardness 19	LC50 (survival)	LC50 = 0.028 mg/L (28.0 µg/L)	Yes	Yes	Shuhaimi-Othman et al. (2013) Malaysia
	Macrobrachium lanchesteri Asian glass shrimp (Palaemonidae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 0.032 mg/L (32.0 µg/L)	Yes	Yes	
	Stenocypris major A freshwater ostracod (Cyprididae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 0.025 mg/L (25.0 µg/L)	Yes	Yes	
	Melanoides tuberculata A snail (Thiaridae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 0.14 mg/L (140.0 µg/L)	Yes	Yes	
	Chironomus javanus A midge larvae (Chironomidae)	Larvae (4^{th} instars)	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 0.17 mg/L (170.0 µg/L)	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
	Nais elinguis An aquatic oligochaete (Naididae)	Adults	Acute - 96 h Other details as above	LC50 (survival)	LC50 = 0.007 mg/L (7.0 µg/L)	Yes	Yes	
21	*Gammarus fossarum* A freshwater amphipod (Gammaridae)	Wild-caught adults (length ca 1 cm)	Chronic – 10 weeks 0.0 vs 1.4 µg/L $CuSO_4 \times 5H_2O$ pH 7.0 Hardness 11.5	Survival	Significantly reduced at 1.4 µg/L Cu (compared to control)	Yes	Yes	Lebrun & Gismondi (2020) France
				Behavioural traits: Respiration (compared to naïve gammarids freshly caught in the field)	Reduced at 1.4 µg/L Cu	Yes	Yes	
				Locomotory activity (moves per minute)	Reduced at 1.4 µg/L Cu	Yes	Yes	
				Feeding rate (leaf dry weight ingested per day)	Reduced at 1.4 µg/L Cu	Yes	Yes	
22	*Hyalella azteca* A freshwater/ brackish water amphipod (Hyalellidae)	7-day old juveniles from a lab culture	Chronic – 14 d 50 - 125 µg/L (nominal) $CuSO_4 \times 5H_2O$ pH 7.5 “moderately hard” water	LC50 (survival) - in the most sensitive treatment combination: stormwater as organic matter source, natural diet, pre-equilibrated diet	LC50 = 44.6 µg/L (based on measured Cu concentrations)	Yes	Yes	Fuad et al. (2024) USA
				EC20 (decrease in body length)	EC20 = 28.7 µg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
					(based on measured Cu concentrations)			
23	*Macrobrachium rosenbergii* Giant river prawn (Palaemonidae)	Post-larvae bought from a local producer (mean weight 0.57 g)	Acute – 48 h 0, 50 & 250 µg/L (nominal) $CuSO_4$ pH not given Hardness not given	Swimming behaviour (speed, motility rate, exploration rate, total distance) Note: A second batch of prawns generally showed higher swimming activity, suggesting that these metrics are not robust in this species (see Supporting Information)	None of the swimming parameters were affected significantly by the Cu treatments	Yes	No	Mena et al. (2024) Costa Rica
24	*Cambaroides dauricus* A crayfish endemic to Eastern Asia (Cambaroididae)	Juveniles from a crayfish farm (weight ca. 8.5 g)	Acute - 96 h 0.01 – 45.8 mg/L $CuSO_4$ x $5H_2O$ pH not given Hardness not given	LC50 (survival)	LC50 = 32.5 mg/L	Yes	Yes	Bao et al. (2020) China
			Subchronic - 14 d 2.06 + 4.12 mg/L Other details as above	Oxygen consumption rate Ammonium excretion rate	Both reduced significantly at 2.06 mg/L	Yes	Yes	
25	*Ceriodaphnia dubia* (a freshwater crustacean) (Daphniidae)	Female neonates < 24 h old (from two laboratory clones)	Subchronic – 7 d Concentration ranges given in a different paper	EC20 (survival) pH ca. 6.5 (4 trials) pH ca. 7.5 (4 trials)	EC20 Survival pH ca. 6.5: 11.0-15.2 µg/L	Yes	Yes	De Schamphelaere et al. (2025)

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			$CuSO_4$ x $5H_2O$ pH 6.5- 8.5 ("natural pH niche") Hardness 58	pH ca. 8.5 (1 trial) Note: "natural pH niche" based on existing experimental and observational data (also included in the paper) EC20 (reproduction – number of juveniles produced) pH ca. 6.5 (4 trials) pH ca. 7.5 (4 trials) pH ca. 8.5 (1 trial)	pH ca. 7.5: 22.0-27.5 µg/L pH 8.55: 30.9 µg/L EC20 reproduction pH ca. 6.5: ca. 12-15 µg/L pH ca. 7.5: ca. 23-27 µg/L pH 8.55: ca. 32 µg/L	Yes Yes Yes Yes Yes	Yes Yes Yes Yes Yes	Belgium & USA
26	*Daphnia exilis* (a North American freshwater crustacean) (Daphniidae)	Female neonates < 24 h old	Acute – 48 h 13-21 µg/L (after earlier range-finding tests) $CuSO_4$ x $5H_2O$ pH not given Hardness "moderate"	LC50 (survival)	LC50 (48 h) = 13.45 µg/L	Yes	Yes	Hernández-Zamora et al. (2023) Mexico
27	*Notodiaptomus iheringi* a Neotropical freshwater copepod (Diaptomidae)	Adults (20-30 days old)	Acute – 48 h 0-30 µg/L $CuCl_2$ pH 7.5-7.7 Hardness 48	LC50 (survival)	LC50 (48 h) = 27.7 µg/L LC10 (48h) = 16.0 µg/L	Yes Yes	Yes Yes	Rocha et al. (2024) Brazil

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			Subchronic – 8 d 16.0 µg/L pH 7.5-7.7 Hardness 48	Reproduction (number of eggs per female & % of viable eggs)	At Cu 16.0 µg/L: More eggs than Control (13.3 eggs/female versus 6.0) – but far more aborted/non-viable eggs (81% vs 20%)	Yes	Yes	
28	*Acanthocyclops americanus* a freshwater copepod (Cyclopidae)	Adults (> 4 months old)	Acute – 48 h 0.0 -10.0 mg/L $CuSO_4$ pH 7.1 Hardness 165	LC50 (survival)	LC50 (48 h) = 2.0 mg/L	Yes	Yes	Sobrino-Figueroa et al. (2020) Mexico
29	*Theodoxus fluviatilis* River nerite - a freshwater snail (Neritidae)	Wild-caught adults (shell length ca. 7 mm)	Chronic – 21 d 4.0 – 39.0 µg/L $CuCl_2 \times 2H_2O$ pH 8.4 Hardness not given	LC50 (survival)	LC50 (21 d) = 16.0 µg/L	Yes	Yes	Rothmeier et al. (2020)
				NOEC (survival)	NOEC (21 d) = 6 µg/L	Yes	Yes	Germany
				Activity (% snails creeping)	Significantly reduced at 15 µg/L	Yes	Yes	
30	*Physa acuta* (a freshwater snail) (Physidae)	Neonates from a laboratory population (72 h old, ca. 0.09 mm long)	Acute – 96 h 0.005-0.034 mg/L $CuCl_2 \times 2H_2O$ pH 7.8 Hardness 180	LC50 & LC10 (survival)	LC50 (96 h) = 162.4 µg/L	Yes	Yes	Crespo & Rossini (2021)
					LC10 (96 h) = 92.2 µg/L	Yes	Yes	Argentina

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			Chronic – 28 d 0.005-0.034 mg/L $CuCl_2 \times 2H_2O$ pH 7.8 Hardness 180	LC50 & LC10 (survival)	LC50 (28 d) = 33.0 µg/L LC10 (28 d) = 28.1 µg/L	Yes Yes	Yes Yes	
				Growth (shell length) (EC50 & EC10)	EC50 (28 d) = 20.1 µg/L EC10 (28 d) = 18.6 µg/L	Yes Yes	Yes Yes	
31	*Planorbella pilsbryi* (a freshwater snail) (Planorbidae)	Adults (shell length 10-14 mm) Juveniles (4-6 weeks old) Embryos (only embryos beginning to enter the two-cell division stage of mitosis)	Acute – 96 h Subchronic – 7 d & 10 d Adults: 0.000-0.091 mg/L Juveniles: 0.000-0.021 mg/L Embryos: 0.000 – 0.845 mg/L $CuSO_4 \times 5H_2O$ pH 7.8-8.8 Hardness 466	Adult survival (LC50, 7 d) Juvenile survival (LC50, 96 h) Embryo hatching success in % (EC50, 10 d)	LC50 (7 d) = 24.0 µg/L LC50 (96 h) = 8-10 µg/L EC50 (10 d) = 78-97 µg/L	Yes Yes Yes	Yes Yes Yes	Osborne et al. (2023) Canada
32	*Planorbella pilsbryi* (a freshwater snail) (Planorbidae)	Parents: adults (shell length 12.4-16.3 mm) Juveniles (4-6 weeks old)	Parental exposures (subchronic - 7 d): 9.1-39.3 µg/L	Growth of parental adults (shell length) after 7 d of Cu exposure	Significantly reduced at 13.8 and 39.3 µg/L compared to Control	Yes	Yes	Osborne et al. (2025) Canada

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			Juvenile exposures (Acute – 96 h):	Juvenile survival (LC50, 96 h)				
			1.4-87.3 µg/L $CuSO_4$ x $5H_2O$ pH 8.2-8.8	Born to parents straight after their 7-d Cu exposure	LC50 = 27.4-36.1 µg/L	Yes	No – patterns variable	(compared to Controls)
			Hardness 466	Born to parents 0-10 d post-exposure	LC50 = 20.5-46.0 µg/L	Yes	No – variable	
				Born to parents 11-20 d post-exposure	LC50 = 22.1-33.4 µg/L	Yes	No - variable	
33	*Girardia tigrina* (a freshwater planarian) (Dugesiidae)	Newborns (3-10 d after hatching, 2-4 mm long)	Acute – 96 h 0.00 – 1.60 mg/L $CuSO_4$ x $5H_2O$	LC50 (survival, 96 h exposure)	Newborns: LC50 (96 h) = 12.0 mg/L	Yes	Yes	Knakievicz & Ferreira (2008) Brazil
		Adults (3-4 months old, 7-10 mm long)	pH not given Hardness 0		Adults: LC50 (96 h) = 42.0 mg/L	Yes	Yes	
		Regenerating planarians (after decapitation)			Regenerating adults: LC50 (96 h) = 48.0 mg/L	Yes	Yes	
			0.05 – 0.80 mg/L	EC50 mobility (72-96 h exposure)	Newborns (after 72 h): EC50 = 0.09 mg/L (90 µg/L)	Yes	Yes	
					Adults (after 96 h): EC50 = 0.27 mg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			0.05 – 0.20 mg/L		Regenerating adults: EC50 = 0.30 mg/L	Yes	Yes	
			Chronic – 35 d Control, 0.05 & 0.20 mg/L	Time needed to regenerate (after 96 h exposure)	Significantly longer at 0.10 mg/L or above	Yes	Yes	
				Fecundity (number of cocoons) & fertility (number of hatchlings)	Both significantly reduced at 0.05 mg/L (= 50 µg/L) or 0.20 mg/L	Yes	Yes	
34	*Palatinus apiculatus* (an autotrophic freshwater dinoflagellate) (Peridiniaceae)	Dinoflagellates from a laboratory culture	"Chronic" – 72 h 0.00 – 1.43 mg/L $CuSO_4$ pH & hardness not given	EC50 (growth) EC20 EC10	EC50 = 0.052 mg/L (52 µg/L) EC20 = 0.018 mg/L (18 µg/L) EC10 = 0.013 mg/L (13 µg/L)	Yes Yes Yes	Yes Yes Yes	Bui et al. (2024) South Korea
35	*Chlorella* spp. (a tropical green microalga) (Chlorellaceae)	Algae in exponential growth mode, harvested from a 5-7 day-old stock culture (isolate 12, CSIRO, isolated initially from Lake Aesake, Papua New Guinea)	"Chronic" – 72 h 0 – 12 µg/L $CuSO_4$ x $5H_2O$ pH 7.3 Hardness 80-90 27 degrees Celsius	EC50 (growth) EC10	EC50 = 2.0 µg/L EC10 = 1.0 µg/L	Yes Yes	Yes Yes	Shakya et al. (2022) Australia/ Papua New Guinea

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
36	*Chlorella* spp. (a tropical green microalga) (Chlorellaceae)	Algae in exponential growth mode, harvested from a 5-6 day-old stock culture (isolate 12, CSIRO, isolated initially from Lake Aesake, Papua New Guinea)	"Chronic" – 72 h 0 – 100 µg/L $CuSO_4$ x $5H_2O$ pH 7.2-7.5 Hardness 80-90 27 degrees Celsius	EC50 (growth)	Without added DOM (0.27 mg C/L DOM): EC50 = 1.9 µg/L With 9.9 mg C/L DOM: EC50 = 63.0 µg/L	Yes Yes	Yes Yes	Macoustra et al. (2020) Australia/ Papua New Guinea
37	*Raphidocelis subcapitata.* (a freshwater microalga) (Elenastraceae)	Algae from a stock culture	"Chronic" – 72 h 0, 33 & 53 µg/L $Cu(NO_3)_2$ pH 7.5 Hardness not given	Growth (number of cells) Chl a content	Reduced significantly at 33 or 53 µg/L Reduced significantly at 53 µg/L	Yes Yes	Yes Yes	Machado & Soares (2024) Portugal
38	Eight different species studied:	Organisms from stock cultures (Culture Collection of Algae and Protozoa)	Chronic – 72 h & 7 d 8 concentrations (ranges differed between species, following range-finders) Cu^{2+} – exact formula not given pH 7.5 Hardness 60					Fettweis et al. (2023) Belgium
	One unicellular cyanobacterium: *Synechococcus elongatus* (Synechococcaceae)	CCAP 1479/1A		EC50 Growth rate (after 3 d) EC50 Total cell yield (after 7 d)	EC50 = 2.7 µg/L EC50 = 2.6 µg/L	Yes Yes	Yes Yes	
	Seven green microalgae:							

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
	Ankistrodesmus falcatus (Selenastraceae)	CCAP 202/14B		EC50 Growth (after 3 d)	EC50 = 12.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 7.8 µg/L	Yes	Yes	
	Chlamydomonas reinhardtii (Chlamydomonadaceae)	CCAP 11/45		EC50 Growth (after 3 d)	EC50 = 22.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 23.0 µg/L	Yes	Yes	
	Chlamydomonas vulgaris (Chlamydomonadaceae)	CCAP 211/11B		EC50 Growth (after 3 d)	EC50 = 60.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 82.0 µg/L	Yes	Yes	
	Desmodesmus subspicatus (Scenedesmaceae)	CCAP 276/20		EC50 Growth (after 3 d)	EC50 = 140.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 140.0 µg/L	Yes	Yes	
	Pseudokirchneriella subcapitata (Selenastraceae)	CCAP 278/4		EC50 Growth (after 3 d)	EC50 = 65.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 39.0 µg/L	Yes	Yes	
	Scenedesmus quadricauda (Scenedesmaceae)	CCAP 276/16		EC50 Growth (after 3 d)	EC50 = 32.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 23.0 µg/L	Yes	Yes	
	Tetraedron minimum (Hydrodictyaceae)	CCAP 273/1		EC50 Growth (after 3 d)	EC50 = 33.0 µg/L	Yes	Yes	
				EC50 Yield (after 7 d)	EC50 = 50.0 µg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
39	*Chlorella* spp. (a tropical green microalga) (Chlorellaceae)	Algae in exponential growth mode, harvested from 5-6 day-old stock cultures at CSIRO (isolated initially from Lake Aesake, Papua New Guinea)	"Chronic" – 72 h 0.46 – 8.8 µg/L Cu^{2+} – exact formula not given pH 7.8-8.1 Hardness 80-90 27 degrees Celsius	Single-species tests: EC50 (growth) EC10	 EC50 = 2.8 µg/L EC10 = 0.7 µg/L	 Yes Yes	 Yes Yes	McKnight et al. (2023) Australia/Papua New Guinea
	Monoraphidium arcuatum (another green microalga) (Selenastraceae)			EC50 (growth) EC10	EC50 = 1.1 µg/L EC10 = 0.5 µg/L	Yes Yes	Yes Yes	
	Pediastrum duplex (another green microalga) (Hydrodictyaceae) *Nannochloropsis*-like sp. (a golden microalga) (Monodopsidaceae)		Multispecies test (*Monoraphidium, Pediastrum & Nannochloropsis* together) 0.31 – 290 µg/L Cu^{2+} – exact formula not given pH 7.5 – 7.7 Hardness 100-120 27 degrees Celsius	Multispecies test: *Monoraphidium* EC50 (growth) EC10 *Pediastrum* EC50 (growth) EC10 *Nannochloropsis* EC50 (growth) EC10	 EC50 = 3.7 µg/L EC10 = 1.4 µg/L EC50 = 3.7 µg/L EC10 = 0.5 µg/L EC50 = 15.0 µg/L EC10 = 13.0 µg/L	 Yes Yes Yes Yes Yes Yes	 Yes Yes Yes Yes Yes Yes	
40	*Microcystis aeruginosa* (a freshwater cyanobacterium) (Microcystaceae)	Cyanobacteria from a stock culture (PCC7806)	"Chronic" – 96 h Cu-only treatment: 0-70 µg/L $CuSO_4 \times 5H_2O$	EC50 (growth)	EC50 = 4.8-5.0 µg/L (in Cu-only treatment)	Yes	Yes	Zhang et al. (2024) Belgium

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
			pH 8.0 Hardness 7.8					
41	*Scenedesmus quadricauda* (a green alga) (Scenedesmaceae)	Algae from a stock culture	Chronic – 8 days 0.00014 – 13.89 mg/L $Cu Cl_2$ x $2H_2O$ pH 7.5, Hardness not given	EC50 (growth) (most ecologically relevant response)	EC50 = 0.31 mg/L (310 µg/L)	Yes	Yes	Nawaz et al. (2025) Slovak Republic
42	*Scenedesmus obliquus* (a green alga) Scenedesmaceae	Algae from a stock culture	"Chronic" – 96 h 0.28 – 1.109 mg/L $Cu (NO_3)_2$ x $2.5H_2O$ pH ca. 7.0, Hardness not given	EC50 (growth) (most ecologically relevant response)	EC50 = 0.26 mg/L (260 µg/L)	Yes	Yes	Lin et al. (2020) USA
43	*Heterocypris incongruens* (a freshwater ostracod) (Cyprididae)	Adult parthenogenetic females; sourced from a wild population in a pool & a commercial toxicity kit	Subchronic: 7-18 d 0, 260 & 460 µg/L (nominal, actual values 97-103% of these) $CuSO_4$ x $5H_2O$ pH not given Hardness 120	Most ecologically informative responses:				Iglikowska et al. (2024) Poland
				Total hatching success (after 14-d exposure)	Reduced significantly at 260 & 460 µg/L	Yes	Yes	
				Mortality of juveniles (within 24 h after emergence from resting eggs)	Increased significantly at 260 & 460 µg/L	Yes	Yes	
				Survival of adults (after 11-d exposure)		Yes	No	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
					Reduced significantly at 260 & 460 µg/L			
44	Seven tropical freshwater species that are local to Kakadu National Park, Australia	Organisms from long-term stock cultures (18 years), except for the mussel: collected from Kakadu NP prior to the tests)	$CuSO_4$ x $5H_2O$ 0.2 – 135 µg/L pH 6.1 – 6.8 Hardness 1.9-3.3 (very soft)	EC50 & EC10 (survival, reproduction or growth) Dissolved Cu concentrations				Trenfield et al. (2022) Australia
	Mogurnda mogurnda (Northern trout gudgeon) (Eleotridae)	24h-old fry	7 days (subchronic)		Growth EC50 = 22.5 µg/L EC10 = 9.6 µg/L	Yes	Yes	
	Velesunio sp. (a mussel) (Hyriidae)	Glochidia <24h old post-release from female	24 h (acute)		Survival LC50 = 6.7 µg/L LC10 = 1.7 µg/L	Yes	Yes	
	Amerianna cumingi (a gastropod) (Planorbidae)	Adults (shell length 10-13 mm)	4 days (acute)		Reproduction EC50 = 8.0 µg/L EC10 = 5.7 µg/L	Yes	Yes	
	Moinodaphnia macleaya (a cladoceran) (Moinidae)	Brood 2 neonates within 7 h of hatching	6 days (acute)		Reproduction EC50 = 13.1 µg/L EC10 = 1.0 µg/L	Yes	Yes	
	Hydra viridissima (Green hydra) (Hydridae)	Hydroids with only 1 bud that is not fully developed	4 days (acute)		Reproduction EC50 = 7.6 µg/L EC10 = 2.5 µg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration (mostly dissolved) & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response?	Clear effect?	Reference & Country
	Chlorella sp. (a green algae) (Chlorellaceae)	4-5 day-old culture within exponential growth phase	72 h ("chronic")		Growth EC50 = 9.1 µg/L EC10 = 1.6 µg/L	Yes	Yes	
	Lemna aequinoctialis (a duckweed) (Araceae)	4 plants each with 3 fronds	4 days ("chronic")		Growth (surface area) EC50 = 12.0 µg/L EC10 = 6.8 µg/L	Yes	Yes	
45	*Chlamydomonas reinhardtii* (a green microalga) (Chlamydomonadaceae)	Algae purchased from a stock culture	"Chronic" – 96 h $CuSO_4$ x $5H_2O$ 0 – 12,000 µg/L (nominal) pH 7.0 Hardness not given	EC50 (growth) (most ecologically relevant response)	EC50 = 8.41 mg/L (8,410 µg/L)	Yes	Yes	Ye et al. (2023) China

2.3. Zinc

For zinc, the concentration ranges detected in Southland rivers during the 2024 wet weather survey (total Zn 1.5 – 27.1 µg L^{-1}); dissolved Zn <1.0 - 23.3 µg L^{-1}) and the 2023 dry weather survey (total Zn <1.1 – 30.0 µg L^{-1}; dissolved Zn <1.0 – 30.0 µg L^{-1}) were very similar, and certainly not higher during the wet weather survey (in contrast to Al and Cu). Therefore, all relevant ecotoxicological studies on zinc are summarised and discussed together in this section.

As demonstrated and discussed in depth in Price et al. (2022, 2023a, 2023b), the toxicity of zinc to freshwater organisms is affected by several water quality parameters, including pH, hardness and dissolved organic carbon (DOC). For example, in Price et al. (2022) zinc concentrations were tested at four hardnesses (5, 31, 93 and 402 mg $CaCO_3$ L^{-1}) and at three pH values (pH 6.7, 7.6 and 8.3) at each level of hardness. This range of pH and hardness was investigated as it covered the 10^{th}–90^{th} percentile range of natural freshwaters in Australian and New Zealand.

For Southland rivers, the abovementioned study by Stauber et al. (2023; see Section 2.1 on aluminium) includes multi-year data on pH values, hardness and DOC at 60 Southland stream and river sites. These data (supplied by Roger Hodson, Environment Southland) are median values (and 10th - 90th percentile range) from 5 years (Feb 2015–Feb 2020) of approximately monthly monitoring at 60 sites. Median pH was 7.5 (range 7.2–7.7), median hardness 36 (range 16-68), and median DOC 3.3 (range 1.5-5.9). These water quality data from Southland need to be kept in mind when interpreting the ecotoxicological studies below, and the findings from studies conducted under strongly different water quality conditions should only be extrapolated with caution to Southland rivers.

2.3.1. New Zealand studies

Three studies investigating zinc toxicity on freshwater biota in New Zealand matched the selection criteria for being included in this review. In one of them, Stauber et al. (2023, Study 1 in Table 3), two organisms were affected by zinc exposure at concentrations similar to those detected in Southland rivers. Laboratory-bred neonates of *Daphnia thomsoni*, a native freshwater microcrustacean, were exposed to zinc for 21 days in five natural New Zealand river waters (pH range <7.0 - 8.1, hardness 9-85). *Daphnia* reproduction was reduced under exposure to dissolved zinc (total neonates/adult: EC20 = 13-78 µg/L; total neonates/surviving adult: EC20 = 22-78 µg/L). Growth of *Raphidocelis subcapitata* (a green microalga with a global distribution) in 72 h was also reduced under zinc exposure in the same five New Zealand river waters (EC20 = 16-68 µg/L). Stream pH and water hardness in some of these five waters were similar to those in the Southland surveys, allowing a direct comparison. Consequently, these findings indicate that the concentrations detected in Southland rivers are likely high enough to be detrimental for two native NZ species belonging to very different taxonomic groups.

In the other two suitable New Zealand studies, neither the native freshwater clam *Sphaerium novaezelandiae* nor the native freshwater mussel *Echyridella menziesii* were affected by zinc exposure at the concentrations measured in Southland rivers (see Studies 2 & 3 in Table 3).

For zinc (and copper), Hickey & Golding (2002) conducted the only existing stream mesocosm study from New Zealand, in which they investigated effects on benthic stream invertebrate communities. However, they only added zinc and copper together as a mixture (in 12 channels with 3 replicates per treatment) at quite high concentrations; therefore, this study had to be excluded from the present review.

2.3.2. Overseas studies

For nine overseas freshwater taxa (and in one community-level outdoor mesocosm experiment), detrimental effects of zinc exposure were found at experimental concentrations within the range detected in Southland rivers. These nine taxa include six animal and three plant species belonging to a broad range of taxonomic groups (a fish, an amphipod, two mussels, a snail, a rotifer, two green algae and one cyanobacterium). Five of the nine species (including three with cosmopolitan distributions) have natural geographical distributions that include temperate (rather than tropical) climates roughly comparable to that of New Zealand, whereas four are tropical. In the mesocosm study, diverse assemblages of phytoplankton, periphyton and zooplankton simulating a subtropical lake environment were strongly affected by zinc exposure for 98 days, thus supporting and lending more weight to the findings of the less realistic laboratory studies on the six model species.

While the toxicity of zinc to freshwater organisms is affected by several water quality parameters, including pH, hardness and dissolved organic carbon (DOC), as discussed at the start of Section 2.3, most of the experiments on these nine overseas taxa were conducted under water quality conditions reasonably similar to those in the two Southland river surveys and/or in the abovementioned study by Stauber et al. (2023) that included water quality data from long-term monitoring in 60 Southland rivers. Consequently, and complementing the findings for the native NZ cladoceran and the native NZ alga presented in Section 2.3.1, the evidence from the nine overseas taxa presented in more detail below and in Table 3 suggests that the zinc contamination concentrations found in the 2023 and 2024 surveys of Southland rivers are high enough to raise concerns regarding potential detrimental effects on the freshwater biota in these rivers. As for copper, this overall assessment is tempered by the cautionary notes that the specific water quality conditions and the geographical distribution of the studied species should also be considered when evaluating to which extent the findings of a given overseas experiment might be extrapolated to the field conditions in Southland rivers.

For juveniles of the fatmucket mussel *Lampsilis siliquoidea*, a North American species, Wang et al. (2020, Study 12 in Table 3, pH 7.6-8.1, hardness 104-107) reported that three growth measures (length per individual, dry weight per individual, combined survivor biomass) were reduced after 12 weeks of exposure to dissolved zinc (length: EC10 = 26 µg/L; dry weight per individual: EC20 = 22 µg/L; total survivor biomass per replicate: EC20 = 21 µg/L. In the same study, reproduction of the North American amphipod *Hyalella azteca* (number of young per female) was reduced when exposed to dissolved zinc for 42 days (EC10 = 29 µg/L).

For *Lecane quadridentate*, a cosmopolitan freshwater rotifer, Torres Guzmán et al. (2010, Mexico, Study 14 in Table 3, pH 7.4-7.8, hardness 80-100), exposed wild-caught rotifers from a Mexican lake to zinc for 96 h. The LC50 for rotifer survival was 123.1 µg/L of dissolved zinc, and the corresponding LOEC was 10 µg/L.

For stock-culture-derived organisms of the fast-growing cyanobacterium *Synechococcus elongatus*, both growth rate (after 3 d, EC50 23.0 µg/L) and total cell yield (after 7 d, EC50 18.0 µg/L) were reduced under exposure to dissolved zinc (Fettweiss et al. 2023, Study 16 in Table 3, pH 7.5, hardness 60).

For the tropical green microalga *Chlorella* spp., Price et al. (2022, Study 19 in Table 3) investigated growth of algae from a stock culture (isolated initially from a lake in Papua New Guinea) during 72-h exposures to dissolved zinc at a range of pH (6.7-8.3) and water hardness (5-402) chosen to cover the 10^{th}–90^{th} percentile range of natural freshwaters in Australian and New Zealand. At hardness 5, the EC50 for growth in 72 h was 6.2-8.7 µg/L dissolved Zn at all three pH values. At hardness 31, the EC50 was 13 µg/L at pH 8.3 but increased to 32 µg/L at pH 6.7. At hardnesses 93 and 402, the corresponding EC50s increased further to 53-184 µg/L.

In the closely related study by Price et al. (2023b, Study 20 in Table 3) on the same species, 72-h zinc exposure tests were run in six different Australian natural freshwaters (pH 6.1–8.2, hardness 3–355, DOC <1–20 mg/L). Here the EC10 for 72-h growth ranged from 6.3 µg/L (at pH 8.0, DOC <1, hardness 11) to 193 µg/L (at pH 7.1, DOC 5.3, hardness 18). In a third study on the same species, Price et al. (2023a, Study 18 in Table 3) ran 72-h zinc exposure tests with or without added DOM from two natural freshwater habitats and one standard, commercial DOM source (pH 6.7-8.3). In this study, the EC10 for 72-h growth without added DOM ranged from 1.3-1.6 µg/L depending on pH, and from 1.8-20.0 µg/L with added DOM.

Growth of *Raphidocelis subcapitata* (a green microalga with a global distribution, see Stauber et al. 2023 from NZ above) in 72 h was also reduced under zinc exposure combined with P-limitation at 10, 30 or 50 µg/L dissolved Zn compared to a control (Rocha & Melão 2025, Study 22 in Table 3, pH 7.0). Without P-limitation, in contrast, zinc treatments had no effect.

Finally, in a study on seven tropical freshwater species from a wide range of taxonomic groups that are all local to Kakadu National Park, Australia, Trenfield et al. (2023, Study 25 in Table 3, pH 6.4-7.0, hardness 3.5-4.5) investigated the effects of exposure to dissolved zinc. The fish *Mogurnda mogurnda* (7-day growth of newly hatched fry, EC10 29.0 µg/L), the mussel *Velesunio angasi* (glochidia exposed to Zn for 24 h, LC10 for survival 21.0 µg/L) and the snail *Amerianna cumingi* (96-h reproduction, EC10 27.0 µg/L) were all negatively affected at Zn concentrations within the range of those measured during the two surveys of Southland rivers.

Mesocosm experiment with long-term zinc exposure

In the only community-based study found in the literature for zinc, Hoang et al. (2021, Study 24 in Table 3) simulated a subtropical lake environment in a 98-day outdoor mesocosm experiment (18 units, round tanks, 76 cm depth x 122 cm diameter; hardness 46-73, pH 7.7-9.2). In this rare long-term study, natural assemblages of phytoplankton, periphyton and zooplankton were initially introduced into the mesocosms by adding water collected from a lake in Florida, then communities were allowed to establish for 5.5 months before treatments started.

After 98 days of exposure to dissolved zinc, responses of phytoplankton (total and subtotal abundance minus cyanobacteria, 2 dominant taxa, diversity), zooplankton (total abundance, 3 dominant taxa, taxon richness) and periphyton (Chlorophyta abundance, the dominant taxon) were assessed. EC50s for Day 98 (when Zn exposure effects were strongest) ranged from 12.2-28.6 µg/L for phytoplankton responses, from 13.2-28.9 µg/L for zooplankton responses (except for taxon richness: 114.8 µg/L), and were 13.9 µg/L for periphyton (Chlorophyta abundance). Notably, most of these EC50s were within the range of the zinc concentrations detected in Southland rivers in the 2023 and 2024 surveys.

Table 3. Summary of single-exposure experiments with dose-response designs on the effects of ***zinc*** exposure on freshwater organisms. Clear, ecologically relevant effects found in acute-exposure experiments at concentrations within the range detected in Southland rivers during the 2024 wet weather survey (total Zn 1.5 – 27.1 µg L^{-1}); dissolved Zn <1.0 - 23.3 µg L^{-1}) are highlighted in blue font. Moreover, such clear effects found in chronic-exposure experiments at concentrations within the range detected in Southland rivers during the 2023 dry weather survey (total Zn (<1.1 – 30.0 µg L^{-1}; dissolved Zn also <1.0 – 30.0 µg L^{-1}) are highlighted in yellow.

LC50 = concentration expected to kill 50% of a group of test animals when administered as a single exposure; EC50 = 50% effect concentration (e.g. reduction in growth compared to control performance); EC20 = 20% effect concentration; EC10 = 10% effect concentration; LOEC = lowest-observed-effect concentration (compared to control treatment).

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
1	*Raphidocelis subcapitata* (a green microalga) (Selenastraceae)	Algae harvested from a stock culture at NIWA Hamilton, New Zealand	"Chronic"–72h 0 – 2000 µg/L (nominal) $ZnSO_4 \times 7H_2O$ Waters from 5 NZ rivers pH <7.0-8.1 Hardness 9-85	EC50, EC20, EC10 (growth) Reference water (ultrapure water with nutrients) 5 natural NZ river waters	Reference water: EC50 = 99 µg/L EC20 = 39 µg/L EC10 = 21 µg/L NZ waters: EC50 = 61-133 µg/L EC20 = 16-68 µg/L EC10 = 6.3-43 µg/L	 Yes Yes Yes Yes Yes Yes	 Yes Yes Yes Yes Yes Yes	Stauber et al. (2023) New Zealand Australia
	Daphnia thomsoni (a freshwater crustacean native to New Zealand) (Daphniidae)	Neonates < 24 h old from a laboratory culture maintained at NIWA Hamilton since 2019 (original animals collected from a pond in Waikato, New Zealand)	Chronic – 21 d 5 – 426 µg/L $ZnSO_4 \times 7H_2O$	EC50, EC20, EC10 (reproduction) Total neonates/adult Total neonates/surviving adult 5 natural NZ river waters (see above)	EC50 = 34-188 µg/L EC20 = 13-78 µg/L EC10 = 8-72 µg/L EC50 = 33-145 µg/L EC20 = 22-78 µg/L EC10 = 17-55 µg/L	Yes Yes Yes Yes Yes Yes	Yes Yes Yes Yes Yes Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
	Ceriodaphnia cf. *dubia* (an Australian freshwater crustacean) (Daphniidae)	Neonates < 24 h old from a laboratory culture maintained since 1991 (original animals collected from a lake in New South Wales, Australia)	Subchronic – 7 d	EC50, EC20, EC10 (survival, reproduction)				
			0 – 500 µg/L (nominal) $ZnSO_4 \times 7H_2O$	Surviving adults	EC50 = 134-324 µg/L	Yes	Yes	
					EC20 = 99-270 µg/L	Yes	Yes	
			Waters from 8 Australian rivers		EC10 = 82-239 µg/L	Yes	Yes	
			pH 5.6-7.5 Hardness <1-412	Total neonates/adult	EC50 = 124-138 µg/L	Yes	Yes	
					EC20 = 73-101 µg/L	Yes	Yes	
					EC10 = 52-83 µg/L	Yes	Yes	
2	*Sphaerium novaezelandiae* (a freshwater clam native to NZ (Sphaeriidae)	Wild-caught adults from a lake (shell length ca. 2 mm)	Acute - 96 h 0 – 5.0 mg/L (nominal) $ZnSO_4 \times 7H_2O$ pH not given Hardness 30	LC50 (survival) (all mussels combined)	LC50 = 1,160 µg/L	Yes	Yes	Phillips & Hickey (2010)
				Three different mussel genotypes	LC50 = 1,100 - 2,400 µg/L (but no significant difference across genotypes)	Yes	Yes	New Zealand
				Reburial time (all genotypes combined) Immediately after 96-h exposure	Significantly slower at 5.0 mg/L than at 0.31 mg/L	Yes	Yes	
				Following 24 h in clean water	Significantly slower at 1.25 and 5.0 mg/L than at lower concentrations	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
3	*Echyridella menziesii* (Kākahi or kāeo) NZ Freshwater Mussel (Hyriidae)	Larvae (glochidia)	Acute - 48 h 10 – 620 µg/L $ZnSO_4 \times 7H_2O$ pH 7.8 Hardness 30	LC50 (survival) NOEC (survival)	LC50 = 128-337 µg/L NOEC = 128-240 µg/L	Yes Yes	Yes Yes	Clearwater et al. (2014) New Zealand
4	*Villosa iris* Rainbow mussel (Unionidae)	Juveniles (age ca. 150 d)	Chronic – 42 d 6.7 & 193 µg/L Zn (no formula given) pH 8.4 Hardness 155 (as in a river with alkaline mine drainage)	Experiment 2 only (more informative) Length increase (growth) Dry weight increase (growth)	 74% reduced at 193 µg/L Zn 83% reduced at 193 µg/L Zn	 Yes Yes	 Yes Yes	Timpano et al. (2022) USA
5	*Pseudunio auricularius* Spengler's freshwater mussel (Margaritiferidae)	Newly transformed juveniles (24 h old)	Acute – 96 h 0 - 800 µg/L (nominal) $ZnCl_2$ pH 7.5 – 8.27 Hardness 160-180	LC50 (survival) LC10 (survival)	LC50 = 267.4 µg/L (hardness 160-180) Estimated for soft water (hardness 42) using a biotic ligand model: LC50 = 94.8 µg/L LC10 = 189.9 µg/L (hardness 160-180)	Yes Yes Yes	Yes Yes Yes	Nakamura et al. (2021) Spain/Portugal

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
6	*Brachymystax lenok* Sharp-snouted lenok (Salmonidae)	Juveniles (ca. 45 days post-hatching, mean length 2.3 cm)	Acute - 96 h 0 – 630 µg/L $Zn(NO_3)_2$ pH 7.29 Hardness 106	LC50 (survival)	LC50 = 222 µg/L	Yes	Yes	Zheng et al. (2023) China
7	*Oncorhynchus mykiss* Rainbow trout (Salmonidae)	Juveniles (fry) (laboratory-reared; 6.7-7.0 cm long)	Acute - 96 h Subchronic – 7 d 0 – not specified $ZnCl_2$ pH 4.7, 5.7 & 7.0 Hardness 9.2 (very soft)	LC50 (survival)	96h - pH 4.7: LC50 = 671.0 µg/L 96h- pH 5.7: LC50 = 97 µg/L 97h - pH 7.0: LC50 = 66 µg/L 7d - pH 4.7: LC50 = 501 µg/L 7d - pH 5.7: LC50 = 97 µg/L 7d - pH 7.0: LC50 = 66 µg/L	Yes Yes Yes Yes Yes Yes	Yes Yes Yes Yes Yes Yes	Cusimano et al. (1986) USA
8	*Channa punctatus* Spotted snakehead - a freshwater fish (Channidae)	Wild-caught juveniles ("fingerlings")	Acute - 96 h 0 – 20 mg/L (nominal) $ZnSO_4 \times 7H_2O$ pH 7.25 Hardness not given	LC50 (survival)	LC50 = 9.47 mg/L	Yes	Yes	Bashir & Samarth (2022) India
9	*Gambusia sexradiata* Mosquitofish (Poeciliidae)	Wild-caught adults from a local wetland	Acute - 96 h 0 – 91.25 mg/L	LC50 (survival)	Salinity 0: LC50 = 25.36 mg/L	Yes	Yes	Pérez-López et al. (2020)

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
			(salinity 0 = freshwater) 0 – 432.84 mg/L (salinity 15 = brackish water) $ZnCl_2$ pH 7.8 Hardness 108.9		Salinity 15: LC50 = 177.91 mg/L	Yes	Yes	Mexico
10	*Danio rerio* Zebrafish (Danionidae)	Fertilised eggs from a laboratory population (exposed from 2 h after hatching to 30 days later)	Chronic – 30 days 0.006 – 4.9 mg/L Zn Cl_2 pH 7.5 Hardness not given	LC50 (survival)	LC50 = 2.31 mg/L (2,310 µg/L)	Yes	Yes	Horie et al. (2020) Japan
				LOEC (survival)	LOEC = 1.50 mg/L (1,500 µg/L)	Yes	Yes	
				Day of hatching post-fertilisation	Delayed significantly at 0.41, 1.5 and 4.9 mg/L	Yes	Yes	
				Growth (after 15 days of exposure)	Reduced significantly at 1.50 mg/L (all fish died at 4.9 mg/L)	Yes	Yes	
11	*Rasbora sumatrana* (a ray-finned fish) (Cyprinidae)	Adults (4-5 cm long)	Acute - 96 h 0.056 – 0.87 mg/L	LC50 (survival)	LC50 = 0.46 mg/L (460 µg/L)	Yes	Yes	Shuhaimi-Othman et al. (2015) Malaysia

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
			$ZnSO_4$ x 7H2O pH 6.5, Hardness 20					
	Poecilia reticulata Guppy (Poeciliidae)	Adults (2-3.5 cm long)	Acute - 96 h 0.1 – 18 mg/L $ZnSO_4$ x 7H2O pH 6.5, Hardness 20	LC50 (survival)	LC50 = 1.05 mg/L (1,050 µg/L)	Yes	Yes	
12	*Lampsilis siliquoidea* (Fatmucket mussel) (Unionidae)	ca. 8-week-old juveniles	Chronic – 4 wk & 12 wk 2.4 – 238 µg/L Zn Cl_2 pH 7.6 – 8.1 Hardness 104-107	Only 12-week results shown (higher toxicity than after 4 weeks)				Wang et al. (2020) USA
				EC20, EC10 (length)	EC20 = 44 µg/L EC10 = 26 µg/L	Yes	Yes	
				EC20, EC10 (dry weight per individual)	EC20 = 22 µg/L EC10 = 14 µg/L	Yes	Yes	
				EC20, EC10 (total survivor biomass per replicate)	EC20 = 21 µg/L EC10 = 13 µg/L	Yes	Yes	
	Hyalella azteca (an amphipod) (Hyalellidae)	7 day-old juveniles	Acute 96-h 0 – 1000 µg/L (nominal) Zn Cl_2 pH & hardness as above	LC50 (survival)	Without feeding: LC50 = 99 µg/L With feeding: LC50 = 194 µg/L	Yes Yes	Yes Yes	
		7 day-old juveniles	Chronic – 42 d 2.5 - 224 µg/L Zn Cl_2	LC20, LC10 (survival)	LC20 = 68 µg/L LC10 = 55 µg/L	Yes	Yes	
				EC20, EC10 (dry weight)	EC20 = 87 µg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
			pH & hardness as above		EC10 = 66 µg/L			
				EC20, EC10 (survivor biomass per replicate)	EC20 = 63 µg/L EC10 = 53 µg/L	Yes	Yes	
				EC290, EC10 (No of young/female)	EC20 = 35 µg/L EC10 = 29 µg/L	Yes	Yes	
13	*Heterocypris incongruens* (a freshwater ostracod) (Cyprididae)	Adult parthenogenetic females; sourced from a wild population in a pool & a commercial toxicity kit	Subchronic: 7-18 d 0, 230 & 410 µg/L) (nominal, actual values 97-103% of these) $ZnSO_4 \times 7H_2O$ pH not given Hardness 120	Most ecologically informative responses:				Iglikowska et al. (2024)
				Total hatching success (after 14-d exposure)	Stimulated slightly (P = 0.05) at 410 µg/L	Yes	Partly – why stimulated?	Poland
				Mortality of juveniles (within 24 h after emergence from resting eggs)	Increased significantly at 230 & 410 µg/L	Yes	Yes	
				Survival of adults (after 11-d exposure)	Not affected by Zn treatments	Yes	No	
14	*Lecane quadridentate* (a freshwater rotifer) (Lecanidae)	Wild-caught rotifers from a lake	Acute - 96 h 31 – 2,000 µg/L $Zn(NO_3)_2$ pH 7.4-7.8 Hardness 80-100)	LC50 (survival)	LOEC 0.01 mg/L = 10 µg/L (extrapolated)	Yes	Yes	Torres Guzmán et al. (2010) Mexico
					LC50 0.1231 mg/L = 123.1 µg/L	Yes	Yes	
15	*Palatinus apiculatus*	Dinoflagellates from a laboratory culture	Chronic – 72 h $ZnSO_4$ 0.00 – 1.62	EC50 (growth) EC20 EC10	EC50 = 0.098 mg/L (98 µg/L)	Yes	Yes	Bui et al. (2024) South Korea

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
	(an autotrophic freshwater dinoflagellate) (Peridiniaceae)		pH & hardness not given		EC20 = 0.033 mg/L (33 µg/L) EC10 = 0.023 mg/L (23 µg/L)	Yes Yes	Yes Yes	
16	Eight different species studied:	Organisms from stock cultures (Culture Collection of Algae and Protozoa)	Chronic – 72 h & 7 d 8 concentrations (ranges differed between species, following range-finders) Zn^{2+} – exact formula not given pH 7.5 Hardness 60					Fettweis et al. (2023) Belgium
	One cyanobacterium: *Synechococcus elongatus* (Synechococcaceae)	CCAP 1479/1A		EC50 Growth rate (after 3 d) EC50 Total cell yield (after 7 d)	EC50 = 23.0 µg/L EC50 = 18.0 µg/L	Yes Yes	Yes Yes	
	Seven green algae:							
	Ankistrodesmus falcatus (Selenastraceae)	CCAP 202/14B		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 910.0 µg/L EC50 = 340.0 µg/L	Yes Yes	Yes Yes	
	Chlamydomonas reinhardtii (Chlamydomonadaceae)	CCAP 11/45		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 970.0 µg/L EC50 = 600.0 µg/L	Yes Yes	Yes Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
	Chlamydomonas vulgaris (Chlamydomonadaceae)	CCAP 211/11B		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 2000.0 µg/L EC50 = 4700.0 µg/L	Yes Yes	Yes Yes	
	Desmodesmus subspicatus (Scenedesmaceae)	CCAP 276/20		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 2400.0 µg/L EC50 = 480.0 µg/L	Yes Yes	Yes Yes	
	Pseudokirchneriella subcapitata (Selenastraceae)	CCAP 278/4		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 200.0 µg/L EC50 = 43.0 µg/L	Yes Yes	Yes Yes	
	Scenedesmus quadricauda (Scenedesmaceae)	CCAP 276/16		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 1600.0 µg/L EC50 = 38.0 µg/L	Yes Yes	Yes Yes	
	Tetraedron minimum (Hydrodictyaceae)	CCAP 273/1		EC50 Growth (after 3 d) EC50 Yield (after 7 d)	EC50 = 56.0 µg/L EC50 = 660.0 µg/L	Yes Yes	Yes Yes	
17	*Scenedesmus obliquus* (a green alga) (Scenedesmaceae)	Algae from a stock culture	"Chronic" – 96 h 2-18 mg/L $ZnSO_4 \times 7H_2O$ pH ca. 7.0 Hardness not given	EC50 (growth) (most ecologically relevant response)	EC50 = 10.13 mg/L (10,130 µg/L)	Yes	Yes	Lin et al. (2020) USA
18	*Chlorella* spp. (a tropical green microalga)	Algae in exponential growth mode,	"Chronic" – 72 h 0 – 5,000 µg/L	EC50 (growth) EC10	No added DOM: EC50 = 48-112 µg/L	Yes	Yes	Price et al. (2023a) Australia/

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
	(Chlorellaceae)	harvested from a 5-7 day-old stock culture (isolated initially from Lake Aesake, Papua New Guinea)	Zn Cl_2 pH 6.7-8.3 Hardness 90 27 degrees Celsius	Without or with added DOM from two natural freshwater habitats and one standard, commercial DOM source	EC10 = 1.3-1.6 µg/L DOM added: EC50 = 17-126 µg/L EC10 = 1.8-20.0 µg/L	Yes Yes Yes	Yes Yes Yes	Papua New Guinea
19	*Chlorella* spp. (a tropical green microalga) (Chlorellaceae)	Algae in exponential growth mode, harvested from a 5-7 day-old stock culture (isolated initially from Lake Aesake, Papua New Guinea)	"Chronic" – 72 h 0 – 5,000 µg/L Zn Cl_2 pH 6.7, 7.6, 8.3 Hardness 5, 31, 93 & 402 27 degrees Celsius	EC50 (growth) EC10 At pH 6.7, 7.6, 8.3 & At hardness 5, 31, 93 & 402	EC50 Hardness 5 pH 6.7 8.7 µg/L (dissolved Zn) pH 8.3 6.2 µg/L Hardness 31 pH 6.7 32 µg/L pH 8.3 13 µg/L Hardness 93 pH6.7 184 µg/L pH 8.3 53 µg/L Hardness 402 pH 6.7 96 µg/L pH 8.3 59 µg/L EC10 Hardness 5 pH 6.7 1.5 µg/L pH8.3 0.85 µg/L Hardness 31	 Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	 Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	Price et al. (2022) Australia/ Papua New Guinea

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
					pH 6.7 3.3 µg/L pH 8.3 1.3 µg/L Hardness 93 pH6.7 4.5 µg/L pH 8.3 3.2 µg/L Hardness 402 pH 6.7 5.3 µg/L pH 8.3 3.9 µg/L	Yes Yes Yes Yes Yes Yes	Yes Yes Yes Yes Yes Yes	
20	*Chlorella* spp. (a tropical green microalga) (Chlorellaceae)	Algae in exponential growth mode, harvested from a 5-7 day-old stock culture (isolated initially from Lake Aesake, Papua New Guinea)	"Chronic" – 72 h 0 – 5,000 µg/L (nominal) Zn Cl_2 pH 6.1 – 8.2 Hardness 3 – 355	EC50 (growth) EC10 Tests run in 6 different Australian natural freshwaters (each buffered or unbuffered) pH 6.1 – 8.2 Hardness 3 – 355 DOC <1 – 20 mg/L	**EC50** Smallest: 50 µg/L (at pH 8.0, DOC <1, hardness 11) Largest: 603 µg/L (at pH 8.1, DOC 4.2, hardness 355) EC10 Smallest: 6.3 µg/L (at pH 8.0, DOC <1, hardness 11) Largest: 193 µg/L (at pH 7.1, DOC 5.3, hardness 18)	Yes Yes Yes Yes	Yes Yes Yes Yes	Price et al. (2023b) Australia/ Papua New Guinea

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
21	*Chlorella vulgaris* (a green microalga with a cosmopolitan distribution) (Chlorellaceae)	Algae in logarithmic growth phase, harvested from a stock culture	"Chronic" – 72 h 0 – 2,000 µg/L (nominal) Zn Cl_2 pH 7.1 Hardness not given	EC50 (growth)	EC50 = 1.53 mg/L (1,530 µg/L)	Yes	Yes	Li et al. (2023) China
22	*Raphidocelis subcapitata* (a green microalga) (Selenastraceae)	Algae in exponential growth phase, harvested from a stock culture	"Chronic" – 72 h 0 – 50 µg/L (nominal) Zn Cl_2 pH 7.0 Hardness not given	Growth (most ecologically informative response) Second stressor: P-limitation (yes/no)	With P-limitation: Growth reduced significantly at 10, 30 or 50 µg/L Zn compared to control Without P-limitation: no effect of Zn treatments	Yes Yes	Yes No	Rocha & Melão (2025) Brazil
23	*Chlamydomonas reinhardtii* (a green microalga) (Chlamydomonadaceae)	Algae purchased from a stock culture	"Chronic" – 96 h 0 – 18,000 µg/L (nominal) $ZnSO_4 \times 7H_2O$ pH 7.0 Hardness not given	EC50 (growth) (most ecologically relevant response)	EC50 = 6.95 mg/L (6,950 µg/L)	Yes	Yes	Ye et al. (2023) China
24	Lake zooplankton, phytoplankton and	Natural assemblages of	Chronic –	Phytoplankton: Total and subtotal abundance	***EC50s on Day 98***			Hoang et al. (2021)

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
	periphyton communities	phytoplankton, periphyton, and zooplankton initially introduced into the mesocosms by adding 18L water collected from a lake in Florida, then communities established for 5.5 months before treatments started	98-d outdoor mesocosm experiment (18 units, round tanks, 76 cm depth x 122 cm diameter) 10, 14, 18, 43, 70 & 174 µg/L (time-weighted means) Zn Cl_2 Water hardness 46-73 pH 7.73-9.19	(minus cyanobacteria), 2 dominant taxa, diversity Zooplankton: Total abundance, 3 dominant taxa, taxon richness Periphyton: Chlorophyta abundance (dominant taxon) Results only shown for Day 98 (strongest effects): EC50 LOEC	*Phytoplankton* Total abundance 20.3 µg/L Subtotal abund. 16.5 µg/L Chlorophyta 15.7 µg/L Chrysophyta 12.2 µg/L Shannon diversity 28.6 µg/L *Zooplankton* Total abundance 24.2 µg/L Rotifera 28.9 µg/L Cladocera 13.2 µg/L Copepoda 17.8 µg/L Taxon richness 114.8 µg/L *Periphyton* Chlorophyta 13.9 µg/L ***LOECs on Day 98*** *Phytoplankton* Total abundance 21 µg/L Subtotal abund. 14 µg/L	 Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	 Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes	USA

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
					Chlorophyta 14 µg/L	Yes	Yes	
					Chrysophyta 14 µg/L	Yes	Yes	
					Shannon diversity 46 µg/L	Yes	Yes	
					Zooplankton			
					Total abundance 21 µg/L	Yes	Yes	
					Rotifera 21 µg/L	Yes	Yes	
					Cladocera 14 µg/L	Yes	Yes	
					Copepoda 21 µg/L	Yes	Yes	
					Taxon richness 21 µg/L	Yes	Yes	
					Periphyton			
					Chlorophyta 14 µg/L	Yes	Yes	
25	Seven tropical freshwater species that are local to Kakadu National Park, Australia	Organisms from long-term stock cultures (18 years), except for the mussel: collected from Kakadu NP prior to the tests)	$ZnSO_4 \times 7H_2O$ 0.2 – 7,200 µg/L pH 6.4 – 7.0 Hardness 3.5-4.5 (very soft)	EC50 & EC10 (survival, reproduction or growth)				Trenfield et al. (2023) Australia
	Mogurnda mogurnda	24h-old fry	7 days (subchronic)		Growth EC50 = 72 µg/L	Yes	Yes	

No.	Species & Country of study	Developmental stage	Duration & Concentration & Metal form	Response variable(s)	Clear Effect?	Ecologically relevant response	Clear Effect?	References
	(Northern trout gudgeon) (Eleotridae)				EC10 = 29 µg/L			
	Velesunio angasi (a mussel) (Hyriidae)	Glochidia <24h old post-release from female	24 h (acute)		Survival LC50 = 52 µg/L LC10 = 21 µg/L	Yes	Yes	
	Amerianna cumingi (a gastropod) (Planorbidae)	Adults (shell length 10-13 mm)	4 days (acute)		Reproduction EC50 = 68 µg/L EC10 = 27 µg/L	Yes	Yes	
	Moinodaphnia macleaya (a cladoceran) (Moinidae)	Brood 2 neonates within 7 h of hatching	6 days (acute)		Reproduction EC50 = 64 µg/L EC10 = 40 µg/L	Yes	Yes	
	Hydra viridissima (Green hydra) (Hydridae)	Hydroids with only 1 bud that is not fully developed	4 days (acute)		Reproduction EC50 = 74 µg/L EC10 = 53 µg/L	Yes	Yes	
	Chlorella sp. (a green algae) (Chlorellaceae)	4-5 day-old culture within exponential growth phase	72 h (“chronic”)		Growth EC50 = 1,867 µg/L EC10 = 286 µg/L	Yes	Yes	
	Lemna aequinoctialis (a duckweed) (Araceae)	4 plants each with 3 fronds	4 days (“chronic”)		Growth (surface area) EC50 = 757 µg/L EC10 = 320 µg/L	Yes	Yes	

3. Approaches for the analysis of Aluminium and other metals in rivers and streams in Southland

The assessment of the results of metals analysed in samples from river water in Southland (Section 1) identified the metals of primary concern to be aluminium (Al), Copper (Cu) and zinc (Zn). Of these three metals, the concentration of Al exceeded the ANZEEC freshwater quality guideline concentration for the protection of aquatic organisms.

In comparison to Cu and Zn, the chemistry of Al is the more complex and its toxicological impacts are the least understood. The following sections of this report therefore primarily discuss Al and refer to Cu and Zn when appropriate.

3.1. The chemistry, distribution and transport of Aluminium in the environment

Aluminium (Al) is the third-most abundant element in rocks (following oxygen and silicon) forming the crust of the earth where it is almost never present in a pure metallic form. Al is classified as a post-transition element within the Boron group (13) of the periodic table. The elements in the boron group are characterized by having three valence electrons and consequently Al forms compounds primarily in the +3 oxidation state. The small and highly charged cationic Al^{3+} forms strong covalent bonds with other elements, and its strong affinity for oxygen is demonstrated by the prevalence in sparingly soluble oxides and aluminosilicates in nature.

Despite its predominance in nature our knowledge of the Al cycle remains poor in comparison to other elements, presumably because it has no known biological function. This absence of biological functionality explains why Al has a toxic effect upon most living organisms, and particularly under acidic conditions where the cationic forms predominate.

The chemistry of Al is very complex and dependent upon pH. It is typically very insoluble, but its solubility can be increased significantly in acidic ($pH<6$) or alkaline ($pH >8$) conditions. Deposition of acid rain upon surface rocks and soil and infiltration into sediment can result in rapid mobilisation and release of Al into soil and sediment porewater where it can be transported into underlying groundwater or migrate into surface waters. The dissolution of Al by acid rain is exacerbated in soil and sediment with poor buffering capacity. The solubility of Al in soil can also be increased by elevated nitrification processes that increase soil acidity and release Al into soil porewater (Lawrence & David 1997). Any ionic Al species released from soil or sediment by acidic conditions can subsequently be complexed by various organic or inorganic ligands of undergo polymerisation to form polynuclear species of Al (Scancar & Milacic 2006).

As demonstrated in previous sections of this report, elevated concentrations of soluble Al species are toxic to a wide range of freshwater organisms. The toxicity of Al in freshwater ecosystems is dependent upon, and complicated by, its chemical speciation or form. To better understand the behaviour and toxicity of Al in freshwater aquatic ecosystems, design appropriate sampling strategies, and optimise methods of analysis, it is necessary to understand how the various species (forms) of Al are distributed and interact with naturally occurring inorganic and organic ligands.

The distribution of Al in nature has been conceptually described as a series of three "pools of Al" consisting of particular, aqueous, and living biomass, interconnected by various pathways or fluxes (Driscoll & Schecher 1990). In the particulate pool, Al is present in soil and sediment in the presence of organic matter and is differentiated by crystalline/mineral forms in equilibria with a second amorphous form from which a third exchangeable form is produced. These latter two forms of Al interact with soil or sediment organic matter.

Upon the influx of water, the exchangeable and amorphous particulate forms of Al within the particulate pool produce an inorganic form of Al within the aqueous pool consisting of numerous inorganic complexes with available anions such as OH^-, F- and SO_4^{2-}. These inorganic complexes interact with dissolved organic carbon (DOC) to produce organic forms of Al comprised of weak and strong complexes with a broad range of organic carbon, from small discrete molecules to macromolecular humic substances. Al has the ability to exchange between inorganic and organic form.

The third pool of living biomass can assimilate Al from the inorganic and organic complexed forms of Al within the second aqueous pool and ultimately release the same or altered forms back into the aqueous pool. Living biomass also provides organic carbon to the particulate pool through decomposition processes.

The speciation or form of Al within the second aqueous pool is strongly influenced by pH and the presence of inorganic and organic complexing ligands (Scancar & Milacic 2006). Below pH 5 mononuclear Al is predominantly present as $Al(H_2O)_6^{3+}$ or Al^{3+} as it is commonly referred to. At less acidic pH greater than 5, Al^{3+} is hydrolysed to produce $Al(H_2O)_5(OH)^{2+}$ (referred to as $Al(OH)^{2+}$) and $Al(H_2O)_4(OH)_2^+$ (referred to as $Al(OH)_2^+$). At neutral pH, Al forms insoluble $Al(OH)_3$ which redissolves in basic solutions exceeding pH 8 to produce $Al(OH)_4^-$.

The speciation of Al is not limited to mononuclear complexes, and it will polymerise at pHs above 5 to form polynuclear Al complexes. These include an unstable dimer $[Al_2(OH)_2(H_2O)_8{}^{4+}$ and the more stable polynuclear complexes $[Al_6(OH)_{12}]^{6+}$, $[Al_{10}(OH)_{22}]^{8+}$ and $[Al_{13}(OH)_{30}]^{9+}$. The formation of these polynuclear Al complexes is enhanced by high temperatures and aging and aggregation of these polymeric forms of Al leads to the formation of insoluble $Al(OH)_3$.

Within soil and sediment porewater various inorganic and organic ligands compete with hydroxide ions to form complexes with Al. In acidic solutions Al can form fluoride and/or sulphate complexes. Moreover, Al can interact with Si, forms weak interactions with bicarbonate (HCO_3^-) and forms sparingly soluble species with PO_4^{3-} that ultimately precipitate as $AlPO_4$.

Al will form complexes with numerous naturally occurring organic ligands. including carboxylic acids with low molecular weight, hydroxycarboxylic acids, phenols, sugar acids and more complex polyphenols. Al can also form stable complexes with humic and fulvic acids, the high-molecular-weight components of humic substances (Gerke 1994, Elkins & Nelson 2002, Takahashi & Dahlgren 2016). Al and other cations bind to humic substances via the numerous oxygen-containing functional groups they contain including carboxyl, phenolic, and alcoholic groups (Gensemer & Playle 1999).

Information of the form of Al in freshwater ecosystems is limited and is principally determined by the application of operationally defined methods of analysis. A number of Al species including precipitated forms are toxic to fish and other aquatic organisms in acidic and circumneutral waters (Gensemer et al. 2018). However, the most toxic forms of Al are the labile mononuclear complexes Al^{3+}, $Al(OH)_2^{1+}$ and $Al(OH)^{2+}$(Bi et al. 2001). For ease of reference, these toxic mononuclear complexes of Al will be collectively described as Al_{Mono} throughout the remaining discussion.

The significance of Al_{Mono} as the key toxic form of Al to aquatic organisms has been demonstrated by its toxicity to salmonid species in freshwater riverine catchments in Canada and Norway (Royset et al. 2005, Dennis & Clair 2012, Sterling et al. 2020).

While measurements of total and total dissolved Al are routinely included within water quality monitoring programmes, they are of limited biological relevance as they do not provide a direct measurement of the highly toxic Al_{Mono} species. Data for total and total dissolved Al have little biological relevance because different Al species differ significantly in their toxicity. The concentration of total and total dissolved Al merely provides a proxy for highly toxic Al_{Mono} species with the assumption that increases or decreases in total and total dissolved aluminium are matched by corresponding increases or decreases in the concentrations of Al_{Mono} species in a natural water sample.

While the speciation of Al affects its distribution and impact in freshwater ecosystems, the impact of Al within freshwater ecosystems is also influenced by extreme and seasonal weather conditions. Elevated concentrations of Al_{Mono} occurring during extreme storm events are attributed to the dilution of base cations as water migrates through shallower and more organic-rich soil layers; increased concentrations of mobile anions (Cl^-, SO_4^{2-} and F^-) facilitating the export of Al_{Mono} into stream and rivers; and lowered pH redissolving accumulated Al in soil and/or sediment in waterways (Sterling et al. 2020). This lowering of pH is exacerbated in the headwaters of forested catchments where rainfall entering soil mobilises low molecular weight components of soil organic carbon including organic acids. It is also generally accepted that the concentration of Al_{Mono} is seasonally elevated during spring snowmelt and autumn rainfall events and seasonally depressed during summer months when the concentration of DOC can be elevated (Sterling et al. 2020). It is therefore important to consider weather and seasonal climatic events leading to peak concentrations of Al_{Mono} within freshwater ecosystems, especially if these coincide with specific life stages of aquatic organisms that are especially vulnerable to Al toxicity.

Because the toxicity of Al in aquatic ecosystems depends upon its form several different methods have been developed to analyse speciated forms of Al, and particularly, the labile and highly toxic mononuclear forms of Al.

3.2. Methods to determine aluminium species in aquatic ecosystems

The quantification of Al species in freshwaters is challenging and very few thorough studies have been reported. Most of the reported data have been obtained from regional studies where natural waters have been impacted by deposition of acid rain and therefore contain elevated concentrations of Al. The analysis methods applied in these studies have limited relevance for rivers and streams in the Southland region of New Zealand where circumneutral pH conditions predominate and the concentrations of Al are lower in comparison.

The complex chemistry of Al species combined with their influence upon pH complicates their analysis. Various Al species can be present within a complex equilibrium in natural waters and many are unstable (Scancar & Milacic 2006).

The quantification of Al speciation in natural waters at trace concentrations is limited by:

- Al not being commonly included in standard metal suites within water quality studies and/or water quality monitoring programs;
- method detection limits being unsuitable for application in studies of natural waters;
- the presence of background Al contamination in chemical reagents, filters, specialist laboratory materials, dust, and air; and
- operational definitions of Al species, and particularly the toxic Al_{Mono} species, varying between research studies and analysis laboratories.

Measurements of total and total soluble Al are obtained by filtering water samples through 0.1 to 0.45 mm pore size filters to remove particulate Al, but their ability to remove colloidal Al varies widely. The results obtained are dependent upon the pore size of the filter and, because the size distribution of particulate Al is continuous, the separation between total particulate and soluble Al is operationally defined rather than absolute. Generally, the smaller the filter pore size the better, but more often than not this has to be balanced against the time it takes to filter samples through smaller pore size filters.

The primary factor limiting the identification of speciated forms of Al in natural waters remains the ability to separate or fractionate Al species into the desired forms for subsequent analysis. The inability to definitively separate speciated forms of Al into specific individual forms means that any separation and/or fractionation of Al species is operationally defined rather than chemically discrete or absolute.

The approaches used to fractionate and analyse speciated forms of Al in natural waters are discussed below with references provided to appropriate review and research articles.

3.2.1. Quantification of reactive Al species in natural waters

Due to the recognised toxicity of Al_{Mono} species various operational methods have been developed to quantify these labile, fast reacting mononuclear and toxic species of Al. These methods are based on fast rates of reactivity with the complexing agents 8-hydroxyquinoline, pyrocatechol violet, alizarin S, 4-nitrocatechol, lumogalliom and Morin, with the resulting Al-complexes detected by colorimetric analysis with UV-spectroscopy or spectrofluorimetry (Dominguez-Renedo et al. 2019). These methods assume that the chemical chelants react rapidly with toxic labile inorganic Al_{Mono} species compared to Al complexed by weak inorganic and/or organic ligands, colloidal and polymeric forms of Al and non-labile Al complexed by strong organic ligands.

The spectroscopic response of the formed Al-complexes is affected by pH and the reaction solutions need to be buffered to the pH that provides the maximum absorbance or fluorescence response. High concentrations of dissolved organic carbon and cations of iron need to be considered as they can interfere in these analyses.

The duration of these complexation reactions is critical to obtaining reproducible results and to ensure the concentration of reactive Al species is not overestimated. Enough time is required to ensure a full reaction is achieved, but extended reaction times can produce overestimates of the concentration of reactive Al species. This results from the complex equilibria between reactive and non-reactive forms of Al within natural waters. The depletion of reactive Al species by complexation can stimulate the release of additional reactive mononuclear species of Al from Al-organic complexes (non-labile monomeric Al) and colloidal polymeric forms of Al. The reaction times typically used in these complexation reaction methods depend upon the complexing agent employed and can vary from 30 seconds to 15 minutes.

These complexing methods provide the advantages of being relatively simple to implement, they use equipment that is inexpensive and common within laboratories, and they can be adapted for use with field portable instruments. They also have the advantage of being adopted for use in automated flow-injection instruments which provide reproducible reaction times, decrease analysis time and increase sample throughput in the laboratory (Andren & Rydin 2009).

The analysis of reactive Al via complexation reaction has previously been applied to quantify reactive Al in New Zealand stream waters. Total reactive Al determined by atomic adsorption spectroscopy following complexation with 8-hydroxyquinoline and extraction with methyl isobutyl ketone was applied to assess the impact of Al upon benthic invertebrates in highly tannic streams on the West Coast of the South Island (Winterbourn & Collier 1987). This study demonstrated a strong positive correlation between the concentration of reactive Al and dissolved organic carbon in the stream waters, with both being negatively correlated with the pH of the stream waters. This study concluded that most of the Al in the stream waters was present in non-toxic organic complexed form.

Other instrumental techniques widely used to quantify Al residues in natural waters, typically total acid digested and total dissolved Al, include flame and graphite atomic adsorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). The latter method is highly selective and has the advantage of being able to detect Al at sub to single unit μg/L concentrations.

To overcome the complexity of Al speciation in natural waters and the effect this has upon quantitating the toxic Al_{Mono} species it is common practice to fractionate environmental samples into defined species prior to quantitating Al.

3.2.2. Fractionation and speciation of Al species in environmental waters.

The approaches discussed below were developed to assess the speciation of Al in water samples as this is the principal medium through which most freshwater aquatic organisms are exposed to Al. Modified versions of these methods have been applied to investigate the speciation of Al in both soil and freshwater sediments, but these are not considered in this review.

Various procedures have been developed to separate and isolate Al species in natural water samples so the more labile and fast-reacting toxic Al_{Mono} species can be quantified. The most-used and widely accepted method to fractionate Al species in natural waters use chelating cation ion-exchange chromatography, usually as columns, where a solution of sample is passed through the exchange media which retains and therefore separates Al_{Mono} species from other polynuclear Al species and organic Al complexes. This fractionation technique introduced by Driscoll (1984) has since been widely adopted and while modifications have been introduced over time all of these related methods apply an indirect approach that operationally defines Al_{Mono} species.

Various cation exchange media have been applied in this separation scheme. Initially hand-packed columns of Chelex-100 resin or Amberlite cation exchange resins were exclusively used, but these media have been complemented and increasingly replaced with the widely available, commercially pre-packed solid-phase extraction and HPLC analytical cation exchange columns.

The broad range of cation exchange resin products that are available today (brands and proprietary adsorbents) has resulted in the absence of a consensus or standardisation of the Driscoll technique. Consequently, reported measurements of Al_{Mono} can be influenced by systematic errors arising from variations in the analytical methods that have been used. Despite these limitations, the Driscoll technique remains the most viable and broadly applied method to quantitate Al_{Mono} species. Whichever specific cation exchange method is applied to measure

Al_{Mono} species, it usually provides an overestimation of the amount of soluble Al_{Mono}. This occurs because whenever a chelating agent, cation exchange resin or complexing agent binds Al^{3+}, there is a shift in equilibria and some additional Al^{3+} is released from inorganic and organic complexes of Al (Gensemer & Playle 1999).

The inorganic speciation of Al can be subsequently calculated using a chemical equilibrium model with inputs of the measured concentration of labile monomeric Al (Al_{Mono}), pH, fluoride and sulphate (Driscoll 1984).

3.2.3. Hyphenated techniques

A fuller description of the advanced hyphenated analytical methods to detect and quantify various forms of Al summarised below can be found in the reviews of Bi et al. (2001) and Scancar & Milacic (2006).

The toxic forms of Al (principally inorganic monomeric Al species (Al_{Mono}) and total monomeric Al can be differentiated and quantified by combining complexing reactions using Flow Injection Analysers (FIA) in combination with electrochemical detection with glass carbon electrodes, hanging mercury drop electrodes differential pulse adsorptive striping voltammetry, amperometric detection etc (Bi et al. 2001).

Other hyphenated analytical methods incorporate combinations of high or fast pressure liquid chromatography with size exclusion (SEC), ion exchange, or reverse-phase columns followed by detection with UV or fluorescence detection, atomic spectroscopy, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Bi et al. 2001, Scancar & Milacic 2006). These advanced analytical methods are primarily used in research laboratories and are not available in commercial testing laboratories or regulatory laboratories undertaking routine water quality testing.

3.2.4. Nuclear magnetic resonance, fluorescence and Infra-red spectroscopy

Nuclear magnetic resonance (NMR), fluorescence and infrared (IR) spectroscopic techniques have been applied to characterise different Al species and quantitatively investigate the equilibria of Al complexes and different Al species in aqueous solution. Aluminium-27 (^{27}Al NMR) spectroscopy has been used to study mononuclear and hydroxy-Al complexes and the formation of polynuclear Al species, formation of complexes with low molecular organic acids, and the formation of Al-NTA-phosphate complexes in solution, and ^{27}Al NMR, fluorescence and IR spectroscopy have been used to study Al complexes with humic and fulvic acids (Scancar & Milacic 2006). The application of these spectroscopic techniques to studying Al forms in natural waters is their limit of detection which are typically in the mg/L concentration versus the mg/L concentrations typically encountered in natural waters. Similar to previously discussed analysis techniques, this analytical capability is often restricted to specialist research laboratories, is expensive to purchase, and in the case of NMR, very expensive to maintain and run.

3.3. The toxicity of Copper and Zinc

The most toxic forms of copper (Cu) and zinc (Zn) in natural waters are the free cationic ions, Cu^{2+} and Zn^{2+}, and toxicity of both metals is reduced when they form inorganic or organic complexes. As summarised in the ANZEEC Freshwater Quality Guidelines:

- the toxicity of Cu and Zn decreases with increasing hardness and alkalinity;
- the levels of dissolved organic matter found in most freshwaters are generally sufficient to remove Cu and Zn toxicity, but often not in very soft waters;
- Copper is strongly adsorbed and Zn is adsorbed by suspended material; and
- Copper complexing is increased at higher pH, but the relationship to toxicity is complex, whereas the toxicity of Zn generally decreases with decreasing pH, at least below pH 8.

In natural waters Cu and Zn are largely present as complexes with natural dissolved organic matter components including humic, fulvic and tannic acids, or adsorbed to colloidal, humic-coated iron and/or manganese oxide particles. Inorganic copper hydroxy species are toxic to aquatic organisms, but these species represent a relatively minor proportion of the dissolved copper within freshwater ecosystems (ANZEEC 2000).

Zinc forms complexes with natural DOM, the stability of which are dependent on the pH, the aqueous concentration of zinc and the presence and concentration of other ions in the waters (Florence & Batley 1977). Alkaline conditions favour the formation of Zn-DOM, $ZnOH^+$ and $ZnCO_3$; the latter complex being more prevalent in waters of increased alkalinity (Wilson 1978).

The removal of zinc from solution via adsorption processes is an important process in natural waters (CCREM 1991). Zinc can be removed from solution via adsorption to iron, aluminium and manganese (oxy)hydroxides and colloidal organic matter (ANZEEC 2000).

The speciation of Cu and Zn in freshwater aquatic ecosystems has some similarity to that of Al, with the result that the same analytical techniques have been applied to identify their form. As discussed above for Al, chelating cation-exchange adsorbents also have applicability to quantifying the toxic bioavailable fraction of Cu and Zn in fresh and marine waters by adsorption and calculation by difference (as per toxic Al species) or by adsorption followed by selective elution (Burgess et al. 1997, Bowles et al. 2006, Dietrich et al. 2013, Price et al. 2023).

3.4. Passive sampling for measuring dissolved phase metals in natural waters

The requirement to obtain consistent chemical measurements of metals and metalloids in natural waters is fundamental to determining the impact and risk they present to aquatic ecosystems and to ensuring water quality regulations are being met. Water quality monitoring programs assessing metals and metalloids largely rely upon grab sampling followed by instrumental analysis to determine total and dissolved concentrations. However, this approach has a number of limitations (Allen et al. 2008) including:

- low probability of capturing both minimum and maximum metal concentrations;
- potential contamination and changes in metal speciation during sample storage and transport;
- the amount of suspended/dissolved colloids and organic matter; and
- sample volume, filtration equipment, and size of the filter.

The results obtained are operationally defined and while this provides comparable results within and between different aquatic catchments and from year to year, the data obtained may be of limited relevance to ambient dissolved or bioavailable metal concentrations within aquatic environments. As previously discussed for Al in natural waters, metals can be present in a variety of forms including free cations, inorganic and organic complexes or sorbed to colloids or suspended particulates (Allen et al. 2008).

The timing of grab sampling-based water quality monitoring is typically scheduled on a calendar-based schedule with little consideration of fluctuations, especially peak contaminant loads, and optimisation of sampling frequency to capture them. Solutions to this problem include the use of dynamic autosamplers that produce representative composite or flow-proportional water samples, and/or passive sampling devices (PSDs) that produce time-weighted average (TWA) concentrations of contaminants in water.

The cost of purchasing, servicing, maintaining, and assuring the security of dynamic autosamplers in the field prohibits their use in large catchment-based studies. In comparison, PSDs are relatively cheap to purchase, can be deployed for several weeks, and only require human intervention during deployment and retrieval, thereby reducing the time and costs of water quality monitoring sample programs.

Additional benefits of PSDs include improved estimations of contaminant concentration trends in natural waters, separating, isolating and concentrating diluted target analytes from large sample volumes, separating target analytes from sample matrix, improving contaminant detection limits, providing a proxy for the bioavailability of contaminants, and in the case of metal contaminants, having the ability to target defined speciated forms. Furthermore, because PSDs are integrative samplers they dramatically reduce the number of grab samples that would otherwise be required for consistent monitoring (Senila 2023).

The most widely used and extensively characterised PSD for sampling metals in water is the diffusive gradient in thin film (DGT) device developed by Davison and Zhang at Lancaster University in 1994 (Davison & Zhang 1994). The DGT device consists of a plastic housing containing a layer of binding agent (complexing reagent) impregnated in a hydrogel that accumulates the analytes, overlaid by a diffusion layer comprising a diffusive hydrogel and an outer filter membrane overlies the binding-layer (DGT Research). Ions diffuse through the filter and diffusive gel to bind and accumulate within the binding-layer. After being retrieved the DGT devise is dismantled in a laboratory, the binding agent/hydrogel is separated and recovered, adsorbed metals are extracted/eluted and analysed - typically by ICP-MS.

The integrative nature of DGT-PSDs can also be a disadvantage as it averages high and low concentrations of analytes, like grab sampling. Other limitations include the need to consider the hydrodynamic properties of the sampling environment and match this with the diffusive properties (thickness) of the diffusion layer. The chemical properties of the water being sampled may influence the diffusion and accumulation of an analyte, in which case it may be necessary to conduct independent in-situ determinations and validation of analyte diffusion coefficients using a similar water chemistry.

Despite these limitations, DGT has become the most widely used method to assess the bioavailable fraction of metals and metalloids in water and sediment within freshwater, estuarine and marine ecosystems, and has become a widely applied method for assessing metals in water quality monitoring programs (Gadd and Milne 2019, Marrugo-Madrid et al. 2021).

With respect to aluminium, which has demonstrated the greatest exceedance of metal toxicity guideline thresholds in Southand rivers and streams, DGT-PSDs have been successfully applied to:

- assessing the speciation and toxicity of Al in acid mine drainage and waterways, estuaries and coastal waters impacted by mining (Balistrieri et al. 2007, Casiot et al. 2009, Gontigo et al. 2016, Shiva et al. 2017, Canavos et al. 2020);
- mapping the release of Al and other elemental solutes during aqueous corrosion of Al alloys (Mukhametzianova et al. 2024);
- assessing the environmental risk of Al and other metals from a deep sea tailings outfall (Sherwood et al. 2009);
- distinguishing total and labile concentrations in wastewater treatment plant effluents (Buzier et al. 2011) and assessing the bioremediation of Al in wastewater treatment plant effluent (Roberts et al. 2018);
- monitoring the bioavailable labile fraction in a drinking water plant (Arnedo et al. 2012);
- determining the labile bioavailable fraction of Al and other metals in natural waters (Larner et al. 2006, Roig et al. 2011, Yabuki et al. 2014, dos Anjos et al. 2017, Schoyen et al. 2017); and
- determining the speciation of Al and other metals in fresh and marine waters (Tonello et al. 2007, Warnken et al. 2007, Warnken et al. 2009, Tonello et al. 2011, Uribe et al. 2011, Ohlander et al. 2012, Liu et al. 2013).

Lastly, and importantly, the labile bioavailable fraction of Al accumulated by DGT-PSD has been demonstrated to predict the toxic inorganic species of Al in acid fresh waters (Al_{Mono}) that accumulates on the gills and produces physiological stress responses in brown trout (Royset et al. 2005). The species of Al accumulated by DGT corresponded to the labile monomeric inorganic forms of Al (highly toxic fraction), with the labile organic fraction being mostly excluded and particulate and colloidal forms being excluded (Royset et al. 2005). Because Al_{Mono} is similarly highly toxic to aquatic invertebrates, the corresponding TWA concentration of Al, Cu, Zn and other metals determined by DGT can be directly compared against their corresponding no-observable effect concentrations of representative aquatic invertebrates to predict both short-term acute and long-term chronic impacts.

Other advantages provided by in-situ DGT assessments of bioavailable metals include overcoming the introduction of potential sample storage artefacts related to the formation of aluminium hydroxides ($Al(OH)_3$ colloids/dissolved polymeric species); the fact that DGT also accumulates and measures a small fraction of labile organically bound Al in water (Royset et al. 2005), therefore providing a broader assessment of bioavailable Al than that obtained by reactive complexing methods; and reducing the error and uncertainty of measurement that is inherent in cation exchange column methods that determine toxic Al_{Mono} species by difference.

3.5. Determination of natural background concentrations of metals in Southland rivers and streams

The increase in the concentration of Al, Cu and Zn in the waters of Southland rivers and streams during wet weather events, and the corresponding increase in the exceedance of their respective ANZEEC water quality Guideline values, especially for total metal concentrations (dissolved and particulate fractions), indicates both dissolved and particulate associated metals are mobilised during wet weather high-flow events.

Presently, the source of the particulate load of these metals during wet weather events, whether they be mobilised from bed sediments within the waterways and/or resulting from particulates sourced from runoff from land, is uncertain.

The catchments of the Oreti and Mataura rivers, being mountains to sea exemplars, provide an opportunity to identify natural background concentrations of metals, inputs into the rivers related to different land uses, and how these influence the profile of metals along the length of the rivers (Environment Southland 2024).

The contribution of metals from contemporary riverine bed sediments into the water column can be determined by sampling and characterising the particle size distribution of bed sediments with complementary measurements of the exchangeable and total metals concentrations, mineralogical analysis of the particles, and if necessary, analysis of organic marker chemicals. The results obtained can subsequently be compared with similar analysis of suspended particulate material obtained from the principal river waters and associated tributaries during wet weather high flow events.

Characterising suspended particulate material and sediment within riverine catchments and relating their sources to different land uses within sub-catchments is more challenging and requires a multi-pronged approach using multiple sources of data. As outlined above for suspended particulates, measurements of exchangeable and total metals concentrations and mineralogical analysis of soils within sub-catchments under different land uses can provide useful baseline data. Other resources available to support this work include the online soil database S-Map that contains data on the soil classification(s), texture (particle size), and soil base chemistry (Bioeconomy Science Institute - S-Map online). Data from S-Map can be complemented by estimates of background concentrations of selected naturally occurring heavy metals across New Zealand (Manaaki Whenua Landcare Research 2023) and/or the direct measurement of heavy metals obtained by sampling soil within defined sub-catchment/land use areas.

Other complimentary methodological approaches that could assist characterising natural background concentrations of heavy metals and their sources into rivers in Southland include:

- analysing sediment and soil samples for specific persistent pesticide residues (DDT isomers and pyrethroid insecticides for dry stock, glyphosate and acid herbicides for dairy farming, hexazinone and terbuthylazine for forestry);
- using Compound Specific Stable Isotope (CSSI) sediment tracers as previously applied to the New River Estuary and Bay of Islands (Gibbs et al. 2014, Swales et al. 2012); and
- analysing sediment and soil samples for n-alkanes and determining their n-alkane index composed of Σ29n-alkanes, Σbiogenic n-alkanes, and Σanthropogenic n-alkanes (Galoski et al. 2019, He et al. 2020, Liu et al. 2022).

A monitoring program to fill current knowledge gaps on background contributions of heavy metals, their sources into riverine systems, and their enhanced mobilisation during severe weather events would ideally:

- span a minimum of least 2 years duration to capture seasonal affects, extreme flow events (low and high), and to provide a suitable number of replicate flow conditions that produces statistically relevant data supporting meaningful outcomes;
- include the sampling of tributary rivers and streams, especially those identified with discrete geology/mineralogy and/or land uses contributing unique signatures of heavy metals and particulates;
- include routine bimonthly (fortnightly) sampling complimented by targeted higher-frequency sampling during extreme weather high flow events;

- include the expanded range of measured water quality parameters, additional analyses specific to quantifying the more toxic and bioavailable fractions of Al, Cu, Zn and other heavy metals (cation exchange fractionation approach and DGT PSDs); and
- incorporate the data produced into an appropriate chemical equilibrium model to elucidate the speciation of heavy metals in river waters throughout the catchment, better predict their potential toxicological impact upon aquatic biota, and identify potential risks associated with the abstraction of water for agriculture use and human consumption.

4. Knowledge gaps and future research needs

For aluminium, copper and zinc, the following research is needed to gain a better understanding of their potential impacts on freshwater biota and ecosystems in Southland's running waters:

- more ecotoxicological laboratory experiments utilising native NZ model species (single-species studies at the population level), especially for aluminium and copper;

- more chronic-exposure ecotoxicological studies at field-realistic exposure concentrations, which focus not just on lethal but also sublethal responses such as growth, reproduction, feeding rates and behaviour;

- more laboratory experiments on NZ species that focus on how water quality variables (toxicity modifying factors) of pH, water hardness, and DOC modify metal toxicity, especially for aluminium and copper;

- more field-realistic outdoor mesocosm experiments that assess the impact of metals at the community and ecosystem levels on NZ species – not just in NZ, but worldwide;

- conduct a thorough review of the methods available to fractionate and quantify the concentration of the toxic labile forms of aluminium (Al_{Mono}), copper and zinc in natural waters, and identify the optimal method(s) for their isolation and analysis;

- subsequently, develop a standardised fractionation method to quantify the toxic labile forms of aluminium (Al_{Mono}), copper and zinc in natural waters;

- assess the use of DGT PSDs to quantify the concentration of the bioavailable toxic fraction of Al, Cu, Zn and other metals in Southland streams and rivers, compare the results obtained with those from the previous identified fractionation method, and confirm the preferred method(s) to quantify the bioavailable toxic fraction of Al, Cu, Zn;

- extended catchment-wide water quality surveys (Section 3.4) assessing the concentration of total, dissolved, bioavailable metal concentrations (DGT), and toxic labile monomeric Al_{Mono} species in Southland streams and rivers, especially under extreme low and high flow conditions (when surface runoff can cause short-term peaks in contaminant concentrations);

- additional water quality parameters to measure in a future monitoring programs specifically total organic carbon, total and dissolved fluoride and sulphate to assist determinations of the speciation of toxic Al species, and silicon as an indicator of contributions of Al from amorphous aluminosilicates;

- review chemical speciation models describing the speciation of heavy metals in natural waters to identify an appropriate model to apply to data produced by a multi-year water quality monitoring program for Southland rivers.

References

Studies cited in Sections 1 & 2

Blakemore K (2023) Metals results from selected River Water Quality Monitoring Sites. Internal memorandum, Environment Southland, 3 July 2023.

Blakemore K (2024) Metals results from selected river water quality sites under wet conditions. Internal memorandum, Environment Southland, 15 August 2024.

Donnachie RL, Johnson AC, Moeckel C, Pereira MG & Sumpter JP (2014) Using risk-ranking of metals to identify which poses the greatest threat to freshwater organisms in the UK. Environmental Pollution 194: 17-23.

Ebner JN (2021) Trends in the application of "omics" to ecotoxicology and stress ecology. Genes 12: 1481.

Forbes VE, Palmqvist A & Bach L (2006) The use and misuse of biomarkers in ecotoxicology. Environmental Toxicology and Chemistry 25: 272–280.

Santos B, Andrade T, Domingues I, Ribeiro R, Soares AM & Lopes I (2021) Influence of salinity on the toxicity of copper and cadmium to Zebrafish embryos. Aquatic Toxicology 241: 106003.

Wan JK, Chu WL, Kok YY & Lee CS. (2021) Influence of polystyrene microplastic and nanoplastic on copper toxicity in two freshwater microalgae. Environmental Science and Pollution Research 28: 33649-33668.

Ecotoxicological studies included in Tables 1-3 of Section 2 (focus on Al, Cu & Zn)

Bao J, Xing Y, Feng C, Kou S, Jiang H & Li X (2020) Acute and sub-chronic effects of copper on survival, respiratory metabolism, and metal accumulation in *Cambaroides dauricus*. Scientific Reports 10: 16700.

Belamy T, Baudrimont M, Cordier M-A, Dassie E, Gourves P-Y & Legeay A (2022) Sensitivity of newly transformed juveniles of the freshwater pearl mussel *Margaritifera margaritifera* to acute toxicity of a wide range of contaminants. Hydrobiologia 849: 2029–2041.

Boyle D, Clark NJ & Handy RD (2020) Toxicities of copper oxide nanomaterial and copper sulphate in early life stage zebrafish: Effects of pH and intermittent pulse exposure. Ecotoxicology and Environmental Safety 190: 109985.

Bui QTN, Kim T, Kim HS, Lee S, Lee S & Ki JS (2024) Sub-lethal effects of metals and pesticides on the freshwater dinoflagellate *Palatinus apiculatus* and environmental implications. Water Environment Research 202496: e11128.

Cardwell AS, Adams WJ, Gensemer RW, Nordheim E, Santore RC, Ryan AC & Stubblefield WA (2018) Chronic toxicity of aluminum, at a pH of 6, to freshwater organisms: empirical data for the development of international regulatory standards/criteria. Environmental Toxicology and Chemistry 37: 36–48, 2018

Clearwater SJ, Thompson KJ & Hickey CW (2014) Acute toxicity of copper, zinc, and ammonia to larvae (Glochidia) of a native freshwater mussel *Echyridella menziesii* in New Zealand. Archives of Environmental Contamination and Toxicology 66: 213-26.

Bálsamo Crespo E & Bulus Rossini G (2021) Comparative assessment of cadmium and copper toxicity to *Physa acuta* (Draparnaud, 1805). Bulletin of Environmental Contamination and Toxicology 107: 378-384.

Bashir S & Samarth M (2022) Effect of sublethal concentration of zinc on fresh water fish *Channa punctatus*. International Journal of Entomology Research 7: 13-18.

Cusimano RF, Brakke DF & Chapman GA (1986) Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences 43: 1497-1503.

Das RK & Nilay Ray N (2022) Acute toxicity of fluoride and aluminium on the freshwater fish, *Cyprinus carpio* L. Indian Journal of Experimental Biology 60: 150-154.

De Schamphelaere KAC, Baken S, Cardwell A, Du Laing G, Nys C, Stubblefield W, Viaene K & Weighman K (2025) The effect of pH on chronic copper toxicity to *Ceriodaphnia dubia* within its natural pH niche. Environmental Toxicology and Chemistry 44: 484-496.

Factor CJB & de Chavez ERC (2012) Toxicity of arsenic, aluminum, chromium and nickel to the embryos of the freshwater snail, *Radix quadrasi* von Möellendorf 1898. Philippine Journal of Science 141: 207-216.

Fettweis A, Hansul S, Schamphelaere K & Smolders E (2023) Metal mixture toxicity of Ni, Cu, and Zn in freshwater algal communities and the correlation of single-species sensitivities among single metals: a comparative analysis. Environmental Toxicology and Chemistry 12: 2666-2683.

Fuad N, Williams R & Vadas TM (2024) Short-term chronic toxicity of copper to *Hyalella azteca:* contrast in terms of equilibrating diet, diet type, and organic matter source. Toxics 12: 608.

Gao S, Sun P, Zhao X, Chang K & Chen W (2023) Waterborne copper exposure decreases fish growth and survival by promoting gills and liver impairments in largemouth bass (*Micropterus salmoides*). Environmental Science and Pollution Research 30: 119204-119216.

Glover CN, Urbina MA, Harley RA & Lee JA (2016) Salinity-dependent mechanisms of copper toxicity in the galaxiid fish, *Galaxias maculatus*. Aquatic Toxicology 174: 199-207.

Gosavi SM, Tapkir SD, Kumkar P, Verma CR & Kharat SS (2020) Act now before its too late: Copper exposure drives chemo-ecology of predator-prey dynamics of freshwater common spiny loach, *Lepidocephalichthys thermalis* (Valenciennes, 1846). Environmental Research 186: 109509.

Hernández-Zamora M, Rodríguez-Miguel A, Martínez-Jerónimo L, & Martínez-Jerónimo F (2023) Combined toxicity of glyphosate (Faena®) and copper to the American cladoceran *Daphnia exilis* — a two-generation analysis. Water 15: 2018.

Hoang TC, Brausch JM, Cichra MF, Phlips EJ, Van Genderen E & Rand GM (2021) Effects of zinc in an outdoor freshwater microcosm system. Environmental Toxicology and Chemistry 40: 2053-2072.

Horie Y, Yonekura K, Suzuki A & Takahashi C (2020) Zinc chloride influences embryonic development, growth, and Gh/Igf-1 gene expression during the early life stage in zebrafish (*Danio rerio*). Comparative Biochemistry and Physiology, Part C 230: 108684.

Iglikowska A, Szwarc A, Bartosik K, Gruba D, Michalak A, Namiotko L, Pouch A, Zaborska A & Namiotko T (2024) Environmental concentrations of metals (Cu and Zn) differently affect the life history traits of a model freshwater ostracod. The European Zoological Journal 91: 687-707.

Iwasaki Y, Cadmus P, Ranville J & Clements WH (2022) Stream mesocosm experiments show no protective effects of calcium on copper toxicity to macroinvertebrates. Environmental Toxicology and Chemistry 41: 1304-1310.

Knakievicz T & Ferreira HB (2008) Evaluation of copper effects upon *Girardia tigrina* freshwater planarians based on a set of biomarkers. Chemosphere 71: 419-428.

Kousar S, Javed M & Ilyas R (2016) Assessment of acute toxicity of aluminum to the four freshwater fish species (*Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*). Pakistan Journal of Agricultural Sciences 53: 491-495.

Kumar M & Srivastava AK (2021) Acute toxicity and ethological responses of fish, *Clarias batrachus* (Linn.) exposed to copper sulfate. International Journal of Zoological Investigations 7: 1022-1027.

Lebrun JD & Gismondi E (2020) Behavioural and biochemical alterations in gammarids as induced by chronic metallic exposures (Cd, Cu and Pb): Implications for freshwater biomonitoring. Chemosphere 257: 127253.

Li Y, Liu S, Ji Z, Sun J & Liu X (2023) Distinct responses of *Chlorella vulgaris* upon combined exposure to microplastics and bivalent zinc. Journal of Hazardous Materials 442: 130137.

Lin Y, Abraham J, Roy Chowdhury A, Su TL, Braida W & Christodoulatos C. (2020) Ecotoxicological response of Scenedesmus obliquus to pure energetic compounds and metal ions found in wastewater streams from munitions manufacturing. Algal Research 48: 101927.

Machado MD & Soares EV (2024) Integration of copper toxicity mechanisms in *Raphidocelis subcapitata*: advancing insights at environmentally relevant concentrations. Toxics 12: 905.

Macoustra GK, Jolley DF, Stauber J, Koppel DJ & Holland A (2020) Amelioration of copper toxicity to a tropical freshwater microalga: Effect of natural DOM source and season. Environmental Pollution 266: 115141.

McKnight KS, Gissi F, Adams MS, Stone S, Jolley D & Stauber J (2023) The effects of nickel and copper on tropical marine and freshwater microalgae using single and multispecies tests. Environmental Toxicology and Chemistry 42: 901-913.

Mathiron AGE, Bertin L, Brosselin V, Delorme N, Duny M, Geffard O & Jubeaux G (2025) Does exposure to zinc, methomyl, and perfluorooctanoic acid alter feeding behavior on MUG® in the freshwater amphipod *Gammarus fossarum*? Environmental Toxicology and Chemistry 44: 1389-1399.

McKay ME, Baseler L, Beblow J, Cleveland M & Marlatt VL (2024) Comparative subchronic toxicity of copper and a tertiary copper mixture to early life stage rainbow trout (*Oncorhynchus mykiss*): impacts on growth, development, and histopathology. Ecotoxicology 33:1-21.

Mena F, Araújo CVM, Echeverría-Sáenz S, Brenes-Bravo G & Moreira-Santos M (2024) Assessing habitat selection in the prawn *Macrobrachium rosenbergii* using the model toxicant copper and colonization as a test endpoint: Does prior exposure determine biochemical and behavioral responses? Aquatic Toxicology 275: 107073.

Nakamura K, Cañete J, Vijuesca D, Guillén N, Sosa C, Mesquita-Joanes F, Sousa R, Ginés E & Sorribas V (2021) Sensitivity of *Pseudunio auricularius* to metals and ammonia: first evaluation. Hydrobiologia 848: 2977-2992.

Nawaz A, Molnárová M, Šotek PE & Fargašová A (2025) Toxicity assessment of Cd and Cu on physicochemical parameters of green microalga *Scenedesmus quadricauda*. Journal of Applied Phycology 37: 431-443.

Naz S, Hussain R, Guangbin Z, Chatha AMM, Rehman ZU, Jahan S, Liaquat M & Khan A (2023) Copper sulfate induces clinico-hematological, oxidative stress, serum biochemical and histopathological changes in freshwater fish rohu (*Labeo rohita*). Frontiers in Veterinary Science 10: 1142042.

Osborne RK, Ijzerman MM, Venier C & Prosser RS (2023) Development of an embryo toxicity test to assess the comparative toxicity of metal exposure on different life stages of freshwater gastropods. Environmental Toxicology and Chemistry 42: 1791-1805.

Osborne RK, Gillis PL & Prosser RS (2025) Parental exposure of the freshwater snail *Planorbella pilsbryi* to copper results in transgenerational effects in offspring. Environmental Toxicology and Chemistry 44: 542-551.

Pérez-López A, Núñez-Nogueira G, Álvarez-González CA, De la Rosa-García S, Uribe-López M, Quintana P & Peña-Marín ES (2020) Effect of salinity on zinc toxicity ($ZnCl_2$ and ZnO nanomaterials) in the mosquitofish (*Gambusia sexradiata*). Environmental Science and Pollution Research 27: 22441-22450.

Phillips NR & Hickey CW (2010) Genotype-dependent recovery from acute exposure to heavy metal contamination in the freshwater *clam Sphaerium novaezelandiae*. Aquatic Toxicology 99: 507-513.

Price GAV, Stauber JL, Jolley DF, Koppel DJ, Van Genderen EJ, Ryan AC & Holland A (2023a) Natural organic matter source, concentration, and pH influences the toxicity of zinc to a freshwater microalga. Environmental Pollution 318: 120797.

Price GAV, Stauber JL, Jolley DF, Koppel DJ, Van Genderen EJ, Ryan AC & Holland A (2023b) Development and validation of multiple linear regression models for predicting chronic zinc toxicity to freshwater microalgae. Environmental Toxicology and Chemistry 42: 2630-2641.

Price GAV, Stauber JL, Holland A, Koppel DJ, Van Genderen EJ, Ryan AC & Jolley DF (2022) The influence of hardness at varying pH on zinc toxicity and lability to a freshwater microalga, *Chlorella* sp. Environmental Science Processes & Impacts 24: 783-793.

Rocha GS & Melão MGG (2025) Impacts of phosphorus limitation in the toxicity of environmental concentrations of lead and zinc in *Raphidocelis subcapitata* (Chlorophyceae). Journal of Applied Phycology 37: 407-417.

Rocha GS, de Palma Lopes LF & Espíndola ELG (2024) Copper and cadmium, isolated and in the mixture, impact the Neotropical freshwater Calanoida copepod *Notodiaptomus iheringi*: A short-term approach with environmental concentrations. Environmental Toxicology and Pharmacology 105: 104326.

Rodrigues GZP, Finkler M, Garcia ALH & Gehlen G (2020) Evaluation of transgenerational effects caused by metals as environmental pollutants in *Daphnia magna*. Environmental Monitoring and Assessment 192: 755.

Rothmeier LM, Martens A, Watermann B, Feibicke M, Kullwatz J & Gergs R (2020) Effects of copper ions on non-target species: a case study using the grazer *Theodoxus fluviatilis* (Gastropoda: Neritidae). Bulletin of Environmental Contamination and Toxicology 105: 62-66.

Salerno J, Gillis PL, Khan H, Burton E, Deeth LE, Bennett CJ, Sibley PK & Prosser RS (2020) Sensitivity of larval and juvenile freshwater mussels (unionidae) to ammonia, chloride, copper, potassium, and selected binary chemical mixtures. Environmental Pollution 256: 113398.

Sánchez-Aceves LM, Pérez-Alvarez I, Gómez-Oliván LM, Islas-Flores H & Barceló D (2021) Developmental alterations, teratogenic effects, and oxidative disruption induced by ibuprofen, aluminum, and their binary mixture on *Danio rerio*. Environmental Pollution 291: 118078.

Santos D, Félix L, Luzio A, Parra S, Cabecinha E, Bellas J & Monteiro SM (2020) Toxicological effects induced on early life stages of zebrafish (*Danio rerio*) after an acute exposure to microplastics alone or co-exposed with copper. Chemosphere 261: 127748.

Shakya M, Silvester E, Rees G, Rajapaksha KH, Faou P & Holland A (2022) Changes to the amino acid profile and proteome of the tropical freshwater microalga *Chlorella* sp. in response to copper stress. Ecotoxicology and Environmental Safety 233: 113336.

Shuhaimi-Othman M, Yakub N, Nur-Amalina R & Abas A (2015) Comparative toxicity of eight metals on freshwater fish. Toxicology and Industrial Health 31: 773-782.

Shuhaimi-Othman M, Nadzifah Y, Nur-Amalina R & Umirah NS (2013) Deriving freshwater quality criteria for copper, cadmium, aluminum and manganese for protection of aquatic life in Malaysia. Chemosphere 90: 2631-2636.

Sobrino-Figueroa A, Álvarez Hernandez SH & Álvarez Silva C (2020) Evaluation of the freshwater copepod *Acanthocyclops americanus* (Marsh, 1983) (Cyclopidae) response to Cd, Cr, Cu, Hg, Mn, Ni and Pb. AIMS Environmental Science 7: 449-463.

Stauber JL, Gadd J, Price GAV, Evans A, Holland A, Albert A, Batley GE, Binet MT, Golding LA, Hickey C, Harford A, Jolley D, Koppel D, McKnight KS, Morais LG, Ryan A, Thompson K, Van Genderen E, Van Dam RA & Warne MSJ (2023) Applicability of chronic multiple linear regression models for predicting zinc toxicity in Australian and New Zealand freshwaters. Environmental Toxicology and Chemistry 42: 2614-2629.

Timpano AJ, Jones JW, Beaty B, Hull M, Soucek DJ & Zipper CE (2022) Combined effects of copper, nickel, and zinc on growth of a freshwater mussel (*Villosa iris*) in an environmentally relevant context. Aquatic Toxicology 242: 106038.

Torres Guzmán F, González FJA & Martínez RR (2010) Implementing *Lecane quadridentata* acute toxicity tests to assess the toxic effects of selected metals (Al, Fe and Zn). Ecotoxicology and Environmental Safety 73: 287-295.

Trenfield MA, Walker SL, Tanneberger C, Kleinhenz LS & Harford AJ. (2022) Development of a site-specific guideline value for copper and aquatic life in tropical freshwaters of low hardness. Environmental Toxicology and Chemistry 41: 2808-2821.

Trenfield MA, Walker SL, Tanneberger C & Harford AJ (2023) Toxicity of zinc to aquatic life in tropical freshwaters of low hardness. Environmental Toxicology and Chemistry 42: 679-683.

Wang L, Hu C, Wang B, Wang H, Wang C, Shu Y, Gao C & Yan Y (2023). Chronic environmentally relevant concentration of copper exposure induces intestinal oxidative stress, inflammation, and microbiota disturbance in freshwater grouper (*Acrossocheilus fasciatus*). Aquatic Toxicology 263: 106702.

Wang N, Kunz JL, Cleveland DM, Steevens JA, Hammer EJ, Van Genderen E, Ryan AC & Schlekat CE (2020) Evaluation of acute and chronic toxicity of nickel and zinc to 2 sensitive freshwater benthic invertebrates using refined testing methods. Environmental Toxicology and Chemistry 39: 2256-2268.

Wang N, Ivey CD, Brunson EL, Cleveland D, Ingersoll CG, Stubblefield WA & Cardwell AS (2018) Acute and chronic toxicity of aluminum to a unionid mussel (*Lampsilis siliquoidea*) and an amphipod (*Hyalella azteca*) in water-only exposures. Environmental Toxicology and Chemistry 37: 61-69.

Ye M, Fang S, Yu Q, Chen J, Li P, Zhang C & Ge Y (2023) Copper and zinc interact significantly in their joint toxicity *to Chlamydomonas reinhardtii*: Insights from physiological and transcriptomic investigations. Science of the Total Environment 905: 167122.

Zhang Q, Demeestere K & De Schamphelaere KAC (2024) Mixture toxicity of the heavy metal copper and the fluoroquinolone antibiotic ciprofloxacin on *Microcystis aeruginosa*. Science of the Total Environment 955: 177144.

Zheng X, Wei C, Fan J, Liu X, Hou Y, Ling J, Wei J, Liu P (2023) Acute toxicity assessment and prediction models of four heavy metals. Toxics 11: 346.

Studies cited in Section 3

Allan, I. J., Knutsson, J., Guigues, N., Mills, G.A.,Fouillac, A.-M. and Greenwood, R. (2008). Chemcatcher® and DGT passive sampling devices for regulatory monitoring of trace metals in surface water. Journal of Environmental Monitoring 10(7): 821-829.

Andrén, C. M. and Rydin, E. (2009). Which aluminium fractionation method will give true inorganic monomeric Al results in fresh waters (not including colloidal Al)? Journal of Environmental Monitoring 11(9): 1639-1646.

ANZEEC (2000) Toxicant default guideline values for aquatic ecosystems — technical briefs:
Copper in freshwater and marine water. https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants/copper-2000
Zinc in freshwater. https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants/zinc-2000

Balistrieri, L.S., Seal, R.R., Piatak, N.M., and Paul, B. (2007). Assessing the concentration, speciation, and toxicity of dissolved metals during mixing of acid-mine drainage and ambient river water downstream of the Elizabeth Copper Mine, Vermont, USA. Applied Geochemistry 22: 930-952.

Bi, S., Yang, X., Zhang, F., Wang, X and Zou, G (2001). Analytical methodologies for aluminium speciation in environmental and biological samples – a review. Fresenius Journal of Analytical Chemistry 370(8): 984-996.

Bioeconomy Science Institute, Manaaki Whenua Landcare Research, S-Map online. https://smap.landcareresearch.co.nz/

Bowles, K.C., Apte, S.C., Batley, G.E., Hales, L.T., and Rogers, N.J. (2006). A rapid Chelex column method for the determination of metal speciation in natural waters. Analytica Chimica Acta 558 (1–2): 237-245.

Burgess, R.M., Charles, J.B., Kuhn, A., Ho, K.T., Patton, L.E. and McGovern, D.G. (1997). Development of a cation-exchange methodology for marine toxicity identification evaluation applications. Environmental Toxicology and Chemistry 16: 1203-1211.

Buzier, R., Tusseau-Vuillemin, M.H., Keirsbulck, M., and Mouchel, J.M. (2011). Inputs of total and labile trace metals from wastewater treatment plants effluents to the Seine River. Physics and Chemistry of the Earth 36: 500-505.

Cánovas, C.R., Basallote, M.D., Borrego, P., Millán-Becerro, R., and Pérez-López, R. (2020). Metal partitioning and speciation in a mining-impacted estuary by traditional and passive sampling methods. Science of the Total Environment 722: 137905.

Casiot, C., Egal, M., Elbaz-Poulichet, F., Bruneel, O., Bancon-Montigny, C., Cordier, M-A., Gomez, E., and Aliaume C. (2009). Hydrological and geochemical control of metals and arsenic in a Mediterranean river contaminated by acid mine drainage (the Amous River, France); preliminary assessment of impacts on fish (*Leuciscus cephalus*). Applied Geochemistry 24: 787-799.

Davison, W and Zhang, H. (1994). In situ speciation measurements of trace components in natural waters using thin-film gels. Nature 367: 546-8.

Díaz, A., Arnedo, R., Céspedes-Sánchez., R, Devesa, R., and Martn-Alonso, J. (2012). Monitoring of (bio)available labile metal fraction in a drinking water treatment plant by diffusive gradients in thin films. Environmental Monitoring and Assessment 184: 539-548.

Dietrich, A.M., Postlethwait, N., and. Gallagher, D.L. (2013). Quantifying Bioavailability and Toxicity of Copper to *Americamysis bahia* - Mysid Shrimp. International Journal of Environmental Science and Development 4 (1): 37-43.

Dennis, I.F. and Clair, T.A. (2012). The distribution of dissolved aluminium in Atlantic salmon (*Salmo salar*) rivers of Atlantic Canada and its potential effect on aquatic populations. Canadian Journal of Fisheries and Aquatic Sciences 69: 1174-1183.

DGT Research, United Kingdom. https://www.dgtresearch.com/#dgt_filters

Domínguez-Renedo, O., Navarro-Cunado, A.M., Ventas-Romay, E. and Alonso-Lomillo, M.A. (2019). Determination of aluminium using different techniques based on the Al(III)-morin complex. Talanta 196: 131-136.

dos Anjos, V.E., Abate, G., and Grassi, M.T. (2017). Determination of labile species of As(V), Ba, Cd, Co, Cr(III), Cu, Mn, Ni, Pb, Sr, V(V), and Zn in natural waters using diffusive gradients in thin-film (DGT) devices modified with montmorillonite. Analytical and Bioanalytical Chemistry 409: 1963-1972.

Driscoll, C. T. (1984). A Procedure for the Fractionation of Aqueous Aluminum in Dilute Acidic Waters. International Journal of Environmental Analytical Chemistry 16(4): 267-283.

Driscoll, C.T and Schecher W.D (1990). The chemistry of aluminium in the environment. Environmental Geochemistry and Health 12 (3): 28-49.

Elkins, K.M and Nelson D. J. (2002). Spectroscopic approaches to the study of the interaction of aluminum with humic substances. Coordination Chemistry Reviews 228 (2): 205-225.

Environment Southland. (2024). Mataura Catchment. Published by Environment Southland Regional Council. https://www.es.govt.nz/repository/libraries/id:26gi9ayo517q9stt81sd/hierarchy/environment/water/Catchment%20science%20summaries/ES_MatauraCatchment.pdf

Gadd, J. and Milne, J. (2019). Monitoring water quality in urban streams and stormwater. Guidance for New Zealand practitioners. NIWA Client Report 2019168AK prepared for Envirolink. MBIE Contract No. CO1X1701.

Gensemer, R. W. and Playle, R. C. (1999). The Bioavailability and Toxicity of Aluminum in Aquatic Environments. Critical Reviews in Environmental Science and Technology 29(4): 315-450.

Galoski, C.E., Jiménez Martínez, A.E., Schultz, G.B., dos Santos, I., and Froehner, S. (2019). Use of n-alkanes to trace erosion and main sources of sediments in a watershed in southern Brazil. Science of The Total Environment 682: 447-456.

Gerke, J (1994). Aluminum complexation by humic substances and aluminum species in the soil solution. Geoderma 63(2): 165-175.

Gibbs, M., Olsen, G., Stewart, M. (2014) New River Estuary sediment sources tracking pilot study. Client report ENS13203 to Southland Regional Council. 39 pp. https://www.es.govt.nz/repository/libraries/id:26gi9ayo517q9stt81sd/hierarchy/environment/water/estuaries/documents/estuarinereports/New%20River%20Estuary%20sediment%20tracking%20pilot%20study.pdf

Gontijo, E.S.J., Watanabe, C.H., Monteiro, A.S.C., et al (2016). Distribution and bioavailability of arsenic in natural waters of a mining area studied by ultrafiltration and diffusive gradients in thin films. Chemosphere 164: 290-298.

He, Y., Yang, C., He, W., Liu, W., and Xu, F. (2020). New insights into spatiotemporal source apportionment of n-alkanes under mixed scenario: A pilot study on Lake Chaohu, China. Science of The Total Environment 742: 140517.

Larner, B.L., Seen, A.J., and Snape, I. (2006). Evaluation of diffusive gradients in thin film (DGT) samplers for measuring contaminants in the Antarctic marine environment. Chemosphere 65: 811–820.

Lawrence, G.B. and David, M. B (1997). Response of aluminum solubility to elevated nitrification ins of a red spruce stand in Eastern Maine. Environmental Science & Technology 31 (3): 825-830.

Liu, R., Lead, J.R., and Zhang, H. (2013). Combining cross flow ultrafiltration and diffusion gradients in thin-films approaches to determine trace metal speciation in freshwaters. Geochimica et Cosmochimica Acta 109:14–26.

Liu, Y., He, Y., Liu, Y., Tao,S., and Liu, W. (2022). Assessing spatiotemporal sources of biogenic and anthropogenic sedimentary organic matter from the mainstream Haihe River, China: Using n-alkanes as indicators. Science of The Total Environment 834: 155382.

Manaaki Whenua Landcare Research. (2023). Determining background soil concentrations of trace elements across New Zealand. Envirolink Grant: 2321-HBRC267. Prepared for Hawkes Bay Regional Council. https://www.envirolink.govt.nz/assets/Envirolink/2321-HBRC267-Determining-background-soil-concentrations-of-trace-elements-across-New-Zealand.pdf

Marrugo-Madrid, S., Turull, M., Zhang, H., and Diez, S. (2021). Diffusive gradients in thin films for the measurement of labile metal species in water and soils: a review. Environmental Chemistry Letters 19(5): 3761-3788.

Mukhametzianova, G., Wager, S.,Eskinja, M., Moshtaghi, M., Mori, G., and Prohaska, T. (2024). Mapping elemental solutes at sub-picogram levels during aqueous corrosion of Al alloys using diffusive gradients in thin films (DGT) with LA-ICP-MS. Analytical and Bioanalytical Chemistry 416(14): 3373-3388.

Öhlander, B., Forsberg, J., Österlund, H. (2012). Fractionation of trace metals in a contaminated freshwater stream using membrane filtration, ultrafiltration, DGT and transplanted aquatic moss. Geochemistry: Exploration, Environment, Analysis 12:303-312.

Price, G.A.V., Stauber, J.L., Jolley, D.F., Koppel, D.J., Van Genderen, E.J., Ryan,A.C., and Holland, A. (2023). Natural organic matter source, concentration, and pH influences the toxicity of zinc to a freshwater microalga. Environmental Pollution 318: 120797.

Roberts, D.A., Shiels, L., Tickle, J., De Nys, R., and Paul N.A. (2018). Bioremediation of Aluminium from the Waste Water of a Conventional Water Treatment Plant Using the Freshwater Macroalga Oedogonium. Water 10(5): 626.

Roig, N., Nadal, M., Sierra, J., Ginebreda, A., Schuhmacher, M., and Domingo, J.L. (2011). Novel approach for assessing heavy metal pollution and ecotoxicological status of rivers by means of passive sampling methods. Environment International 37: 671-677.

Røyset, O., Rosseland, B.O., Kristensen, T., Kroglund, F., Garmo, O.A., and Steinnes, E. (2005). Diffusive Gradients in Thin Films Sampler Predicts Stress in Brown Trout (*Salmo trutta* L.) Exposed to Aluminum in Acid Fresh Waters. Environmental Science & Technology 39(4): 1167-1174.

Scancar, J. and Milacic R (2006). Aluminium speciation in environmental samples: a review. Analytical and Bioanalytical Chemistry 386(4): 999-1012.

Schoyen, M., Allan, I.J., Ruus, A., Havardstun, J., Hjermann, D.O., and Beyer, J. (2017). Comparison of caged and native blue mussels (*Mytilus edulis* spp.) for environmental monitoring of PAH, PCB and trace metals. Marine Environmental Research 130: 221–232.

Senila, M. (2023). Metal and metalloid monitoring in water by passive sampling – A review. Reviews in Analytical Chemistry 42(1): 1-17.

Sherwood, J.E., Barnett, D., Barnett, N.W., Dover, K., Howitt, J., Ii, H., Kew, P., and Mondon, J. (2009) Deployment of DGT units in marine waters to assess the environmental risk from a deep sea tailings outfall. Analytica Chimica Acta 652:215-223.

Shiva, A.H., Teasdale, P.R., Welsh, D.T, and Bennett,.W.W. (2017). Evaluation of the DGT technique for selective measurement of aluminium and trace metal concentrations in an acid drainage-impacted coastal waterway. Environmental Science: Processes and Impacts 19: 742-751.

Sterling, S. M., McLeod, S., Rotteveel, L., Hart, K., Clair, T. A., Halfyard, E. A., and O'Brien, N. L (2020). Ionic aluminium concentrations exceed thresholds for aquatic health in Nova Scotian rivers, even during conditions of high dissolved organic carbon and low flow. Hydrology and Earth System Sciences 24(10): 4763-4775.

Swales, A.; Gibbs, M.; Hewitt, J.; Hailes, S.; Griffiths, R.; Olsen, G.; Ovenden, R.; Wadhwa, S. (2012). Sediment sources and accumulation rates in the Bay of Islands and implications for macro-benthic fauna, mangrove and saltmarsh habitats. NIWA Client Report HAM2012–048 prepared for Northland Regional Council, 132 pp. https://www.nrc.govt.nz/resource-library-summary/research-and-reports/coastal/sedimentation-reports/bay-of-islands-sedimentation-report/

Takahashi, T and Dahlgren, R.A. (2016). Nature, properties and function of aluminum-humus complexes in volcanic soils. Geoderma 263: 110-121.

Tonello, P.S., Rosa, A.H., Abreu, C.H., and Menegario, A.A. (2007). Use of diffusive gradients in thin films and tangential flow ultrafiltration for fractionation of Al(III) and Cu(II) in organic-rich river waters. Analytica Chimica Acta 598:162-168.

Tonello, P.S., Goveia, D., Rosa, A.H., Fraceto, L.F., and Menegario, A.A. (2011). Determination of labile inorganic and organic species of Al and Cu in river waters using the diffusive gradients in thin films technique. Analytical and Bioanalytical Chemistry. 399:2563-2570.

Warnken, K.W., Davison, W., Zhang, H., Galceran, J., and Puy, J. (2007). In situ measurements of metal complex exchange kinetics in freshwater. Environmental Science and Technology 41:3179-3185.

Warnken, K.W., Lawlor, A.J., Lofts, S., Tipping, E., Davison, W., and Zhang, H. (2009). In situ speciation measurements of trace metals in headwater streams. Environmental Science and Technology 43:7230-7236.

Winterbourn, M.J., and Collier, K.J. (1987). Distribution of benthic invertebrates in acid, brown water streams in the South Island of New Zealand. Hydrobiologia 153: 277-286.

Yabuki, L.N.M., Colaço, C.D., Menegário, A.A., Domingos, R.N., Kiang, C.H., and Pascoaloto, D. (2014). Evaluation of diffusive gradients in thin films technique (DGT) for measuring Al, Cd Co, Cu, Mn, Ni, and Zn in Amazonian rivers. Environmental Monitoring and Assessment 186(2): 961-969.