NIWA
Taihoro Nukurangi

Chronic sensitivity of juvenile Canterbury mudfish (*Neochanna burrowsius*) and periphyton (*Rhizoclonium* sp.) to boron

Prepared for Bathurst Resources Ltd

June 2018

Authors/Contributors:
Hickey, C.W.
Thompson, K.J.
Bell, S.
Arnold, J.

For any information regarding this report please contact:
Dr C.W. Hickey
Principal Scientist

+64-7-856 1713
chris.hickey@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
PO Box 11115
Hamilton 3251

Phone +64 7 856 7026

NIWA Client Report No:	2018199HN
Report date:	June 2018
NIWA Project:	BRL18202

Contents

Tables

Figures

Reviewed and Approved for release by:

David Roper

Formatting checked by

A.Wadhwa

Executive summary

Bathurst Coal Ltd operates the Canterbury Coal Mine (CCM) which is located in the Malvern Hills which are situated along the foothills of the Southern Alps at the western edge of the Canterbury Plains. It is an opencast coal mine which has been developed over previous underground workings that were worked until 2003 when opencast mining commenced. Coal mining has been virtually continuous in the Malvern Hills coalfield since the underground Homebush mine opened in 1872, with at least 87 separate opencast and underground coal mines in the area.

Bathurst commissioned NIWA to develop site-specific water quality guidelines for boron which are applicable to the CCM receiving waters. Chronic toxicity testing was undertaken with two locally-relevant species, a fish and an alga, to supplement the boron toxicity database. A site-specific guideline was then undertaken to derive boron values considered suitable for application to the CCM site.

This report documents the results of chronic toxicity measurements for boron the Canterbury mudfish (*Neochanna burrowsius*) and a filamentous alga (*Rhizoclonium* sp.). The chronic tests were of 40 days duration for the mudfish, measuring toxicity endpoints of survival, growth (length and weight) and condition. The threshold toxicity for the mudfish was 10.2 g boron m^{-3}. The 7-day chronic test for the alga measured biomass (as chlorophyll *a*). The threshold toxicity for the alga was 1.7 g boron m^{-3}.

Approaches for site-species guideline derivation commonly use multiple components, including: (i) using local reference water quality data, (2) using biological effects data from laboratory-based toxicity testing, and (3) using biological effects data from field surveys. This assessment used a site-specific modification to the toxicity database as informed by the local habitats and biological monitoring data.

Based on the nature of the receiving water environments, being low energy stream and wetland habitats downstream of the CCM discharge, the site-specific guideline derivation excluded the microalgae which would not be considered critical for threshold sensitivity protection in this type of receiving water. Filamentous algae and rooted macrophytes are the predominant plant species in these habitats.

The site-specific database comprised 20 species which included the Canterbury mudfish data and filamentous alga, together with data for five macrophyte species. The threshold sensitivity for the filamentous algae was at the 8th percentile of the species sensitivity distribution (SSD) and the Canterbury mudfish was at the 66th percentile. The most sensitive species in the site-specific SSD is a duckweed (threshold sensitivity 1.4 g m^{-3}) and the least sensitive a fish (Eastern rainbowfish, 102 g m^{-3}).

The site-specific guideline values are:

Site-specific guideline values for boron for application to CCM receiving waters.

Site-specific guideline value type	Boron (freshwater) toxicity guideline value (g m^{-3})
High conservation value systems (99% species protection)	0.8
Slightly to moderately disturbed systems (95% species protection)	1.6
Highly disturbed systems	
(90% species protection)	2.3
(80% species protection)	3.4

Guideline applicability to the CCM site. The catchments in the local area surrounding the CCM have numerous historic coal mines with seeps leaching boron to streams and wetlands. Additionally, the area has large scale forestry and farming operations with stock access to waterways. The monitoring data for these streams indicates relatively depauperate communities which reflect a low habitat quality.

Based on multiple indicators the local receiving water conditions would be considered “highly disturbed systems” in the narrative used by ANZECC (2000) to describe guideline types. Thus, a protection threshold for boron of 90% equating to 2.3 g boron m^{-3} would be considered appropriate for application to the receiving waters around the CCM operations.

1 Introduction

1.1 Background

Bathurst Coal Ltd operates the Canterbury Coal Mine (CCM) which is located in the Malvern Hills which are situated along the foothills of the Southern Alps at the western edge of the Canterbury Plains.

It is an opencast coal mine which has been developed over previous underground workings that were worked until 2003 when opencast mining commenced. Coal mining has been virtually continuous in the Malvern Hills coalfield since the underground Homebush mine opened in 1872, with at least 87 separate opencast and underground coal mines in the area.

1.2 Brief

To develop a site-specific water quality guideline for boron for Canterbury Coal Mine (CCM) discharges. This specifically relates to a brief to address conditions specified in Resource Consent CRC1700541 (email Hamish McLauchlan, Bathurst Resources Ltd, 25 May 2017).

Resource Consent CRC170541 was granted with the following conditions:

Condition 14 boron limits:
Boron 1.5 mg/L – three month rolling median*
**Until modified in accordance with Conditions 16. to 21.*

Condition16 **Amendments to Boron Trigger Value**
The Consent Holder may request amendments to the Boron trigger value as listed in Condition 14. Any request shall occur only after the Consent Holder carries out a programme of work to develop a site-specific trigger value in the Tara Stream. The programme shall include:

a. Further detailed environmental chemistry and ecological studies on the Tara Stream and streams in the vicinity of the site (including local streams unaffected by the Consent Holder's activities) to establish background boron levels and sensitive benchmark organisms present in those streams;

b. Laboratory based toxicological studies using local sensitive benchmark organisms occurring in the streams and site water.

Best practice scientific evaluation of that data and the development of site specific trigger values for boron that is based on the Australian and New Zealand Guidelines for Fresh and Marine Water Quality 2000 methodology.

The initial phase of this study involved reviewing the available water quality and biological monitoring data to provide the basis for species selection and deriving the site-specific boron guidelines. A meeting was held with ECan staff on 19 October to discuss the issue and potential species which might be used in laboratory boron toxicity studies. A site visit was also undertaken on 19 October to inspect Tara Stream, Bush Gully Stream and various seep sites associated with historic mine workings.

The study design report recommended (Hickey 2017):

1. Chronic boron toxicity testing with the Canterbury Mudfish;
2. Laboratory testing for boron sensitivity for periphyton biofilms; and
3. Derivation of a site-specific guideline for boron which is suitable for application to streams in the region of the CCC operations.

The proposed study design was accepted by ECan on 18 January 2018 (email from Paul Murney to Campbell Robertson, Bathurst).

2 Methods

2.1 Canterbury mudfish

A methodology for the captive management of the Canterbury mudfish (*Neochanna burrowsius*) (Figure 2-1) has been published (O'Brien and Dunn 2005). The juvenile fish were reared by Dr Leanne O'Brien at her fish farm facility at Dunsandel to a stage suitable to initiate the toxicity testing. The juvenile fish were then transported to NIWA's laboratory in Christchurch to perform the testing.

Figure 2-1: Canterbury mudfish. Image: http://www.rodmorris.co.nz/ from https://www.doc.govt.nz/nature/native-animals/freshwater-fish/non-migratory-galaxiids/canterbury-galaxias/

2.1.1 Canterbury mudfish: laboratory testing procedures

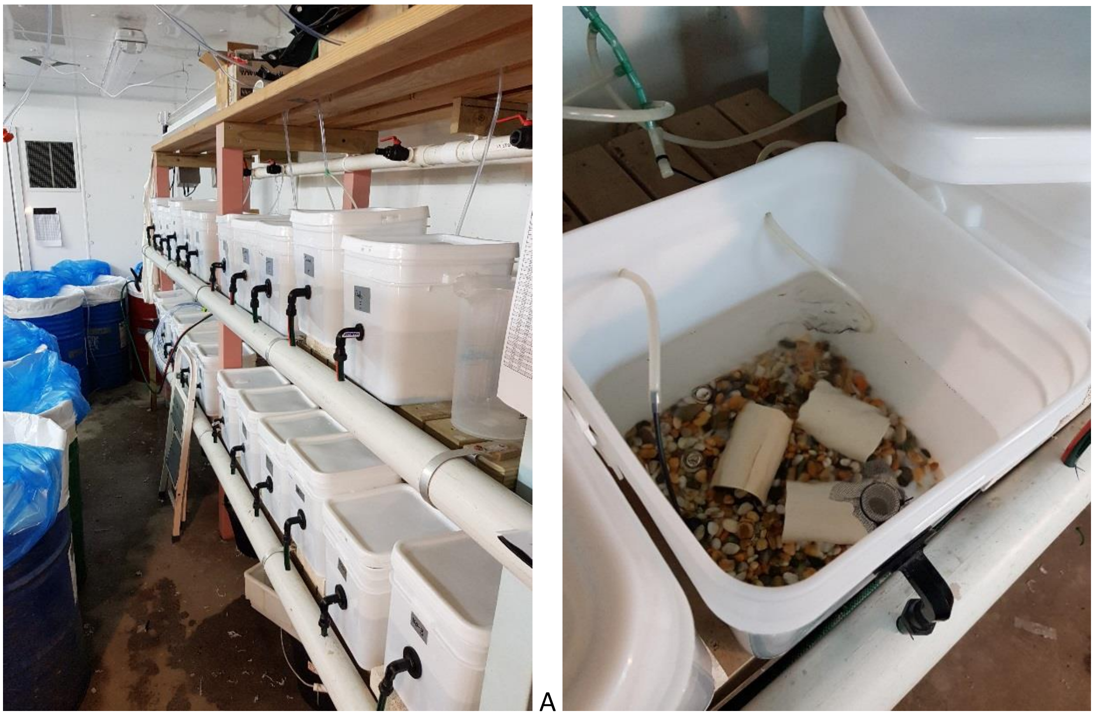
All testing procedures would be undertaken following NIWA's standard operating procedures for fish toxicity testing (NIWA 2005), and in compliance with the toxicity laboratories animal ethics approvals for the holding and testing procedures.

An initial laboratory acclimation period of 12 days was used to equilibrate the fish to the water quality, feeding regime and tank conditions. The tank habitat was modified for the mudfish to provide refugia and a bed substratum in the tanks comprising of aquarium stones (per-washed) and shelter/refugia made of half-round PVC pipe. These habitat modifications were found to calm the fish and improve the tank behaviour by reducing the previous surface swimming behaviour. The apparatus used for flow-through chronic testing with Canterbury mudfish is shown in Figure 2-1.

The chronic tests were conducted according to standard procedures and the methods summarised in Table 2-1 using flow-through conditions for the duration of the 40 day test exposure.

Table 2-1: Summary of test conditions: chronic tests using juvenile Canterbury mudfish (*Neochanna burrowsius*).

Test species:	Canterbury Mudfish
Test type:	Chronic (40 d), flow through
Test material:	Boron in the form Boric Acid, H_3BO_3; CAS No. 10043-35-3
Reference method:	OECD (2000)
Test protocol:	NIWA SOP 28.1 (NIWA 2005); OECD (2000)
Test initiation:	24/2/2018
Test organisms:	*Neochanna burrowsius*; juveniles; mean 0.78 g, 54 mm;
Organism source:	Fish farm, Dunsandel (Dr Leanne O'Brien, Ichthyo-niche). 12/02/2018
Organism conditioning:	On arrival fish treated with recommended dosage of API Stress Coat® and Brooklands Wunder Tonic prior to test initiation. Held for 12 d in Christchurch city tap water with bloodworm feeding prior to test initiation.
Organisms/Container:	10
Organism loading:	0.98 g L^{-1} (initial)
Nominal test concentrations:	Control, 0.2, 0.5, 5, 20, 50 mg L^{-1} Boron
Replicates:	3 for controls, 3 for treatments
Dilution water:	Christchurch city tap water
Test duration:	40 days
Sample pre-treatment:	Nil
Test chambers:	15 L bucket with aquarium stones and fish shelters (half-round PVC pipe)
Test volume:	8 L
Test type:	Flow through with 3 water exchanges per day
Test temperature:	15 ⍰ 1°C
Aeration:	Continuous moderate aeration at >100 bubbles min^{-1}
Feeding during test:	Frozen bloodworms at 5% of body weight daily
Lighting:	16:8h light: dark, low light
Chemical data:	Temperature, pH, dissolved oxygen, ammoniacal-N, boron, hardness, nitrate-N
Effect measured:	Survival, growth (length, weight, condition)
Test acceptability criteria:	Control survival at least 90%
Minimum Significant Difference (MSD):	Survival: 11.2%; growth (length): 6.9%; growth (weight): 23.5%; growth (condition): 17.1%
Test acceptability:	**Achieved**



A B

Figure 2-2: Apparatus used for chronic testing of Canterbury mudfish. A: Flow-through testing system used for chronic fish test. B: Internal view of fish habitat.

2.1.2 Periphyton: laboratory test procedures

Chronic tests were conducted with the filamentous periphyton (*Rhizoclonium* sp.) (Figure 2-3). *Rhizoclonium* are filamentous green algae with cells that are large, long and cylindrical (Figure 2-3). They are common in unshaded stony streams and rivers during summer low flows (Biggs and Kilroy 2000; Landcare Research 2018). Another filamentous green algal species (*Spirogira* sp.) was the dominant species in the stream samples collected in October 2017, and in the repeat sampling in March 2018. However, *Rhizoclonium* was the filamentous algal species which developed in the spring-fed habitat used to develop the filamentous algae for test initiation. Both *Spirogira* and *Rhizoclonium* are filamentous algal species which develop in slow-flowing, open (unshaded) streams and often occur where there are point sources of elevated nutrient concentrations, such as from groundwater inputs (Biggs and Kilroy 2000). The *Rhizoclonium* culture used for these toxicity tests was nearly a monoculture with few other algal species present (K. Safi, NIWA, pers comm).

The tests were performed using a uniform initial mass of filamentous algae as the inoculum. The filamentous algal growths at the completion of the experiment are shown in Figure 2-4.

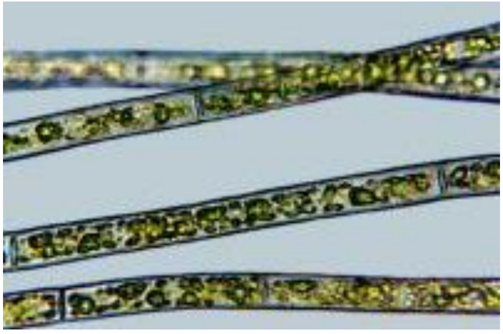


Figure 2-3: ***Rhizoclonium* sp. (Chlorophyta) filamentous algae.** Magnification 450x (from Landcare Research (2018))

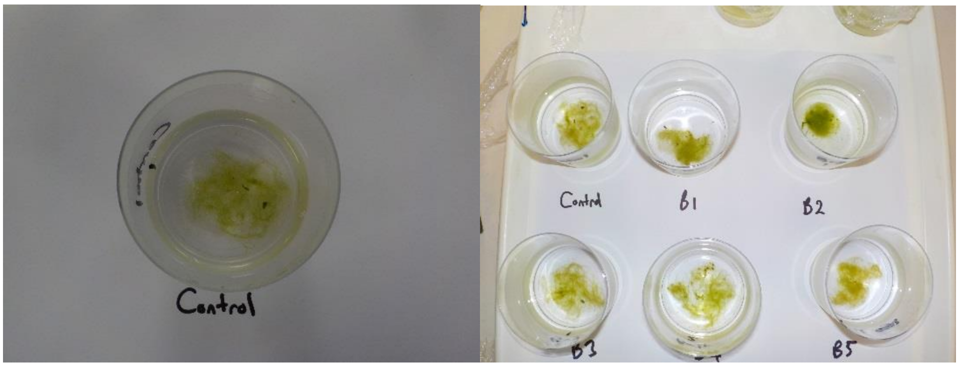
Tests were conducted according to standard procedures and the methods summarised in Table 2-2. All tests were conducted in plastic vessels to eliminate potential for release of dissolved boron from borosilicate glass. The growth median used for these tests was BG-11 algal culture medium which has been found to perform well for growth of filamentous algae (*Spirogira* sp., Flores-Moya et al. (2005)). The concentration of boron in the BG-11 nutrient media was 0.5 g m^{-3}.

Table 2-2: Summary of test conditions: chronic tests using periphyton (*Rhizoclonium* sp.).

Test species:	Periphyton
Test type:	Chronic (7 d), static renewal
Test material:	Boron in the form Boric Acid, H_3BO_3; CAS No. 10043-35-3
Reference method:	Flores-Moya et al. (2005)
Test protocol:	ISO 8692 (2012) (modified)
Test initiation:	18/6/18
Test organisms:	*Rhizoclonium* sp. (Identification by K. Safi, NIWA)
Organism source:	Fernhollow Spring
Organism conditioning:	Held 72 h in BG11 algal culture medium prior to test initiation
Organisms/Container:	0.02 g
Nominal test concentrations:	Control, 0.32, 1, 10, 32, 100 mg L^{-1} Boron
Replicates:	5 for controls, 3 for treatments
Dilution water:	BG11 algal culture medium (Flores-Moya et al. 2005)
Test duration (nominal):	7 days
Sample pre-treatment:	Nil
Test chambers:	250 mL polyethylene cups covered with cling film
Test volume:	100 mL
Test type:	Static renewal (twice per week)
Test temperature:	25 ± 1°C
Aeration:	Nil
Lighting:	24 h light; 100 µE
Mixing:	Shaker table at 100 rpm
Chemical data:	Temperature, pH, dissolved oxygen, conductivity, boron (water and tissue)

Effect measured:	Growth inhibition measured as chl *a* (acetone extraction – using tissue grinder, spectrophotometric absorption (APHA 2012))
Test acceptability criteria:	>30% biomass growth
Endpoint:	7 days
Minimum Significant Difference (MSD):	42%
Test acceptability:	**Achieved**

Figure 2-4: Apparatus used for chronic testing of periphyton. Photo at completion of test showing boron concentration range (B1 through B5) for a single replicate of each test concentration.



2.2 Chemicals and analyses

The boric acid used for all experiments was Merck Emsure® with an assay specification of 99.5-100.5%.

All confirmatory chemical analyses for boron were undertaken by Hill Laboratories, Hamilton. Their analytical detection limit was 0.005 g m^{-3}. Analyses for total ammoniacal-N and nitrate-N were also undertaken by Hill Laboratories.

3 Results

All of the tests met the acceptance criteria specified in the test methods. The average measured boron concentrations were used for all statistical calculations for the chronic fish tests. The nominal boron concentrations were used for the statistical calculations for the chronic algal tests as the results for the confirmatory chemical analyses were not available at the time of preparation of this report.

The durations of the fish and periphyton tests qualify as chronic data based on the updated ANZECC guideline derivation guidance (Batley et al. 2014; Warne et al. 2015).

The results of the tests are summarised below with the detailed analytical data, test results and statistical analysis provided in the appendices.

3.1 Chronic sensitivity of Canterbury mudfish to boron

The tests were undertaken using flow through conditions with a nominal two turnovers per day based on flow measurements of the peristaltic dosing pumps. All tanks were dosed from batch tanks which were intermittently refilled and dosed with stock solution of borate. Water samples for chemical analysis were collected approximately weekly as a composited sample from the exposure tanks. The chemical analysis data for the 40 day chronic fish test is summarised in Table 3-1 with details provided in Appendix A. The five boron concentrations ranged from 0.19 g m^{-3} to 55 g m^{-3} based on a geometric dilution series. The measured boron concentrations are used for all statistical calculations. The average pH concentrations in the treatments was 7.94 in the control, decreasing slightly to 7.42 in the highest boron concentrations. Some occasional high pH measurements were recorded during the test, particularly in the control and lowest boron treatment (i.e., pH >8.3, Appendix A). We would not expect that such elevated pH conditions could occur given the flow through conditions and the absence of aquatic plants in the tanks. These data are identified in Appendix A and we consider may have been calibration or measurement errors associated with the instrument calibration. The average ammoniacal-N concentration in the treatments ranged from 0.09 to 0.17 g NH_4-N m^{-3}, with all values falling markedly below the ANZECC (2000) guideline value for ammoniacal-N at pH 8 of 0.9 g NH_4-N m^{-3}. The average nitrate concentrations were in the range 0.20 to 0.24 g m^{-3} and were comparable to the influent dilution water concentration. These values are markedly less than the 'A-band' toxicity values (MfE 2014) and would not expect to contribute to measured toxicity.

The initial size of the fish averaged 53 mm (SD = 3.9 mm, CV = 7.4%) and weight 0.76 g (SD = 0.21, CV = 27.6%) (Table 3-2) based on a sub-sample of 15 fish measured at test initiation (Appendix B). All fish were randomly allocated to treatments at the initiation of the test.

The 40 d chronic fish test met the survival criteria in the control treatment with 93% survival. Survival was high in all treatments with 100% survival in the maximum concentration of 55 g B m^{-3} (Table 3-2). The control fish at the completion of the test had an average length of 59 mm (i.e., an indicative 8.8% increase in length) and an average weight of 1.07 g (i.e., an indicative 40% increase in weight) – with CV of 27.5% being comparable with the initial measurements. The fish condition factor was initially 0.96 and increased slightly in the control to 1.01 at the completion of the test (Table 3-2). The data for the individual measurements from the replicated test exposures is provided in Appendix B.

Table 3-1: Summary means for boron concentrations and water quality over the 40 d chronic fish test. See Appendix A for analytical data.

Boron concentration		pH	Dissolved Oxygen	Temp.	Conductivity	Ammoniacal-N	Nitrate-N	Hardness
(Nominal) g Boron m^{-3}	(Measured median) g Boron m^{-3}		g m^{-3}	°C	µS cm^{-1}	g NH_4-N m^{-3}	g NO_3-N m^{-3}	g $CaCO_3$ m^{-3}
0	0.02	7.94	10.1	14.3	104.5	0.17	0.24	43
0.2	0.187	7.90	10.0	14.4	104.3	0.11	0.20	43
0.5	0.53	7.74	10.1	14.4	103.0	0.13	0.23	43
5	5.8	7.62	10.0	14.4	100.9	0.12	0.23	43
20	18	7.56	10.1	14.2	101.2	0.09	0.22	43
50	55	7.42	10.0	14.3	100.2	0.16	0.21	45

The summary statistics shown in Table 3-2 are from the ANOVA multiple comparison results shown in Appendix C. The results indicate a statistically significant response (*P* <0.05) for the lowest test concentration based on an average length reduction of 9.4% and a weight reduction of 25%. Both of these values are greater than the calculated minimum statistical difference (MSD) values for these single tests of 6.9% and 23.5 respectively – indicating that that level of effect would be considered significant based on the replicate numbers and the endpoint variability over the experimental treatment. However, both the length and weight endpoints do not show a concentration-response relationship with boron with the 10-fold concentration increase from 0.19 to 18 g m^{-3} – with each showing comparable levels of nominal effect relative to the control treatment (Table 3-2). Based on the lack of a concentration-response, the threshold for boron effect would be considered to occur at the concentration prior to the increasing effect at 55 g boron m^{-3}. Fish showed a significant reduction in both weight and condition between the 18 g m^{-3} and 55 g m^{-3} boron concentrations (by 19.8% and 12.5% respectively).

The summary statistics for the chronic endpoints are shown in Table 3-3. The regression analysis of the concentration-response relationships indicates a threshold effect concentration of around 20 g m^{-3}, with the ANOVA threshold effect concentration (TEC) values of 10.2 g m^{-3} for both length and weight growth measures. The more conservative value of 10.2 g m^{-3} was selected as the chronic endpoint value for incorporation into the site-specific guideline calculations.

Table 3-2: Summary means for chronic fish growth and condition data. '*' indicates statistically significant compared with control treatment (P <0.05); '[*]' indicates statistically significant result which is less than the method detection limit based on the minimum significant difference (MSD) for the test (see Table 2-1 for MSD values).

Treatment, boron concentration (g m^{-3})	Survival (%)	Length (mm) (SD)	Wet weight (g) (SD)	Condition factor (SD)
Initial measurements	-	53 (3.90)	0.76 (0.21)	0.96 (0.1)
Control 0.02	93.3	59 (5.02)	1.07 (0.29)	1.01 (0.12)
0.187	100	53 (4.37)*	0.80 (0.19)*	1.03 (0.09)
0.53	100	54 (5.31)*	0.84 (0.23)	1.04 (0.11)
5.8	96.7	54 (6.42)	0.84 (0.30)	1.04 (0.10)
18	100	53 (6.37)*	0.81 (0.28)*	1.04 (0.13)
55	100	51 (7.10)*	0.65 (0.32)*	0.91 (0.19)

Table 3-3: Results of chronic Canterbury mudfish (*Neochanna burrowsius*) test. Statistical results are based on measured boron concentrations. Bold indicates the most sensitive chronic endpoints.

Organism	Hardness	Concentration of Boron (g B m^{-3})					Control
	g $CaCO_3$ m^{-3}	EC_{50} [a] (95% CL)	EC_{10} [a] (95% CL)	NOEC [a]	LOEC [a]	TEC [a]	%
Canterbury mudfish (juvenile)	43						
– 40 d survival		>55	>55	55	>55	n/a	93
– 40 d growth (length)		>55	20.5	5.8	18	**10.2**	
– 40 d growth (weight)		>55	ca. 20	5.8	18	**10.2**	
– 40 d condition		>55	47.5	55	>55	n/a	

See Table 2-1 for test conditions.
[a] NOEC: No observed effect concentration; LOEC: Lowest observed effect concentration; TEC: Threshold effect concentration = geometric mean of NOEC and LOEC concentrations.

3.2 Chronic sensitivity of periphyton to boron

The filamentous periphyton were grown under laboratory conditions in a high nutrient medium for a 7 day chronic test. The boron concentration was elevated in the control treatment to 0.5 g m^{-3} using this nutrient media with five nominal concentrations covering the range from 0.82 g m^{-3} to 100 g m^{-3} (Table 3-4). The nominal boron concentrations were used for statistical analysis of effects concentrations as analytical results were not available at the time of reports. The control pH was 7.25 with the lowest pH of 7.04 in the highest test concentration. The electrical conductivity was elevated compared with natural surface waters and would be at levels comparable with groundwater seeps. The media contains relatively high concentrations of phosphate buffer to maintain a stable pH for the duration of the test when high biomass levels of algae for the algal inoculum.

Table 3-4: Summary of chemical analyses for periphyton test. Initial water quality measurements.

Boron concentration (Nominal)[a] g Boron m^{-3}	pH	Dissolved Oxygen g m^{-3}	Temp. °C	Conductivity µS cm^{-1}
0.5	7.25	8.90	25.0	1997
0.82	7.31	8.90	25.0	2043
1.5	7.29	8.80	25.0	2042
10.5	7.27	8.80	25.0	2037
32.5	7.20	8.80	25.0	2026
100	7.04	8.80	25.0	1992

[a] Results pending for measured concentrations

The alga chronic endpoints are based on biomass measures as chlorophyll *a* with results provided in Appendix D with a summary of toxicity results shown in Table 3-5. The statistical summary for the tests is provided in Appendix F. The filamentous algae growth was a 53% increase in biomass based on the chlorophyll *a* measurement. The mean grown inhibition measurements showed minimal response to 1 g m^{-3} followed by a progressive increase at higher concentrations (Table 3-5).

The variability within treatments ranged widely with CV values from 1.4% to 49% (for the 10.5 g m^{-3} concentration) and a MSD value for the test of 36% (Appendix F). This high MSD results in a NOEC and LOEC values based on the ANOVA statistical testing (Table 3-6). The regression analysis of the concentration-response relationship gave a threshold EC_{10} value of 1.7 g m^{-3}. Based on visual inspection of the dose-response relationship (Appendix F), this EC_{10} value would be representative of the threshold for growth reduction. Based on this analysis the EC_{10} value of 1.7 g m^{-3} was used for the site-specific guideline derivation.

Table 3-5: Summary means for chronic periphyton inhibition data. '*' indicates statistical significant based on ANOVA comparison with control. Note: The measured chlorophyll *a* concentration is normalised to the measured initial weight for each treatment.

Treatment	Initial weight alga (g)	Chl *a* µg/g	Mean growth inhibition (%)
Initial inoculum measurement	0.0203	2133	
Control	0.0204	3267	
0.32	0.0203	3114	4.68
1	0.0202	3116	4.62
10	0.0203	2264	30.7
32	0.0206	1246	61.9*
100	0.0201	806	75.3*

Table 3-6: Results of chronic periphyton (*Rhizoclonium* sp.) test. Statistical results are based on nominal concentrations. Bold indicated threshold value used for site-specific guideline derivation.

Organism	Concentration of Boron (g Boron m^{-3})					
	EC_{50} [a] (95% CL)	EC_{20} [a] (95% CL)	EC_{10} [a] (95% CL)	NOEC [b]	LOEC [b]	TEC [b]
Periphyton						
– 7 d growth inhibition	22.0 (10.7-45.0)	4.3 (0.55-11.0)	**1.7** (n/a-4.6)	10.5	32.5	18.5

See Table 2-2 for test conditions.
[a] EC_N: Concentration causing a N% effect relative to the controls. A lower value indicates greater toxicity.
[b] NOEC: No observed effect concentration; LOEC: Lowest observed effect concentration; TEC: Threshold effect concentration

4 Discussion

Canterbury mudfish

This study has successfully completed the first chronic toxicity test with the Canterbury mudfish. While protocols for hatching and rearing the Canterbury mudfish had been established (O'Brien and Dunn 2005), there was no experience in undertaking a standardised toxicity testing procedure. The initial fish rearing for this testing was undertaken by Dr O'Brien at her fish rearing facility in Dunsandel. This early life-stage rearing has specific dietary requirements and uncertainties regarding survival and relative growth rates of large numbers of fish under standardised conditions. For these reasons it was considered desirable to initiate the toxicity tests with juvenile mudfish which had moved on to a larger dietary intake consisting of blood-worms. The juvenile mudfish were also size screened prior to transfer to the NIWA laboratory facilities to better standardise the initial conditions.

The fish were held in the NIWA Christchurch laboratories for 12 days acclimation to the laboratory conditions and the standard high dietary feed level designed to achieve a statistically significant growth during the test period. Achieving a high growth rate is a balance between test temperature (with higher temperatures giving higher growth rates), fish density, feeding rate and test duration. The temperature for these tests was held at 15°C to minimise potential disease risk. A dietary feeding rate of 5% of their body weight per day was used based on our experience with chronic tests with galaxiids (Hickey et al. 2013). Based on their initial relatively high weight variability a duration of 40 days was considered necessary to obtain a suitable weight gain to differentiate the treatments. The test conditions for mudfish were modified from the normal laboratory tanks to include aquarium gravels and refugia (half-round PVC pipes) to provide habitat within the tanks. The modification to include these habitat components in all of the test tanks results in fish which were less affected by the laboratory conditions.

The 40 day test gave good survival of the fish and achieved an acceptable growth rate of 40% in wet weight from the initial fish. This allowed for a differentiation of the weight as an endpoint for the chronic effects. As there was no significant reduction in survival or concentration-response trend in survival up to the maximum boron exposure concentration of 55 g m^{-3}, the growth (length and weight) and condition measures provided the basis for determining the chronic effect threshold.

The fish showed a statistically significant reduction in length (by 9.4%) and weight (by 25.6%) between the control and the lowest test concentration of boron (0.19 g m^{-3}). However, for the 10-fold range of increasing boron concentration to 18 g m^{-3} there was no concentration-response showing a response to the increasing boron concentration. There does not appear to be any basis or this difference between the control and the lower concentrations – either based on the initial fish inoculum or on the water quality conditions. The threshold for boron effect was based on the statistical measure for length and weight reduction at 10.2 g boron m^{-3}. This value was used for the site-specific guideline derivation.

Periphyton

The dominant filamentous algae observed in the streams and seeps in the catchments around CCM was *Spirogira* sp. However, a closely related species (*Rhizoclonium* sp.) grew as a near mono-culture in the spring which was used to establish the inoculum for the tests. Both *Spirogira* sp. and *Rhizoclonium* sp. are filamentous algae which inhabit relatively low flow, open (high-light) and high nutrient environments (Biggs and Kilroy 2000), so would be considered suitable for this site-specific assessment.

Filamentous algae are not routinely used for toxicity bioassays. This is both because of the difficulties of having a standard initial inoculum, establishing uniform standardised culture conditions and in measuring the growth endpoint. Thus, there are no standard toxicity testing procedures to follow for undertaking these filamentous algal toxicity tests. A primary consideration in undertaking these tests was to use a media that was suitable for optimising the growth of these high-nutrient species. We used a media recommended by Flores-Moya et al. (2005) (BG-11 medium) based on their successful use in growing *Spirogira* sp.. The BG-11 media has a relatively high boron concentration (0.5 g m^{-3}) which we incorporated into the nominal concentrations used for the statistical calculations.

The *Rhizoclonium* sp. grew well over the 7 day chronic test duration at 25 °C and achieved a 50% increase in biomass (measured as chlorophyll *a*). The variability in growth of the filamentous algae was relatively high (CV up to 49% in the three replicates), indicating the greater number of treatment replicates would be desirable for method standardisation. However, the regression analysis of the concentration-response relationship provided a threshold value of 1.7 g boron m^{-3} for use in the site-specific guideline derivation.

4.1 Site-specific guideline derivation

4.1.1 Background to updating the boron guideline

The boron guidelines for freshwater are currently being revised as part of the updating of the ANZECC (2000) water quality guidelines. An updated boron guidelines derivation was undertaken in 2016 (Binet et al. 2016) following the revised derivation procedures (Batley et al. 2014; Warne et al. 2015). This boron guideline revision is still in draft stage and awaiting receipt of expert review comments. The proposed revised default guideline values (GVs) are given in Appendix G are derived from the application of the statistical model fitting to the species sensitivity distribution (SSD).

The boron SSD is based on chronic toxicity data available for 22 species covering 8 taxonomic groups, comprising green microalgae (2), diatoms (2), macrophytes (5), cladocerans (2), amphipod (1), bivalves (1), fish (7) and amphibians (2). The most sensitive species are microalgae (green algae and diatom species), with the most sensitive fish (Zebrafish) occurring at the 18th percentile of the SSD (see SSD in Appendix G).

Site-specific guideline derivations have been consented for boron by other regional councils in New Zealand. For example, Waikato Regional Council associated with the disposal of fly ash at the Rotowaro mine for boron are:

- 90% protection: B <5.4 mg/L
- 95% protection: B <3.5 mg/L
- 99% protection: B <1.3 mg/L

These site-specific guidelines incorporated new data on the sensitivity of a key native plant species (*Potamogeton ocreatus*) which was present in the receiving waters of that mine discharge (Golder

Associates 2010; Golder Associates 2010). The sensitivity data for this macrophyte species is included in the proposed updated guidelines (Binet et al. 2016).

4.1.2 Site-specific guideline for boron

Generic water quality guidelines (WQGs) are science-based numerical concentrations that represent the level of risk that the community is willing to take based on what it believes the environment can withstand and the ecosystem condition it is prepared to accept (ANZECC 2000). Methods by which site-specific WQGs can be derived vary from simple modifications of the relevant generic WQG to completely new derivations based on site-specific physicochemical data and/or local biological effects data (van Dam et al. 2014). Approaches for site-species guideline derivation commonly use multiple components, including: (i) using local reference water quality data, (2) using biological effects data from laboratory-based toxicity testing, and (3) using biological effects data from field surveys (van Dam et al. 2014). The basis for developing the site-specific guidelines will be assessed relative to these three approaches.

1 Local reference water quality

Boron is ubiquitous in the environment, occurring as a trace element of igneous rocks and is commonly found in sedimentary rocks derived from marine waters. Natural weathering of rocks is a major source of B in the environment, and the amount released depends on the surrounding geology. Concentrations of boron in surface freshwaters are typically <0.5 mg/L, depending on the geochemical nature of the drainage catchment (Binet et al. 2016). In New Zealand rivers with low or no geothermal influence, concentrations of boron range from <0.5 to 410 µg/L, with a geometric mean of 16 µg/L (Deely 1997). Boron is also elevated in concentration in New Zealand coals (Sim and Lewin 1975; Craw et al. 2006).

Boron is an essential nutrient for higher plants, but its essentiality to other taxonomic groups (including microalgae) is species specific (Binet et al. 2016).

In freshwater ecosystems, boric acid accounts for approximately 95% of the dissolved B, whereas the borate ion is approximately 5% (Stumm and Morgan 1995). Boric acid is moderately soluble in water and behaves as a very weak Lewis acid. The behaviour of boric acid in water systems depends on other parameters such as temperature, pressure, pH and ionic strength. Chemical speciation of boric acid varies with acidity according to the flowing equilibrium equation:

$$B(OH)_3 + H_2O \leftrightarrow B(OH)_4^- + H^+; \ pK_a = 9.15 \text{ at } 25°C$$

The effect of pH on boron toxicity is not consistent, but targeted pH-related boron data were limited to just two crustacean species. Targeted acute toxicity studies for pH ranges of 6.5, 7.5 and 8.5 found increasing toxicity for one species (cladoceran, *Ceriodaphnia dubia*) with decreasing pH, but no effect for an amphipod (Soucek et al. 2011).

While there was no information available on the effect of water quality parameters on boron toxicity to macrophytes, boron accumulation by the aquatic duckweed, *Lemna minor*, has been shown to be pH-dependant such that higher concentrations of boron are accumulated at lower pH (Frick 1985).

Thus, while changes in local pH in the streams would not be expected to have a significant effect of boron toxicity to aquatic species in circum-neutral streams, there may be greater effects for some species in streams receiving acid drainage.

2 Biological effects from field surveys

The range in boron concentrations in streams is limited, with highest concentrations occurring in seeps (Hickey 2017). Additionally, the macroinvertebrate and fish populations are limited and likely affected by other habitat stressors associated with agricultural and forestry land-use practices (Golder Associates 2014).

For these reasons, it is not practical to use natural gradients to robustly establish tolerance and effect thresholds on macroinvertebrate or fish communities. However, the elevated boron concentrations present in a range of seeps indicates a tolerance of local filamentous algal communities for elevated boron concentrations. Proliferations of filamentous algae were observed growing in the seeps during the site visit on 19th October 2017 (Hickey 2017).

3 Biological effects data from laboratory-based toxicity testing

The species sensitivity distribution provides the basis for a site-specific guideline derivation and for numeric GV calculation. Two approaches can be used for the site-specific derivation: (i) selection of species included in the generic SSD – either by selection for native or resident species, or removal of species which would not otherwise be present in a specific environment (e.g., lake-dwelling species for riverine exposures); or (ii) supplementing the SSD database with key ecologically important species present at the site.

The SSD database of 22 species contains representatives of 8 taxonomic groups (Appendix G), however, no native species recorded in the monitoring data for the Waianiwaniwa Valley streams is included. The native macrophyte Blunt pondweed (*Potamogeton ochreatus*) is not resident in the streams, though some duckweed species (*Lemna* sp.) are likely to be present. The cladoceran *Ceriodaphnia dubia* is present in slow-flowing waters in New Zealand and rainbow trout are possibly present in the greater catchment. Rainbow trout are often a sensitive species and as such are a surrogate species for other fish present in the catchment.

The most sensitive species in the SSD are microalgae, including diatoms and microalgae, from a study undertaken by DSIR laboratories in 1985 (Wilkinson 1985). These studies used boron-spiked natural lake waters to measure growth rates and maximum yield for four boron concentrations (0 (control lake water), 1, 3, 10 and 30 mg/L). The data used in the SSD were the no observed effect concentration (NOEC) values for growth for these species and based on nominal (i.e., not measured) boron concentrations. Tests were also undertaken in borosilicate glass vessels which may have leased an unknown amount of boron into the test treatments. No regression relationships were reported for this study, so the preferred low-effect threshold values (e.g., EC_{10}) as recommended by the revised derivation procedure (Warne et al. 2015), could not be included in this derivation. Further review of the suitability of this data is expected as part of the technical review of these draft guidelines.

The planktonic microalgae would not be considered key primary producers which require a high level of protection (i.e., a NOEC concentration threshold) for site-specific consideration in these streams. The key in stream primary producers are emergent grasses and raupo (Golder Associates 2014), with periphyton communities growing on submerged macrophytes, gravels and woody-debris supporting the food-chain. Available additional information for macrophyte species is included in Table 4-1 to summarise chronic threshold and higher effects (i.e., chronic EC_{50}) concentrations.

The chronic EC_{50} values for various aquatic macrophytes vary widely, with values in the range 20-40 g/m^3 for duckweed, pondweed and milfoils which could be considered representative of a range of species which could be considered representative of some of the species which would inhabit low-energy stream and wetland habitats. Plants require boron as an essential element for growth and show a variety of physiological responses to both low boron (Dell and Huang 1997), with highest naturally occurring concentrations of soil boron are in soils derived from marine evaporites and marine argillaceous sediment (Nable et al. 1997). Nable et al. (1997) report that diagnosing boron toxicity in plants, either by visible symptoms or tissue analysis has limited applicability. Based on this analysis of macrophyte data is appears that while some species have sensitive endpoints, other species/endpoints are highly tolerant of boron exposure.

Table 4-1: Aquatic macrophyte sensitivity data for chronic (long-term) boron exposure.

Taxonomic group (Phylum)	Species	Life stage	Duration (d)	Toxicity measure (Test endpoint)	Toxicity value (g/m^3)	Estimated chronic NOEC (g/m^3) [a]	Reference [b]
Macrophyte (Angiosperm)	*Egeria densa* (Brazilian waterweed)	Apical stem cutting	28	NOEC (biomass)	6.1	6.1	1
	Lemna disperma (Duckweed)	NR	7	EC10 (growth)	1.4	1.4	2
	Lemna gibba (Gibbous duckweed)	3-frond clones	7	NOEC (growth)	8	8	3
	Lemna gibba (Gibbous duckweed)	3-frond clones	7	EC50 (growth)	18.6		3
	Potamogeton ochreatus (Blunt pondweed)	Apical stem cutting	30	IC10 (shoot growth)	4.9	4.9	4
	Potamogeton ochreatus (Blunt pondweed)	Apical stem cutting	30	EC50 (shoot growth)	11.3		4
	Potamogeton ochreatus (Blunt pondweed)	Apical stem cutting	30	NOEC (shoot weight)	7.5		4
	Myriophyllum spicatum (Eurasian milfoil)		32	EC50 (shoot weight)	41.3		5
	Myriophyllum spicatum (Eurasian milfoil)		32	EC50 (shoot growth)	33		5
	Myriophyllum spicatum (Eurasian milfoil)		32	EC50 (root length)	29.3		5
	Myriophyllum spicatum (Eurasian milfoil)		32	EC50 (root weight)	27.6		6
	Spirodella polyrrhiza (duckweed)		10	EC50 (abnormal fronds)	17.1		6
	Spirodella polyrrhiza (duckweed)		10	EC50 (frond number)	14.3		6
	Spirodella polyrrhiza (duckweed)		10	EC50 (growth)	11.7		6
	Ranunculus penicillatus (Buttercup)		21	EC50 (photosynthesis)	10		7
	Elodea canadensis (waterweed)		21	EC50 (photosynthesis)	5		7
	Myriophyllum alterniflorum (water milfoil)		21	EC50 (photosynthesis)	5		7

Notes: [a] Data used in revised boron guideline derivation (Binet et al. 2016); [b] 1, Thompson (1987); 2, Acqua Della Vita (2014); 3, Gur et al. (2016); 4, Golder Associates (2010); 5, Stanley (1974); 6, Davis et al. (2002); 6, Nobel (1981).

The SSD includes sensitivity data for five macrophyte species which would be considered suitable surrogates for the species present in these receiving waters. Therefore, no specific testing for macrophyte sensitivity was proposed for this study. However, testing was proposed to establish the periphyton community with data to be included from this study (see following section).

Invertebrates. The SSD is based on data for four invertebrate species (2 cladocerans; 1 amphipod and 1 bivalve). There are no potentially sensitive EPT species[1] present in these streams. Species present would be expected to be protected by the sensitive surrogate species included in the SSD used for the guideline derivation.

Fish. The SSD is based on seven fish species, with the Zebrafish embryos the most sensitive species equating to the 18th percentile of the sensitivity distribution (1.8 mg/L, Appendix G). The chronic sensitivity of rainbow trout is at the 40th percentile of the distribution with a toxicity value of 6.2 mg/L. This range of sensitive fish species would generally be expected to provide a moderately-high confidence in the level of protection for fish species present in the streams.

The presence of the endangered Canterbury mudfish (*Neochanna* sp.) in the receiving streams raises concerns for the potential sensitivity of this species to elevated boron concentrations. There are no galaxiid fish species in the boron database and so the relative sensitivity of this group of species to boron exposure is unknown. On this basis, it was recommended that the chronic sensitivity of the Canterbury mudfish to boron be determined (Hickey 2017).

4.1.3 Site-specific guideline derivation calculations

The boron data for the site-specific boron guideline derivation for the CCM discharges is summarised in Table 4-2. This data was supplemented with the threshold sensitivity data for the Canterbury mudfish and the filamentous algal species. As discussed above, the data excluded from the site-specific guideline derivation are the microalgae which would not be considered critical for threshold sensitivity protection in the low energy stream and wetland habitats constituting the receiving water habitats downstream of the CCM discharge.

The SSD plot for the site-specific guideline is shown in Figure 4-1 for taxonomic groups with the numeric guideline values given in Table 4-3. Figure G-1 shows that SSD for the species with the model output data shown in Figure G-2. The threshold sensitivity for the filamentous algae was at the 8th percentile of the SSD and the Canterbury mudfish was at the 66th percentile. The most sensitive species in the site-specific SSD is a duckweed (threshold sensitivity 1.4 g m^{-3}) and the least sensitive a fish (Eastern rainbowfish, 102 g m^{-3}).

The site-specific guideline value for 95% species protection is 1.6 g boron m^{-3}, with lower protection GVs for 90% protection at 2.3 g m^{-3} and 80% protection at 3.4 g m^{-3} (Table 4-3).

Guideline applicability to the CCM site. The catchments in the local area surrounding the CCM have numerous historic coal mines with seeps leaching boron to streams and wetlands (Hickey 2017). Additionally, the area has large scale forestry and farming operations with stock access to waterways. Areas of significant sediment runoff were observed during the field visit in October 2017

[1] EPT = Ephemeroptera, mayflies; Plecoptera, stoneflies; Trichoptera, caddisflies.

(C. Hickey, pers. obs.). The monitoring data for these streams indicates relatively depauperate communities which reflect a low habitat quality (Golder Associates 2014).

Based on multiple indicators the local receiving water conditions would be considered "highly disturbed systems" in the narrative used by ANZECC (2000) to describe guideline types. Thus, a protection threshold for boron of 90% equating to 2.3 g boron m^{-3} would be considered appropriate for application to the receiving waters around the CCM operations.

Table 4-2: Summary of single chronic toxicity value for each species used to derive the default guideline values for dissolved boron in freshwater and for site-specific guideline derivation. Final data table from draft boron guideline derivation Binet et al. (2016) with site-specific data added for Canterbury mudfish and filamentous algae (shaded). Strikethrough indicates microalgal data not included in site-specific guideline calculation.

Taxonomic group (Phylum)	Species	Life stage	Duration (d)	Toxicity measure (Test endpoint)	Chronic toxicity value (mg/L)	Note
Amphibian (Chordata)	*Anaxyrus fowleri* (Fowlers toad)	Embryo	7.5	LC10 (mortality and development)	41	
	Rana pipiens (Leopard frog)	Embryo	7.5	LC10 (mortality and development)	29	
Fish (Chordata)	*Carassius auratus* (Goldfish)	Embryo	7	LC10 (mortality)	17	
	Danio rerio (Zebrafish)	Embryo	34	NOEC (mortality)	1.8	
	Ictalurus punctatus (Channel catfish)	Embryo	9	LC10 (mortality)	14	
	Melanotaenia splendida (Eastern rainbowfish)	Embryo	12	LC10 (mortality)	102	
	Micropteris salmoides (Largemouth bass)	Embryo	11	LC10 (mortality)	6.0	
	Oncorhynchus mykiss (Rainbow trout)	Embryo	28	LC10 (mortality)	6.2	
	Pimephales promelas (Fathead minnow)	Embryo	32	LC10 (mortality)	12	
	***Neochanna burrowsius* (Canterbury mudfish)**	**Juveniles**	**40**	**TEC (growth: length, weight)**	**10.2**	**This study**
Bivalve (Mollusca)	*Lampsilis siliquoidea* (Fatmucket clam)	Juvenile	21	NOEC (biomass)	10	
Macro-crustacean (Arthropoda)	*Hyalella azteca* (amphipod)	Juvenile	42	NOEC (reproduction)	6.6	
Micro-crustacean (Arthropoda)	*Ceriodaphnia dubia* (Water flea)	Neonate	7	NOEC (reproduction)	5.6	
	Daphnia magna (Water flea)	Neonate	14	NOEC (reproduction)	2.4	
Macrophyte (Angiosperm)	*Egeria densa* (Brazilian waterweed)	Apical stem cutting	28	NOEC (biomass)	6.1	
	Lemna disperma (Duckweed)	NR	7	EC10 (growth)	1.4	
	Lemna gibba (Gibbous duckweed)	3-frond clones	7	NOEC (growth)	8	
	Lemna minor (Common duckweed)	3-frond clones	7	NOEC (growth)	8	
	Potamogeton ochreatus (Blunt pondweed)	Apical stem cutting	30	IC10 (shoot growth)	4.9	
Green microalga (Chlorophyta)	*Chlorella pyrenoidosa*	Late log phase culture	14	NOEC (growth)	~~0.4~~	
	Pseudokirchneriella subcapitata	NR	4	NOEC (growth)	~~2.8~~	
Green filamentous alga (Chlorophyta)	***Rhizoclonium* sp.**	**NR**	**7**	**EC10 (growth)**	**1.7**	**This study**

Diatom (Bacillariophyta)	*Cyclotella* sp	NR	4-14	NOEC (biomass)	10
	Navicula sp	NR	4-16	NOEC (biomass)	1.0

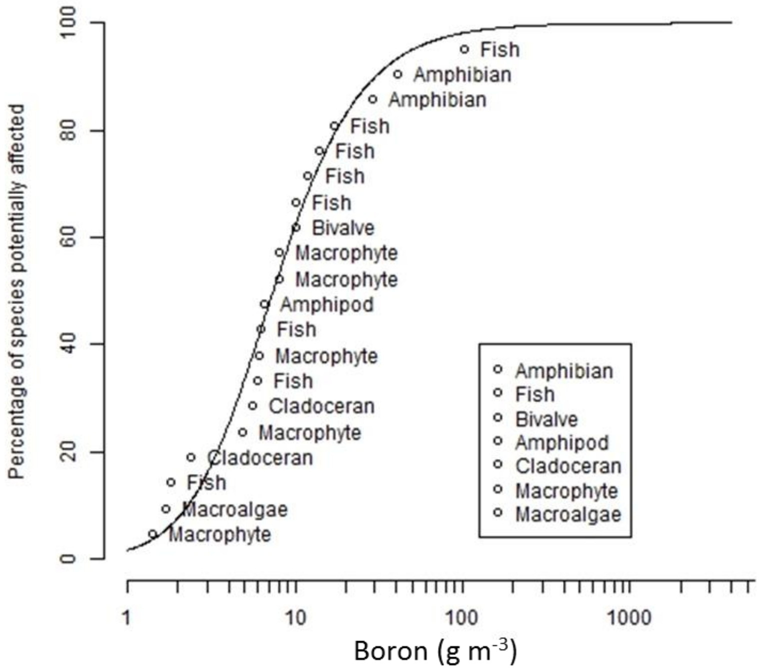


Figure 4-1: Boron species sensitivity distribution for site-specific guideline derivation: taxa data. See Table 4-2 for species data.

Table 4-3: Site-specific guideline values for boron for application to CCM receiving waters.

Site-specific guideline value type	Boron (freshwater) toxicity guideline value (g m^{-3})
High conservation value systems (99% species protection)	0.8
Slightly to moderately disturbed systems (95% species protection)	1.6
Highly disturbed systems	
(90% species protection)	2.3
(80% species protection)	3.4

5 Acknowledgements

We are grateful to Leanne O'Brien for rearing and supply of Canterbury mudfish and to Phil Jellyman (NIWA) for his assistance in facilitating the testing at NIWA's Christchurch facilities.

6 Glossary of abbreviations and terms

ANZECC	Australian and New Zealand Environment and Conservation Council.
ARMCANZ	Agricultural and Resource Management Council of Australia and New Zealand
CAS	Chemical Abstracts Service.
Chronic toxicity	Lingering or continuing for a long time; often for periods from several weeks to years. Can be used to define either the exposure of an aquatic species or its response to an exposure (effect). Chronic exposure typically includes a biological response of relatively slow progress and long continuance, often affecting a life stage.
EC_{50} (median effective concentration)	The concentration of material in water that is estimated to be effective in producing some lethal or growth response in 50% of the test organisms. The EC_{50} is usually expressed as a time-dependent value (e.g., 24 hour or 96 hour LC_{50}).
Endpoint	Measured attainment response, typically applied to ecotoxicity or management goals.
ESEC	Ecologically Significant Effects Concentrations.
Guideline (water quality)	Numerical concentration limit or narrative statement recommended to support and maintain a designated water use.
H_3BO_3	Boric acid
Hardness	Hard water is water that has high mineral content. Water hardness is generally determined by the concentration of the common cations calcium and magnesium and expressed as equivalent calcium carbonate ($CaCO_3$).
LC_{50}	Median lethal concentration.
LOEC (Lowest observed effect concentration)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
NPS-FW	National Policy Statement on Freshwater.
NO_3^-	Nitrate ion.
NO_3-N	Nitrate-nitrogen.
NO[A]EL	No observed [adverse] effects level.
NOEC (No observed effect concentration)	The highest concentration of a toxicant at which no statistically significant effect is observable, compared to the controls; the statistical significance is measured at the 95% confidence level.
Species	A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group.
SSD	Species Sensitivity Distribution.
Standard (water quality)	An objective that is recognised in enforceable environmental control laws of a level of government.
Toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
Toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical).
Trigger value (TV)	These are the concentrations (or loads) of the key performance indicators measured for the ecosystem, below which there exists a low risk that adverse biological (ecological) effects will occur. They indicate a risk of impact if exceeded and should 'trigger' some action, either further ecosystem specific investigations or implementation of management/remedial actions.
Water quality criteria	Scientific data evaluated to derive the recommended quality of water for various uses.

7 References

Acqua Della Vita (2014) Condomine River - DTA of boron and treated CSG water. No. *Final report*. Acqua Della Vita, Queensland, Australia, pp. 34.

ANZECC (2000) Australian and New Zealand guidelines for fresh and marine water quality. National Water Quality Management Strategy Paper No. 4, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australia.

APHA (2012) Standard Methods for the Examination of Water and Wastewater. 22nd ed. American Public Health Association, Washington, DC, USA.

Batley, G.E.; van Dam, R.A.; Warne, M.S.; Chapman, J.C.; Fox, D.R.; Hickey, C.W.; Stauber, J.L. (2014) Technical Rationale for Changes to the Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants. Updated May 2017. No. CSIRO Land and Water Report Prepared for the Council of Australian Government's Standing Council on Environment and Water (SCEW), Sydney, Australia. pp. 43.

Biggs, B.J.; Kilroy, C. (2000) Stream periphyton monitoring manual. Prepared for the New Zealand Ministry for the Environment by NIWA, Christchurch.

Binet, M.T.; Batley, G.E.; Hickey, C.W.; Golding, L.A.; Adams, M.S. (2016) Guidelines for the protection of aquatic ecosystems, toxicant trigger values: Boron – Freshwater. Australian and New Zealand guidelines for fresh and marine water quality. Draft July 2016. No. Council of Australian Governments Standing Council on Environment and Water, Canberra, ACT, Australia. pp. 19.

Craw, D.; Rufaut, C.G.; Haffert, L.; Todd, A. (2006) Mobilisation and attenuation of boron during coal mine rehabilitation, Wangaloa, New Zealand. *Science of the Total Environment,* 368: 444–455.

Davis, S.M.; Drake, K.D.; Maier, K.J. (2002) Toxicity of boron to the duckweed, *Spirodella polyrrhiza*. *Chemosphere,* 48: 615–620.

Deely, J. (1997) Metal in the Bay of Plenty rivers 1996. No. 97/6. Bay of Plenty Regional Council, Whakatane, NZ. pp.

Dell, B.; Huang, L.B. (1997) Physiological response of plants to low boron. *Plant and Soil,* 193: 103–120.

Flores-Moya, A.; Costas, E.; Banares-Espana, E.; Garcia-Villada, L.; Altamirano, M.; Lopez-Rodas, V. (2005) Adaptation of *Spirogyra insignis* (Chlorophyta) to an extreme natural environment (sulphureous waters) through preselective mutations. *New Phytologist,* 166: 655–661.

Frick, H. (1985) Boron tolerance and accumulation in the duckweed, *Lemna minor*. *Journal of Plant Nutrition,* 8: 1123–1129.

Golder Associates (2010) Effects of Boron on the growth and condition of *Potamogeton ochreatus*. No. SOLIDHTY024. Golder Associates (NZ) Limited report for Solid Energy New Zealand Ltd, pp. 45.

Golder Associates (2010) Recalculation of site specific ANZECC (2000) trigger values for boron. No. 0878110737. Golder Associates (NZ) Limited report for Solid Energy New Zealand Ltd, pp. 20.

Golder Associates (2014) Aquatic baseline assessment of ecological values of streams in the Wainiwaniwa Valley. No. Golder Associates report for Canterbury Coal (2013) Ltd, pp. 36.

Gur, N.; Turker, O.C.; Bocuk, H. (2016) Toxicity assessment of boron (B) by *Lemna minor* L. and *Lemna gibba* L. and their possible use as model plants for ecological risk assessment of aquatic ecosystems with boron pollution. *Chemosphere*, 157: 1-9.

Hickey, C.W. (2017) Derivation of site-specific water quality guideline for boron for Canterbury Coal Mine (CCM) discharges. No. BRL18201. *NIWA report* to Bathurst Resources Ltd, pp. 18.

Hickey, C.W.; Thompson, K.J.; Albert, A.; Martin, M.L. (2013) Chronic sensitivity of juvenile inanga (*Galaxias maculatus*) and early life-stage rainbow trout (*Oncorhynchus mykiss*) to nitrate. No. HBR13207; HAM2013-012. *NIWA report* for Hawke's Bay Regional Council, pp. 122.

ISO 8692 (2012). Water quality – fresh water algal growth inhibition test with unicellular green algae. No. 8692. International Organization for Standardization, Geneva, Switzerland. pp. 21.

Landcare Research (2018). *Rhizoclonium* sp. (Chlorophyta) filamentous algae (https://www.landcareresearch.co.nz/resources/identification/algae/identification-guide/interpretation/indicator-taxa/poor-streams/rhizoclonium).

Ling, N.; O'Brien, L.K.; Miller, R.; Lake, M. (2013). A revised methodology to survey and monitor New Zealand mudfish. No. DOCDM-452382. Department of Conservation, Wellington (unpublished). pp. 45.

MfE (2014). National Policy Statement for Freshwater Management 2014. Updated August 2017 to incorporate amendments from the National Policy Statement for Freshwater (http://www.mfe.govt.nz/publications/fresh-water/national-policy-statement-freshwater-management-2014-amended-2017). No. Ministry for the Environment, Wellington. pp. 47.

Nable, R.O.; Banuelos, G.S.; Paull, J.G. (1997) Boron toxicity. *Plant and Soil,* 193: 181–198.

NIWA (2005) Standard Operating Procedure 28.1. Freshwater fish (*Oncoryhynchus mykiss*) acute toxicity test protocol (Revision 4). No. 28.1. *National Institute of Water and Atmospheric Research* (NIWA), Hamilton, protocol prepared by L.A. Golding, Hamilton, New Zealand. pp. 39.

Nobel, W. (1981) To the Effect of Boron Pollution on Submerged Softwater-Macrophytes. (Zum EinfluB von Bor auf submerse Weichwasser-Makrophyten). *Angewandte Botanik – Journal of Applied Botany.,* 55: 501–514.

O'Brien, L.; Dunn, N. (2005) Captive management of mudfish *Neochanna* (Teleostei: Galaxiidae) spp. No. DOC Research & Development Series 205. Department of Conservation, pp. 29.

OECD. (2000) OECD guideline for the testing of chemicals: fish, juvenile growth test. No. TG 215. Organisation for Economic Co-operation and Development, Paris, France. pp. 16.

Sim, P.G.; Lewin, J.F. (1975) Potentially toxic metals in New Zealand coals. *New Zealand Journal of Science*, 18: 635–641.

Soucek, D.J.; Dickinson, A.; Koch, B.T. (2011) Acute and Chronic Toxicity of Boron to a Variety of Freshwater Organisms. *Environmental Toxicology and Chemistry*, 30: 1906–1914.

Stanley, R.A. (1974) Effect of 2,4-D and Various Salts on Eurasian Watermilfoil. *Weed Science*, 22: 591–594.

Stumm, W.; Morgan, J.J. (1995) Aquatic chemistry: chemical equilibria and rates in natural waters. Wiley-Interscience, New York.

Thompson, K. (1987) Studies of the toxicity of boron and of Huntly West Mine waste waters to the aquatic macrophyte *Egeria densa*. No. A technical report prepared by the University of Waikato for the Water Quality Centre of the Ministry of Works and Development, Hamilton. pp. 64.

van Dam, R.A.; Humphrey, C.L.; Harford, A.J.; Sinclair, A.; Jones, D.R.; Davies, S.; Storey, A.W. (2014) Site-specific water quality guidelines: 1. Derivation approaches based on physicochemical, ecotoxicological and ecological data. *Environmental Science and Pollution Research*, 21: 118–130.

Warne, M.S.; Batley, G.E.; van Dam, R.A.; Chapman, J.C.; Fox, D.R.; Hickey, C.W.; Stauber, J.L. (2015) Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants. Updated May 2017. No. Prepared for the Council of Australian Government’s Standing Council on Environment and Water (SCEW) Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland. pp. 46.

Wilkinson, V. (1985) Boron tolerance by freshwater algae. No. Taupo Research Laboratory Report no. 80. Taupo Research Laboratory, Department of Scientific and Industrial Research, Taupo, New Zealand. pp. 30+.

Appendix A Measured water quality data for chronic fish test

Table A-1: Water quality measurements for chronic fish test.

Date	Boron concentration		pH	Dissolved Oxygen	Temp.	Conductivity
	(Nominal)	(Measured: Median)				
	g Boron m^{-3}	g Boron m^{-3}		g m^{-3}	°C	µS cm^{-1}
24/02/2018	0	0.02	7.65	9.63	16.2	100.2
	0.2	0.187	7.69	9.21	16.5	101.4
	0.5	0.53	7.88	9.32	16.4	101.2
	5	5.8	7.73	9.28	16.4	101.0
	20	18	7.51	9.26	15.5	98.6
	50	55	7.30	9.34	16.7	99.2
26/02/2018	0	0.02	7.31	10.03	14.3	100.6
	0.2	0.187	8.68*	10.15	14.3	107.4
	0.5	0.53	8.20	10.28	14.1	107.1
	5.0	5.8	7.68	10.28	14.1	107.0
	20	18	7.55	10.25	14.1	105.1
	50	55	7.34	10.26	14.1	102.4
28/02/2018	0	0.02	8.44*	10.03	14.1	96.2
	0.2	0.187	8.16	10.20	14.3	99.3
	0.5	0.53	8.02	10.18	14.4	97.7
	5	5.8	7.87	10.14	14.0	101.1
	20	18	7.81	10.28	14.0	99.8
	50	55	7.67	10.13	14.1	93.9
2/03/2018	0	0.02	7.44	10.22	14.0	92.4
	0.2	0.187	7.23	9.48	14.0	98.0
	0.5	0.53	7.43	10.26	13.9	92.0
	5	5.8	7.53	10.34	13.8	91.3
	20	18	7.47	10.31	13.8	89.9
	50	55	7.52	10.38	13.8	89.6
9/03/2018	0	0.02	7.81	10.34	14.0	108.8
	0.2	0.187	7.74	10.34	14.0	104.5
	0.5	0.53	7.64	10.29	14.1	105.1
	5	5.8	7.55	9.89	14.9	95.3
	20	18	7.76	10.21	14.0	104.0
	50	55	7.66	9.77	14.0	100.6
16/03/2018	0	0.02	8.45*	10.10	14.0	109.6
	0.2	0.187	8.13*	10.19	14.1	104.8
	0.5	0.53	7.94	10.15	14.1	106.1
	5	5.8	7.71	9.81	14.0	104.1
	20	18	7.66	10.21	13.8	103.1
	50	55	7.69	9.76	13.9	100.7
23/03/2018	0	0.02	8.34*	10.18	14.2	112.3
	0.2	0.187	7.84	10.34	14.3	102.7
	0.5	0.53	7.51	10.18	14.3	98.5
	5	5.8	7.29	10.22	14.3	99.9
	20	18	7.14	10.19	14.3	101.0
	50	55	7.08	10.24	14.2	102.3

Date	Boron concentration		pH	Dissolved Oxygen	Temp.	Conductivity
	(Nominal)	(Measured: Median)				
	g Boron m^{-3}	g Boron m^{-3}		g m^{-3}	°C	µS cm^{-1}
30/03/2018	0	0.02	7.98	10.17	14.2	108.2
	0.2	0.187	7.72	10.21	14.1	105.8
	0.5	0.53	7.25	10.27	14.2	106.3
	5	5.8	7.56	10.08	14.2	105.6
	20	18	7.50	10.08	14.1	100.2
	50	55	7.07	9.60	14.1	104.8
5/04/2018	0	0.02	8.02	10.09	14.1	112.0
	0.2	0.187	7.90	10.01	14.1	114.4
	0.5	0.53	7.83	10.02	14.2	113.4
	5	5.8	7.70	10.12	14.1	103.0
	20	18	7.60	10.03	14.1	109.3
	50	55	7.47	10.06	14.0	108.5

'*'Indicates pH value higher than expected under flow-through conditions and tanks without algal growths. Possible calibration of measurement error. Measured boron monitoring data shown in Table A-3.

Table A-2: Chemical monitoring data for water hardness.

	Hardness g/m³ as CaCO3				Treatment		
Day	Date	Control 0	0.2 mg/L	0.5 mg/L	5 mg/L	18 mg/L	55 mg/L
0	24/02/2018	41	-	-	-	-	-
6	2/03/2018	42					
13	9/03/2018						
20	16/03/2018	43					
20	16/03/2018	42	43	43	43	43	45
27	23/03/2018						
34	30/03/2018	43					
40	5/04/2018	43					
	Average	**42.3**	**43**	**43**	**43**	**43**	**45**

Table A-3: Chemical monitoring data for boron.

	Boron g/m³				Treatment		
Day	Date	Control 0	0.2 mg/L	0.5 mg/L	5 mg/L	18 mg/L	55 mg/L
6	2/03/2018	0.016	0.23	0.66	6.2	16.8	66
13	9/03/2018	0.017	0.187	0.48	3.1	16.7	48
20	16/03/2018	0.02	0.176	0.55	6	19.7	62
34	30/03/2018	0.019	0.187	0.53	5.8	23	55
40	5/04/2018	0.019	0.188	0.53	5.2	18	50
	Average	**0.0182**	**0.1936**	**0.55**	**5.26**	**18.84**	**56.2**
	St Dev	0.002	0.021	0.067	1.264	2.622	7.694
	Median	0.019	0.187	0.53	5.8	18	55
	Geometric Mean	0.02	0.19	0.55	5.11	18.70	55.78

Table A-4: Chemical monitoring data for ammoniacal-N.

	Total ammoniacal-N g/m³			Treatment			
Day	Date	Control 0	0.2 mg/L	0.5 mg/L	5 mg/L	18 mg/L	55 mg/L
0	24/02/2018	0.013	0.016	< 0.010	< 0.010	< 0.010	0.04
6	2/03/2018	0.162	0.055	0.082	0.069	0.082	0.147
13	9/03/2018	0.174	0.07	0.06	0.027	0.062	0.049
20	16/03/2018	0.25	0.107	0.127	0.54	0.27	0.25
27	23/03/2018	0.23	0.195	0.162	0.095	0.32	0.21
34	30/03/2018	0.124	0.119	0.141	0.2	0.094	0.155
40	5/04/2018	0.58	0.14	0.166	0.145	0.085	0.2
	Average	**0.22**	**0.10**	**0.12**	**0.18**	**0.15**	**0.15**
	St Dev	0.177	0.059	0.043	0.187	0.112	0.080
	Median	0.17	0.11	0.13	0.12	0.09	0.16
	Geometric Mean	0.147	0.080	0.115	0.119	0.123	0.125

Table A-5: Chemical monitoring data for nitrate-N.

	Nitrate-N g/m³			Treatment			
Day	Date	Control 0	0.2 mg/L	0.5 mg/L	5 mg/L	18 mg/L	55 mg/L
0	24/02/2018	0.26	0.14	0.26	0.25	0.16	0.11
6	2/03/2018	0.21	0.2	0.2	0.2	0.2	0.19
13	9/03/2018	0.96	0.25	0.37	0.25	0.96	0.26
20	16/03/2018	0.2	0.23	0.23	0.21	0.22	0.21
27	23/03/2018	0.24	0.23	0.24	0.24	0.25	0.24
34	30/03/2018	0.22	0.13	0.13	0.13	0.16	0.14
40	5/04/2018	0.24	0.18	0.22	0.23	0.31	0.28
	Average	**0.333**	**0.194**	**0.236**	**0.216**	**0.323**	**0.204**
	St Dev	0.277	0.046	0.072	0.042	0.286	0.062
	Median	0.24	0.2	0.23	0.23	0.22	0.21
	Geometric Mean	0.279	0.189	0.226	0.211	0.262	0.195

Appendix B Chronic Canterbury mudfish survival, growth and condition data

Table B-1: Survival data for chronic test with Canterbury mudfish.

Treatment		Number surviving								Final	Final Survival %
	Rep	24 Feb	3 Mar	7 Mar	10 Mar	17 Mar	18 Mar	24 Mar	31 Mar	5 Apr	
Control 0.02 ppm	1	10	10	10	10	10	10	10	10	10	100
	2	10	10	9	9	9	9	9	9	9	90
	3	10	10	9	9	9	9	9	9	9	90
0.187 ppm	1	10	10	10	10	10	10	10	10	10	100
	2	10	10	10	10	10	10	10	10	10	100
	3	10	10	10	10	10	10	10	10	10	100
0.53 ppm	1	10	10	10	10	10	10	10	10	10	100
	2	10	10	10	10	10	10	10	10	10	100
	3	10	10	10	10	10	10	10	10	10	100
5.8 ppm	1	10	10	10	10	10	9	9	9	9	90
	2	10	10	10	10	10	10	10	10	10	100
	3	10	10	10	10	10	10	10	10	10	100
18 ppm	1	10	10	10	10	10	10	10	10	10	100
	2	10	10	10	10	10	10	10	10	10	100
	3	10	10	10	10	10	10	10	10	10	100
55 ppm	1	10	10	10	10	10	10	10	10	10	100
	2	10	10	10	10	10	10	10	10	10	100
	3	10	10	10	10	10	10	10	10	10	100

Table B-2: Chronic fish survival summary.

Sample ID	Measured Concentration (g m^{-3})	Replicate	Number surviving	Survival %	Treatment mean %
Control	0.02	1	10	100	93.3
	0.02	2	9	90	
	0.02	3	9	90	
mg Boron L^{-1}	0.187	1	10	100	100
	0.187	2	10	100	
	0.187	3	10	100	
mg Boron L^{-1}	0.53	1	10	100	100
	0.53	2	10	100	
	0.53	3	10	100	
mg Boron L^{-1}	5.8	1	9	90	96.7
	5.8	2	10	100	
	5.8	3	10	100	
mg Boron L^{-1}	18	1	10	100	100
	18	2	10	100	
	18	3	10	100	
mg Boron L^{-1}	55	1	10	100	100
	55	2	10	100	
	55	3	10	100	

Table B-3: Chronic fish growth and condition data. Condition factor calculated using the formula of Ling et al. (2013).

Treatment	Rep	Fish #	Date	Length (mm)	Wet Weight (g)	Condition Factor
Time 0		1	23/2/18	60	1.096	0.98
Time 0		2	23/2/18	50	0.6733	1.06
Time 0		3	23/2/18	61	1.1478	0.98
Time 0		4	23/2/18	50	0.5295	0.83
Time 0		5	23/2/18	58	0.9882	0.98
Time 0		6	23/2/18	57	1.0655	1.12
Time 0		7	23/2/18	55	0.7431	0.87
Time 0		8	23/2/18	50	0.5702	0.90
Time 0		9	23/2/18	50	0.542	0.85
Time 0		10	23/2/18	50	0.6016	0.95
Time 0		11	23/2/18	55	0.6719	0.79
Time 0		12	23/2/18	55	0.7801	0.92
Time 0		13	23/2/18	53	0.8361	1.10
Time 0		14	23/2/18	52	0.6972	0.97
Time 0		15	23/2/18	44	0.4517	1.06
Control 0.02	1	1	5/4/18	64	1.2353	0.91
Control 0.02	1	2	5/4/18	61.5	1.1843	0.98
Control 0.02	1	3	5/4/18	65	1.5084	1.06
Control 0.02	1	4	5/4/18	54	0.8293	1.03
Control 0.02	1	5	5/4/18	58	0.5855	0.58
Control 0.02	1	6	5/4/18	52	0.8361	1.17
Control 0.02	1	7	5/4/18	53	0.797	1.05
Control 0.02	1	8	5/4/18	53	0.7213	0.95
Control 0.02	1	9	5/4/18	53	0.7818	1.03
Control 0.02	1	10	5/4/18	50	0.6463	1.02
Control 0.02	2	1	5/4/18	66	1.2689	0.85
Control 0.02	2	2	5/4/18	65	1.6	1.12
Control 0.02	2	3	5/4/18	63	1.5495	1.20
Control 0.02	2	4	5/4/18	57	0.9974	1.05
Control 0.02	2	5	5/4/18	63	1.1687	0.90
Control 0.02	2	6	5/4/18	56	0.9563	1.06
Control 0.02	2	7	5/4/18	60	1.1635	1.04
Control 0.02	2	8	5/4/18	55	0.9501	1.11
Control 0.02	2	9	5/4/18	53	0.7241	0.95
Control 0.02	2	10	5/4/18			
Control 0.02	3	1	5/4/18	60	1.0757	0.96
Control 0.02	3	2	5/4/18	64	1.2825	0.94
Control 0.02	3	3	5/4/18	68	1.4837	0.90
Control 0.02	3	4	5/4/18	61	1.3855	1.18
Control 0.02	3	5	5/4/18	55	0.9686	1.14
Control 0.02	3	6	5/4/18	56	0.9656	1.07
Control 0.02	3	7	5/4/18	62	1.1861	0.96
Control 0.02	3	8	5/4/18	54	0.7619	0.95
Control 0.02	3	9	5/4/18	58	1.0718	1.07
Control 0.02	3	10	5/4/18			
0.187	1	1	5/4/18	58	0.854	0.85
0.187	1	2	5/4/18	60	1.088	0.98
0.187	1	3	5/4/18	51	0.7496	1.11
0.187	1	4	5/4/18	52	0.6263	0.87

Treatment	Rep	Fish #	Date	Length (mm)	Wet Weight (g)	Condition Factor
0.187	1	5	5/4/18	50	0.6677	1.05
0.187	1	6	5/4/18	53	0.8487	1.12
0.187	1	7	5/4/18	53	0.6898	0.91
0.187	1	8	5/4/18	50	0.588	0.93
0.187	1	9	5/4/18	48	0.6491	1.16
0.187	1	10	5/4/18	45	0.4766	1.04
0.187	2	1	5/4/18	57	0.8556	0.90
0.187	2	2	5/4/18	60	1.2664	1.14
0.187	2	3	5/4/18	58	1.1032	1.10
0.187	2	4	5/4/18	57	0.964	1.01
0.187	2	5	5/4/18	50.5	0.7293	1.11
0.187	2	6	5/4/18	57	0.8741	0.92
0.187	2	7	5/4/18	51	0.7499	1.11
0.187	2	8	5/4/18	55	0.9177	1.08
0.187	2	9	5/4/18	62	1.0428	0.85
0.187	2	10	5/4/18	53	0.8135	1.07
0.187	3	1	5/4/18	47	0.5111	0.97
0.187	3	2	5/4/18	51	0.7318	1.08
0.187	3	3	5/4/18	56	0.9394	1.04
0.187	3	4	5/4/18	48	0.6082	1.09
0.187	3	5	5/4/18	48	0.6052	1.08
0.187	3	6	5/4/18	52	0.7378	1.03
0.187	3	7	5/4/18	55	0.8609	1.01
0.187	3	8	5/4/18	56	1.0546	1.17
0.187	3	9	5/4/18	54	0.8561	1.06
0.187	3	10	5/4/18	47	0.5002	0.95
0.53	1	1	5/4/18	61	1.2159	1.04
0.53	1	2	5/4/18	61	0.8371	0.71
0.53	1	3	5/4/18	56	0.9394	1.04
0.53	1	4	5/4/18	57	1.1388	1.20
0.53	1	5	5/4/18	63.5	1.2236	0.92
0.53	1	6	5/4/18	48	0.602	1.08
0.53	1	7	5/4/18	48.5	0.782	1.35
0.53	1	8	5/4/18	52	0.7155	1.00
0.53	1	9	5/4/18	49	0.6166	1.03
0.53	1	10	5/4/18	53	0.7063	0.93
0.53	2	1	5/4/18	53	0.8373	1.10
0.53	2	2	5/4/18	58	1.0625	1.06
0.53	2	3	5/4/18	57	1.0592	1.11
0.53	2	4	5/4/18	57	1.096	1.15
0.53	2	5	5/4/18	55	0.9041	1.06
0.53	2	6	5/4/18	50.5	0.6776	1.04
0.53	2	7	5/4/18	50	0.6508	1.03
0.53	2	8	5/4/18	48	0.6434	1.15
0.53	2	9	5/4/18	46	0.5083	1.04
0.53	2	10	5/4/18	46	0.5257	1.07
0.53	3	1	5/4/18	57	1.00	1.05
0.53	3	2	5/4/18	64	1.333	0.98
0.53	3	3	5/4/18	52	0.6732	0.94
0.53	3	4	5/4/18	50.5	0.6442	0.98
0.53	3	5	5/4/18	63	1.1152	0.86

Treatment	Rep	Fish #	Date	Length (mm)	Wet Weight (g)	Condition Factor
0.53	3	6	5/4/18	47	0.6196	1.18
0.53	3	7	5/4/18	53	0.7739	1.02
0.53	3	8	5/4/18	52.5	0.8361	1.13
0.53	3	9	5/4/18	53	0.74	0.97
0.53	3	10	5/4/18	48	0.568	1.02
5.8	1	1	5/4/18	63	1.2721	0.98
5.8	1	2	5/4/18	65	1.5193	1.06
5.8	1	3	5/4/18	51	0.8225	1.22
5.8	1	4	5/4/18	51	0.7774	1.15
5.8	1	5	5/4/18	58	0.9333	0.93
5.8	1	6	5/4/18	64	1.4999	1.10
5.8	1	7	5/4/18	64	1.2146	0.89
5.8	1	8	5/4/18	53	0.6695	0.88
5.8	1	9	5/4/18	46	0.5295	1.08
5.8	1	10	5/4/18			
5.8	2	1	5/4/18	58	1.0309	1.03
5.8	2	2	5/4/18	46	0.5228	1.07
5.8	2	3	5/4/18	61	1.1307	0.96
5.8	2	4	5/4/18	48	0.532	0.95
5.8	2	5	5/4/18	46	0.5123	1.04
5.8	2	6	5/4/18	52	0.7303	1.02
5.8	2	7	5/4/18	47	0.606	1.16
5.8	2	8	5/4/18	53	0.7268	0.96
5.8	2	9	5/4/18	51	0.6394	0.95
5.8	2	10	5/4/18	45	0.4496	0.98
5.8	2	11	5/4/18	58	1.0309	1.03
5.8	2	30	5/4/18	46	0.5228	1.07
5.8	3	1	5/4/18	50	0.7913	1.25
5.8	3	2	5/4/18	64	1.2038	0.88
5.8	3	3	5/4/18	48	0.6227	1.11
5.8	3	4	5/4/18	57	0.8581	0.90
5.8	3	5	5/4/18	45	0.5133	1.12
5.8	3	6	5/4/18	52	0.7185	1.00
5.8	3	7	5/4/18	58	1.1438	1.14
5.8	3	8	5/4/18	51	0.7734	1.15
5.8	3	9	5/4/18	50	0.693	1.09
5.8	3	10	5/4/18	53	0.7367	0.97
18	1	1	5/4/18	65	1.4082	0.99
18	1	2	5/4/18	52	0.7767	1.08
18	1	3	5/4/18	53	0.7843	1.03
18	1	4	5/4/18	67	1.6071	1.02
18	1	5	5/4/18	60	1.043	0.94
18	1	6	5/4/18	61	1.0345	0.88
18	1	7	5/4/18	44	0.3969	0.93
18	1	8	5/4/18	49	0.5468	0.92
18	1	9	5/4/18	46	0.6848	1.40
18	1	10	5/4/18	53	0.7283	0.96
18	2	1	5/4/18	51	0.759	1.12
18	2	2	5/4/18	53	0.8018	1.05
18	2	3	5/4/18	51	0.6302	0.93
18	2	4	5/4/18	48	0.669	1.20

Treatment	Rep	Fish #	Date	Length (mm)	Wet Weight (g)	Condition Factor
18	2	5	5/4/18	52	0.7132	1.00
18	2	6	5/4/18	55	0.8743	1.03
18	2	7	5/4/18	56	0.9215	1.02
18	2	8	5/4/18	50	0.8157	1.28
18	2	9	5/4/18	52	0.7303	1.02
18	2	10	5/4/18	46	0.4742	0.97
18	3	1	5/4/18	45	0.5172	1.13
18	3	2	5/4/18	55	0.8511	1.00
18	3	3	5/4/18	67	1.3945	0.89
18	3	4	5/4/18	56	0.8592	0.95
18	3	5	5/4/18	47	0.5742	1.10
18	3	6	5/4/18	58	1.1664	1.16
18	3	7	5/4/18	54	0.6862	0.85
18	3	8	5/4/18	55	0.8816	1.03
18	3	9	5/4/18	44	0.5133	1.20
18	3	10	5/4/18	46	0.5877	1.20
55	1	1	5/4/18	53	[2]	[3]
55	1	2	5/4/18	53	1.1423	1.50
55	1	3	5/4/18	62	1.123	0.91
55	1	4	5/4/18	43	0.2856	0.72
55	1	5	5/4/18	54	0.7455	0.93
55	1	6	5/4/18	40	0.2896	0.91
55	1	7	5/4/18	47	0.4741	0.90
55	1	8	5/4/18	46	0.3398	0.69
55	1	9	5/4/18	40	0.2849	0.89
55	1	10	5/4/18	56	0.7337	0.81
55	2	1	5/4/18	65	1.3125	0.92
55	2	2	5/4/18	54	0.9366	1.16
55	2	3	5/4/18	51	0.7586	1.12
55	2	4	5/4/18	57	1.0021	1.05
55	2	5	5/4/18	58	0.8178	0.81
55	2	6	5/4/18	50	0.531	0.84
55	2	7	5/4/18	51	0.635	0.94
55	2	8	5/4/18	45	0.4891	1.07
55	2	9	5/4/18	48	0.5924	1.06
55	2	10	5/4/18	54	0.8781	1.09
55	3	1	5/4/18	56	0.7767	0.86
55	3	2	5/4/18	61	1.2262	1.04
55	3	3	5/4/18	40	0.2415	0.76
55	3	4	5/4/18	46	0.5162	1.05
55	3	5	5/4/18	60	0.7302	0.65
55	3	6	5/4/18	55	0.7261	0.85
55	3	7	5/4/18	40	0.2036	0.64
55	3	8	5/4/18	50	0.5462	0.86
55	3	9	5/4/18	45	0.3244	0.71
55	3	10	5/4/18	42	0.2507	0.68

[2] Data not recorded
[3] Data cannot be calculated

Table B-4: Summary endpoint and physico-chemical data for test with Canterbury mudfish.

Boron			Endpoint (means)				Physico-chemical (means)			
Nominal	Measured (median)		Survival	Weight	Length	Condition	Cond	DO	pH	Temp
g Boron m^{-3}	g Boron m^{-3}	Rep	%	g	mm		µS cm^{-1}	g m^{-3}		˚C
Control	0.02	1	100	0.9125	56	0.98	104.5	10.09	7.94	14.3
		2	90	1.1532	60	1.03				
		3	90	1.1313	60	1.02				
0.2	0.187	1	100	0.7238	52	1.00	104.3	10.01	7.90	14.4
		2	100	0.9317	56	1.03				
		3	100	0.7405	51	1.05				
0.5	0.53	1	100	0.8777	55	1.03	103.0	10.11	7.74	14.4
		2	100	0.7965	52	1.08				
		3	100	0.8299	54	1.01				
5	5.8	1	90	1.0265	57	1.03	100.9	10.02	7.62	14.4
		2	100	0.6881	51	1.01				
		3	100	0.8055	53	1.06				
20	18	1	100	0.9011	55	1.01	101.2	10.09	7.56	14.2
		2	100	0.7389	51	1.06				
		3	100	0.8031	53	1.05				
50	55	1	100	0.6021	49	0.92	100.2	9.95	7.42	14.3
		2	100	0.7953	53	1.01				
		3	100	0.5542	50	0.81				

Appendix C Summary statistics for chronic juvenile Canterbury Mudfish test

Survival

CETIS Analytical Report

Report Date:	26 Jun-18 12:18 (p 7 of 8)
Test Code:	BRL18202 Fish | 14-2937-5961

Larval Fish 40-d Survival and Growth Test — **NIWA Ecotoxicology**

Analysis ID:	12-7892-1287	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.9.0
Analyzed:	26 Jun-18 12:16	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	07-4221-8500	**Test Type:**	Survival-Growth	**Analyst:**	K Thompson
Start Date:	24 Feb-18	**Protocol:**	Not Applicable	**Diluent:**	Christchurch Tapwater
Ending Date:	05 Apr-18	**Species:**	Neochanna burrowsius	**Brine:**	Not Applicable
Duration:	40d 0h	**Source:**	Ichthyo-niche	**Age:**	
Sample ID:	12-3616-8333	**Code:**	BRL18202 Fish	**Client:**	Bathurst Resources Ltd
Sample Date:	24 Feb-18	**Material:**	Boron	**Project:**	Special Studies
Receipt Date:		**Source:**	Solution made by NIWA		
Sample Age:	n/a	**Station:**	Lab Solution		

Data Transform	Alt Hyp	Trials	Seed	TST b	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	n/a	n/a	n/a	55	> 55	n/a		11.2%

Unequal Variance t Two-Sample Test

Control vs	Conc-mg/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water	0.187	-2	2.92	0.159	2	CDF	0.9082	Non-Significant Effect
	0.53	-2	2.92	0.159	2	CDF	0.9082	Non-Significant Effect
	5.8	-0.7071	2.353	0.181	3	CDF	0.7348	Non-Significant Effect
	18	-2	2.92	0.159	2	CDF	0.9082	Non-Significant Effect
	55	-2	2.92	0.159	2	CDF	0.9082	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.030986	0.006197	5	2.1	0.1357	Non-Significant Effect
Error	0.035412	0.002951	12			
Total	0.066398		17			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	12.8	5.064	1.8E-04	Unequal Variances
Variances	Mod Levene Equality of Variance Test	0.8	8.746	0.5876	Equal Variances
Distribution	Anderson-Darling A2 Normality Test	2.278	3.878	<1.0E-37	Non-Normal Distribution
Distribution	D'Agostino Skewness Test	1.68E-14	2.576	1.0000	Normal Distribution
Distribution	Kolmogorov-Smirnov D Test	0.3333	0.2344	1.1E-05	Non-Normal Distribution
Distribution	Shapiro-Wilk W Normality Test	0.8085	0.8546	0.0020	Non-Normal Distribution

Survival Rate Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.02	D	3	0.9333	0.7899	1.0000	0.9000	0.9000	1.0000	0.0333	6.19%	0.00%
0.187		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-7.14%
0.53		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-7.14%
5.8		3	0.9667	0.8232	1.0000	1.0000	0.9000	1.0000	0.0333	5.97%	-3.57%
18		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-7.14%
55		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-7.14%

Angular (Corrected) Transformed Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.02	D	3	1.303	1.07	1.537	1.249	1.249	1.412	0.05432	7.22%	0.00%
0.187		3	1.412	1.411	1.413	1.412	1.412	1.412	0	0.00%	-8.34%
0.53		3	1.412	1.411	1.413	1.412	1.412	1.412	0	0.00%	-8.34%
5.8		3	1.358	1.124	1.591	1.412	1.249	1.412	0.05432	6.93%	-4.17%
18		3	1.412	1.411	1.413	1.412	1.412	1.412	0	0.00%	-8.34%
55		3	1.412	1.411	1.413	1.412	1.412	1.412	0	0.00%	-8.34%

008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

Larval Fish 40-d Survival and Growth Test — **NIWA Ecotoxicology**

Analysis ID:	12-7892-1287	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.9.0
Analyzed:	26 Jun-18 12:16	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Survival Rate Detail

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0.02	D	1.0000	0.9000	0.9000
0.187		1.0000	1.0000	1.0000
0.53		1.0000	1.0000	1.0000
5.8		0.9000	1.0000	1.0000
18		1.0000	1.0000	1.0000
55		1.0000	1.0000	1.0000

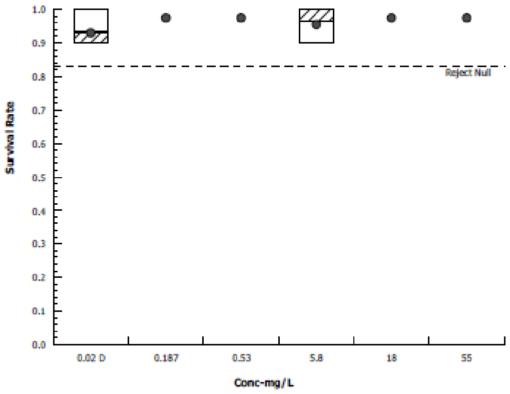
Angular (Corrected) Transformed Detail

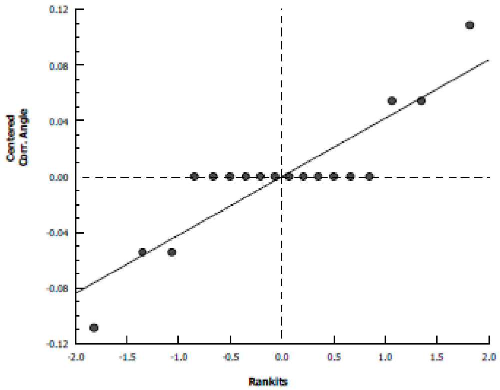
Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0.02	D	1.412	1.249	1.249
0.187		1.412	1.412	1.412
0.53		1.412	1.412	1.412
5.8		1.249	1.412	1.412
18		1.412	1.412	1.412
55		1.412	1.412	1.412

Survival Rate Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0.02	D	10/10	9/10	9/10
0.187		10/10	10/10	10/10
0.53		10/10	10/10	10/10
5.8		9/10	10/10	10/10
18		10/10	10/10	10/10
55		10/10	10/10	10/10

Graphics





Condition

CETIS Analytical Report

Report Date: 29 Jun-18 14:49 (p 1 of 3)
Test Code: BRL18202 Fish | 14-2937-5961

Larval Fish 40-d Survival and Growth Test — **NIWA Ecotoxicology**

Analysis ID:	10-8654-7979	**Endpoint:**	Condition	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 14:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	07-4221-8500	**Test Type:**	Survival-Growth	**Analyst:**	K Thompson
Start Date:	24 Feb-18	**Protocol:**	Not Applicable	**Diluent:**	Christchurch Tapwater
Ending Date:	05 Apr-18	**Species:**	Neochanna burrowsius	**Brine:**	Not Applicable
Duration:	40d 0h	**Source:**	Ichthyo-niche	**Age:**	

Sample ID:	12-3616-8333	**Code:**	BRL18202 Fish	**Client:**	Bathurst Resources Ltd
Sample Date:	24 Feb-18	**Material:**	Boron	**Project:**	Special Studies
Receipt Date:		**Source:**	Solution made by NIWA		
Sample Age:	n/a	**Station:**	Lab Solution		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	704281	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
EC5	29.37	16.97	n/a
EC10	47.54	11.76	n/a
EC15	>55	n/a	n/a
EC20	>55	n/a	n/a
EC25	>55	n/a	n/a
EC40	>55	n/a	n/a
EC50	>55	n/a	n/a

Condition Summary

			Calculated Variate						
Conc-mg/L	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0.02	D	3	1.009	0.9772	1.032	0.01649	0.02857	2.83%	0.00%
0.187		3	1.026	1.001	1.05	0.01402	0.02429	2.37%	-1.69%
0.53		3	1.041	1.013	1.08	0.02018	0.03495	3.36%	-3.14%
5.8		3	1.035	1.011	1.061	0.01458	0.02525	2.44%	-2.56%
18		3	1.043	1.014	1.062	0.01453	0.02516	2.41%	-3.29%
55		3	0.9121	0.8108	1.007	0.05666	0.09813	10.76%	9.63%

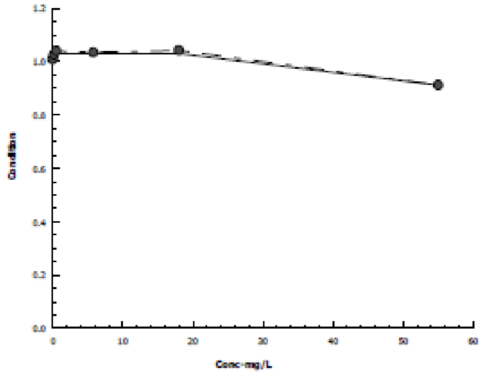
Condition Detail

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0.02	D	0.9772	1.032	1.019
0.187		1.001	1.028	1.05
0.53		1.03	1.08	1.013
5.8		1.033	1.011	1.061
18		1.014	1.062	1.051
55		0.9189	1.007	0.8108

008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

Larval Fish 40-d Survival and Growth Test					**NIWA Ecotoxicology**
Analysis ID:	10-8654-7979	**Endpoint:**	Condition	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 14:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics



Length

CETIS Analytical Report

Report Date: 26 Jun-18 12:18 (p 3 of 8)
Test Code: BRL18202 Fish | 14-2937-5961

Larval Fish 40-d Survival and Growth Test — **NIWA Ecotoxicology**

Analysis ID:	18-3967-6472	**Endpoint:**	Mean Length-mm	**CETIS Version:**	CETISv1.9.0
Analyzed:	26 Jun-18 12:17	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	07-4221-8500	**Test Type:**	Survival-Growth	**Analyst:**	K Thompson
Start Date:	24 Feb-18	**Protocol:**	Not Applicable	**Diluent:**	Christchurch Tapwater
Ending Date:	05 Apr-18	**Species:**	Neochanna burrowsius	**Brine:**	Not Applicable
Duration:	40d 0h	**Source:**	Ichthyo-niche	**Age:**	
Sample ID:	12-3616-8333	**Code:**	BRL18202 Fish	**Client:**	Bathurst Resources Ltd
Sample Date:	24 Feb-18	**Material:**	Boron	**Project:**	Special Studies
Receipt Date:		**Source:**	Solution made by NIWA		
Sample Age:	n/a	**Station:**	Lab Solution		

Data Transform	Alt Hyp	Trials	Seed	TST b	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	n/a	n/a	n/a	5.8	18	10.22		6.9%

Unequal Variance t Two-Sample Test

Control vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water	0.187*	2.958	2.353	4.364	3	CDF	0.0298	Significant Effect
	0.53*	3.514	2.353	3.339	3	CDF	0.0195	Significant Effect
	5.8	2.263	2.353	5.262	3	CDF	0.0543	Non-Significant Effect
	18*	3.606	2.353	3.656	3	CDF	0.0183	Significant Effect
	55*	4.598	2.353	4.044	3	CDF	0.0097	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	101.602	20.3203	5	3.836	0.0262	Significant Effect
Error	63.5749	5.29791	12			
Total	165.176		17			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	1.382	15.09	0.9262	Equal Variances
Variances	Levene Equality of Variance Test	0.815	5.064	0.5614	Equal Variances
Variances	Mod Levene Equality of Variance Test	0.2262	8.746	0.9379	Equal Variances
Distribution	Anderson-Darling A2 Normality Test	0.5279	3.878	0.1817	Normal Distribution
Distribution	D'Agostino Skewness Test	0.8343	2.576	0.4041	Normal Distribution
Distribution	Kolmogorov-Smirnov D Test	0.1684	0.2344	0.1955	Normal Distribution
Distribution	Shapiro-Wilk W Normality Test	0.9409	0.8546	0.3000	Normal Distribution

Mean Length-mm Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.02	D	3	58.64	53.72	63.55	59.78	56.35	59.78	1.143	3.38%	0.00%
0.187		3	53.15	46.87	59.43	52	51.4	56.05	1.46	4.76%	9.35%
0.53		3	53.65	50.03	57.27	54	52.05	54.9	0.8411	2.72%	8.50%
5.8		3	53.57	45.3	61.84	52.8	50.7	57.22	1.922	6.21%	8.63%
18		3	53.03	48.5	57.56	52.7	51.4	55	1.053	3.44%	9.55%
55		3	50.73	45.21	56.26	49.5	49.4	53.3	1.284	4.38%	13.48%

Mean Length-mm Detail

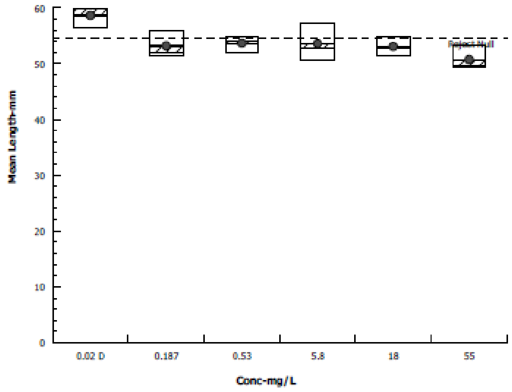
Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0.02	D	56.35	59.78	59.78
0.187		52	56.05	51.4
0.53		54.9	52.05	54
5.8		57.22	50.7	52.8
18		55	51.4	52.7
55		49.4	53.3	49.5

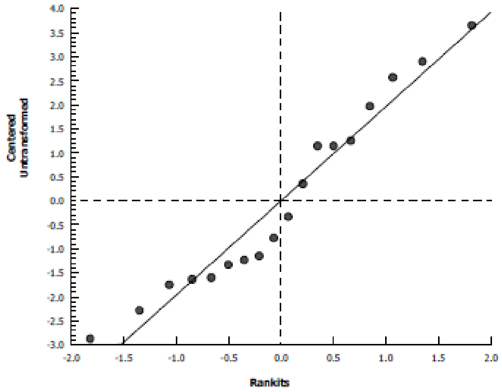
008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

Larval Fish 40-d Survival and Growth Test | NIWA Ecotoxicology

Analysis ID:	18-3967-6472	**Endpoint:**	Mean Length-mm	**CETIS Version:**	CETISv1.9.0
Analyzed:	26 Jun-18 12:17	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Graphics





Weight

CETIS Analytical Report

Report Date:	29 Jun-18 14:49 (p 3 of 3)	**Test Code:**	BRL18202 Fish | 14-2937-5961

Larval Fish 40-d Survival and Growth Test — **NIWA Ecotoxicology**

Analysis ID:	08-6990-3030	**Endpoint:**	Mean Weight-g	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 14:48	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	07-4221-8500	**Test Type:**	Survival-Growth	**Analyst:**	K Thompson
Start Date:	24 Feb-18	**Protocol:**	Not Applicable	**Diluent:**	Christchurch Tapwater
Ending Date:	05 Apr-18	**Species:**	Neochanna burrowsius	**Brine:**	Not Applicable
Duration:	40d 0h	**Source:**	Ichthyo-niche	**Age:**	
Sample ID:	12-3616-8333	**Code:**	BRL18202 Fish	**Client:**	Bathurst Resources Ltd
Sample Date:	24 Feb-18	**Material:**	Boron	**Project:**	Special Studies
Receipt Date:		**Source:**	Solution made by NIWA		
Sample Age:	n/a	**Station:**	Lab Solution		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	703949	200	Yes	Two-Point Interpolation

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC50	>55	n/a	n/a

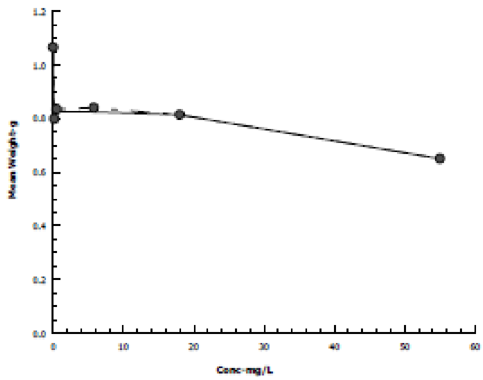
Mean Weight-g Summary

			Calculated Variate						
Conc-mg/L	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.02	D	3	1.066	0.9125	1.153	0.07682	0.1331	12.49%	0.00%
0.187		3	0.7987	0.7238	0.9316	0.06667	0.1155	14.46%	25.06%
0.53		3	0.8347	0.7965	0.8777	0.02357	0.04082	4.89%	21.67%
5.8		3	0.84	0.6881	1.026	0.0992	0.1718	20.45%	21.18%
18		3	0.8144	0.7389	0.9011	0.04714	0.08165	10.03%	23.58%
55		3	0.6505	0.5542	0.7953	0.07371	0.1277	19.63%	38.96%

Mean Weight-g Detail

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0.02	D	0.9125	1.153	1.131
0.187		0.7238	0.9316	0.7405
0.53		0.8777	0.7965	0.83
5.8		1.026	0.6881	0.8055
18		0.9011	0.7389	0.8031
55		0.6021	0.7953	0.5542

Graphics



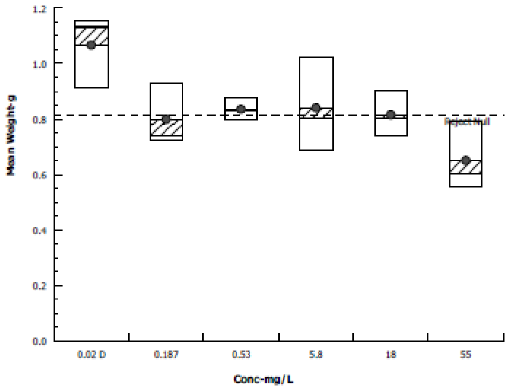
008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

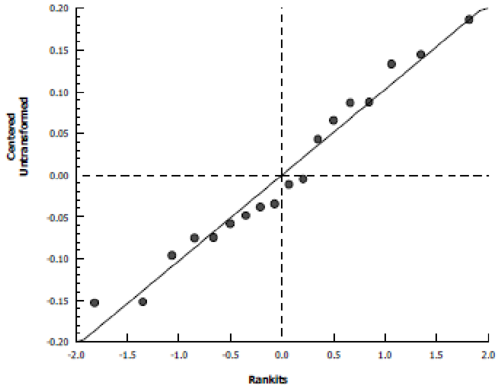
CETIS Analytical Report		**Report Date:**	26 Jun-18 12:18 (p 6 of 8)
		Test Code:	BRL18202 Fish | 14-2937-5961

Larval Fish 40-d Survival and Growth Test — **NIWA Ecotoxicology**

Analysis ID:	06-4848-7501	**Endpoint:**	Mean Weight-g	**CETIS Version:**	CETISv1.9.0
Analyzed:	26 Jun-18 12:17	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Graphics





Appendix D Chronic periphyton growth inhibition data

Table D-1: Chronic periphyton growth inhibition data.

Sample ID	Nominal Concentration g m^{-3}	Rep.	Dilution factor	A750b	A665b	A645b	A630b	A750a	A665a	Initial Weight alga (g)	Chl *a* µg/g Initial sample
Time 0		1	5	6	113	55	31	6	70	0.0204	3021
Time 0		2	5	2	95	44	23	3	58	0.0206	2643
Time 0		3	5	0	66	30	15	1	40	0.0210	1842
Time 0		4	5	1	66	30	15	2	39	0.0200	2006
Time 0		5	5	3	77	36	20	3	47	0.0201	2139
Time 0		6	5	2	47	22	12	2	29	0.0200	1290
Time 0		7	5	4	72	34	19	4	44	0.0198	2026
Time 0		8	5	1	65	30	15	2	39	0.0209	1851
Time 0		9	5	1	80	36	19	2	48	0.0199	2376
Control	0	1	5	7	106	54	31	7	66	0.0203	2824
	0	2	5	4	130	62	32	5	78	0.0199	3817
	0	3	5	4	147	71	36	4	85	0.0204	3934
	0	4	5	2	125	59	30	3	75	0.0208	3514
	0	5	5	3	79	39	21	4	48	0.0204	2248
mg Boron L^{-1}	0.32	1	5	2	109	50	26	3	66	0.0202	3121
	0.32	2	5	18	136	72	47	17	87	0.0200	3439
	0.32	3	5	4	104	51	28	4	64	0.0206	2783
mg Boron L^{-1}	1	1	5	2	107	52	26	3	65	0.0201	3066
	1	2	5	5	112	55	30	5	69	0.0203	3035
	1	3	5	2	114	51	27	3	69	0.0203	3247
mg Boron L^{-1}	10	1	5	5	42	23	14	5	27	0.0206	1043
	10	2	5	2	94	45	24	2	58	0.0203	2541
	10	3	5	8	117	59	34	9	73	0.0201	3208
mg Boron L^{-1}	32	1	5	0	42	21	10	1	25	0.0205	1258
	32	2	5							0.0203	[4]
	32	3	5	6	52	29	18	6	34	0.0209	1234
mg Boron L^{-1}	100	1	5	3	32	18	11	3	21	0.0199	792
	100	2	5	8	38	25	18	8	27	0.0201	784
	100	3	5	6	37	23	16	6	25	0.0204	843

[4] Sample spilt, no result

Appendix E Chronic chlorophyll *a* data for test with periphyton

Table E-1: Chronic growth inhibition periphyton using chlorophyll *a*.

Sample ID	Nominal Concentration g m^{-3}	Replicate	Initial Weight alga g	Chl *a* µg/g	Growth Inhibition %	Mean Growth Inhibition %
Time 0		1	0.0204	3021		
Time 0		2	0.0206	2643		
Time 0		3	0.0210	1842		
Time 0		4	0.0200	2006		
Time 0		5	0.0201	2139		
Time 0		6	0.0200	1290		
Time 0		7	0.0198	2026		
Time 0		8	0.0209	1851		
Time 0		9	0.0199	2376		
Control	0	1	0.0203	2824		
	0	2	0.0199	3817		
	0	3	0.0204	3934		
	0	4	0.0208	3514		
	0	5	0.0204	2248		
mg Boron L^{-1}	0.32	1	0.0202	3121	4.46	4.68
	0.32	2	0.0200	3439	-5.27	
	0.32	3	0.0206	2783	14.83	
mg Boron L^{-1}	1	1	0.0201	3066	6.17	4.62
	1	2	0.0203	3035	7.09	
	1	3	0.0203	3247	0.61	
mg Boron L^{-1}	10	1	0.0206	1043	68.06	30.69
	10	2	0.0203	2541	22.22	
	10	3	0.0201	3208	1.80	
mg Boron L^{-1}	32	1	0.0205	1258	61.49	61.86
	32	2	0.0203	[5]		
	32	3	0.0209	1234	62.22	
mg Boron L^{-1}	100	1	0.0199	792	75.75	75.32
	100	2	0.0201	784	76.00	
	100	3	0.0204	843	74.20	

[5] Sample spilt, no result

Appendix F Summary statistics for chronic periphyton test

CETIS Analytical Report

Report Date: 29 Jun-18 15:22 (p 1 of 2)
Test Code: BRL18202 Phyto | 17-4822-8169

EC Alga Growth Inhibition Test — **NIWA Ecotoxicology**

Analysis ID:	16-2349-5547	**Endpoint:**	Chlorophyll a	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 15:21	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes
Batch ID:	17-5541-1048	**Test Type:**	Cell Growth	**Analyst:**	S Bell
Start Date:	18 Jun-18	**Protocol:**	Not Applicable	**Diluent:**	Algal Culture Media
Ending Date:	25 Jun-18	**Species:**	Rhizoclonium sp.	**Brine:**	Not Applicable
Duration:	7d 0h	**Source:**	Field Collected	**Age:**	
Sample ID:	13-3615-7840	**Code:**	BRL18202 Phyto	**Client:**	Bathurst Resources Ltd
Sample Date:	18 Jun-18	**Material:**	Boron	**Project:**	Special Studies
Receipt Date:		**Source:**	Solution made by NIWA		
Sample Age:	n/a	**Station:**	Lab Solution		

Data Transform	Alt Hyp	Trials	Seed	TST b	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	n/a	n/a	n/a	10.5	32.5	18.47		35.8%

Bonferroni Adj t Test

Control	vs	Conc-mg/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Control Alga me		0.82	0.3462	2.65	1169	6	CDF	1.0000	Non-Significant Effect
		1.5	0.3423	2.65	1169	6	CDF	1.0000	Non-Significant Effect
		10.5	2.273	2.65	1169	6	CDF	0.1016	Non-Significant Effect
		32.5*	3.998	2.65	1340	5	CDF	0.0038	Significant Effect
		100.5*	5.577	2.65	1169	6	CDF	2.2E-04	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	17097600	3419510	5	9.368	5.8E-04	Significant Effect
Error	4745350	365027	13			
Total	21842900		18			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	20	15.09	0.0013	Unequal Variances
Variances	Levene Equality of Variance Test	5.119	4.862	0.0082	Unequal Variances
Variances	Mod Levene Equality of Variance Test	3.478	6.632	0.0576	Equal Variances
Distribution	Anderson-Darling A2 Normality Test	0.845	3.878	0.0295	Normal Distribution
Distribution	D'Agostino Skewness Test	1.452	2.576	0.1464	Normal Distribution
Distribution	Kolmogorov-Smirnov D Test	0.2271	0.2288	0.0110	Normal Distribution
Distribution	Shapiro-Wilk W Normality Test	0.9204	0.8605	0.1152	Normal Distribution

Chlorophyll a Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.5	AM	5	3267	2380	4154	3514	2248	3934	319.6	21.87%	0.00%
0.82		3	3114	2299	3930	3121	2783	3439	189.6	10.54%	4.68%
1.5		3	3116	2832	3401	3066	3035	3247	66.13	3.68%	4.62%
10.5		3	2264	-489.7	5018	2541	1043	3208	640.1	48.96%	30.69%
32.5		2	1246	1093	1399	1246	1234	1258	12.04	1.37%	61.86%
100.5		3	806.4	727.3	885.6	792.1	784.2	842.9	18.4	3.95%	75.32%

Chlorophyll a Detail

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0.5	AM	2824	3817	3934	3514	2248
0.82		3121	3439	2783		
1.5		3066	3035	3247		
10.5		1043	2541	3208		
32.5		1258	1234			
100.5		792.1	784.2	842.9		

008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

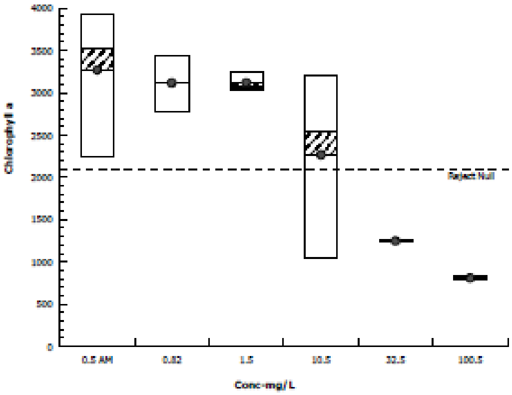
CETIS Analytical Report

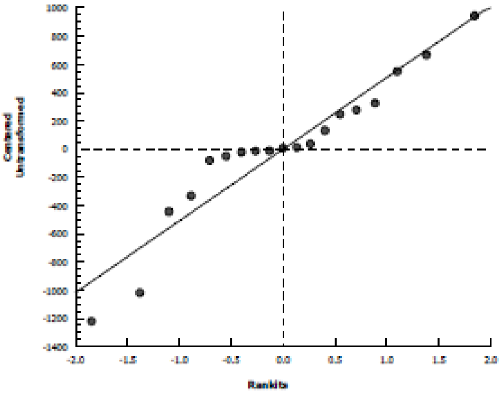
Report Date: 29 Jun-18 15:22 (p 2 of 2)
Test Code: BRL18202 Phyto | 17-4822-8169

EC Alga Growth Inhibition Test | **NIWA Ecotoxicology**

Analysis ID:	16-2349-5547	**Endpoint:**	Chlorophyll a	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 15:21	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Graphics





008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

CETIS Analytical Report

Report Date: 29 Jun-18 15:22 (p 1 of 2)
Test Code: BRL18202 Phyto | 17-4822-8169

EC Alga Growth Inhibition Test — **NIWA Ecotoxicology**

Analysis ID:	12-3582-0799	**Endpoint:**	Chlorophyll a	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 15:21	**Analysis:**	Nonlinear Regression (NLR)	**Official Results:**	Yes
Batch ID:	17-5541-1048	**Test Type:**	Cell Growth	**Analyst:**	S Bell
Start Date:	18 Jun-18	**Protocol:**	Not Applicable	**Diluent:**	Algal Culture Media
Ending Date:	25 Jun-18	**Species:**	Rhizoclonium sp.	**Brine:**	Not Applicable
Duration:	7d 0h	**Source:**	Field Collected	**Age:**	
Sample ID:	13-3615-7840	**Code:**	BRL18202 Phyto	**Client:**	Bathurst Resources Ltd
Sample Date:	18 Jun-18	**Material:**	Boron	**Project:**	Special Studies
Receipt Date:		**Source:**	Solution made by NIWA		
Sample Age:	n/a	**Station:**	Lab Solution		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Normal: ω=1	Off: μ*=μ	None	None

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
5	-118.8	245.2	246.4	0.7500	Yes	0.09832	3.411	0.9596	Non-Significant Lack of Fit

Point Estimates

Level	mg/L	95% LCL	95% UCL
IC5	0.6904	n/a	3.07
IC10	1.661	n/a	4.549
IC15	2.861	0.1538	7.476
IC20	4.308	0.5522	11.07
IC25	6.04	1.225	15.1
IC40	13.64	5.508	29.78
IC50	21.96	10.72	44.98

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	3384	343	2657	4111	9.866	<1.0E-37	Significant Parameter
γ	0.8511	0.3389	0.1326	1.57	2.511	0.0232	Significant Parameter
δ	21.96	10.32	0.07599	43.85	2.127	0.0493	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Lack of Fit	107700	35890	3	0.09832	0.9596	Non-Significant
Model	1.32E+08	43980000	3	145	<1.0E-37	Significant
Pure Error	4745000	365000	13			
Residual	4853000	303300	16			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance Test	20	11.07	0.0013	Unequal Variances
	Mod Levene Equality of Variance	3.478	3.687	0.0576	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9527	0.9007	0.4393	Normal Distribution
	Anderson-Darling A2 Normality Te	0.4989	2.492	0.2139	Normal Distribution

Chlorophyll a Summary

			Calculated Variate						
Conc-mg/L	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.5	AM	5	3267	2248	3934	319.6	714.7	21.87%	0.00%
0.82		3	3114	2783	3439	189.6	328.4	10.54%	4.68%
1.5		3	3116	3035	3247	66.13	114.5	3.68%	4.62%
10.5		3	2264	1043	3208	640.1	1109	48.96%	30.69%
32.5		2	1246	1234	1258	12.04	17.03	1.37%	61.86%
100.5		3	806.4	784.2	842.9	18.4	31.87	3.95%	75.32%

008-327-988-5 CETIS™ v1.9.0.8 Analyst:________ QA:________

CETIS Analytical Report

Report Date:	29 Jun-18 15:22 (p 2 of 2)
Test Code:	BRL18202 Phyto | 17-4822-8169

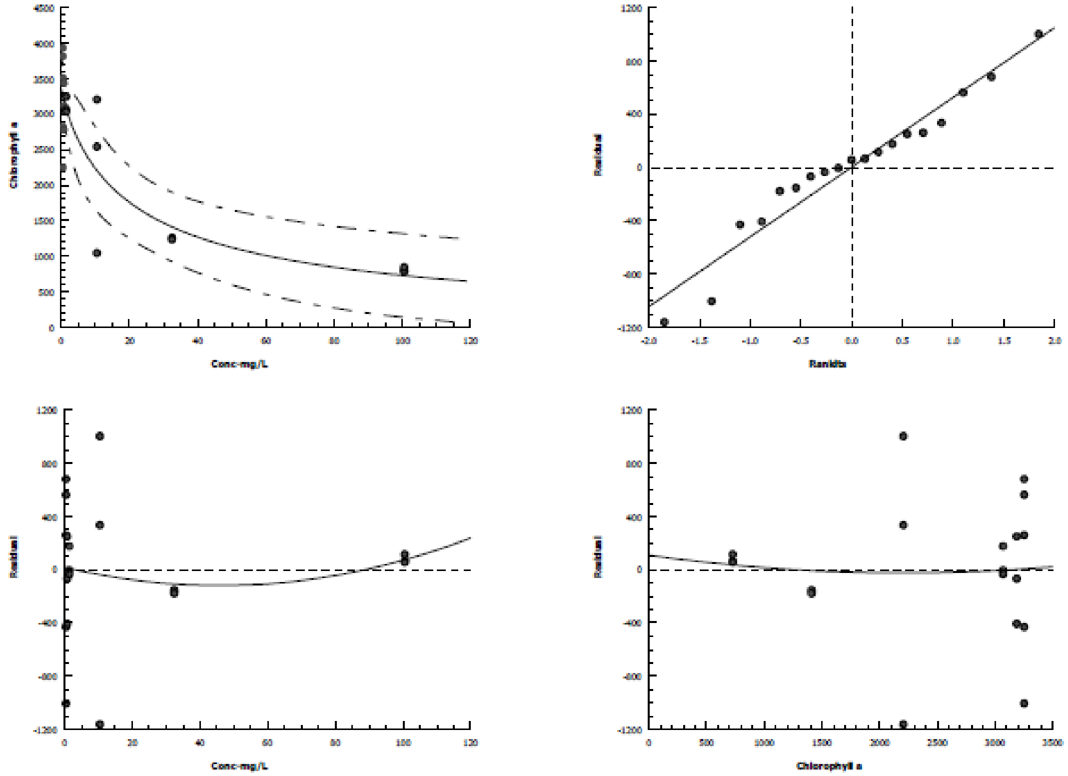
EC Alga Growth Inhibition Test — **NIWA Ecotoxicology**

Analysis ID:	12-3582-0799	**Endpoint:**	Chlorophyll a	**CETIS Version:**	CETISv1.9.0
Analyzed:	29 Jun-18 15:21	**Analysis:**	Nonlinear Regression (NLR)	**Official Results:**	Yes

Chlorophyll a Detail

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0.5	AM	2824	3817	3934	3514	2248
0.82		3121	3439	2783		
1.5		3066	3035	3247		
10.5		1043	2541	3208		
32.5		1258	1234			
100.5		792.1	784.2	842.9		

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Normal [ω=1]



Appendix G Updated ANZECC guideline for boron

The ANZECC guideline for boron is currently being revised and updated with new data published since 1998 and following the latest derivation procedures. The document is still awaiting completion of the technical review before finalising the derivation.

For this assessment, this updated guideline was used as the basis for the initial boron toxicity screening. Information summarised is the Executive summary, default guideline tables, the species sensitivity distribution and summary of chronic toxicity values from this updated guideline.

Reference:

Binet, M.T., Batley, G.E., Hickey, C.W., Golding, L.A., Adams, M.S. (2016) Guidelines for the protection of aquatic ecosystems, toxicant trigger values: Boron – Freshwater. *Australian and New Zealand guidelines for fresh and marine water quality.* Draft July 2016. No. Council of Australian Governments Standing Council on Environment and Water, Canberra, ACT, Australia: 19.

Summary

Boron is widely distributed in the environment as a natural constituent of minerals, in particular in clay-rich sedimentary rocks, coal, shale, and in some soils. Highest boron concentrations are found in marine sediments and as a consequence marine waters have boron near 5 mg/L. By comparison concentrations of boron in surface freshwaters are typically <0.5 mg/L, depending on the geochemical nature of the drainage catchment.

Since the last revision of the freshwater boron guideline values (GVs) for toxicity in 2000, errors were identified in the derivation and new data have become available. The revised GV is significantly higher than the current value (changing from 0.37 mg B/L to 0.83 mg B /L for 95% species protection).

High reliability GVs for boron in freshwaters were derived from 22 chronic (long-term) toxicity data, comprising seven fish, two amphibians, three crustaceans, one bivalve, five macrophytes, two green microalgal species and two diatoms.

The default GVs for a range of protection levels are:

Default guideline value type	Boron (freshwater) toxicity guideline value (mg/L)
Reliability	***Very high***
High conservation value systems (99% species protection)	0.24
Slightly to moderately disturbed systems (95% species protection)	0.83
Highly disturbed systems	
(90% species protection)	1.4
(80% species protection)	2.6

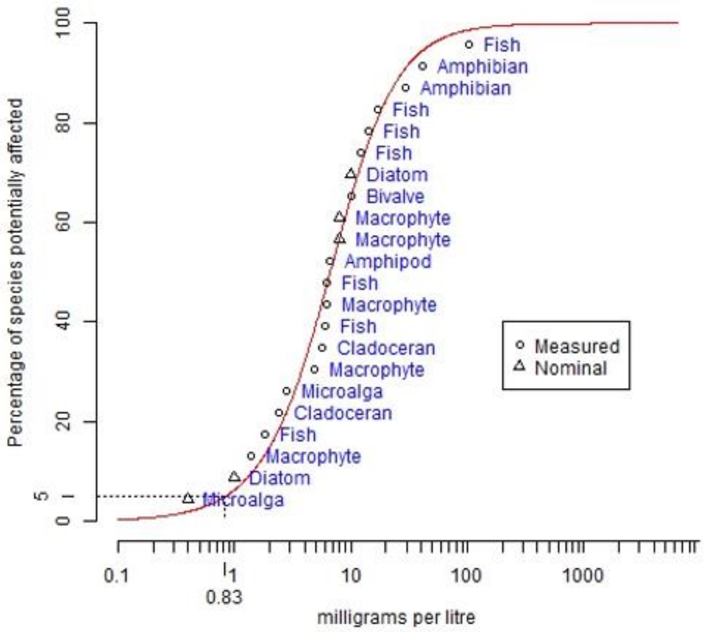


Figure 1 Cumulative frequency distribution (from Burrlioz 2.0©) for boron.

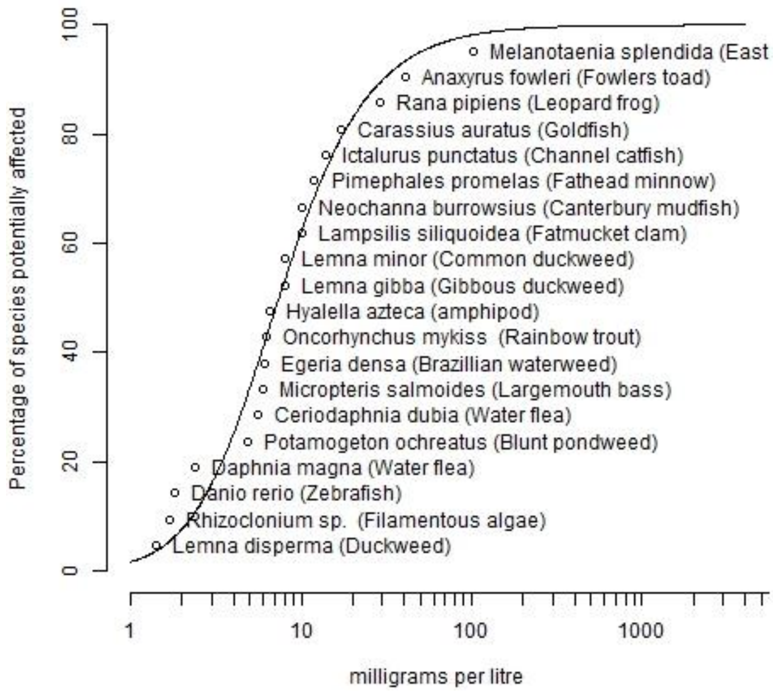


Figure G-1: Boron species sensitivity distribution for site-specific guideline derivation: species data. See Table 4-2 for species data.

Boron (site-specific)

```
Units: milligrams per litre
Model: Burr type III

Protection level information
Protect. level      Guideline Value      lower 95% CI         upper 95% CI
99%                 0.8                  0.3                  2.4
95%                 1.6                  1.1                  3.5
90%                 2.3                  1.5                  4.3
80%                 3.4                  2.2                  5.8

notes: Taxa group
```

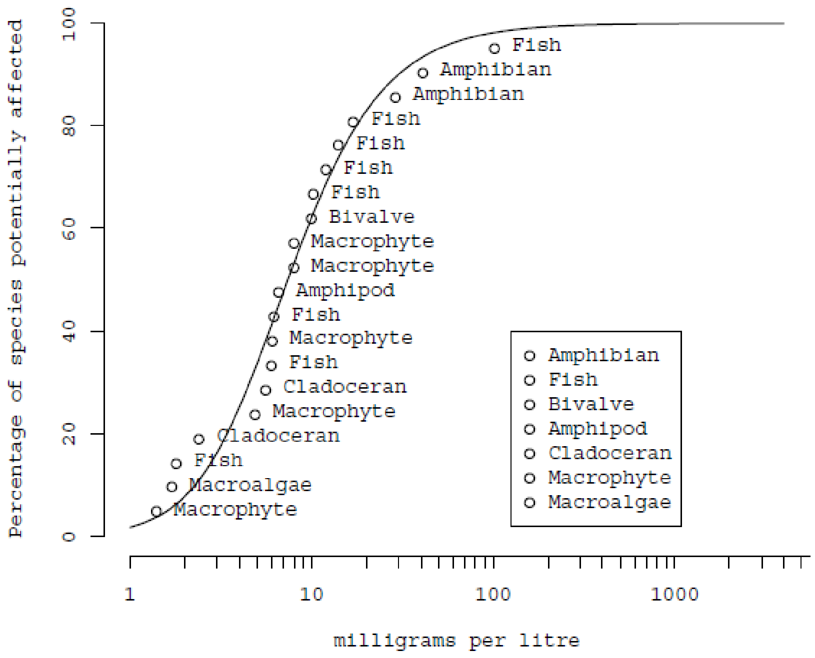


Figure G-2: Boron species sensitivity distribution for site-specific guideline derivation: taxonomic group and calculated protection levels. See Table 4-2 for species data.