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Lake Okaro and Rerewhakaaitu Fish Health Monitoring: Okaro Z2G1 Trial

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EXECUTIVE SUMMARY

On 27 September 2007, 112 T of modified zeolite (Z2G1; Blue Pacific Minerals, Matamata, NZ) was applied to Lake Okaro. Two fish collections were timed around the application to assess potential changes in fish health associated with exposure to the Z2G1 product. Lake Rerewhakaaitu was used as a reference site for purposes of comparison. Two fish species, the rainbow trout (*Oncorhynchus mykiss*) and the common bully (*Gobiomorphus cotidianus*), and one invertebrate, the koura (*Paranephrops planifrons*) were chosen for monitoring in this study. A broad selection of specific and non-specific endpoints were measured to investigate general and toxicant-linked changes in fish physiology and general health. Analysis of trout and koura tissue chemistry revealed that Z2G1-derived Al was not readily bioavailable to these species. Obvious changes in trout gill histopathology were not observed, suggesting no obvious impact of Z2G1 on this endpoint in this species. Differing haematological changes were observed for trout and bully between sampling periods. Changes in haematology are likely to be progressive and acclimatory, and were presumably influenced by changes in fish physiology and lake condition. An unexplained osmoregulatory disturbance as indicated by increases in plasma ion concentration for trout sampled after the Z2G1 application was observed in Lake Okaro trout. Based on the results of this study, it is concluded that there were no obvious negative impacts on fish health owing to Z2G1 exposure.

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1. INTRODUCTION

In Environment Bay of Plenty's Water and Land Plan (2002), water quality targets were set for each of the Rotorua Lakes. Initially five lakes (Okareka, Okaro, Rotorua, Rotoiti and Rotoehu) that were not meeting water quality targets were scheduled for action plans to examine ways of improving water quality in these lakes. This has recently been extended to nine lakes (including Tarawera, Rotoma, Tikitapu and Okataina) which have below target water quality, with a view to eventually developing action plans for all the Rotorua Lakes.

Due to early signs of deteriorating lake water quality (Hamilton 2003; Scholes 2004), the Lake Okaro Catchment Management Action Plan (EBOP 2004) was the first to be developed. This Action Plan outlined a number of options to reduce nutrient inputs such as sewage reticulation, pastoral retirement and nutrient adsorbing-mineral applications. Okareka was the first lake to receive whole-lake mineral applications over three successive years with the intent to bind up internal P (McIntosh 2007). The Lake Okaro Action Plan (EBOP 2006), jointly developed by Environment Bay of Plenty, Rotorua District Council and Te Arawa Lakes Trust is the second in the Rotorua Lakes restoration programme.

Lake Okaro is categorised as super- or hypertrophic; possessing the highest trophic level index (TLI; 5.6) of all the Rotorua lakes, experiencing severe algal blooms, seasonal water stratification and periods of hypolimnetic oxygen depletion. Reasons for the declining water quality in Lake Okaro have been attributed largely to pastoral land use and catchment development. The surrounding land has been farmed since the 1950s with greater than 95% of the catchment now as pasture. Progressive water quality declines have been ongoing since the 1970s.

The Lake Okaro Action plan set a number of goals to improve lake water quality by reducing lake nutrient loads by 910 kg of N and 20 kg of P. To date, a number of management/remediation options have been employed:

- Alum application in December 2003

- Wetland construction initiated in 2005
- Ongoing riparian protection through land retirement, and stream and lake fencing to prevent livestock access
- Sediment capping with nutrient adsorbing material in September 2007
- Ongoing evaluation of best management practices

On the grounds that half of lake P and a third of N is estimated to originate from lake sediment, the decision was made in the Lake Okaro Action Plan to cap the lake sediment with a nutrient adsorbing product. A number of effective nutrient adsorbing media have been assessed for potential application in the Rotorua Lakes (Yang et al. 2004). A selection of sediment capping materials such as modified zeolite, Phoslock^{TM}, iron slag and allophane were tested by in situ mesocosm trials in Lake Okaro during 2005 with inconclusive results (Özkundakci 2006; Zheng 2006; Özkundakci and Hamilton 2007; Yang et al. 2007). More recent laboratory-scale trials have shown modified zeolite (Z2G1; Blue Pacific Minerals, Matamata, NZ) to be highly effective at adsorbing P from Lake Okaro sediments (Gibbs et al. 2007). Another advantage of Z2G1 is that it readily binds both P and N.

Modified zeolite (Z2G1) is a proprietary product produced by amending natural zeolite with an aluminosilicate polymer. Although tightly bound to the zeolite, the presence of Al in Z2G1 represents potential for toxicity under certain conditions (Gensemer and Playle 1999). However, acute and sub-chronic toxicity studies have shown Z2G1 to be minimally toxic with only slight toxicity observed in fish and algae (Martin and Hickey 2007). Martin and Hickey suggest that the inhibition of algal growth may have even been due to P limitation rather than direct toxicity.

In September 2007, 112 T of Z2G1 was applied to Lake Okaro with an estimated 350 g/m^2 coverage/capping of the lake sediment. The purpose of this study was to assess in situ health of trout, common bully and koura in relation to this event.

2. MATERIALS AND METHODS

2.1 Fish Collection

Two fish collections were timed around the Z2G1 application; one prior to and one following (Table 1). In order to provide an environmental risk assessment, fish were sampled from Lakes Okaro and Rerewhakaaitu for comparison. Rainbow trout (*Oncorhynchus mykiss*) and common bully (*Gobiomorphus cotidianus*) were selected for general health assessments; the koura (*Paranephrops planifrons*) for sediment- and product-associated metal accumulation.

Table 1. Sampling dates for fish over the study period.

		Sampling Dates	
Lake	Fish	Pre-application	Post-application
Okaro	Trout	23/08/2007	20/11/2007
	Bully	28/08/2007*	20/11/2007
	Koura	27/08/2007*	10/10/2007*
Rerewhakaaitu	Trout	30/08/2007	21/11/2007
	Bully	31/08/2007	21/11/2007
	Koura	13/09/2007	22/11/2007

*Supplied by NIWA

Rainbow trout were captured using six gill nets set around each lake during daylight hours. Nets were checked hourly by working the length of the net and removing fish immediately. Common bully were captured using approximately 15 Gee-minnow traps set in 5 m of water. Minnow traps were set late in the afternoon, left over-night and checked the following morning.

As koura are not found in Lake Okaro, they were collected from Lake Rotoiti by NIWA staff to be used for additional biota monitoring studies. Prior to the Z2G1 application, koura were caged in Lake Okaro. After 2 weeks, cages were retrieved and a sub-sample (n = 8; 5 male, 3 female) were retained for elemental analyses. Another sub-

sample (n = 7; 5 male, 2 female) of koura were retrieved in October, approximately 3 weeks after the Z2G1 application. Lake Rerewhakaaitu koura were collected by SCUBA diving.

2.2 Necropsy

Trout were sampled on shore within 10 min of removal from the nets. A 4-5 mL sample of blood was taken by caudal venipuncture using heparinised syringes and stored on ice until processing. Fish were sacrificed by a blow to the head prior to being weighed, measured and necropsied. The liver, gonad and spleen were removed and weighed. Whole livers were placed in Whirlpack^{TM} storage bags and stored on ice until they could be frozen at -20°C for elemental analysis. Sub-samples of gill and spleen tissues were removed, placed in histocasettes and fixed in 10% neutral buffered formalin.

Upon capture, common bully were immediately transported back to the laboratory. Fish were first anaesthetised with MS-222 (0.1 g/L). Approximately 15-40 µL of blood was taken by caudal venipuncture using heparinised syringes and processed immediately. Fish were sacrificed by an overdose of anaesthetic, then weighed and measured. Liver and gonads were removed and weighed. Koura were chilled with an ice slurry for 30 min before being weighed and measured for total length. Hepatopancreas (digestive gland) and tail muscle tissue were removed and frozen at -20°C for metals analysis.

2.3 Haematology

Haematological variables generally included whole blood haemoglobin concentration (Hb), haematocrit (Hct), red blood cell count (RBCC), mean red cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), total white cell count (WBCC), and differential leukocyte counts (DWBC). Leukocyte counts were not undertaken on common bully due to limited sample volume.

2.3.1 Haemoglobin

Haemoglobin was determined by the cyanmethaemoglobin method as described by Dacie and Lewis (1991). Two microlitres of whole blood was added to 1.0 mL of a

modified Drabkin's reagent in a 1.5 mL centrifuge tube and mixed thoroughly. The supernatant was aspirated and its absorbance measured using a Metertek SP-830 spectrophotometer at 540 nm.

2.3.2 Haematocrit

Haematocrit was determined using the microcapillary method. Well-mixed whole blood was drawn into 1-5 µL micro-haematocrit tubes (Drummond Scientific Company, USA), sealed with critoseal and centrifuged for 5 min at 12,000 rpm. Hematocrit was calculated as the percentage of packed red cell volume.

2.3.3 Flow cytometric analysis of trout whole blood

Owing to their relatively small size, inconsistent blood volumes were collected from common bully preventing analysis by both traditional and flow cytometric methods. For this study, total red (RBC) and total white blood cell (WBC) counts were determined by flow cytometry at Scion (Rotorua) for trout only. Anticoagulated whole blood (10 µL) was suspended in a TruCount tube (BD Biosciences) containing 3.986 ml minimum essential medium (Gibco) with 0.25% BSA (Sigma). The TruCount tube contained an accurately known pre-dosed number of fluorescent beads. Immediately after cell dispersion, 4 µL of 0.5 mg/mL dihexyloxacarbocyanine, DiOC6(3) (Molecular Probes) in dimethylsulphoxide (Sigma) was added, and then the sample was incubated on ice for 30 min in the dark. Flow cytometry counts were performed on a FACSVantage SE DiVa flow cytometer (BD Biosciences) equipped with a 488 nm laser, powered at 300 mW. Forward scatter (FSC), side scatter (SSC) and fluorescence were measured in the 500-560 nm wavelength range (FL1). The detector photomultiplier voltages were set at 200, 300 and 500 mV respectively, and were viewed in logarithmic mode. Threshold was set at channel 1000 on the FSC detector. The instrument sheath fluid was IsoTon II (Beckman-Coulter). Sample flow was adjusted to yield a count rate of 2000 events/s. Data was displayed on SSC vs. FL1 dot plots, and gates were set around the RBC, WBC and fluorescent bead populations to define each group. A total of 500 fluorescent beads were counted. Cell count/mL for RBCs and WBCs was determined using the following formula:

$$\text{cells/ml} = \frac{\text{cell count x total bead count (TruCount tube)}}{\text{actual bead count (500) x blood volume (0.01 ml)}}$$

2.3.4 Common bully RBC counts

For common bully, 2 µL of whole blood was mixed with 98 µL of red cell diluting fluid and stored at 4°C. Red blood cell counts were made using images of RBCs on a haemocytometer captured at 10x magnification using an AxioCam HRC camera and a Leica DMRD microscope. ImagePro Plus software was used to count cells after enhancement and filtering of images.

2.3.5 Trout differential leukocyte counts

Blood smears were prepared using 2 µL of well-mixed whole blood on glass slides. Air-dried smears were fixed in absolute methanol and stained with a Leishman-Giemsa solution. The stained smears were cover-slipped using Clarion mounting medium (Sigma-Aldrich), and examined by light microscopy under oil emersion at 400x magnification. For each slide, areas were randomly chosen and 100 leukocytes were counted and differentiated into three different types (lymphocytes, granulocytes and thrombocytes) based on their morphology.

2.4 Histology

Preserved trout gill and spleen samples were processed at Gribbles Animal Pathology Laboratory (Hamilton, NZ). Gill tissue samples were first decalcified in dilute formic acid for 1 h. Approximately 5 µm sections of gill and spleen tissue were mounted on slides and stained with haematoxylin-eosin. Gill tissue slides were scanned at low magnification (50 x) to examine and estimate the distribution of large or severe lesions. Lesions were identified according to Mallat (1985) and ranked on a scale of 0-3, corresponding to none, low, moderate or severe frequency. Digital spleen images of 10 microscope fields were taken at 100 x magnification using an AxioCam HRC camera and a Leica DMRD microscope. Melano-macrophage centres (MMCs) in spleen tissue were measured using ImagePro Plus® software (Media Cybernetics Inc., Silver Springs, MD) by filtering out non-pigmented material and MMCs less than three cells in

size. Total MMC area was expressed as a percentage of the total area of spleen tissue examined.

2.5 Tissue elemental analysis

A suite of 28 elements were measured in trout and koura tissue samples based on USEPA (1987) methods. In brief, hepatopancreas and muscle tissue samples were digested using tetramethylammonium hydroxide, heat and mixing. The colloidal suspension was then partially oxidized by the addition of hydrogen peroxide and metals solubilized by acidification with nitric acid and heating. Samples were diluted and filtered prior to analysis by inductively-coupled plasma mass spectrometry (ICP-MS; Department of Chemistry, Waikato University, Hamilton, NZ). All tissue element concentrations were determined on a wet weight basis.

2.6 Statistical methods

Body weight (condition factor), liver, gonad and spleen size data were analysed using analysis of covariance (ANCOVA) on base-10 logarithmically transformed variables, with body size (length or weight) as the covariate. Haematology data were analysed by analysis of variance (ANOVA). DWBC counts and MMCs were measured as percentages; data were arcsine transformed prior to analysis (Sokal and Rohlf, 1973). All statistical hypothesis tests were performed using lake and sampling period as factors.

Although statistical comparisons using ANCOVA were completed on body, liver, gonad and spleen weights, data are presented as somatic indices for greater ease of comparison. Gonado-somatic index (GSI) was calculated from gonad weight and body weight as [gonad weight / (body weight – gonad weight)] x 100. Liver- and spleen-somatic indices (LSI and SSI) were calculated in the same manner, substituting gonad weight for the other organs. Fulton's condition factor (K) was calculated as [(body weight – organ weights) /length^3] x 100.

All statistical analyses were performed using STATISTICA v8.0 software. The critical level of statistical significance for all tests was $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical lake properties

Physicochemical lake water quality data for July 2007 to March 2008 was supplied by Environment Bay of Plenty scientist Paul Scholes and has been summarised in Figs. 1-3 to illustrate the large seasonal changes that occur in these lakes. The efficacy of the Z2G1 product is outside the scope of this study; therefore, nutrient data are not included in this report and will be presented elsewhere. Substantial temperature and dissolved oxygen (DO) changes were recorded in both lakes over the monitoring period (Fig. 1). Commencing in late spring, Lake Okaro water began to temperature stratify. By early summer, significant deoxygenation of the bottom waters accompanied increased surface water temperatures. Lake Rerewhakaaitu did not appear to stratify over the same period. Although some variable changes in DO were recorded, homogenous temperature increases occurred throughout the entire Lake Rerewhakaaitu water column. Lake Rerewhakaaitu water pH was relatively unchanged, while elevated pH in the Lake Okaro surface water was observed during the late spring and summer months (Fig. 2). Total Al in Lake Okaro water varied over time (Fig. 3). In general, average water Al concentration did not change from month-to-month, although increased surface water Al was observed in the months immediately following Z2G1 application.

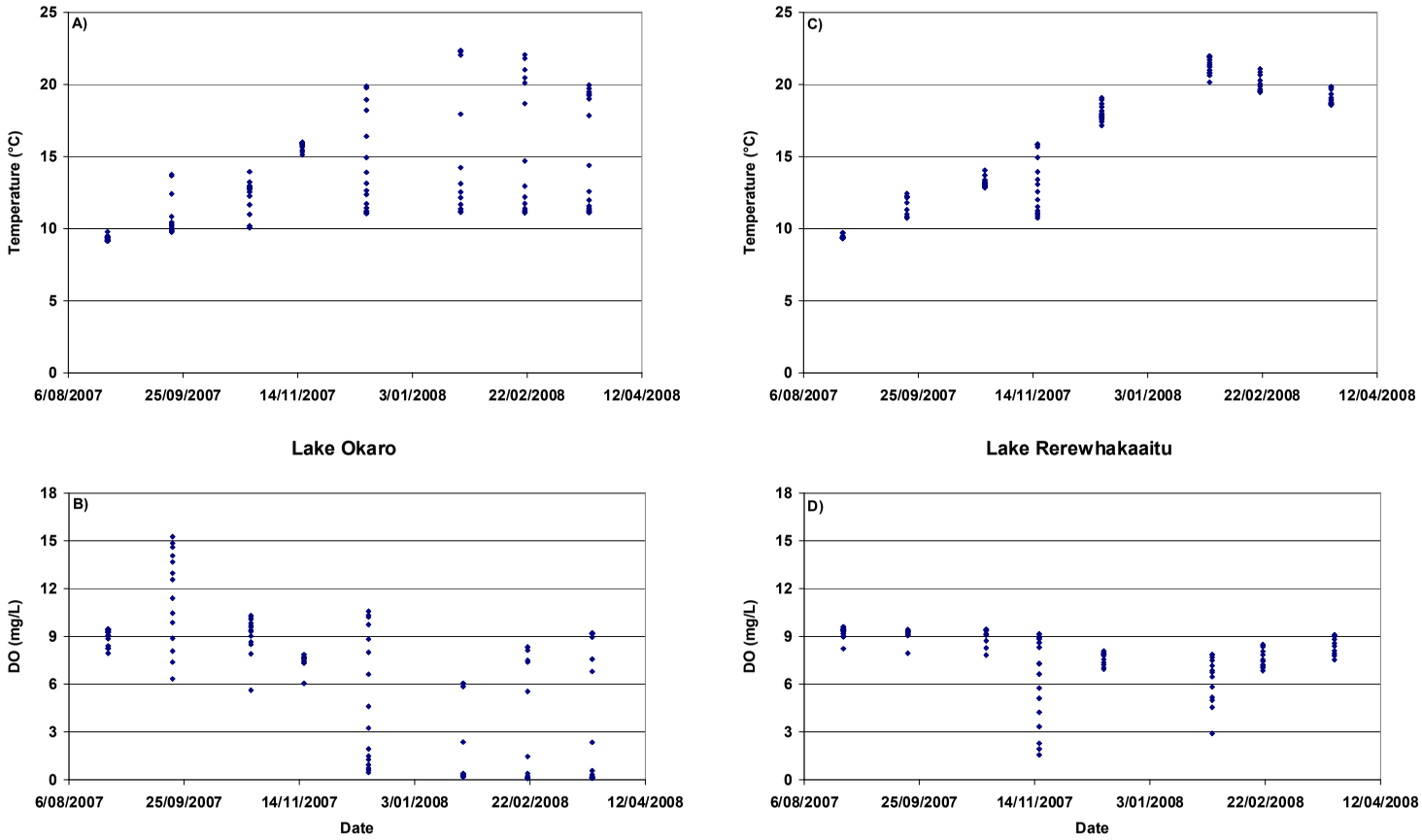
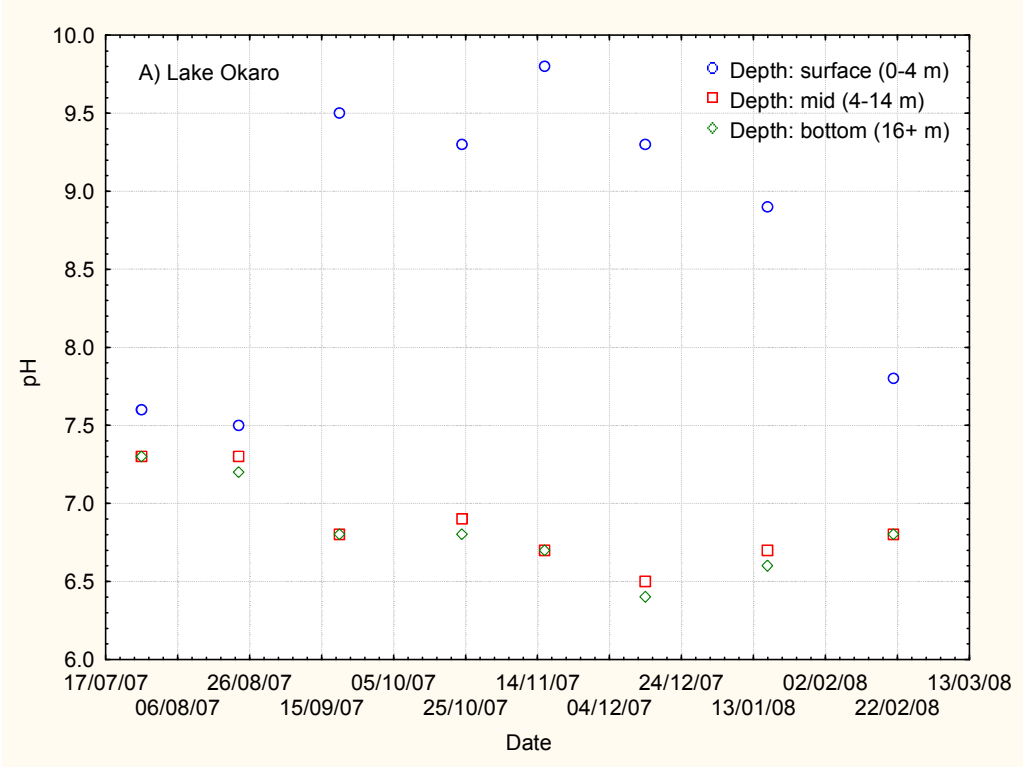


Fig. 1. Lake Okaro (A, B) and Rerewhakaaitu (C, D) temperature and dissolved oxygen (DO) data at 1 m intervals collected from July 2007 to March 2008 by Environment Bay of Plenty.



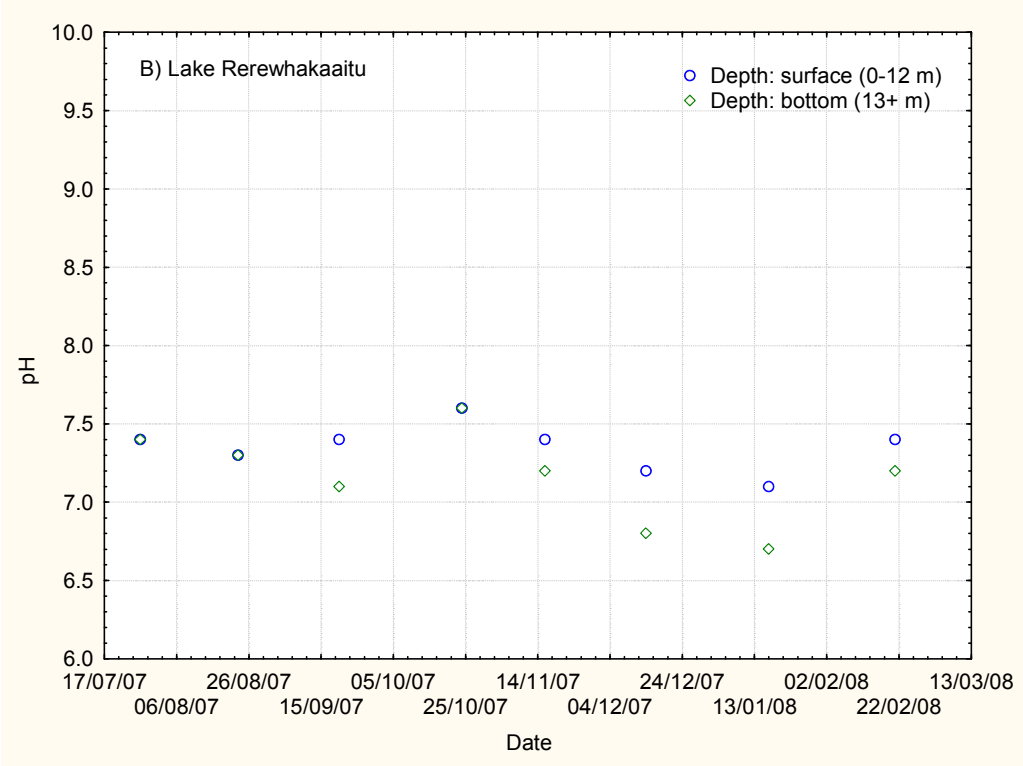


Fig. 2. Lake Okaro A) and B) Rerewhakaaitu pH data at water depth ranges of 0-4, 4-14 and 16+ m (Okaro) and 0-12 and 13+ m (Rerewhakaaitu) collected from July 2007 to March 2008 by Environment Bay of Plenty.

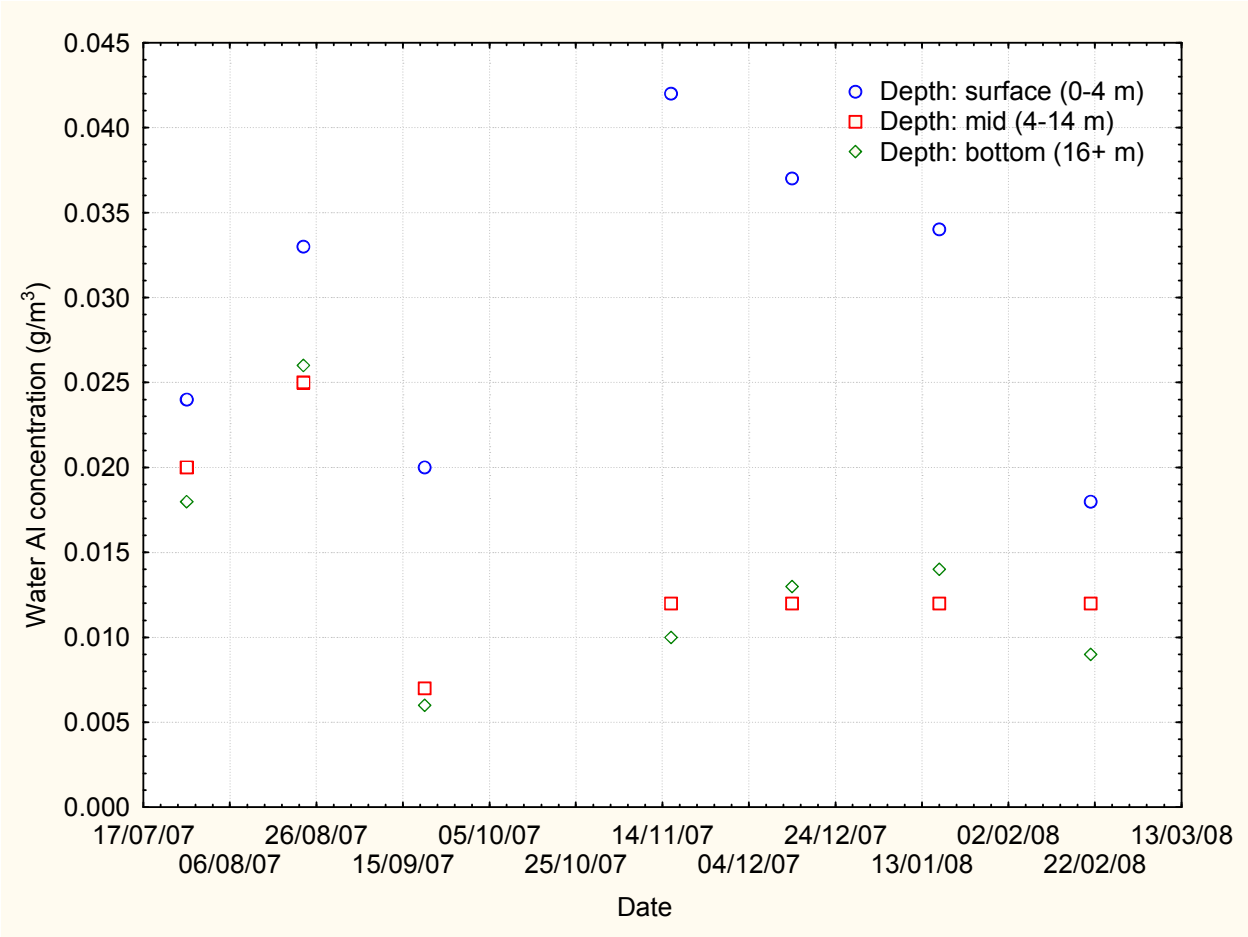


Fig. 3. Lake Okaro water Al concentrations (g/m^3) at water depth ranges of 0-4, 4-14 and 16+ m collected from July 2007 to March 2008 by Environment Bay of Plenty.

3.2 Fish surveys

In August, attempts were made to survey locations around Lakes Okaro and Rerewhakaaitu for smelt and common bully abundance estimates. However, beach seining was compromised by an abundance of filamentous benthic algae. Because so few data were obtained from both lakes at this time, these results have been omitted from this report. As several other simultaneous fish monitoring studies were being conducted (e.g. NIWA koura and common bully studies), a decision was made not to repeat the surveys and instead focus on the health assessment component of this study. However, to facilitate possible future monitoring, a summary of previous Lake Okaro surveys performed between 2003 and 2005 (van den Heuvel and Landman unpublished data) have been summarised and included in Appendix 7.1.

3.3 Physiological indices

General physiological parameters have been summarised and tabulated for ease of comparison in Tables 2-4.

Rainbow trout were relatively abundant and easily captured by gill netting. Roughly equal numbers of male and female trout were captured during the August sampling period in both lakes, followed by a distinct female-bias in Lake Okaro during November (Table 2). Trout from both lakes were in relatively good condition, although slightly decreased condition factor of Rerewhakaaitu trout was observed from August to November. A mixture of immature, mature and spawned fish were observed in both lakes during August, with most fish beginning to recover as shown by trends of increasing LSI, except for Okaro males, the general absence of ripe fish and early signs of gonadal recrudescence in females by November. A small but insignificant increase in mean spleen size was observed for males from August to November in Lake Okaro. However, all results for Okaro males in November are compromised by the limited sample size.

Common bully appeared to be anecdotally abundant in both lakes based on relative ease of capture by minnow trapping. Lake Okaro bullies appeared outwardly healthy, while virtually all those from Rerewhakaaitu were extensively covered by an unidentified

parasite appearing as black spots/cysts on the body and fins. Opposite trends of increasing and decreasing condition factor between August and November were observed in Lake Okaro and Rerewhakaaitu bullies, respectively (Table 3). Between-lake differences in both condition factor and LSI coincided with differences in reproductive synchrony between populations. In this instance, most Rerewhakaaitu females possessed large, ripe ovaries during August with most spawning presumably occurring between August and November. An obvious peak in GSI for Okaro females was not evident and spawning may have taken place either side of each sampling event.

These data are relatively consistent with previous trout and common bully monitoring performed between 2003 and 2005 (summarised in Appendix 7.2).

Table 2. Mean (SEM, n) of size, somatic indices and melano-macrophage centre areas for male and female rainbow trout (*Oncorhynchus mykiss*) in Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. (*) Asterisks indicate significant difference ($p < 0.05$) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	August		November		Statistical Hypothesis	
	Okaro	Rerewhakaaitu	Okaro	Rerewhakaaitu	Lake	Period
Males						
Length (mm)	513 (7, 15)	516 (12, 13)	484 (23, 3)	567 (7, 8)		
Weight (g)	1589 (67, 15)	1527 (87, 13)	1277 (101, 3)	1704 (46, 8)		
Condition	1.17 (0.03, 15)	1.11 (0.04, 13)	1.13 (0.07, 3)	0.94 (0.05, 8)		
GSI (%)	2.98 (0.29, 14)	2.49 (0.18, 12)	0.87 (0.58, 2)	0.60 (0.14, 8)		*
LSI (%)	0.86 (0.02, 15)	0.79 (0.04, 13)	0.78 (0.05, 3)	1.05 (0.06, 8)	Interaction	
SSI (%)	0.13 (0.01, 14)	0.13 (0.02, 13)	0.19 (0.05, 3)	0.14 (0.02, 8)		
MMC (% area)	0.073 (0.022, 15)	0.227 (0.046, 12)	0.527 (0.141, 3)	0.581 (0.116, 8)		*
Females						
Length (mm)	487 (20, 10)	484 (10, 10)	475 (10, 18)	526 (8, 12)		
Weight (g)	1310 (128, 10)	1340 (93, 10)	1245 (78, 18)	1478 (50, 12)		
Condition	1.14 (0.09, 10)	1.17 (0.05, 10)	1.14 (0.02, 18)	1.03 (0.05, 12)		
GSI (%)	0.31 (0.06, 2)	0.24 (0.06. 5)	0.36 (0.05, 16)	0.42 (0.05, 12)		
LSI (%)	0.73 (0.06, 10)	0.91 (0.07, 10)	0.80 (0.03, 18)	1.27 (0.06, 12)	Interaction	
SSI (%)	0.10 (0.01, 10)	0.09 (0.01, 10)	0.09 (0.01. 18)	0.11 (0.01, 12)		
MMC (% area)	0.074 (0.023, 11)	0.207 (0.051, 12)	0.262 (0.028, 18)	0.447 (0.063, 12)	*	*

Table 3. Mean (SEM, n) of size and somatic indices for male and female common bully (*Gobiomorphus cotidianus*) in Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. (*) Asterisks indicate significant difference ($p < 0.05$) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	August		November		Statistical Hypothesis	
	Okaro	Rerewhakaaitu	Okaro	Rerewhakaaitu	Lake	Period
Males						
Length (mm)	65.4 (1.4, 13)	66.7 (1.2, 10)	70.2 (2.1, 9)	64.7 (1.8, 12)		
Weight (g)	3.22 (0.26, 13)	4.18 (0.26, 10)	4.56 (0.33, 9)	3.03 (0.22, 12)		
Condition	1.10 (0.02, 13)	1.33 (0.04, 10)	1.25 (0.03, 9)	1.06 (0.03, 12)	Interaction	
GSI (%)	0.83 (0.07, 12)	1.79 (0.14, 10)	0.89 (0.08, 9)	1.01 (0.10, 12)	Interaction	
LSI (%)	1.82 (0.14, 13)	3.47 (0.33, 10)	1.55 (0.10, 9)	2.55 (0.12, 12)	*	*
Females						
Length (mm)	65.9 (2.6, 7)	72.1 (1.5, 10)	75.7 (1.6, 11)	62.0 (2.3, 8)		
Weight (g)	3.40 (0.50, 7)	6.09 (0.32, 10)	5.61 (0.36, 11)	3.20 (0.42, 8)		
Condition	1.08 (0.06, 7)	1.30 (0.04, 10)	1.18 (0.02, 11)	1.11 (0.04, 8)	Interaction	
GSI (%)	3.23 (1.07, 7)	21.04 (0.87, 10)	6.41 (1.78, 11)	14.03 (0.94, 8)	*	
LSI (%)	1.83 (0.18, 7)	3.26 (0.16)	2.02 (0.28, 11)	3.02 (0.18, 8)	*	

Table 4. Mean (SEM, n) and *range* weight and length data for koura (*Paranephrops planifrons*) sampled from Lakes Rotoiti (translocated to and caged in Lake Okaro) and Rerewhakaaitu during August and November 2007.

	August		November	
	Okaro	Rerewhakaaitu	Okaro	Rerewhakaaitu
Males				
Total length (mm)	88.2 (1.9, 5)	126.4 (6.8, 5)	71.6 (8.4, 5)	124.2 (9.9, 5)
	84-95	*111-149*	*62-105*	*87-238*
Carapace length (mm)	28.4 (0.8, 5)	22.4 (2.0, 5)	23.0 (2.3, 5)	41.8 (3.8, 5)
	26-31	*19-30*	*20-32*	*27-47*
Weight (g)	16.75 (1.18, 5)	56.95 (9.31, 5)	9.24 (4.02, 5)	48.75 (8.67, 5)
	13.77-21.02	*35.79-88.86*	*4.21-25.24*	*16.84-61.86*
Females				
Total length (mm)	83.3 (3.8, 3)	107.6 (3.0, 5)	79.5 (11.5, 2)	98.2 (4.9, 5)
	77-90	*96-114*	*68-91*	*85-110*
Carapace length (mm)	26.3 (0.9, 3)	17.0 (0.8, 5)	24.5 (3.5, 2)	30.2 (2.3, 5)
	25-28	*14-19*	*21-28*	*24-26*
Weight (g)	13.95 (1.90, 3)	29.84 (2.68, 5)	9.76 (4.08, 2)	22.20 (4.16, 5)
	11.21-17.61	*18.67-34.66*	*5.68-13.84*	*10.92-31.21*

3.4 Haematology

Changes in the haematology of both species were observed over the monitoring period. For both male and female Okaro trout there were significant decreases in Hct, but not for RBCC or whole blood haemoglobin between August and November (Table 5). This suggests the presence of smaller, immature cells in November as further indicated by decreases in MCV (Table 5). A similar trend was seen for Rerewhakaaitu trout where lower Hct was also observed in November, although this change coincided with reduced whole blood Hb in males and females, and fewer RBCs in males. A different pattern of change emerged for bullies from both lakes. From August to November, significant increases in RBCC, Hb, Hct and MCV were seen in Okaro bullies, while Rerewhakaaitu fish showed only minor changes in some of the measured parameters (Table 6). These results may implicate an increased turnover of RBCs in trout with numerous, smaller immature erythrocytes predominating, while more numerous and larger erythrocytes predominate in bullies.

Changes in red cell haematology are most likely associated with changes in environmental conditions or altered stress responsiveness. Lochmiller et al. (1989) showed that changes in haematological variables for striped bass (*Morone saxatilis*), such as greater RBC numbers and increased Hct, Hb and MCH in autumn and winter months correlate well with changes in both water temperature and dissolved oxygen. In another study on rainbow trout exposed to artificial seasonal conditions, it was found that during simulated winter conditions larger, mature erythrocytes with increased Hb were produced, while under simulated spring and summer conditions, similar numbers of smaller erythrocytes with less haemoglobin predominated (Houston et al. 1996). These authors suggested that for rainbow trout, a reduction in viscosity-based circulatory cost may be more advantageous than an elevation in blood oxygen-carrying capacity under summer conditions. The differences between species in the current study may represent different strategies for adapting to changing environmental conditions. For the less active, benthic common bully, increased circulatory costs associated with increased cell numbers and volume may be less significant than for active pelagic species such as the trout.

There was an increase in total WBCC between August and November in Okaro trout of both sexes (Table 5). This may potentially indicate a lake-specific immune response in these fish, although the relative proportions of leukocytes (i.e. DWBC) did not differ between lake populations in November. Total and DWBC have been shown to respond to stress and changes in water quality (Tierney et al. 2004). In August, the tail-end of the trout spawning season, relatively fewer lymphocytes were observed in both trout populations, particularly for Lake Okaro, which subsequently increased in all groups by November. In fish, during periods of development, a trade-off between reproduction and self-maintenance has been suggested (Kortet et al. 2003). Depression of lymphocyte numbers (lymphocytopenia) associated with sexual maturity and spawning season has been described for brown trout (*Salmo trutta*) (Pickering 1986; Pickering and Pottinger 1987). Similar observations have been made of wild rainbow trout populations during the spawning months in Lakes Okareka and Tikitapu (Landman and Ling 2006; Landman et al. 2007).

Table 5. Mean (SEM, n) red and white blood parameters for male and female rainbow trout (*Oncorhynchus mykiss*) in Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. (*) Asterisks indicate significant difference ($p < 0.05$) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	August		November		Hypothesis	
	Okaro	Rerewhakaaitu	Okaro	Rerewhakaaitu	Lake	Period
Males						
Hct (%)	43.6 (2.2, 15)	39.0 (3.0, 13)	34.4 (2.0, 3)	25.3 (1.6, 8)	*	*
RBCC (x 10^{12} cells/L)	1.16 (0.06, 15)	1.05 (0.07, 13)	1.15 (0.08, 3)	0.79 (0.05, 8)	*	
Hb (g/L)	96.9 (4.4, 15)	79.8 (4.6, 13)	91.0 (9.6, 3)	60 (4.9, 8)	*	*
MCV (fl)	376 (8, 15)	368 (6.5, 13)	301 (12, 3)	321 (8.2, 8)		*
MCH (pg/cell)	84.2 (2.6, 15)	78.1 (4.4, 13)	79.3 (5.6, 3)	77.8 (7.8, 8)		
MCHC (g/L)	224 (6, 15)	212 (11.3, 13)	266 (29, 3)	241 (20.4, 8)		*
WBCC (x 10^{10} cells/L)	2.31 (0.12, 15)	2.50 (0.28, 13)	4.17 (0.83)	2.60 (0.18, 8)	Interaction	
Lymphocyte (%)	37.9 (2.1, 8)	60.2 (9.5, 6)	95.3 (2.6, 3)	88.8 (2.8, 6)	Interaction	
Granulocyte (%)	31.8 (4.3, 8)	18.3 (4.6, 6)	1.7 (1.2, 3)	7.2 (2.5, 6)	Interaction	
Thrombocyte (%)	30.4 (2.9, 8)	21.5 (8.3, 6)	3.0 (1.5, 3)	4.0 (1.9, 6)		*
Females						
Hct (%)	42.6 (2.4, 10)	35.5 (2.1, 10)	39.1 (1.3, 18)	30.0 (1.1, 12)	*	*
RBCC (x 10^{12} cells/L)	1.21 (0.07, 10)	1.08 (0.05, 10)	1.30 (0. 04, 18)	0.96 (0.05, 12)	Interaction	
Hb (g/L)	97.7 (8.6, 10)	73.9 (5.8, 10)	95.3 (2.5, 18)	62.2 (3.3, 12)	*	
MCV (fl)	354 (11, 10)	327 (9.8, 10)	303 (8, 18)	316 (6.7, 12)	Interaction	
MCH (pg/cell)	79.7 (4.3, 10)	67.8 (3.4, 10)	73.8 (1.7, 18)	64.9 (2.1, 12)	*	
MCHC (g/L)	227 (13, 10)	208 (9.8, 10)	247 (8, 18)	206 (5.5, 12)	*	
WBCC (x 10^{10} cells/L)	2.71 (0.28, 10)	3.81 (0.27, 10)	5.38 (0.43, 18)	2.46 (0.25, 12)	Interaction	
Lymphocyte (%)	36.0 (13.1, 3)	69.8 (7.9, 4)	88.3 (2.2, 10)	88.6 (2.4, 5)	Interaction	
Granulocyte (%)	13.7 (8.2, 3)	10.5 (3.1, 4)	9.9 (2.5, 10)	5.4 (0.6, 5)		
Thrombocyte (%)	50.3 (19.1, 3)	19.8 (5.0, 4)	1.8 (0.6, 10)	6.0 (1.9, 5)	Interaction	

Table 6. Mean (SEM, n) red blood cell parameters for male and female common bully (*Gobiomorphus cotidianus*) in Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. (*) Asterisks indicate significant difference ($p < 0.05$) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	August		November		Hypothesis	
	Okaro	Rerewkakaaitu	Okaro	Rerewkakaaitu	Lake	Period
Males						
Hct (%)	18.4 (1.4, 13)	26.7 (1.8, 10)	44.2 (4.3, 9)	29.2 (1.9, 12)	Interaction	
RBCC (x 10^{12} cells/L)	0.69 (0.05, 13)	0.94, 0.05, 10)	1.39 (0.12, 9)	1.02 (0.07, 12)	Interaction	
Hb (g/L)	37.6 (2.2, 13)	44.4 (2.4, 10)	77.1 (4.2, 9)	62.2 (3.3, 12)	Interaction	
MCV (fl)	299 (4, 13)	302 (11, 10)	349 (10, 9)	315 (4, 12)	Interaction	
MCH (pg/cell)	62.7 (2.8, 13)	51.2 (2.8, 10)	62.7 (3.3, 9)	68.4 (3.2, 12)	Interaction	
MCHC (g/L)	210 (9, 13)	170 (8, 10)	181 (10, 9)	218 (11, 12)	Interaction	
Females						
Hct (%)	22.9 (1.7, 7)	30.1 (2.3, 10)	42.0 (3.1, 11)	32.2 (2.3, 8)	Interaction	
RBCC (x 10^{12} cells/L)	0.83 (0.03, 7)	1.02 (0.05, 10)	1.28 (0.08, 11)	1.13 (0.08, 8)	Interaction	
Hb (g/L)	307 (9, 7)	314 (13, 10)	350 (9, 11)	319 (4, 8)	Interaction	
MCV (fl)	43.2 (2.4, 7)	48.5 (4.1, 10)	68.7 (3.4, 11)	58.9 (2.6, 8)		*
MCH (pg/cell)	59.4 (5.1, 7)	50.7 (3.3, 10)	57.9 (1.8, 11)	60.0 (3.8, 8)	Interaction	
MCHC (g/L)	195 (18, 7)	162 (9, 10)	166 (6, 11)	188 (13, 8)		

3.5 Tissue metal accumulation

As a possible marker of exposure to the Z2G1 product, trout and koura tissues were analysed for the presence of Al. Although low concentrations of Al were found in the liver and hepatopancreas tissues of trout and koura, accumulation of Al was less evident in Lake Okaro biota (Appendix 7.3). These results suggest Z2G1-derived Al was either not readily bioavailable, or uptake and subsequent depuration had already occurred between the Z2G1 application in September and sampling of animals in November. The lack of accumulation is not surprising as Al is known to be a specific surface-active toxicant with minimal penetration into the bloodstream and accumulation in the tissues (Gensemer and Playle 1999; Wood 2001). However, in the absence of internal accumulation, potential Al toxicity may still occur at other sites such as the gills and is discussed further in Sections 3.6 and 3.7.

Of all the elements measured in the tissues, changes in Hg concentrations with sampling period were the most noteworthy for both trout and koura. Approximately 2- to 3-fold increases in tissue Hg concentrations were found in Lake Okaro trout from August to November (Figs. 4-5; Appendix 7.3). At the beginning of the study, Rotoiti koura introduced into Lake Okaro also had high concentrations of tissue Hg (4.90 mg/kg wet weight in the hepatopancreas; 13.1 mg/kg in flesh; Fig. 6; Appendix 7.3). Significant Hg depuration occurred by October with the complete removal from the hepatopancreas and a reduction from 13.1 to 2.4 mg/kg in the flesh. The majority of accumulated Hg in aquatic biota is obtained through the diet (Wiener and Spry 1996). Therefore, the lack of continued accumulation in the Lake Okaro koura may be linked to limited foraging capacity as a result of caging in this study.

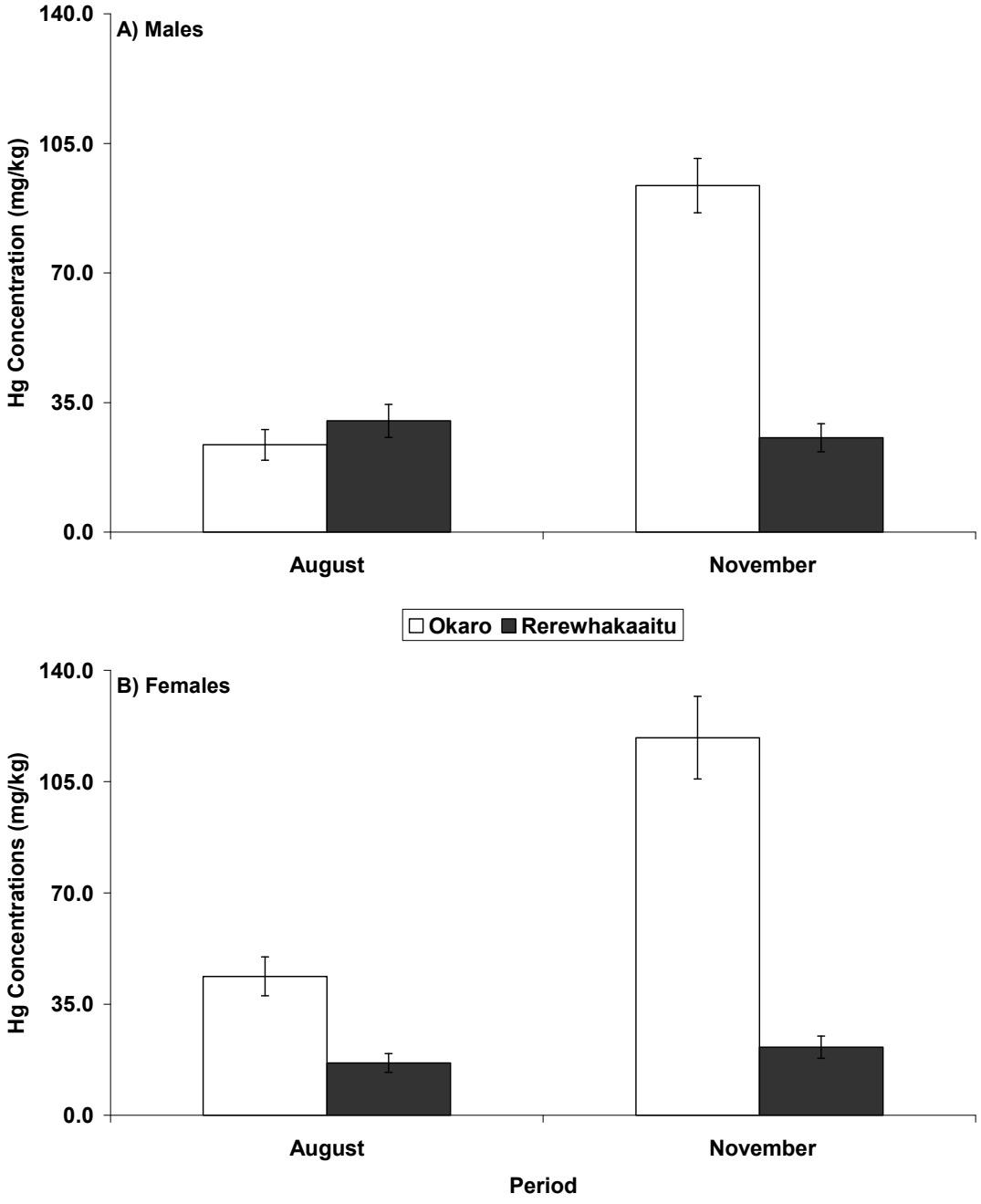


Fig. 4. Mean liver mercury (Hg) concentrations (mg/kg wet weight) in A) Male and B) Female rainbow trout (*Oncorhynchus mykiss*) from Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. Error bars indicate standard error of the mean.

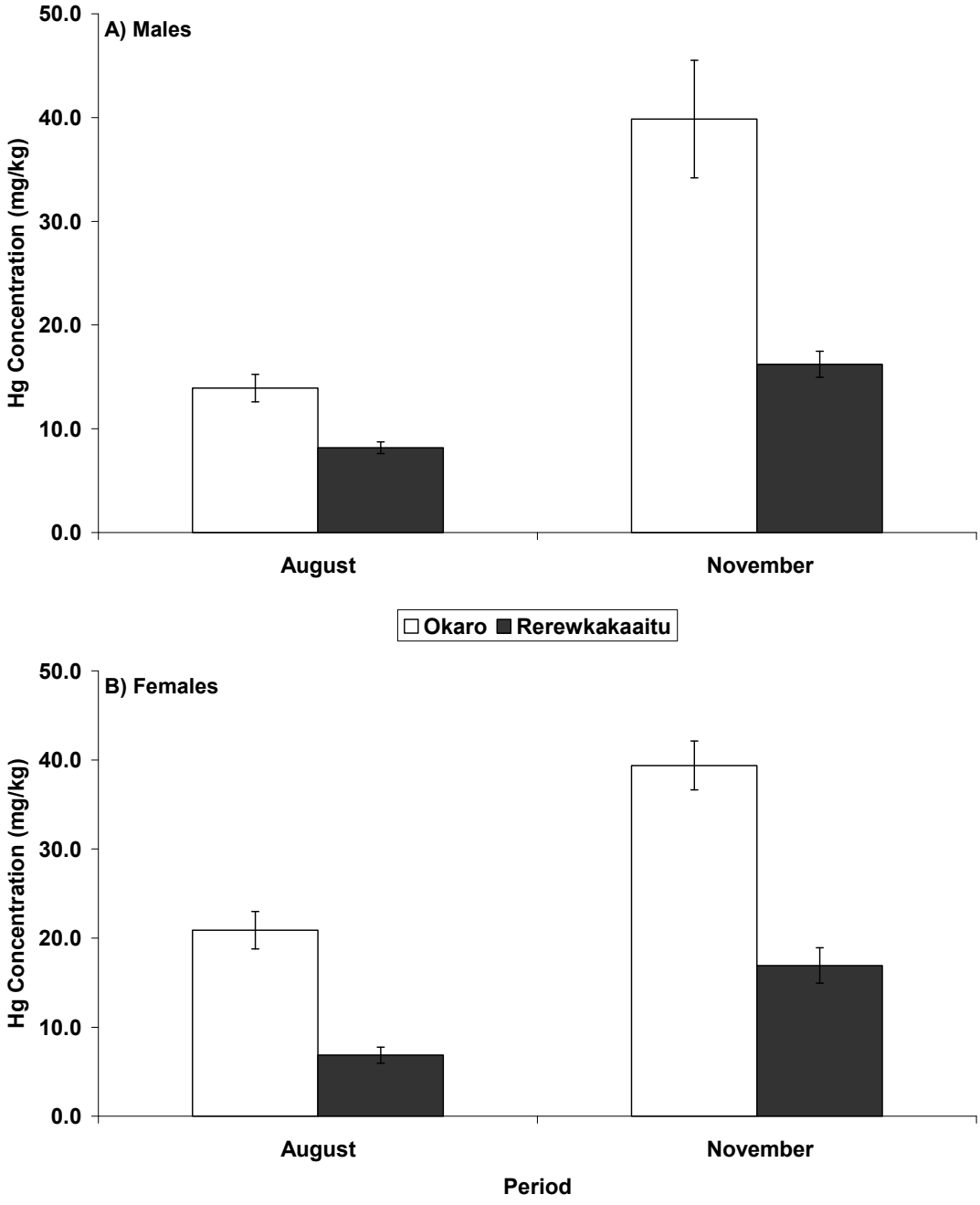


Fig. 5. Mean flesh mercury (Hg) concentrations (mg/kg wet weight) in A) Male and B) Female rainbow trout (*Oncorhynchus mykiss*) from Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. Error bars indicate standard error of the mean.

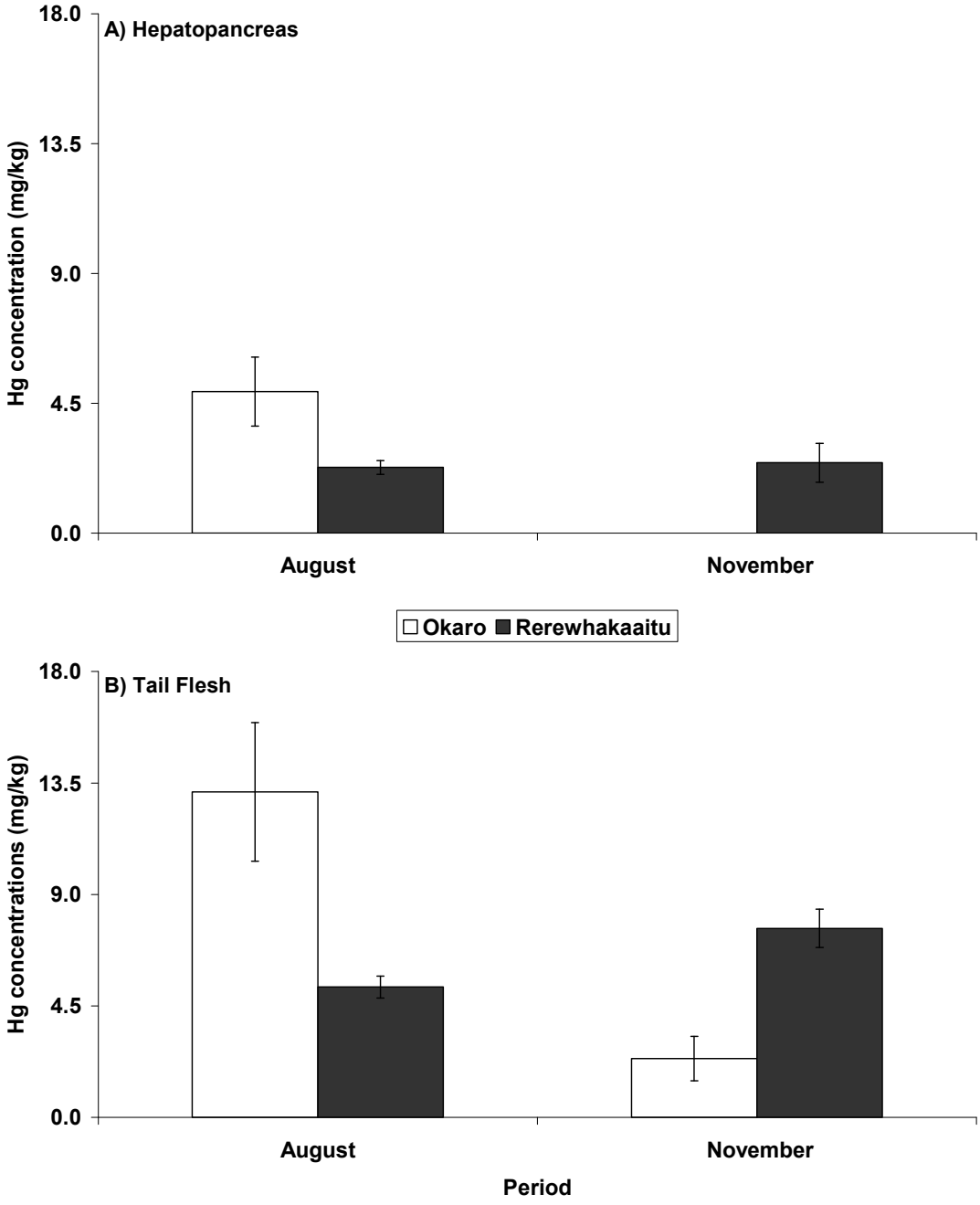


Fig. 6. Mean mercury (Hg) concentrations (mg/kg wet weight) in A) Hepatopancreas and B) Tail flesh tissues of koura (*Paranephrops planifrons*) from Lakes Rotoiti (translocated to and caged in Lake Okaro) and Rerewhakaaitu during August and November 2007. Error bars indicate standard error of the mean.

3.6 Histology

Splenic MMC densities increased in trout from both lakes from August to November (Fig. 7; Table 2). With the exception of males in November, mean MMC areas were consistently higher in Rerewhakaaitu trout. Melano-macrophages centres are aggregations of pigment containing cells found in the haemopoietic tissues of the spleen and kidney. These MMCs have various roles associated with iron recycling, toxin metabolism and immune function (Agius and Roberts 2003). Increased MMCs have been linked to exposure to organic pollutants (Fishelson 2006), sediment contamination and environmental stressors such as hypoxia (Fournie et al. 2001), and parasitisation (De Vico et al. 2008). Given that Rerewhakaaitu fish possessed equal or greater mean MMC areas, it is unlikely that the observed increased between August and November in Okaro trout was linked to the Z2G1 application. These centres are also associated with natural processes such as aging, starvation, nutritional imbalance and temperature stress (Wolke 1992). Therefore, it is more likely that the increases in MMCs in both trout populations were linked to seasonal changes in lake water quality (e.g. temperature, DO, food availability), potentially associated changes in haematology such as increased red cell turnover, or changes in seasonal physiology (e.g post-spawning recovery).

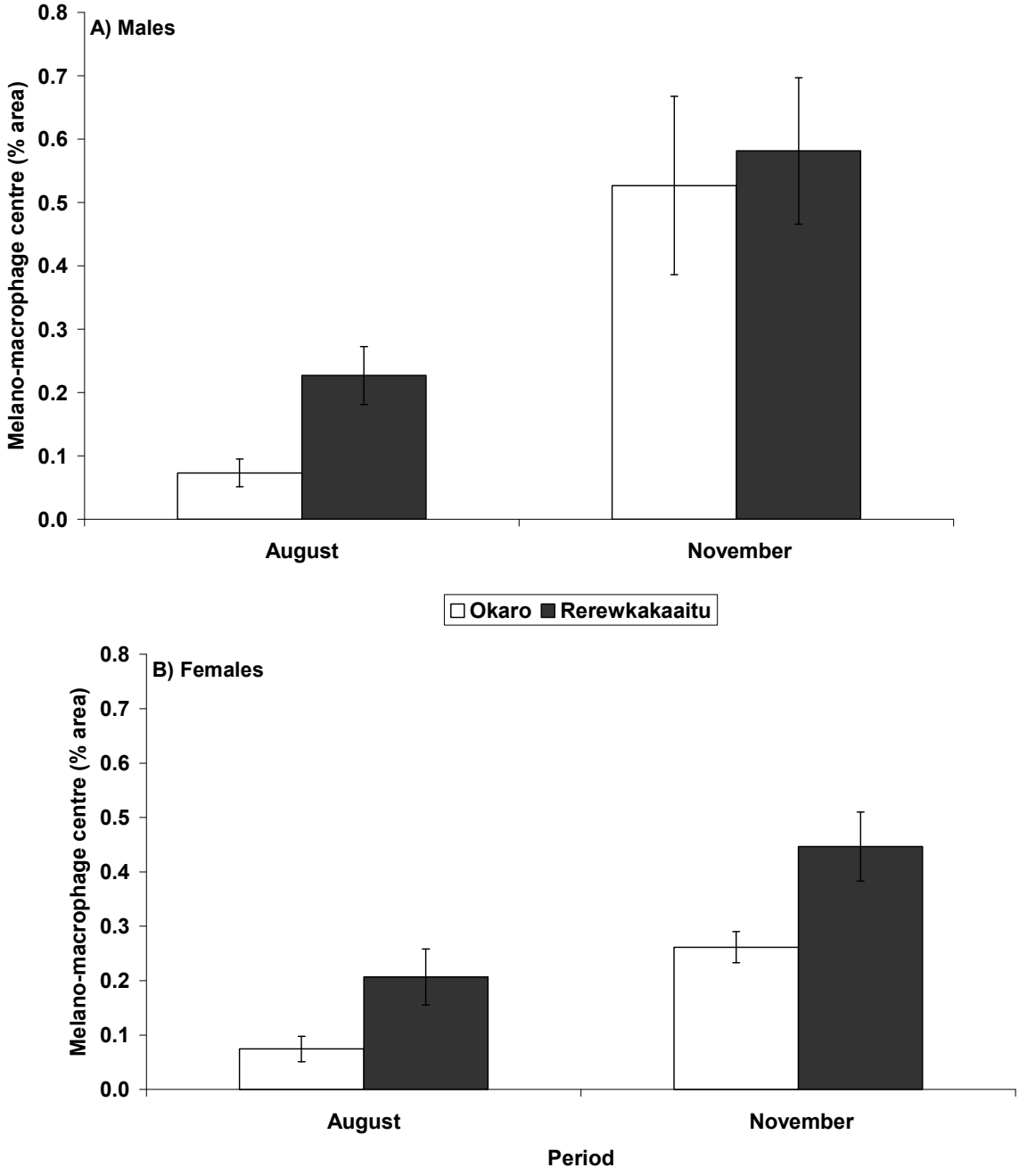


Fig. 7. Mean percentage area of melano-macrophage centres (MMCs) in splenic tissue of A) Male and B) Female rainbow trout (*Oncorhynchus mykiss*) from Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. Error bars indicate standard error of the mean.

As has been observed in previous studies (Landman et al. 2007), low levels of clubbing at the tips of the secondary lamellae, lifting of the lamellar epithelia, lamellar fusion, hyperplasia and vascular congestion were commonly observed in gill specimens (Fig. 8). Severity of lesions were typically low to moderate with no examples showing extreme damage or change, and major differences were not evident between lake populations or between August and November sampling. The gills are important multifunctional organs responsible for respiration, ionoregulation, acid-base regulation and excretion (Wood 2001). Because the fish gill may represent in excess of 50% of the total fish surface area, the gills are a primary target of toxicant action. The gill is the primary site of Al-related toxicity and Al is known as a specific surface-active toxicant (Gensemer and Playle 1999; Wood 2001).

The lack of obvious effects as measured by general gill histopathology may be expected for two possible reasons. Firstly, for fish that survive the first few days of Al exposure, recovery of resting respiratory and ionoregulatory homeostasis can occur (Wood 2001). Alternatively, the short residency time of a rapidly settling capping material in the water column would be unlikely to have any major implications for respiratory toxicity in pelagic fish species such as trout. However, for benthic organisms such as the common bully and koura that are more likely to have contact with the material or any potential free/dissolved Al near the sediment/water interface, this may be of greater significance. Indeed, accumulation at the gill surface following aqueous Al exposure at neutral pH has been demonstrated in freshwater crayfish species (*Pacifiasticus leniusculus*) in the absence of internal accumulation (Ward et al. 2006). Future studies should take this into consideration by specifically examining for Al deposits on the gill and more detailed quantification of specific cellular changes, such as proliferation of chloride and mucous cells.

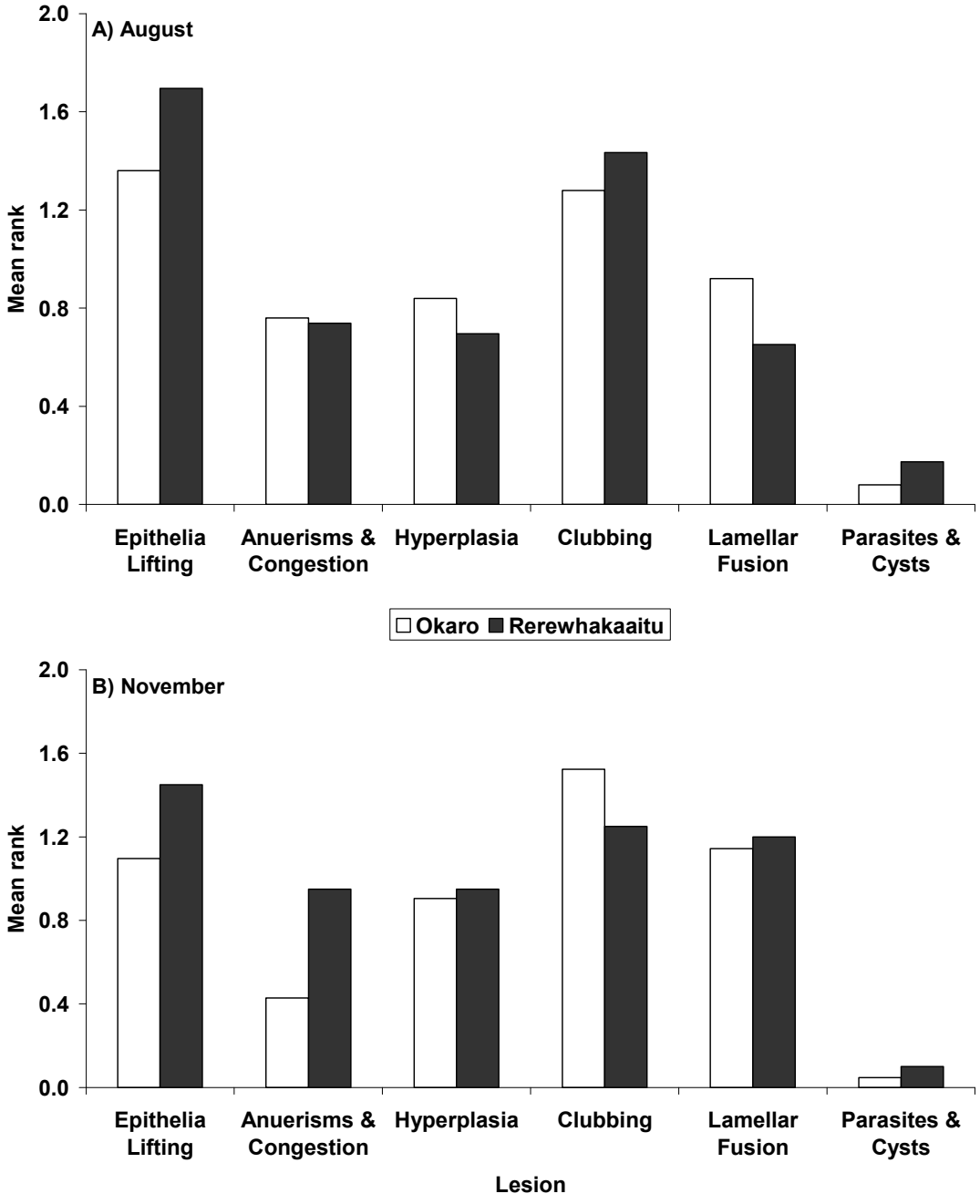


Fig. 8. Gill histopathological observations for rainbow trout (*Oncorhynchus mykiss*) from Lakes Okaro and Rerewhakaaitu sampled during A) August and B) November 2007. Frequency and severity of lesions were ranked from 0-3, corresponding to none (0), low (1), moderate (2) and severe (3). Data are presented as a mean score of the ranks for each lesion type.

3.7 Plasma ions

Trout plasma ions were measured to indicate any possible osmoregulatory disruption due to toxicant exposure. Significant changes and differences in plasma ion concentrations were observed between sampling periods and lakes (Table 7). Notable increases in plasma Na^+, Cl^-, Mg^{2+} and Ca^{2+} were observed in Okaro trout from August to November. With the exception of plasma K^+, changes in plasma ion status were generally not evident in Rerewhakaaitu trout.

Ionoregulatory toxicity of aluminium typically occurs in the lower pH range (i.e. < 4.8), well below current lake pH, and large losses of plasma Na^+ and Cl^- followed by lesser amounts of both K^+ and Ca^{2+} are normally expected (Wood 2001). Despite initial losses of plasma Na^+ and Cl^- following a stressful episode (net confinement), Postlethewaite and McDonald (1995) demonstrated that compensatory changes in ion uptake may occur following recovery from stress. In response to physiological stress, chronic soft water and some toxicant exposures, a number of compensatory responses may occur at the gill, such as proliferation of chloride cells which are the primary site of Ca^{2+} and Cl^- uptake (McCormick 1990; Perry 1997; Wood 2001). It is generally agreed that the consequences of cell proliferation is a thickening of the lamellar diffusion barrier which may result in changes in ion and gas transfer (Perry 1998; Wood 2001). In essence, greater numbers of these transport cells result in greater ion uptake, coupled with decreased diffusive efflux due to a reduction in branchial ionic permeability.

As increased accumulation of Hg was clearly demonstrated in Okaro trout, it also worth considering other possible toxicants (e.g. Hg, ammonia, nitrite). For example, Hg exposure is known to result in net ion losses in freshwater species and ion increases in marine species (Wood 2001). However, it has been observed in at least one example that sub-chronic exposure (14 d) of freshwater brook trout (*Salvelinus fontinalis*) to Hg (2.9 µ/L) resulted in improved osmoregulation shown by increased plasma Na^+ and Cl^- concentrations (Christensen et al. 1977 in Wood 2001).

As has been previously observed, considerable variation in plasma K^+ may occur in rainbow trout captured by gill netting (Landman et al. 2007). In general, K^+ levels increased between August and November, particularly for Rerewhakaaitu trout.

Unfortunately, the interpretation of plasma K^+ is confounded by significant releases from the muscle tissue following strenuous exercise (Holk and Lykkeboe 1998). Varied degrees of strenuous exercise are expected in trout captured by gill-netting, which may have been further affected by increased water temperatures in November.

These results clearly indicate some form of ionoregulatory disturbance in Lake Okaro trout. Increases in some tissue electrolytes (Na^+ and Ca^{2+}) in Okaro koura may also point to some generalised ionoregulatory effect of the Z2G1 application or some seasonal lake-specific effect. Examination of physical and chemical lake properties over the monitoring period may help to resolve this question.

Table 7. Mean (SEM, n) blood plasma ion concentrations (mM) for male and female rainbow trout (*Oncorhynchus mykiss*) in Lakes Okaro and Rerewhakaaitu sampled during August and November 2007. (*) Asterisks indicate significant difference ($p < 0.05$) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	August		November		Hypothesis	
	Okaro	Rerewhakaaitu	Okaro	Rerewhakaaitu	Lake	Period
Males						
Na^+ (mM)	175.3 (3.3, 15)	179.0 (3.1, 13)	194.0 (7.3, 3)	179.0 (11.4, 7)		
K^+ (mM)	0.73 (0.23, 15)	1.02 (0.38, 13)	3.03 (0.67, 3)	5.56 (1.08, 7)	Interaction	
Cl^- (mM)	130.1 (4.1, 15)	131.1 (1.6, 13)	147.9 (6.1, 3)	140.1 (2.9, 7)		*
Mg^{2+} (mM)	1.06 (0.04, 15)	1.43 (0.07, 13)	1.49 (0.08, 3)	1.40 (0.12, 7)	*	*
Ca^{2+} (mM)	1.71 (0.05, 15)	2.05 (0.06, 13)	2.37 (0.12, 3)	1.94 (0.14, 7)	*	*
Females						
Na^+ (mM)	172.3 (6.4, 10)	176.9 (2.8, 10)	197.7 (3.7, 18)	173.8 (2.9, 12)	Interaction	
K^+ (mM)	0.77 (0.13, 10)	0.55 (0.29, 10)	1.56 (0.35, 18)	4.87 (0.93, 12)	Interaction	
Cl^- (mM)	124.9 (5.1, 10)	128.0 (2.1, 10)	139.4 (2.0, 18)	143.2 (3.7, 12)		*
Mg^{2+} (mM)	1.06 (0.06, 10)	1.35 (0.09, 10)	1.75 (0.05, 18)	1.51 (0.07, 12)	*	*
Ca^{2+} (mM)	1.88 (0.11, 10)	2.28 (0.17, 10)	2.90 (0.09, 18)	2.13 (0.08, 12)	*	*

4. CONCLUSIONS

In the November sampling period after the Z2G1 application, a number of changes were observed in the haematology, blood and tissue chemistry, and some tissue histology. Results of field-based fish health studies are often difficult to interpret due to numerous confounding factors such as changes in water temperature and dissolved oxygen, unknown changes in other water quality parameters (e.g. chemistry) and seasonal changes in fish physiology. The monitored lakes undergo significant seasonal transformations, particularly in the highly eutrophic Lake Okaro where large temperature and dissolved oxygen shifts occur. Throughout the year, fish also undergo normal changes in biology, such as during reproductive development and spawning which can also affect a number of physiological endpoints. Changes in some measured endpoints, such as haematology are likely to be progressive and acclimatory and will expectedly occur at different times of the year and between lakes of varying condition. However, tissue chemistry results are less ambiguous and this study was able to demonstrate that Z2G1-derived Al was not readily bioavailable to lake biota. Although Al can still be toxic to fish without being internally bioavailable, obvious changes in trout gill histopathology were not observed during this study, suggesting no obvious impact of Z2G1 exposure at least on this endpoint in this species. An osmoregulatory disturbance, as indicated by increased plasma ion concentrations, was demonstrated for Lake Okaro trout. The pattern of this response was opposite to that expected following acute Al exposure. However, a response to general stress or a compensatory response to toxicant exposure cannot be ruled out. It is concluded that there were no obvious negative impacts on fish health owing to Z2G1 exposure based on the results of this study.

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7. APPENDICES

7.1 Historical fish survey data

Table A1. Mean (SEM, n) length (cm) and weight (g) of common bully (*Gobiomorphus cotidianus*) and smelt (*Retropinna retropinna*) subsamples from 6 beach seines around Lake Okaro during three consecutive years (2003-2005)

			Length (cm)		Weight (g)	
Species	Period	n	Mean	sem	Mean	sem
Bully	2003	78	2.46	0.09	0.34	0.04
	2004	94	2.76	0.08	0.30	0.03
	2005	113	2.34	0.04	0.14	0.01
Smelt	2004	79	4.52	0.08	0.78	0.04
	2003	89	3.37	0.08	0.31	0.03
	2005	112	4.90	0.08	0.93	0.05

Table A2. Lake Okaro seine survey data with total fish numbers captured at each of the 6 sampling sites.

	Common bully	Smelt
Site 1	983	150
Site 2	148	1715
Site 3	1470	2708
Site 4	1690	30
Site 5	1103	1180
Site 6	1830	330

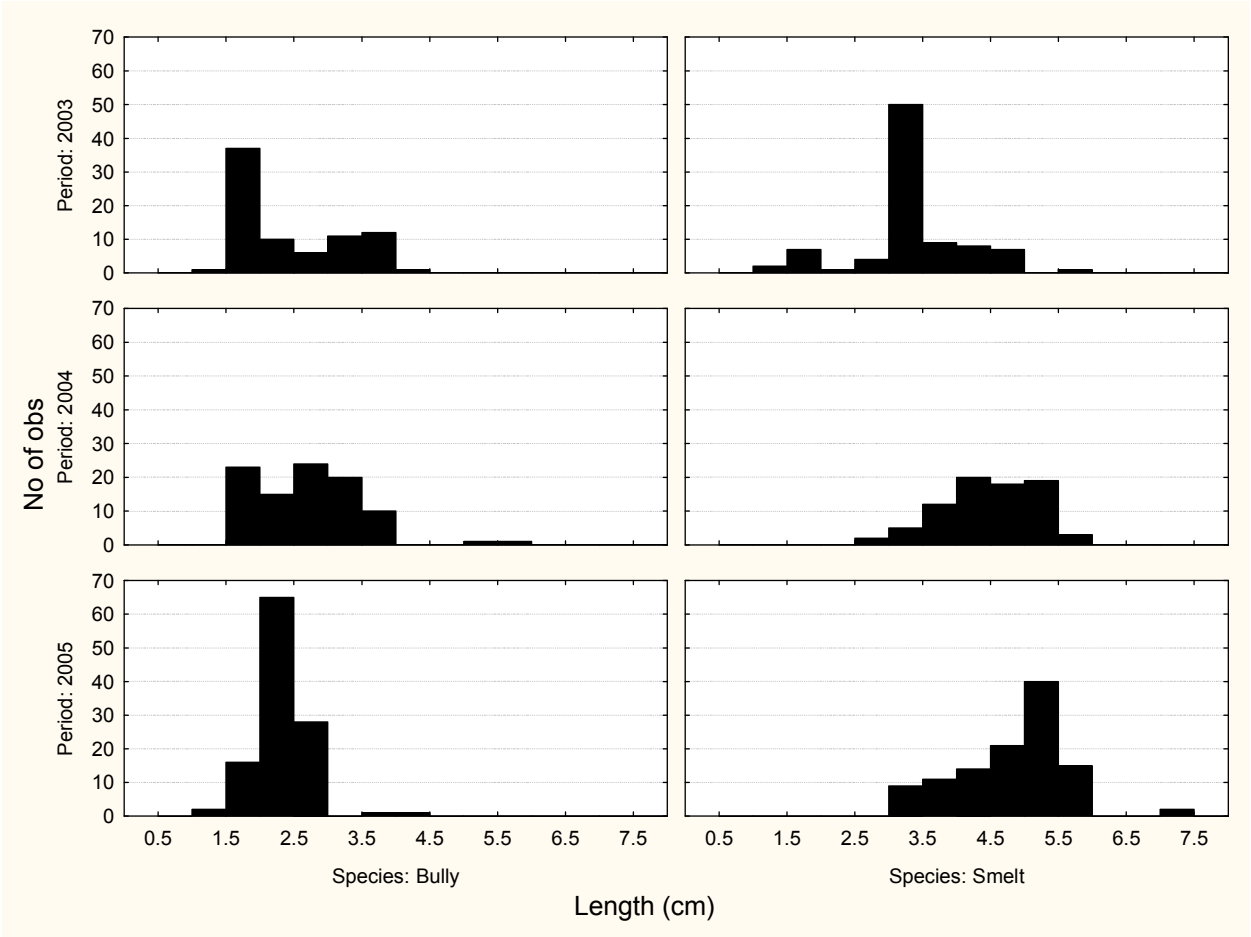

Fig. A1. Common bully (*Gobiomorphus cotidianus*) and smelt (*Retropinna retropinna*) length (cm) distributions from subsamples of 6 beach seine samples from Lake Okaro.

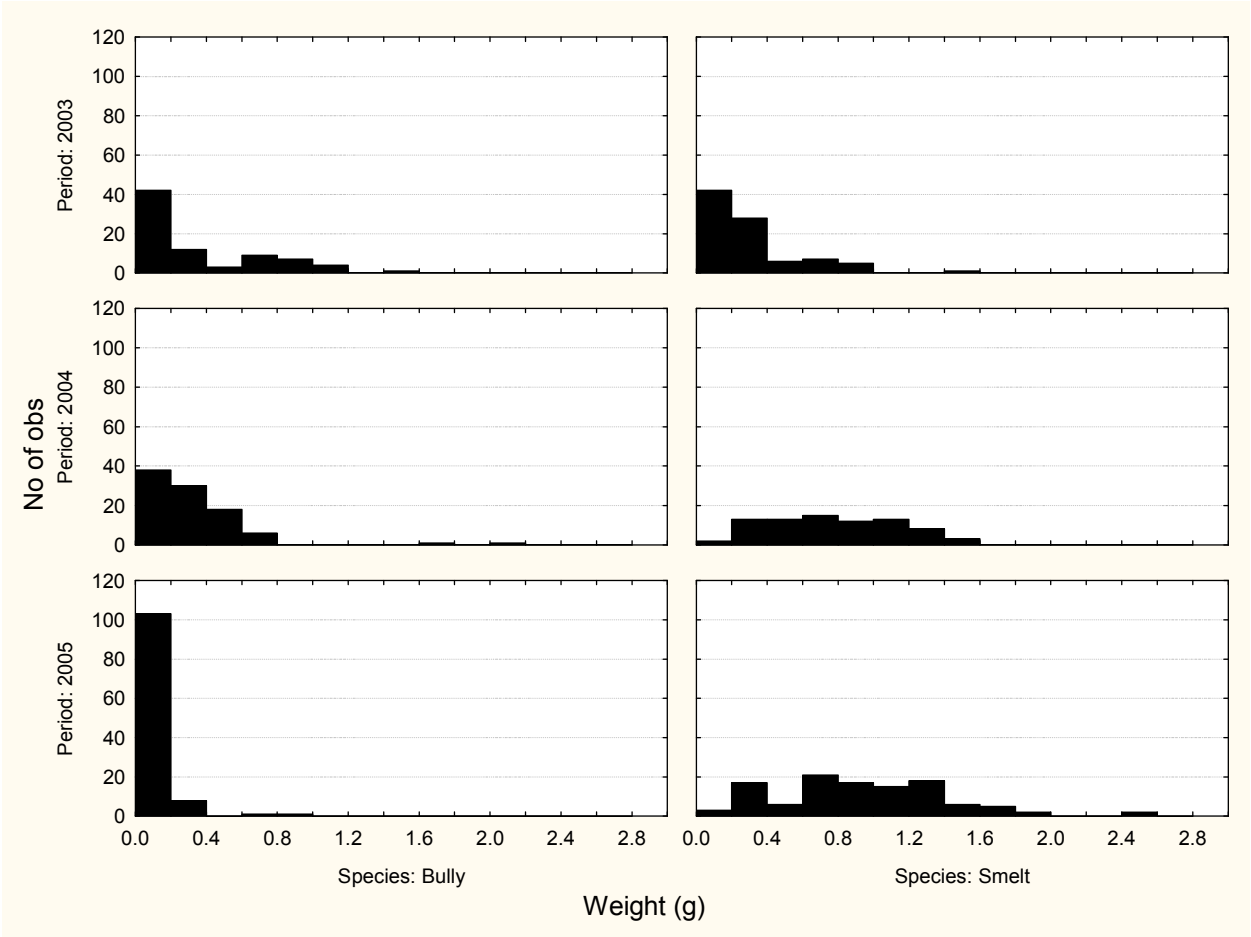

Fig. A2. Common bully (*Gobiomorphus cotidianus*) and smelt (*Retropinna retropinna*) weight (g) distributions from subsamples of 6 beach seine samples from Lake Okaro.

7.2 Historical fish health data

Table A3. Mean (SEM, n) of size and somatic indices male and female common bully (*Gobiomorphus cotidianus*) in Lake Okaro captured by over night minnow trapping in December each year over 2003, 2004 and 2005.

			Length (cm)		Weight (g)		Condition		GSI		LSI	
Sex	Period	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Female	2003	20	5.50	0.10	1.95	0.12	1.06	0.02	5.84	0.60	2.39	0.13
	2004	20	6.54	0.14	3.59	0.27	1.15	0.02	4.95	0.47	2.95	0.17
	2005	20	6.00	0.16	2.38	0.27	0.98	0.07	6.49	0.96	2.19	0.16
Male	2003	22	5.79	0.15	2.56	0.20	1.23	0.02	0.97	0.09	1.83	0.11
	2004	19	6.54	0.10	3.62	0.18	1.25	0.03	0.73	0.04	1.71	0.14
	2005	20	6.01	0.17	2.47	0.23	1.07	0.04	0.86	0.11	1.65	0.16

Table A4. Mean (SEM, n) of size and somatic indices male and female rainbow trout (*Oncorhynchus mykiss*) in Lake Okaro camptured by gill-netting during daylight hours during December of 2004 and 2005.

			Length (cm)		Weight (g)		Condition		LSI		SSI			GSI	
Sex	Period	n	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	GSI n	Mean	Std.Err.
Female	2004	21	42.9	0.90	870.0	40.4	1.09	0.03	0.797	0.038	0.083	0.009	20	0.530	0.027
	2005	12	46.1	0.69	1148.3	30.5	1.16	0.04	0.955	0.071	0.128	0.019	10	0.489	0.045
Male	2004	24	40.6	0.93	800.1	44.3	1.16	0.02	0.716	0.026	0.094	0.013	0	immature	
	2005	10	48.4	1.22	1267.0	77.2	1.11	0.05	0.903	0.037	0.108	0.012	3*	0.568	0.182

*7/10 immature fish

7.3 Trout and koura tissue metal data

Table A5. Trout liver metals suite. Data as **mean** ± sem (mg/kg wet weight) tissue concentrations. BDL = below detection limits.

Lake	Period	n	Na 23	Mg 24	Al 27	P 31	K 39	Ca 43	Fe 54	Mn 55	Cu 65	Zn 68	Se 82	Ag 109	Hg 202	Tl 205	U 235
Males																	
Okaro	August	15	**1302**	**129**	**1.33**	**1815**	**2534**	**25.5**	**361**	**2.27**	**33.3**	**46.8**	**3.25**	**BDL**	**23.6**	**0.032**	**BDL**
			41	2	0.02	42	26	0.9	55	0.28	10.8	7.9	0.57		4.1	0.006	
	November	3	**1174**	**185**	**BDL**	**3375**	**2979**	**27.7**	**385**	**1.49**	**24.1**	**24.9**	**2.62**	**BDL**	**93.6**	**BDL**	**BDL**
			38	6		135	88	0.6	78	0.08	5.8	0.7	1.06		7.3		
Rerewkakaaitu	August	13	**1682**	**139**	**2.23**	**2138**	**2485**	**34.1**	**597**	**1.71**	**62.8**	**60.8**	**1.41**	**0.88**	**30.0**	**0.027**	**0.018**
			75	5	0.19	89	54	3.6	85	0.18	19.4	8.4	0.32	0.14	4.5	0.002	0.002
	November	8	**1160**	**173**	**2.74**	**3036**	**2786**	**28.8**	**941**	**1.48**	**76.4**	**21.6**	**0.98**	**BDL**	**25.5**	**BDL**	**0.007**
			84	9	0.75	149	182	1.7	145	0.09	29.7	1.0	0.15		3.8		0.001
Females																	
Okaro	August	10	**1229**	**165**	**3.24**	**2334**	**2811**	**28.5**	**387**	**1.47**	**11.3**	**29.5**	**1.48**	**BDL**	**43.7**	**0.028**	**BDL**
			63	6	0.86	70	111	2.3	96	0.11	3.3	3.0	0.50		6.1	0.005	
	November	18	**1150**	**192**	**BDL**	**3640**	**3067**	**35.0**	**480**	**1.37**	**38.4**	**29.8**	**3.47**	**BDL**	**118.8**	**BDL**	**BDL**
			28	3		57	65	3.4	53	0.05	7.8	0.6	0.64		13.0		
Rerewkakaaitu	August	10	**1497**	**168**	**4.12**	**2699**	**2842**	**87.5**	**492**	**1.13**	**66.1**	**24.8**	**2.16**	**0.67**	**16.5**	**0.024**	**0.009**
			54	3	1.28	63	83	34.0	113	0.09	32.2	1.0	0.87	0.16	2.9	0.003	0.001
	November	12	**1179**	**181**	**2.37**	**3224**	**2975**	**33.8**	**682**	**1.54**	**42.9**	**21.2**	**0.76**	**BDL**	**21.5**	**BDL**	**0.012**
			51	5	0.27	78	91	2.2	138	0.13	13.3	0.7	0.05		3.5		0.001

Table A6. Trout flesh metals suite. Data as **mean** ± sem (mg/kg wet weight) tissue concentrations. BDL = below detection limits.

Site	Period	n	Na 23	Mg 24	Al 27	P 31	K 39	Ca 43	Cu 65	Zn 68	Hg 202	U 235
Males												
Okaro	August	15	**461**	**209**	**1.77**	**1569**	**3758**	**122**	**0.39**	**3.38**	**13.9**	**BDL**
			32	10	0.18	63	108	47	0.02	0.22	1.3	
	November	3	**327**	**287**	**BDL**	**2646**	**4493**	**278**	**0.24**	**3.87**	**39.8**	**BDL**
			38	22		178	217	201	0.02	0.16	5.7	
Rerewhakaaitu	August	13	**349**	**218**	**BDL**	**1582**	**3944**	**200**	**0.29**	**2.10**	**8.2**	**0.017**
			32	8		59	64	62	0.02	0.12	0.6	0.002
	November	8	**577**	**257**	**1.48**	**2479**	**4229**	**167**	**0.36**	**4.56**	**16.2**	**BDL**
			81	11	0.21	92	158	84	0.07	0.53	1.3	
Females												
Okaro	August	10	**375**	**240**	**1.39**	**1712**	**3959**	**68**	**0.31**	**3.38**	**20.9**	**BDL**
			47	11	0.05	45	62	22	0.03	0.30	2.1	
	November	18	**278**	**304**	**BDL**	**2599**	**4139**	**156**	**0.29**	**3.71**	**39.4**	**BDL**
			11	6		71	49	77	0.02	0.15	2.7	
Rerewhakaaitu	August	10	**246**	**259**	**BDL**	**1731**	**4011**	**184**	**0.23**	**2.68**	**6.9**	**0.016**
			29	10		42	79	49	0.01	0.41	0.9	0.002
	November	12	**386**	**296**	**1.49**	**2608**	**4328**	**131**	**0.31**	**4.10**	**16.9**	**BDL**
			24	9	0.22	50	74	21	0.03	0.19	2.0	

Table A7a. Koura hepatopancreas metals suite. Data as **mean** ± sem (mg/kg wet weight) tissue concentrations. BDL = below detection limits.

Lake	Period	n	Na 23	Mg 24	Al 27	P 31	K 39	Ca 43	Cr 53	Fe 54	Mn 55	Co 59
Okaro	August	8	**2120**	**169**	**BDL**	**1318**	**2082**	**267**	**BDL**	**51.2**	**61.5**	**BDL**
			62	6		40	52	16		3.9	6.7	
	November	7	**3053**	**211**	**BDL**	**1665**	**2041**	**532**	**BDL**	**BDL**	**45.6**	**BDL**
			130	15		118	79	68			10.3	
Rerewhakaaitu	August	10	**2211**	**262**	**2.10**	**1573**	**2219**	**205**	**BDL**	**60.6**	**26.3**	**0.498**
			112	15	0.50	53	40	10		3.5	6.7	0.082
	November	10	**1506**	**160**	**6.42**	**1829**	**2246**	**190**	**BDL**	**59.7**	**16.4**	**0.559**
			86	13	2.89	82	118	16		8.7	3.7	0.068

Table A7b. Koura hepatopancreas metals suite continued.

Lake	Period	n	Cu 65	Zn 68	As 75	Se 82	Sr 88	Cd 111	Ba 137	Hg 202	U 235
Okaro	August	8	**10.4**	**36.3**	**1.65**	**BDL**	**2.28**	**BDL**	**2.37**	**4.90**	**0.157**
			3.2	8.7	0.12		0.20		0.33	1.20	0.016
	November	7	**8.6**	**28.2**	**BDL**	**BDL**	**3.49**	**BDL**	**2.96**	**BDL**	**0.109**
			1.3	13.1			0.32		0.58		0.012
Rerewhakaaitu	August	10	**23.8**	**73.5**	**0.71**	**0.471**	**2.91**	**0.559**	**3.76**	**2.28**	**0.022**
			9.4	12.2	0.06	0.022	0.39	0.038	0.76	0.24	0.003
	November	10	**9.4**	**37.4**	**0.62**	**0.380**	**1.66**	**0.297**	**1.79**	**2.44**	**0.028**
			1.1	4.7	0.05	0.006	0.14	0.049	0.30	0.67	0.006

Table A8a. Koura tail flesh metals suite. Data as **mean** ± sem (mg/kg wet weight) tissue concentrations. BDL = below detection limits.

Lake	Period	n	Na 23	Mg 24	Al 27	P 31	K 39	Ca 43	Cr 53	Fe 54	Mn 55	Co 59
Okaro	August	8	**1532**	**251**	**BDL**	**1426**	**2883**	**198**	**1.58**	**BDL**	**2.66**	**BDL**
			91	14		51	118	10	1.02		0.25	
	November	7	**2228**	**292**	**4.55**	**2019**	**2742**	**326**	**0.98**	**BDL**	**6.38**	**BDL**
			123	6	1.18	85	55	33	0.05		1.75	
Rerewhakaaitu	August	10	**1378**	**257**	**BDL**	**1671**	**2838**	**152**	**0.13**	**BDL**	**0.36**	**BDL**
			46	4		57	50	9	0.00		0.04	
	November	10	**858**	**296**	**2.20**	**2520**	**3202**	**119**	**BDL**	**BDL**	**0.44**	**BDL**
			32	10	0.39	39	87	9			0.06	

Table A8b. Koura tail flesh metals suite continued.

Lake	Period	n	Cu 65	Zn 68	As 75	Se 82	Sr 88	Cd 111	Ba 137	Hg 202	U 235
Okaro	August	8	**3.18**	**11.8**	**1.51**	**BDL**	**0.668**	**BDL**	**BDL**	**13.1**	**BDL**
			0.15	0.7	0.25		0.068			2.8	
	November	7	**3.38**	**16.0**	**BDL**	**BDL**	**1.367**	**BDL**	**BDL**	**2.4**	**BDL**
			0.25	1.7			0.136			0.9	
Rerewhakaaitu	August	10	**3.27**	**12.5**	**BDL**	**BDL**	**0.348**	**BDL**	**BDL**	**5.3**	**0.009**
			0.25	0.6			0.031			0.4	0.001
	November	10	**3.42**	**11.3**	**BDL**	**BDL**	**0.271**	**BDL**	**BDL**	**7.6**	**0.010**
			0.45	0.5			0.027			0.8	0.002