Lake Okareka and Tikitapu Fish Health Monitoring 2006

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EXECUTIVE SUMMARY

Phoslock^{TM} is a lanthanum-amended bentonite clay product that can remove dissolved phosphorus from the water column and cap sediments to reduce phosphate release. Large-scale applications of Phoslock^{TM} have been carried out in 2005 and 2006 as part of the Lake Okareka management plan. Fish health monitoring in Lake Okareka following the 2005 Phoslock^{TM} application identified concerning changes in fish health but these could not be directly attributed to Phoslock^{TM} exposure. This study was undertaken to examine fish health following the 2006 application and included an additional benthic species (koura) and a control lake (Tikitapu) for comparative purposes to delineate seasonal or lake-specific changes in fish health.

Results indicated that trout and koura in Lake Okareka accumulated greater bioavailable lanthanum following Phoslock^{TM} application but concentrations of this element were very low in their flesh and consumption of these species poses minimal or no significant risk to human health. Significant changes in some physiological parameters between lakes and over time (pre- and post-Phoslock^{TM} application) are primarily attributable to differences in reproductive timing of fish in both lakes and differences in the mean size of bullies and koura in each lake. The significant deterioration in fish health observed during the 2005 post-application monitoring period was not repeated in 2006.

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1.0 INTRODUCTION

In the Lake Okareka Catchment Management Plan (2003), methods were proposed to reduce nitrogen and phosphorus levels in Lake Okareka. Several interim remediation options were proposed until other solutions such as sewage reticulation, modifications to land use and wetland renovation could be enacted. Objections to the hyperlimnetic discharge lead to an alternative intermediate *in situ* treatment option. In 2005, the first of three annual nutrient adsorbing mineral applications was performed on Lake Okareka. The intention of the mineral applications was to reduce phosphorus load by 0.1 tonnes per year.

Phoslock^{TM} is a lanthanum-amended bentonite clay product that can remove dissolved phosphorus from the water column and can be used to form a reactive capping layer to intercept nutrients released from sediments (Haghseresht 2004). Large-scale Phoslock^{TM} applications have been performed in Western Australia on the Swan and Canning Rivers (Douglas et al. 1999; Robb et al. 2003) and now in New Zealand on Lake Okareka (McIntosh 2006).

Laboratory and field monitoring studies (Stauber and Binet 2000; Martin and Hickey 2004) have shown that Phoslock^{TM} poses little risk to algae, cladocera and fish. Although the lanthanum ingredient may be potentially toxic to fish and aquatic invertebrates, studies on this product suggest that the lanthanum element is strongly bound to the bentonite and is of little toxicological risk in the environment (Haghseresht 2004). During the 2005 Okareka fish health monitoring (Landman et al. 2006a), changes in haematology and gill histopathology indicated a potential decline in fish health after the Phoslock^{TM} application. However, the cause of this fish health change could not be resolved.

A supplementary health assessment of Lakes Okareka and Tikitapu (Blue Lake) rainbow trout (*Oncorhynchus mykiss*) was completed in April 2006

(Landman et al. 2006b). During the second evaluation period, Lake Okareka trout health was found to have improved over the period from October 2005 to April 2006. In general, trout from both lakes were in good health, and presented similar haematological profiles and gill histopathology.

The current study is a continuation of the Lake Okareka fish health monitoring program initiated in 2005, primarily examining the 2006 mineral application. In this study, rainbow trout, common bully (*Gobiomorphus cotidianus*) and koura (*Paranephrops planifrons*) were investigated in Lake Okareka. The adoption of nearby Lake Tikitapu as a reference site was done for comparative purposes to help discriminate potential seasonal and mineral exposure effects on fish health.

2.0 METHODS

2.1 Fish collection

Twenty tonnes of Phoslock^{TM} was applied to Lake Okareka in June 2006. This was approximately two months earlier than the previous year. Fish capture and sampling was timed around the 2006 application. Sampling of fish from Lakes Okareka and Tikitapu (Blue Lake) was conducted over three periods; one period prior to the mineral application and two periods after application.

Pre-mineral application rainbow trout (*Oncorhynchus mykiss*) sampling was performed in April as part of the trout baseline/recovery study (Landman et al. 2006b). Common bully (*Gobiomorphus cotidianus*) and koura (*Paranephrops planifrons*) were not collected during the April trout health assessment. Pre-mineral application bully and koura collections were performed in the week (12-17 June) prior to the mineral application. All species were then collected at approximately two weeks (15-16 July 2006) and two months (11-15 September) post-mineral application.

Rainbow trout were captured using six gill nets set around each lake during daylight hours (Fig. 1). Nets were checked hourly by working the length of the net and removing all fish immediately. Common bully were captured using approximately 30 Gee-minnow traps set in 5-15 m of water. Minnow traps were set late in the afternoon, left over-night and checked the following morning. Where insufficient numbers were obtained, traps were removed and reset in another location. Koura were collected by Aquatek (Tauranga, NZ) and Waikato University (Hamilton, NZ) SCUBA divers.

Fig. 1. Nick Ling (left) and Jeroen Brys (right) setting a gill net by boat on Lake Tikitapu (Blue Lake).

2.2 Necropsy

Rainbow 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 (Fig. 2) 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 storage bags and stored on ice until they could be frozen at -20°C for lanthanum analysis. Subsamples of gill and spleen tissues were removed, placed in histocasettes and fixed in 10% neutral buffered formalin. Trout heads were removed, numbered and stored at -20°C for later sampling. Common bullies were immediately transported back to the laboratory after capture. Fish were first anaesthetised with MS-222 (0.1 g L^{-1}). Approximately 20-100 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 in 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 assessments were performed on trout and bully blood. Haematocrit (Hct; packed red cell volume) was determined by the microcapillary method. Two microlitres of whole blood was added to 1 mL of Drabkin's solution and whole blood haemoglobin determined spectrophotometrically at 540 nm. Total red blood cell counts (RBCCs) were made by adding 10 μL of whole blood to 4 mL of IsotonII solution (Beckman Coulter) in Truecount tubes (BD Biosciences) and measured with a Becton Dickinson FACSVantage flow cytometer. Two microlitres of whole blood was mixed with 98 μL of red cell diluting fluid and stored on ice for manual count validation. Manual RBCCs were made using images of RBCs on a haemocytometer captured at 100 x magnification using an AxioCam HRC camera and a Zeiss Axioplan 2 light microscope. ImagePro Plus® software (Media Cybernetics Inc., Silver Springs, MD) was used to count cells after enhancement and filtering of images. Haematometric indices; mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were all calculated. All haematology was performed according to standard methods (Wintrobe 1934; Dacie and Lewis 1991).

Fig. 2. Nick withdrawing a rainbow trout blood sample by caudal venipuncture.

2.4 Histology

Preserved 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 hematoxylin-eosin. Gill tissue slides were scanned at low magnification (50 x) to examine and estimate the distribution of large or severe lesions. Up to 20 fields of view were examined at 200-400 x magnification for finer cellular detail. 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 Zeiss Axioplan 2 light 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, darkly stained 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 metals analysis

2.5.1 Trout lanthanum

Frozen rainbow trout liver and flesh tissue samples were sent to RJ Hill Laboratories (Hamilton, NZ) for the determination of lanthanum. Samples were subjected to a nitric/hydrochloric acid digestion and lanthanum determined by inductively coupled plasma mass spectrometry (ICP-MS).

2.5.2 Koura metal suite

A suite of metals were measured in koura hepatopancreas and muscle tissue samples based on USEPA (1987) methods. Brielfly, 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 ICP-MS (Department of Chemistry, Waikato University, Hamilton, NZ).

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. Tissue metals analysis was also performed by ANCOVA using weight and length as separate covariates to control for effects of fish size. Haematology data were analysed by analysis of variance (ANOVA). Significant differences were further defined using Tukey's post-hoc test. Because differential white cell counts were measured as proportions of various cell types, data were arcsine transformed (Sokal and Rohlf 1973) prior to analysis.

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 v6.1 software. The critical level of statistical significance for all tests was $\alpha = 0.05$.

3.0 RESULTS AND DISCUSSION

Over the 2006 fish health monitoring period, rainbow trout, common bully and koura were captured from Lakes Okareka and Tikitapu. Time of year (season) influenced both fishing effort and sex ratios of rainbow trout. Trout capture was generally more difficult during April and September. Coinciding with spawning season, trout were presumably more active in the July period when fishing was easiest. A male bias was observed in both lakes that was most obvious in September when only 3 out of 18 trout captured in Okareka and none out of 20 in Tikitapu were female. Common bully and koura were relatively abundant in Lake Okareka, and while present in Tikitapu, approximately twice the effort (i.e. 2x fishing days) was required to obtain the appropriate sample sizes for these species.

3.1 Physiology

General physiological parameters have been summarized and tabulated for ease of comparison in Tables 1-3. Weight and length ranges of male and female koura in Okareka were consistent over the monitoring period. More varied size ranges were obtained for Tikitapu koura and these were generally smaller and lighter per unit length.

Somatic growth and energy storage in fish species can be measured in terms of weight, length, condition (weight per unit length) and liver size. Condition and liver somatic index are expected to be influenced by seasonal changes in temperature, food availability, photoperiod and reproduction. In the current study, fish condition was generally consistent in both fish species across the three sampling periods (Fig. 3), apart from female common bully. Female condition increased over the three sampling periods, except in Tikitapu females where reduced condition was measured in September.

Table 1. Mean (SEM, n) of size and somatic indices in male and female rainbow trout. Asterisks indicate significant difference ($p < 0.05$) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	April		July		September		Hypothesis	
	Okareka	Tikitapu	Okareka	Tikitapu	Okareka	Tikitapu	Lake	Period
Males								
Length (mm)	520 (6, 15)	509 (9, 15)	536 (7, 14)	493 (9, 11)	528 (6, 15)	511 (7, 20)		
Weight (g)	1458 (58, 15)	1419 (85, 15)	1631 (69, 14)	1478 (74, 11)	1467 (56, 15)	1511 (81, 20)		
Condition (*K*)	1.01 (0.03, 15)	1.01 (0.02, 15)	1.00 (0.03, 14)	1.18 (0.09, 11)	0.97 (0.02, 15)	1.09 (0.04, 20)		
GSI	1.79 (0.32, 15)	4.04 (0.46, 15)	2.84 (0.23, 14)	2.39 (0.29, 11)	2.03 (0.18, 14)	2.23 (0.19, 20)	interaction	
LSI	0.83 (0.05, 15)	0.98 (0.06, 15)	0.72 (0.02, 14)	1.01 (0.05, 10)	0.65 (0.03, 15)	0.76 (0.03, 20)	*	*
SSI	0.13 (0.02, 14)	0.16 (0.02, 15)	0.15 (0.02, 14)	0.20 (0.02, 11)	0.13 (0.02, 15)	0.18 (0.02, 20)	*	
Females								
Length (mm)	488 (8, 9)	493 (9, 8)	506 (9, 10)	496 (8, 9)	522 (9, 3)			
Weight (g)	1315 (42, 9)	1463 (55, 8)	1478 (92, 10)	1486 (82, 9)	1435 (135, 3)			
Condition (*K*)	1.06 (0.03, 9)	1.08 (0.05, 8)	0.94 (0.05, 10)	1.06 (0.03, 9)	1.00 (0.09, 3)			
GSI	5.49 (1.20, 9)	11.68 (1.41, 8)					*	
LSI	1.19 (0.07, 9)	1.31 (0.07, 8)	0.56 (0.05, 10)	0.91 (0.09, 9)	0.68 (0.07, 3)		*	*
SSI	0.10 (0.02, 9)	0.08 (0.01, 8)	0.06 (0.01, 9)	0.14 (0.02, 9)	0.06 (0.03, 3)		interaction	

Table 2. Mean (SEM, n) of size and somatic indices in male and female common bully. Asterisks indicate significant difference (p < 0.05) in overall ANCOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	June		July		September		Hypothesis	
	Okareka	Tikitapu	Okareka	Tikitapu	Okareka	Tikitapu	Lake	Period
Males								
Length (mm)	61.0 (1.57, 6)	56.8 (0.95, 11)	71.6 (3.38, 12)	60.3 (2.22, 7)	81.9 (2.80, 8)	68.6 (2.11, 14)		
Weight (g)	2.27 (0.14, 6)	1.96 (0.10, 11)	5.08 (1.11, 12)	2.43 (0.32, 7)	6.99 (0.87, 8)	3.96 (0.68, 14)		
Condition (*K*)	0.98 (0.03, 6)	1.03 (0.04, 11)	1.15 (0.07, 12)	1.03 (0.04, 7)	1.19 (0.04, 8)	1.09 (0.04, 14)		
GSI	0.85 (0.05, 6)	1.27 (0.32, 11)	0.82 (0.12, 12)	0.82 (0.10, 7)	0.68 (0.07, 8)	0.82 (0.16, 14)		
LSI	1.22 (0.09, 6)	2.38 (049, 11)	2.33 (0.21, 12)	3.49 (0.29, 7)	2.81 (0.22, 8)	2.66 (0.12, 14)	*	*
Females								
Length (mm)	63.2 (1.42, 13)	61.6 (3.30, 9)	68.5 (2.58, 10)	62.9 (1.85, 12)	79.2 (2.79, 12)	68.7 (1.33, 6)		
Weight (g)	2.52 (0.16, 13)	2.80 (0.63, 9)	3.98 (0.60, 10)	3.03 (0.32, 12)	7.13 (0.89, 12)	3.21 (0.30, 6)		
Condition (*K*)	0.95 (0.02, 13)	1.03 (0.04, 9)	1.09 (0.05, 10)	1.10 (0.02, 12)	1.26 (0.10, 12)	0.90 (0.03, 6)	interaction	
GSI	1.84 (0.18, 13)	1.77 (0.26, 9)	3.37 (0.36, 10)	3.35 (0.36, 12)	7.70 (1.43, 12)	5.62 (1.18, 6)		*
LSI	1.58 (0.10,13)	3.73 (0.44, 9)	2.68 (0.47, 10)	3.77 (0.16, 12)	2.41 (0.18, 12)	2.45 (0.28, 6)	interaction	

Table 3. Mean (SEM, n) and *range* of koura weight and length data over the monitoring period.

	June		July		September	
	Okareka	Tikitapu	Okareka	Tikitapu	Okareka	Tikitapu
Males						
Weight (g)	80.1 (7.21, 6)	63.7 (13.66, 7)	61.0 (7.87, 7)	10.1 (1.75, 6)	62.2 (11.13, 5)	14.8 (3.48, 8)
	58.4 - 106.2	*22.1 - 103.4*	*35.9 - 86.3*	*6.0 - 17.2*	*39.2 - 98.4*	*2.5 - 25.1*
Total length (mm)	143.2 (4.25, 6)	124.6 (13.62, 7)	127.6 (4.33, 7)	74.7 (3.75, 6)	132.2 (7.74, 5)	79.5(7.97, 8)
	127 - 158	*60 - 158*	*114 - 142*	*64 - 89*	*115 - 157*	*46 - 97*
Females						
Weight (g)	42.6 (10.37, 3)	20.9 (3.34, 3)	55.5 (15.70, 3)	28.5 (18.46, 4)	50.5 (4.62, 5)	38.0 (11.62, 6)
	21.8 - 53.7	*16.4 - 27.5*	*28.1 - 82.4*	*9.2 - 83.9*	*41.9 - 67.1*	*19.1 - 94.8*
Total length (mm)	105.00 (24.01, 3)	92.67 (4.70, 3)	126.00 (12.74, 3)	96.75 (20.09, 4)	126.40 (3.50, 5)	113.17 (10.23, 6)
	57 - 130	*87 - 102*	*103 - 147*	*75 - 157*	*116 - 137*	*89 - 156*

Liver somatic index (LSI) was a more sensitive measure of energy storage and mobilization in both fish species given that changes in liver size were observed between sampling periods (Fig. 4). Liver somatic index was also typically greater in Tikitapu fish compared to Okareka. Decreasing trout LSI was measured from April to September, while common bully LSI generally increased from June to July then decreased in September. The changes in female bully corresponded with changes in condition. The changes in LSI are presumably linked to spawning and subsequent recovery in trout, and sexual maturation and growth of the gonads in the common bully.

The majority of trout captured during April were maturing adults in various stages of gonadal development. At this time, Tikitapu trout appeared to be slightly more advanced as only one immature fish was captured in Tikitapu compared to five immature fish in Okareka. Male gonado-somatic index differed between lakes in April but was similar by July and into September (Fig. 5A). All female trout captured were also ripe or partially spawned by July. Simultaneous, progressive development of the gonads in female common bully shows relatively synchronous reproductive timing between lakes (Fig. 5B). This was not observed in male common bully as testis sizes did not change between the sampling periods.

A complex pattern of spleen size changes were observed with distinct differences between male and female trout (Fig. 6). Male spleen somatic index (SSI) was statistically uniform in each lake population, although modestly higher in July. Opposite changes in female spleen size were shown as decreased SSI from April to July in Okareka and increased SSI in Tikitapu trout.

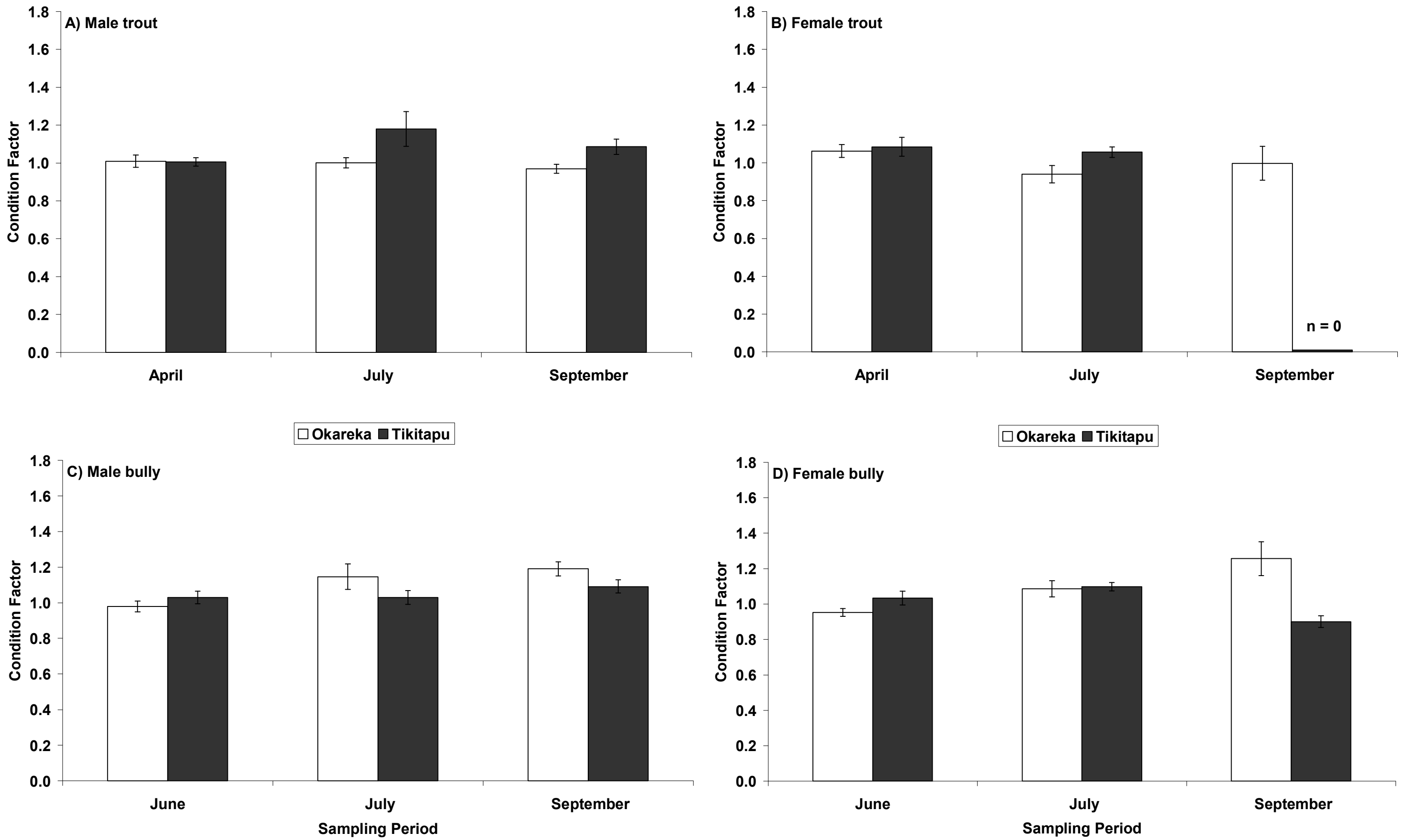

Fig. 3. Condition factor (weight per unit length) of A) male trout, B) female trout, C) male bully and D) female bully. Condition was consistent between lake populations at each sampling period, except for female common where reduced condition was found for Tikitapu females in September.

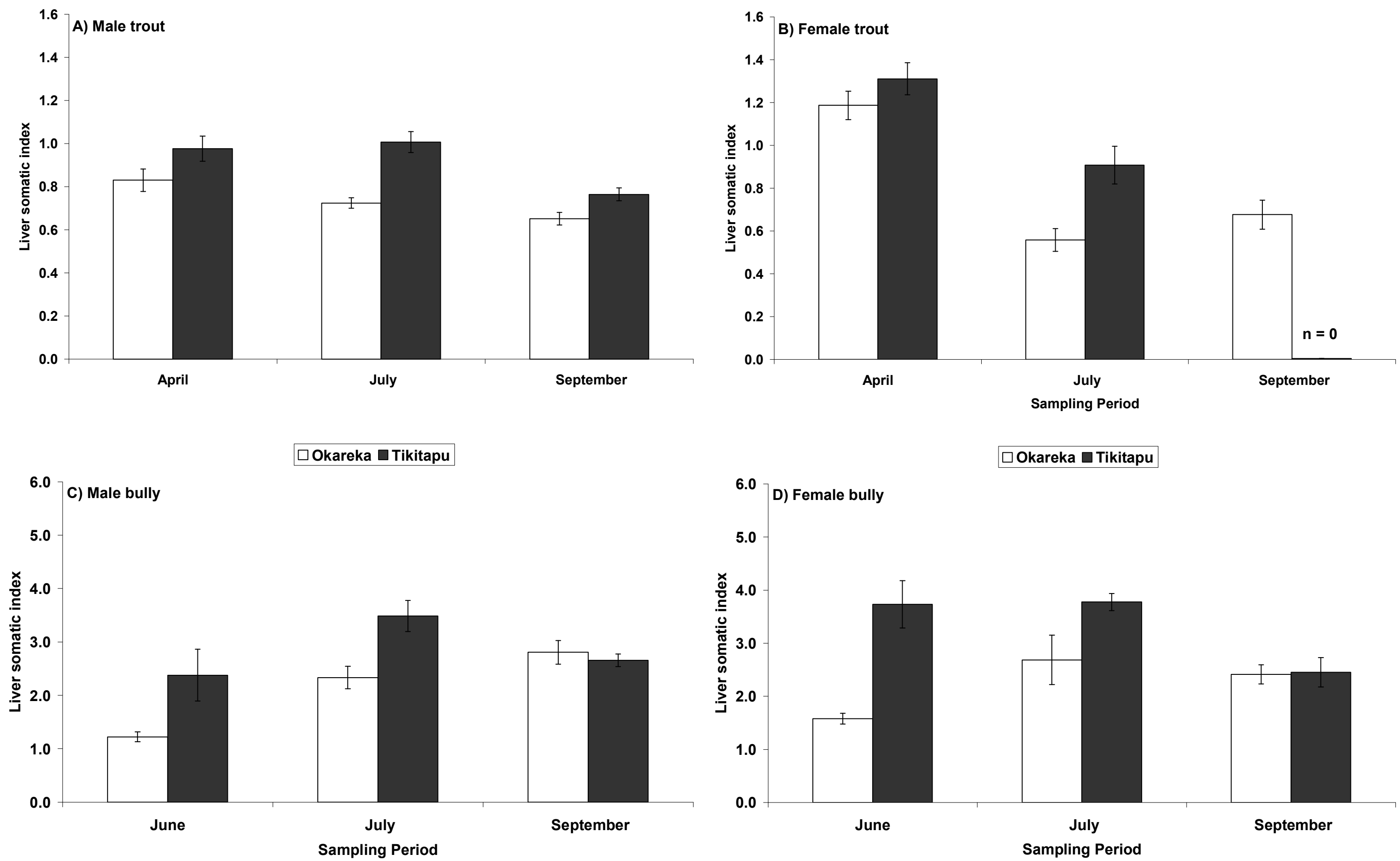

Fig. 4. Mean liver somatic index (LSI) of A) male trout, B) female trout, C) male bully and D) female bully. Liver size was generally greater in Tikitapu fish. Decreasing liver size was measured in male and female trout from April to September, while common bully liver size generally increased from June to July. Error bars indicate standard error of the mean.

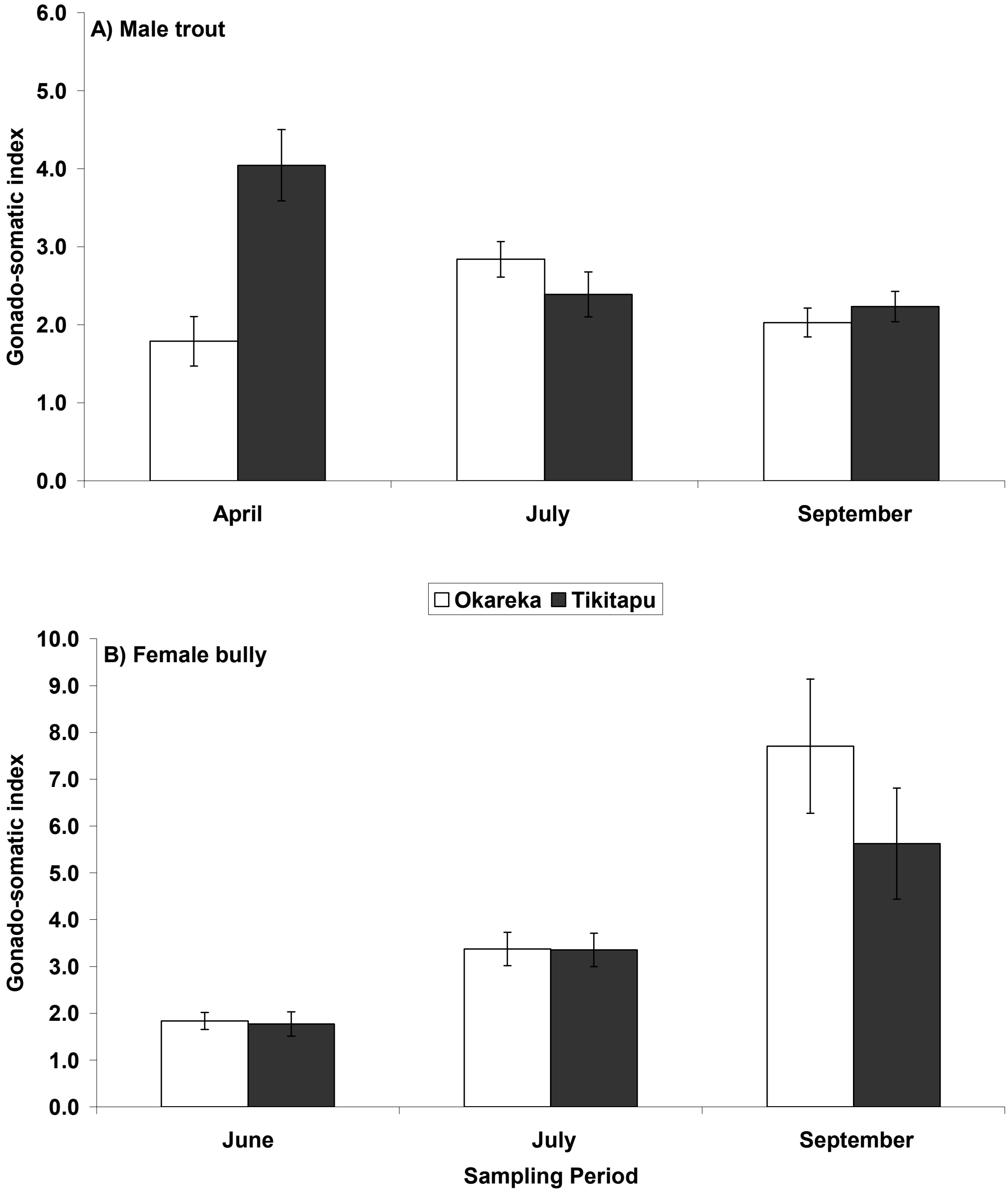

Fig. 5. Mean gonado-somatic index (GSI) of A) male trout and B) female bully. Differences in male trout GSI were evident initially in April but not distinguishable from July to September. Simultaneous progressive increases in female bully GSI were observed from June to September. Error bars indicate standard error of the mean.

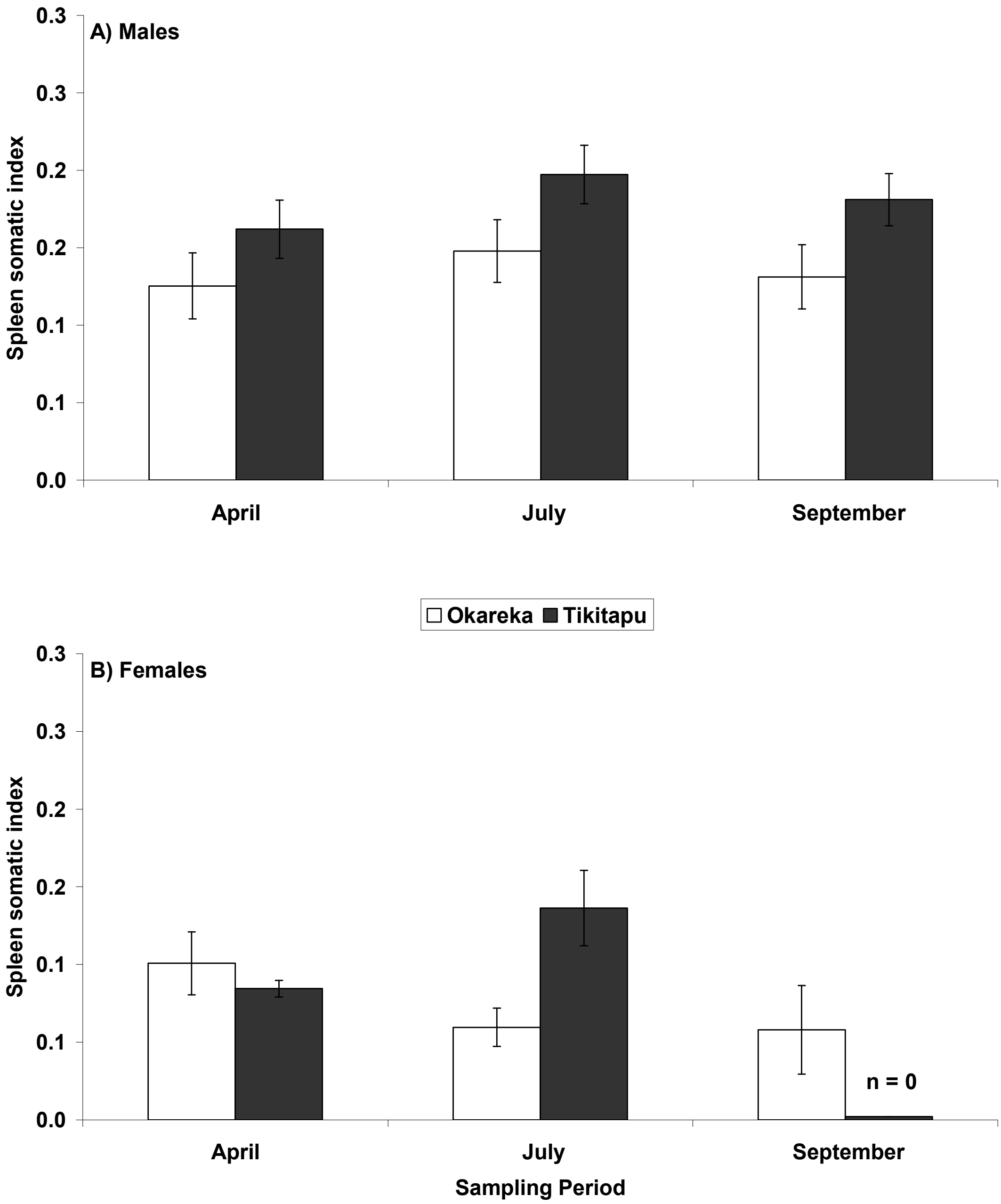

Fig. 6. Mean spleen somatic index (SSI) of A) male and B) female trout. Spleen size was slightly greater in Tikitapu males throughout the monitoring. An interaction was observed as opposite changes in female spleen size from April to July. Error bars indicate standard error of the mean.

3.2 Haematology

Changes in male and female trout haematology were observed, most notably between sampling periods rather than between lakes (Table 4). Haematocrit was the only parameter that did not change with period, site or sex. Circulating red blood cell (RBC) numbers decreased from April to July in both sexes but increased again by September in males. Opposite trends of increasing and decreasing haemoglobin in Okareka and Tikitapu males was found over the three sampling periods from April to September. No changes in haemoglobin were observed in female trout. The observation of depressed RBC numbers in male trout during spawning was unexpected. Increases in circulating RBCs have been shown to coincide with elevated androgen levels in maturing male brown trout compared to female or immature male fish (Pottinger and Pickering 1987). Similar observations have recently been made in maturing rainbow trout (van den Heuvel et al. unpublished data). Nevertheless, current haematological changes appear to be seasonal in origin as they were generally mirrored in both lake populations. Accessory haematological parameters expectedly coincided with changes in RBCs and haemoglobin concentration. In female trout, increases in MCHC, MCH and MCV also coincided with the reductions in RBC count from April to July. Similar increases in MCHC, MCH and MCV were also observed for male trout from April to July, which then generally decreased by September. Such changes are presumably compensatory, aimed at alleviating the potential negative effects of reduced RBCs or the increased energetic demands associated with spawning activity.

Different haematological trends were seen in the common bully (Table 4). Reduced haematocrit was measured in male common bully in the July sampling period that was not matched in females. Circulating RBC numbers were consistent in male bullies from June to September. Small increases in female RBC count were observed from June to September. Haemoglobin levels decreased from June to July and remained consistent through to September in male and female bullies from both lakes. Interactions between lake and sample period were observed for MCHC, MCH and MCV. General

Table 4. Mean (SEM, n) blood parameters for male and female rainbow trout. Asterisks indicate significant difference ($p < 0.05$) in overall ANOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	April		July		September		Hypothesis	
	Okareka	Tikitapu	Okareka	Tikitapu	Okareka	Tikitapu	Lake	Period
Males								
Hct (%)	42.5 (3.5, 10)	45.7 (1.8, 12)	45.2 (1.6, 9)	37.5 (1.7, 11)	45.6 (2.1, 14)	40.4 (2.2, 30)		
RBCC ($\times10^{12}$ cells/L)	1.26 (0.09, 10)	1.30 (0.05, 12)	1.05 (0.06, 9)	0.89 (0.05, 11)	1.22 (0.04, 14)	1.13 (0.05, 20)		*
Hb (g/L)	92.0 (7.5, 10)	100.5 (4.6, 12)	106.9 (2.7, 9)	93.5 (4.5, 11)	100.8 (4.4, 14)	81.3 (3.7, 20)	interaction	
MCH (pg/cell)	71.9 (3.9, 10)	77.8 (2.1, 12)	104.0 (4.4, 9)	109.0 (8.7, 11)	83.0 (3.0, 14)	72.0 (1.2, 20)	interaction	
MCHC (g/L)	219 (7, 10)	220 (4, 12)	237 (4, 9)	251 (9, 11)	222 (5, 14)	206 (7, 20)		*
MCV (fl)	329 (16, 10)	355 (10, 12)	438 (14, 9)	436 (35, 11)	377 (16, 14)	358 (13, 20)		*
Lymphocyte (%)	71.6 (5.2, 10)	76.8 (4.0, 12)	68.9 (4.0, 9)	52.0 (5.9, 11)	68.7 (4.8, 14)	71.4 (3.4, 17)		*
Granulocyte (%)	12.0 (2.1, 10)	13.7 (3.5, 12)	27.7 (4.5, 9)	45.4 (5.5, 11)	21.3 (3.1, 14)	18.6 (2.3, 17)	interaction	
Thrombocyte (%)	16.4 (4.4, 10)	9.6 (1.9, 12)	3.4 (1.2, 9)	2.6 (1.4, 11)	10.0 (2.4, 14)	9.9 (2.1, 17)		*
Females								
Hct (%)	43.1 (4.2, 7)	48.3 (1.1, 6)	44.6 (1.5, 8)	46.4 (2.6, 5)	34.0 (6.4, 3)	-		
RBCC ($\times10^{12}$ cells/L)	1.27 (0.08, 7)	1.36 (0.06, 6)	1.12 (0.01, 8)	1.05 (0.10, 5)	1.05 (0.09, 3)	-		*
Hb (g/L)	94.0 (8.5, 7)	104.4 (3.6, 6)	105.5 (4.5, 8)	113.8 (5.8, 5)	87.6 (15.7, 3)	-		
MCH (pg/cell)	73.8 (5.2, 7)	77.0 (2.5, 6)	98.9 (10.4, 8)	110.0 (9.2, 5)	84.8 (18.7, 3)	-		*
MCHC (g/L)	220 (5, 7)	217 (8, 6)	236 (6, 8)	247 (11, 5)	265 (36, 3)	-		*
MCV (fl)	338 (28, 7)	358 (20, 6)	430 (61, 8)	454 (22, 5)	321 (49, 3)	-		*
Lymphocyte (%)	77.8 (7.3, 6)	76.2 (7.0, 6)	62.4 (7.2, 8)	66.8 (11.6, 4)	86.5 (8.5, 2)	-		
Granulocyte (%)	14.6 (3.9, 6)	12.0 (4.5, 6)	28.5 (6.1, 8)	17.5 (6.5, 4)	2.5 (2.5, 2)	-		
Thrombocyte (%)	12.0 (4.4, 6)	11.8 (3.6, 6)	9.1 (2.0, 8)	15.8 (5.4, 4)	11.0 (6.0, 2)	-		

Table 5. Mean (SEM, n) blood parameters for male and female common bully. Asterisks indicate significant difference ($p < 0.05$) in overall ANOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	June		July		September		Hypothesis	
	Okareka	Tikitapu	Okareka	Tikitapu	Okareka	Tikitapu	Lake	Period
Males								
Hct (%)	32.6 (3.2, 6)	29.7 (2.6, 10)	24.9 (1.7, 12)	25.3 (1.8, 7)	32.1 (2.3, 8)	26.6 (1.9, 14)		*
RBCC ($\times 10^{12}$ cells/L)	1.04 (0.05, 4)	0.78 (0.06, 9)	1.16 (0.09, 7)	0.62 (0.05, 7)	1.02 (0.13, 5)	0.68 (0.07, 7)	*	
Hb (g/L)	81.1 (8.5, 6)	68.2 (3.6, 11)	62.6 (4.0, 12)	52.4 (2.8, 7)	48.2 (4.1, 8)	47.7 (2.0, 14)	*	*
MCH (pg/cell)	83.1 (4.4, 4)	86.3 (6.5, 9)	60.2 (6.3, 7)	89.5 (10.8, 7)	45.5 (2.8, 5)	68.2 (4.5, 7)	*	*
MCHC (g/L)	248 (10, 6)	246 (15, 10)	260 (18, 12)	211 (16, 7)	151 (9, 8)	189 (14, 14)	interaction	
MCV (fl)	326 (27, 4)	335 (19, 8)	209 (17, 7)	423 (31, 7)	334 (24, 5)	436 (36, 7)	interaction	
Females								
Hct (%)	26.6 (1.4, 13)	35.2 (2.1, 9)	29.0 (2.6, 7)	28.4 (1.3, 12)	33.2 (2.3, 12)	31.5 (6.5, 6)		
RBCC ($\times 10^{12}$ cells/L)	0.85 (0.04, 12)	0.85 (0.05, 7)	0.92 (0.04, 2)	0.62 (0.02, 12)	1.19 (0.11, 6)	0.90 (0.26, 3)	*	*
Hb (g/L)	72.5 (3.0, 14)	79.2 (5.2, 9)	52.4 (7.8, 8)	52.7 (1.9, 12)	51.7 (2.9, 12)	49.2 (5.8, 6)		*
MCH (pg/cell)	86.8 (6.4, 12)	94.5 (8.0, 7)	60.1 (3.4, 2)	84.8 (3.0, 12)	45.5 (4.3, 6)	52.4 (3.1, 2)		*
MCHC (g/L)	277 (14, 13)	230 (17, 9)	217 (21, 7)	189 (10, 12)	158 (6, 12)	182 (30, 6)	*	*
MCV (fl)	304 (12, 11)	398 (32, 7)	225 (52, 2)	457 (19, 12)	293 (40, 6)	287 (4, 2)	interaction	

trends of decreasing MCHC and MCH coincided with lower haemoglobin levels. Similar trends in MCV changes were observed for male and female bullies. Mean red cell volumes decreased in Okareka and increased in Tikitapu in the July period. In general, RBC numbers and haemoglobin levels were slightly lower in Tikitapu bullies. The relationship between body size and respiratory demand is worthy of consideration here. Although common bully body size was variable between sample groups, Okareka bullies were up to 2-fold heavier in some cases (Table 2). Thus, body size differences help to explain some of the observed differences in haematology, such as greater RBCs and haemoglobin levels in the larger Okareka fish. Similar to trout, most haematological parameters were particularly influenced by sampling period.

Changes in differential white blood cell counts of male trout were observed between the three sampling periods (Table 4). A reduced ratio of lymphocytes was found in July samples that was most pronounced in the Tikitapu males. The reductions in lymphocyte numbers also coincided with increases in granulocyte numbers and a smaller but significant decrease in thrombocytes. Female differential white cell counts did not mirror the changes in males, although a near significant increase in the proportion of granulocytes from April to July is noteworthy. The changes in differential white cell counts occurred primarily between sampling periods which were mirrored in both lake populations. Changes in humoral immune parameters have previously been demonstrated where lymphocytopenia (depression of lymphocyte numbers) was shown in sexually mature male brown trout compared to immature fish (Pickering 1986). The increased proportion of granulocytes is presumably a function of both fewer lymphocytes and also greater numbers of granulocytes.

3.3 Histology

The density of splenic melano-macrophage centres (MMCs) was significantly greater in the April samples (Fig. 7). Greater MMC area was also found in Okareka spleen samples compared to Tikitapu in the April period. Melano-macrophage centres in fish are aggregations of pigment-containing cells found primarily within the haemopoietic tissues of the spleen and kidney, having various roles associated with iron recycling, toxin metabolism and immune function (Agius and Roberts 2003). These centres have also been linked to natural processes such as aging, starvation, nutritional imbalance and temperature stress (Wolke 1992). Recent research has shown MMCs to be useful indicators in fish of exposure to degraded environments such as hypoxia and sediment contamination (Fournie et al. 2001). Greater splenic MMC areas in both lake trout populations prior to the Phoslock^{TM} application implicate normal, seasonal changes in lake quality or fish physiology.

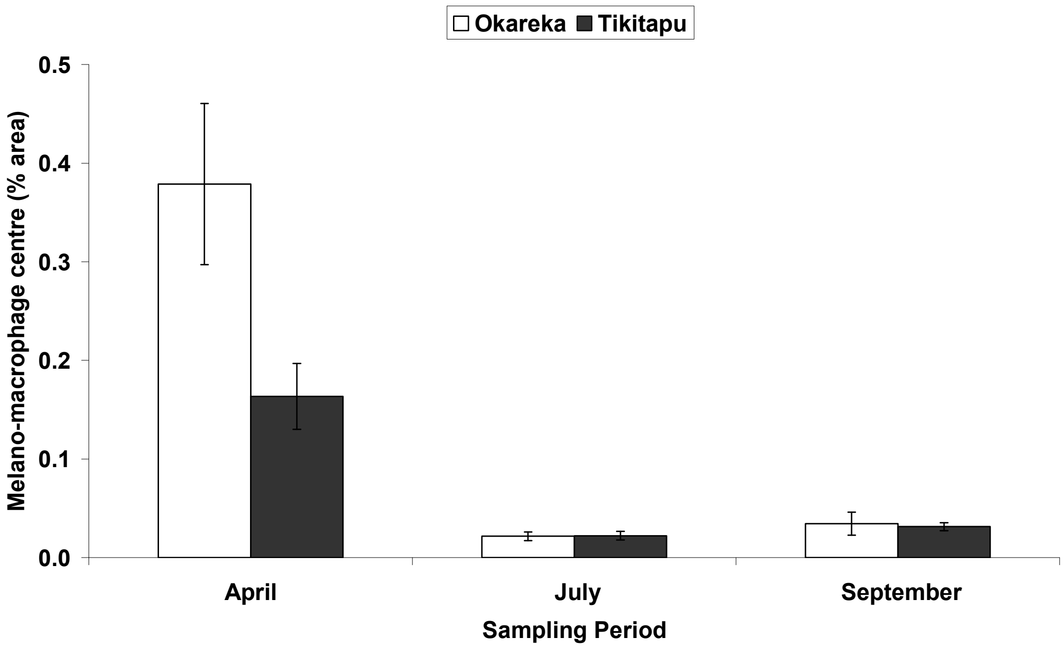

Fig. 7. Density of melanomacrophage centres (MMCs) in splenic tissue of rainbow trout. Greatest MMC densities were measured in both trout populations in the April sampling period. Differences between site were not distinguishable from July to September. Error bars indicate standard error of the mean.

It has been suggested that even in the absence of other observed effects, gill histopathology might still be a relevant early-warning monitoring tool for the health of fish populations (Lease et al. 2003). In the current study, there were no clear histopathological differences in the gills of male or female trout from either lake over the three sampling periods. Examples of several gross lesion types are presented in Fig. 8. Parasites, cysts and vascular congestion were generally uncommon. Low to moderate severity of clubbing or curling at the tips of the secondary lamellae was observed in most fish, regardless of site. Epithelial lifting was also relatively common in most gill samples, suggesting preservation or sectioning artifact. The incidence and severity of other lesions are typically low and there were no consistent signs of tissue reaction, irritation or damage.

Several changes in trout plasma ion concentrations were found over the study period (Table 6). Gills are the main extra-renal osmoregulatory organ in freshwater fish and plasma ions were measured to indicate any possible osmoregulatory disruption in Lake Okareka fish due to mineral exposure. Lanthanum is a known antagonist of calcium channels and has been shown to affect the transport of chloride and calcium ions in fish gills, binding specifically to the apical surface of chloride cells (Perry 1998). Furthermore, Eddy and Bath (1979) observed significant loss of plasma sodium and chloride in response to lanthanum exposure. The significant decline in plasma chloride in Lake Okareka fish immediately following Phoslock™ application may be due to effects of lanthanum on gill osmoregulation, however, plasma chloride declined to comparable levels in Tikitapu fish in September indicating major seasonal changes in plasma levels of this anion that may be associated with reproductive physiology. Chloride strongly influences acid-base regulation in fish and an increased anion gap in fish has been implicated in increased mortality of fish following exhaustive exercise. However, it is not possible to conclusively ascribe the observed changes in plasma ions to Phoslock™ exposure or to predict any adverse impact of these responses without background data on seasonal and reproductive physiology of lacustrine trout or the chronic physiological responses of fish to lanthanum exposure under controlled conditions.

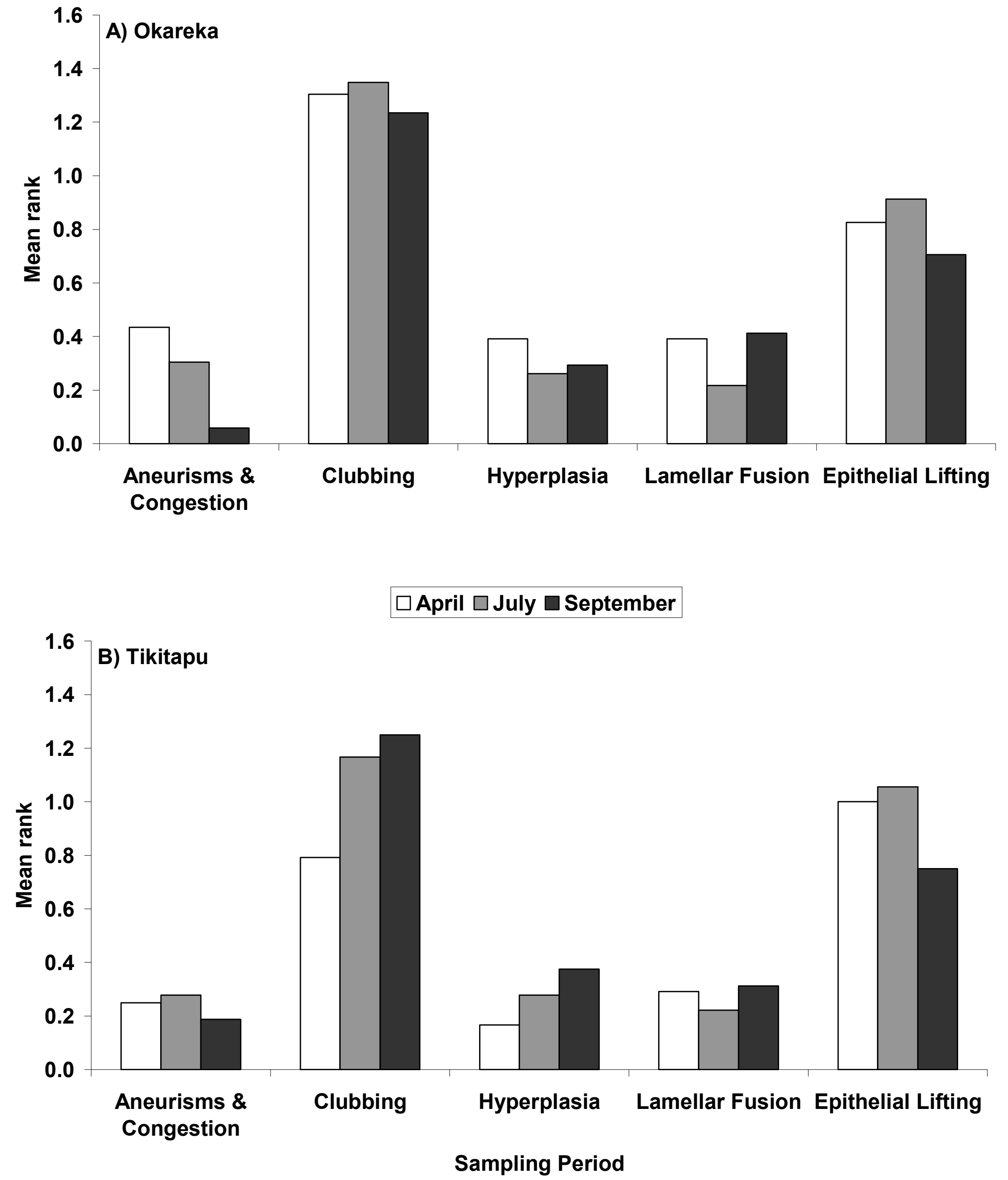

Fig. 8. Gill histopathology observations for rainbow trout from A) Okareka and B) Tikitapu. Frequency and severity of histolpathological lesions were ranked from 0-3, corresponding to none (0), low (1), moderate (2) and severe (3). Data are presented as a mean score of the ranks for each lesion category.

Table 6. Mean (SEM, n) blood plasma ions for male and female rainbow trout. Asterisks indicate significant difference ($p < 0.05$) in overall ANOVA factors for lake and sampling period. Statistical interactions are between lake and sampling period.

	April		July		September		Hypothesis	
	Okareka	Tikitapu	Okareka	Tikitapu	Okareka	Tikitapu	Lake	Period
Males								
Na^+ (mM)	120.2 (3.2, 11)	102.2 (5.0, 11)	170.3 (4.3, 9)	181.4 (7.5, 11)	129.2 (3.9, 14)	142.1 (4.7, 20)	interaction	
K^+ (mM)	1.91 (0.81, 11)	0.97 (0.30, 11)	0.29 (0.03, 9)	0.45 (0.21, 11)	0.35 (0.03, 14)	0.70 (0.25, 20)		*
Cl^- (mM)	131.5 (4.1, 11)	118.4 (1.5, 11)	73.3 (3.1, 9)	110.1 (7.0, 11)	55.4 (3.0, 14)	51.3 (4.3, 20)	interaction	
Mg^{2+} (mM)	0.86 (0.08, 11)	0.73 (0.06, 11)	0.84 (0.05, 9)	1.01 (0.05, 11)	0.56 (0.03, 14)	0.69 (0.04, 20)	interaction	
Ca^{2+} (mM)	1.42 (0.14, 11)	1.28 (0.12, 11)	1.32 (0.06, 9)	1.52 (0.05, 11)	0.88 (0.07, 14)	0.97 (0.07, 20)		*
Females								
Na^+ (mM)	112.2 (5.1, 7)	113.3 (3.8, 6)	170.7 (5.7, 8)	185.2 (7.3, 5)	119.3 (4.8, 3)	-		*
K^+ (mM)	2.49 (1.18, 7)	1.45 (0.57, 6)	0.19 (0.03, 8)	0.18 (0.03, 5)	0.21 (0.02, 3)	-		*
Cl^- (mM)	124.1 (4.5, 7)	120.5 (4.4, 6)	72.4 (2.8, 8)	113.0 (5.1, 5)	45.1 (7.7, 3)	-	interaction	
Mg^{2+} (mM)	1.02 (0.17, 7)	0.93 (0.07, 6)	0.84 (0.05, 8)	1.26 (0.12, 5)	0.48 (0.06, 3)	-	interaction	
Ca^{2+} (mM)	2.03 (0.34, 7)	2.19 (0.19, 6)	1.65 (0.16, 8)	1.82 (0.15, 5)	0.73 (0.13, 3)	-		

3.4 Tissue metals

Lanthanum was not detected in trout flesh collected during the 2005 monitoring period (Landman et al. 2006a). Similarly in 2006, trout flesh lanthanum could not be detected. Concentrations slightly above the limit of detection (0.002 mg/kg) were measured in the flesh of only three out of 99 samples analysed, ranging from 0.0029-0.0095 mg/kg in these samples. The three flesh samples with detectable lanthanum were from Lake Okareka trout in July immediately after the mineral application. Although lanthanum was generally not found in trout flesh tissue, this element is known to accumulate in the internal organs and structures of fish (Hao et al. 1996). Accordingly, detectable levels of lanthanum were found in the livers of all trout captured from both lakes (Fig. 9). Significant increases in liver lanthanum concentration were measured by September in male Okareka trout compared to insignificant increases in Tikitapu. Lake and sampling period changes were observed in female trout as liver lanthanum concentration increased from April to July in both populations, although there was greater overall accumulation in the Okareka population.

Similar patterns of lanthanum accumulation were observed in koura flesh and hepatopancreas (liver equivalent) tissues (Fig. 10). Although greater tail flesh lanthanum concentrations were measured in Okareka koura, concentrations in both lake koura populations were generally low. Significant increases in hepatopancreas lanthanum concentrations were measured in Okareka koura immediately following the mineral application in July and increased further by September. A modest increase in hepatopancreas lanthanum was observed in Tikitapu koura in July, but subsequently decreased by September. In addition to lanthanum, a full suite of metals were measured in the koura tissues. Twenty four out of 30 elements measured were found at detectable levels in most tissue samples. These data have been summarized and presented in Appendix 2. With few exceptions (e.g. Mg, P, K, Cr and Hg), most elements were found in greater concentrations in the hepatopancreas compared to tail flesh. Although not directly related to this particular study, analysis of this data showed contrasting changes and differences with sampling period and lake population for a large number of elements and serves primarily to highlight the seasonal and temporal differences for other measured endpoints.

Accumulation of lanthanum in the koura tissues and trout liver reveals the presence of bioavailable lanthanum sources in both lakes. The higher lanthanum concentrations in Okareka biota demonstrates greater lanthanum bioavailability in this lake. The significant increase in koura hepatopancreas and trout liver lanthanum two weeks and two months, respectively after the Lake Okareka Phoslock^{TM} application implies increased lanthanum bioavailability due to greater lake levels originating from the mineral product. Although lanthanum is known to have a variety of effects such as binding to proteins, enzymes and phosphate, and competing with calcium binding sites (Das et al. 1988), there is a paucity of literature on biologically relevant lanthanum concentrations relating to chronic toxicity in aquatic organisms. Therefore, the levels found in the trout and koura cannot be linked to harmful consequences in this study and assessment of chronic exposure and mechanisms of depuration would be valuable.

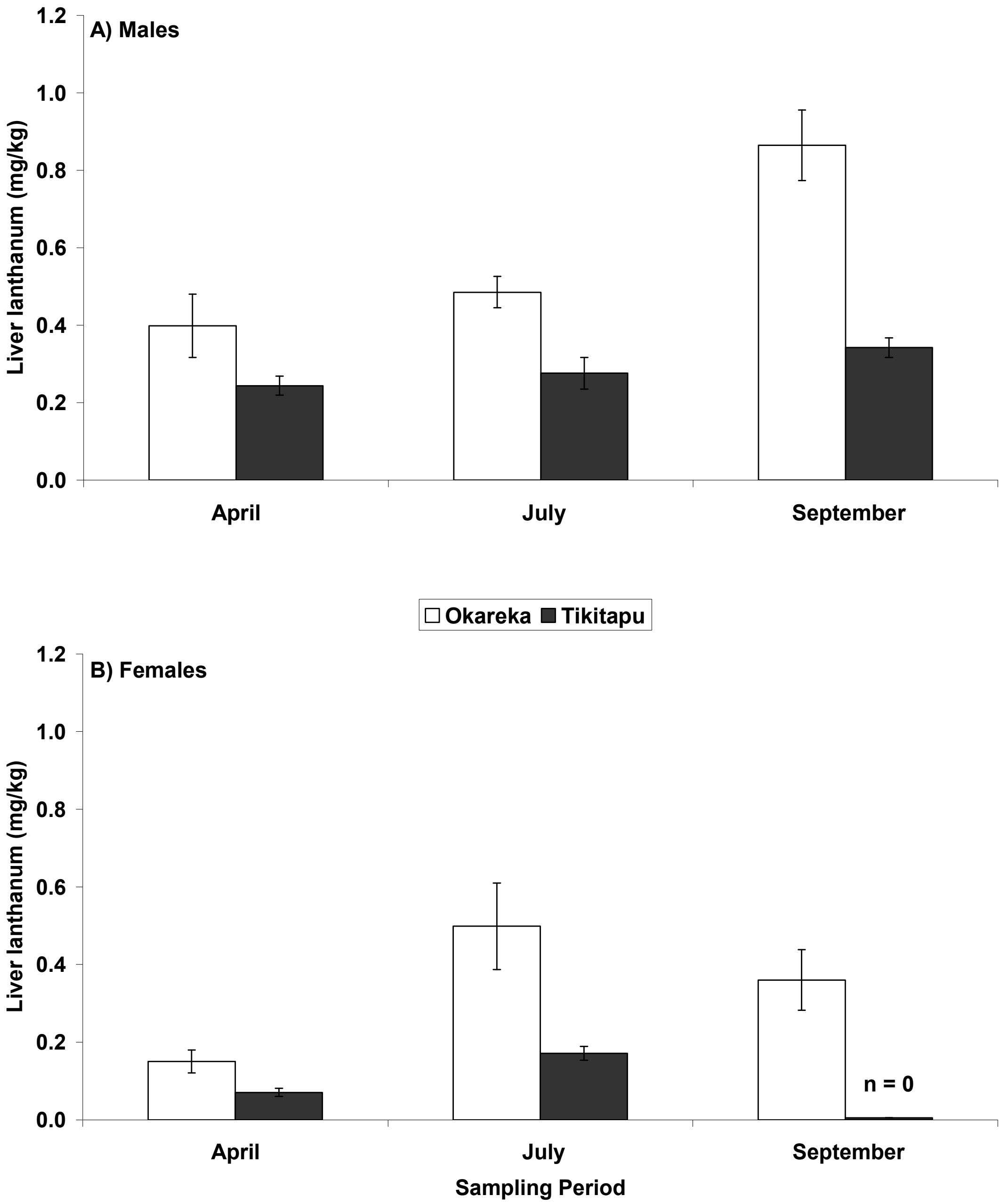

Fig. 9. Liver lanthanum concentration (mg/kg) in A) male and B) female rainbow trout. Greatest lanthanum concentrations were measured in Okareka trout livers. Significant liver concentrations increases were measured from April to September in males and from April to July in females. Error bars indicate standard error of the mean.

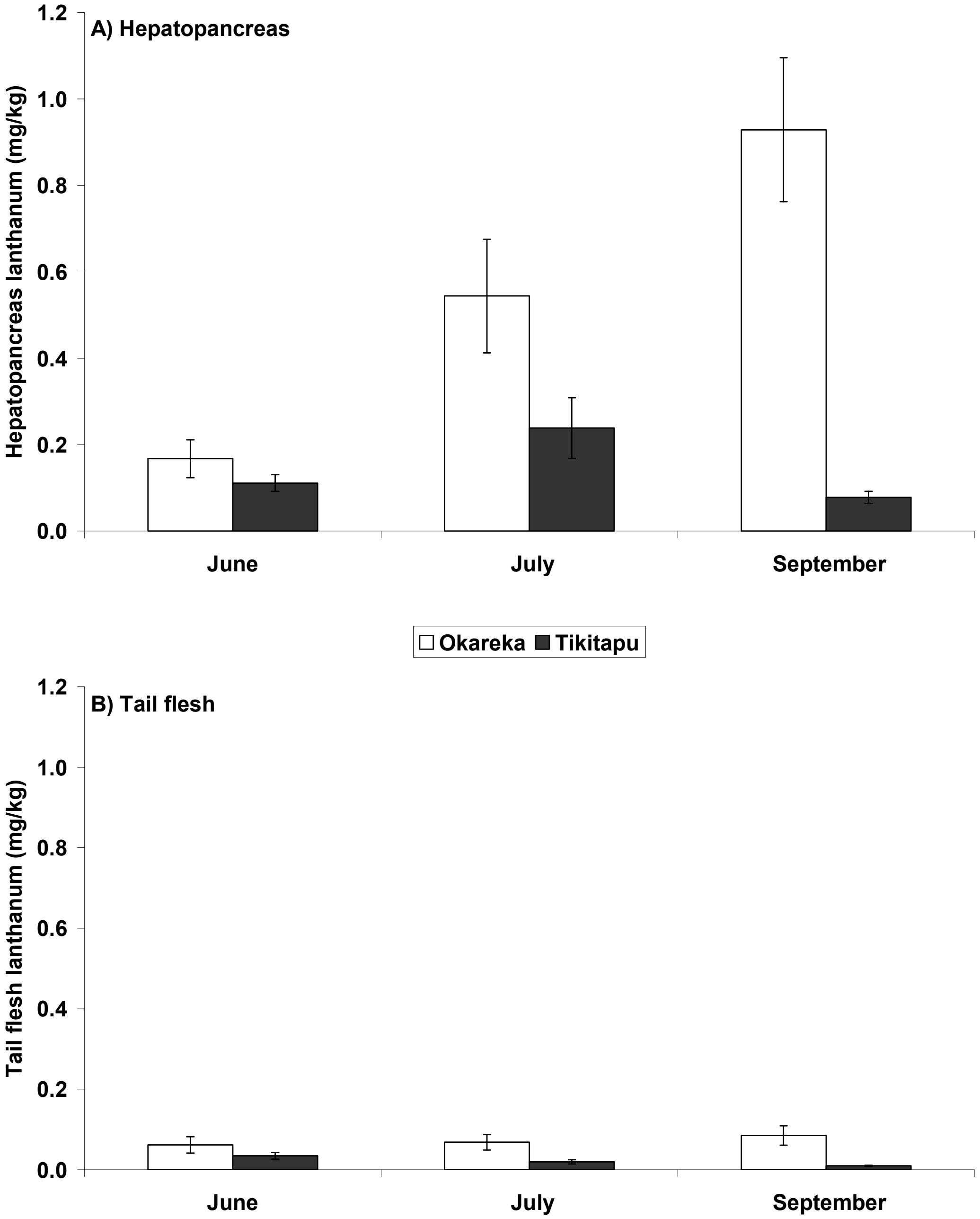

Fig. 10. Koura lanthanum concentration (mg/kg) in A) hepatopancreas and B) tail flesh tissues. Greatest lanthanum concentrations were measured in the hepatopancreas where significant increases were found in Okareka koura following the mineral application. Error bars indicate standard error of the mean.

4.0 CONCLUSIONS

During the 2005 monitoring period, changes in spleen, haematology and histopathology were observed in rainbow trout and common bully. These changes indicated a potential decline in general fish health during that monitoring period which could not be easily attributed to mineral exposure, lake quality or season. A more precautionary approach was employed in the current study. By using Tikitapu as a reference site, we were able to demonstrate seasonal changes in fish physiology that were generally mirrored in both lakes.

Although there is evidence of exposure to greater bioavailable lanthanum in Lake Okareka trout, there was no corresponding evidence of any obvious decline in the health of any species sampled following Phoslock™ application in 2006. Most of the differences observed between lakes Okareka and Tikitapu can be attributed to temporal differences in reproductive synchrony or allometric effects related to the differences in mean size of bullies and koura in these lakes.

5.0 ACKNOWLEDGEMENTS

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6.0 REFERENCES

Agius C and Roberts RJ. 2003. Melano-macrophage centres and their role in fish pathology. Journal of Fish Diseases 26: 499-509.

Dacie JV and Lewis SM. 1991. Practical Haematology, Churchill Livingstone, London, England. pp. 37-85.

Das T, Sharma A and Geeta T. 1988. Effects of lanthanum in cellular systems. Biological Trace Element Research 18: 201-228.

USEPA. 1987. Determination of Metals in Fish Tissue by Inductively Coupled Plasma – Atomic Emission Spectrometry. EPA Method 200.11, Revision 1.3, April 1987.

Douglas GB, Adeney JA and Robb MS. 1999. A novel technique for reducing bioavailable phosphorus in water and sediments. International Association of Water Quality Conference on Diffuse Pollution, pp. 517-523. International Assoc. Water Quality Diffuse Pollution Conference. 16-20 May, 1999, Perth, WA.

Eddy FB and Bath RN. 1979. Effects of lanthanum on sodium and chloride fluxes in the goldfish *Carassius auratus*. Journal of Comparative Physiology B 129: 145-149.

Fournie JW, Summers JK, Courtney LA and Engle VD. 2001. Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. Journal of Aquatic Animal Health 13: 105-116.

Haghseresht F. 2004. The use of Phoslock^{TM} in reducing filterable reactive phosphorus level in water bodies: an overview of the properties of Phoslock^{TM} and its performance in improving water quality. March 2004 report, Integrated Mineral Technology Holdings Ltd.

Hao S, Xiaorong W, Zhaozhe H, Chonghua W, Liansheng W, Lemei D, Zhong L and Yijun C. 1996. Bioconcentration and elimination of five light rare earth elements in carp (*Cyprinus carpio* L.). Chemosphere 33: 1475-1483.

Lake Okareka Catchment Management Action Plan. 2003. Environment Bay of Plenty Environmental Publication 2003/23.

Landman MJ, van den Heuvel MR and Stuthridge TR. 2006a. Lake Okareka Fish Health monitoring 2005. Scion Report prepared for Environment Bay of Plenty. 12 April 2006.

Landman MJ, Ling N and Stuthridge TR. 2006b. Lake Okareka and Tikitapu Fish Health Monitoring 2006: Trout Baseline Study. Scion Report prepared for Environment Bay of Plenty. 31 May 2006.

Lease HM, Hansen JA, Bergman HL and Meyer JS. 2003. Structural changes in gills of Lost River suckers exposed to elevated pH and ammonia concentrations. Comparative Biochemistry and Physiology Part C 134: 491-500.

Mallat J. 1985. Fish gill structural changes induced by toxicants and other irritants, a statistical review. Canadian Journal of Fisheries and Aquatic Sciences 42: 630-648.

Martin ML and Hickey CW. 2004. Determination of HSNO ecotoxic thresholds for granular Phoslock™ (Eureka 1 formulation) phase 1: acute toxicity. NIWA client report: HAM2004-137.

McIntosh J. 2006. Phoslock application – Lake Okareka. Environment Bay of Plenty Environmental Publication 2006/06.

Perry SF. 1998. The chloride cell: structure and function in the gills of freshwater fishes. Annual Review of Physiology 59: 325-347.

Pickering AD. 1986. Changes in blood cell composition of the brown trout, *Salmo trutta* L., during the spawning season. Journal of Fish Biology 29: 335-347.

Pottinger TG and Pickering AD. 1987. Androgen levels and erythrocytosis in maturing brown trout, *Salmo trutta* L. Fish Physioly Biochemistry 3: 121-126.

Robb M, Greenop B, Goss Z, Douglas G and Adeney J. 2003. Application of Phoslock^{TM}, an innovative phosphorus binding clay, to two Western Australian waterways: preliminary findings. Hydrobiologica 494: 237-243.

Sokal, RR, Rohlf FJ. 1973. Introduction to Biostatistics. W. H. Freeman and Company, San Francisco.

Stauber JL and Binet MT. 200. Canning River Phoslock^{TM} field trial – Ecotoxicity testing final report; CSIRO Report ET317R.

van den Heuvel MR, Landman MJ, West DW and Finley M. 2006. Altered physiology of rainbow trout in response to modified energy intake combined with pulp and paper effluent exposure. Canadian Journal of Fisheries and Aquatic Sciences, In review

Wintrobe MM. 1934. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Hematologica 51: 32-49.

Wolke RE. 1992. Piscine macrophage aggregates: a review. Annual Review of Fish Diseases 2: 91-108.

APPENDIX 1 TROUT LIVER LANTHANUM REPORTS

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Client: *Scion*
Address: *Private Bag 3020, ROTORUA*
Contact: *Michael Landman*

Laboratory No: *416611*
Date Registered: *1/05/2006*
Date Completed: *5/05/2006*
Page Number: *1 of 2*

Client's Reference: Fish Liver Lanthanum

The results for the analyses you requested are as follows:

Sample Type: Biological Materials, Fish/shellfish tissue

Sample Name	Lab No	Lanthanum (mg/kg as rcvd)
OKT601	416611/1	0.135
OKT602	416611/2	0.094
OKT603	416611/3	0.350
OKT604	416611/4	0.381
OKT605	416611/5	0.614
OKT607	416611/6	0.343
OKT608A	416611/7	0.126
OKT608B	416611/8	0.080
OKT609	416611/9	0.158
OKT610	416611/10	1.37
OKT611	416611/11	0.208
OKT612	416611/12	0.162
OKT613	416611/13	0.133
OKT614	416611/14	0.507
OKT615	416611/15	0.103
OKT616	416611/16	0.098
OKT617	416611/17	0.340
OKT618	416611/18	0.195
OKT619	416611/19	0.225
OKT620	416611/20	0.317
OKT621	416611/21	0.402
OKT622	416611/22	0.658
OKT623	416611/23	0.243
OKT624	416611/24	0.085
BLT601	416611/25	0.046
BLT602	416611/26	0.113
BLT603	416611/27	0.226

Sample Name	Lab No	Lanthanum (mg/kg as rcvd)
BLT604	416611/28	0.356
BLT605	416611/29	0.162
BLT606	416611/30	0.059
BLT607	416611/31	0.033
BLT608	416611/32	0.103
BLT609	416611/33	0.343
BLT610	416611/34	0.297
BLT611	416611/35	0.226
BLT612	416611/36	0.231
BLT613	416611/37	0.195
BLT614	416611/38	0.357
BLT615	416611/39	0.248
BLT616	416611/40	0.079
BLT617	416611/41	0.089
BLT618	416611/42	0.403
BLT619	416611/43	0.105
BLT620	416611/44	0.117
BLT621	416611/45	0.044
BLT622	416611/46	0.262
BLT623	416611/47	0.108

Summary of Methods Used and Detection Limits

The following table(s) gives a brief description of the methods used to conduct the analyses for this job.
The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Substance Type: Biological Materials

Parameter	Method Used	Detection Limit
Biological Materials Digest	Nitric/hydrochloric acid digestion.	N/A
Lanthanum	Nitric/hydrochloric acid digestion. ICP-MS determination.	0.002 mg/kg as rcvd

Analyst's Comments:

These samples were collected by yourselves and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the submitter.

Roger Haslemore B.Sc., Ph.D., MNZIC
Food and Industrial Client Services Manager

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ANALYSIS REPORT

Client:	Scion	**Lab No:**	600655	Version 1
Contact:	Landman, Michael	**Date Registered:**	04-Aug-2006	
	c/o Scion	**Date Reported:**	09-Aug-2006	
	Private Bag 3020	**Quote No:**	30082	
	ROTORUA	**Order No:**		
		Client Reference:	Lanthanum in trout tissue	
		Submitted By:	Landman, Michael	

Sample Type: Fish						
	Sample Name:	OKT 25	OKT 26	OKT 27	OKT 28	OKT 29
	Lab Number:	600655.1	600655.2	600655.3	600655.4	600655.5
Lanthanum	mg/kg as rcvd	1.3	0.58	0.74	0.76	0.45
Sample Type: Fish						
	Sample Name:	OKT 30	OKT 31	OKT 32	OKT 33	OKT 34
	Lab Number:	600655.6	600655.7	600655.8	600655.9	600655.10
Lanthanum	mg/kg as rcvd	0.83	0.35	0.37	0.096	0.44
Sample Type: Fish						
	Sample Name:	OKT 35	OKT 36	OKT 37	OKT 38	OKT 39
	Lab Number:	600655.11	600655.12	600655.13	600655.14	600655.15
Lanthanum	mg/kg as rcvd	0.43	0.47	0.47	0.23	0.43
Sample Type: Fish						
	Sample Name:	OKT 40	OKT 41	OKT 42	OKT 43	OKT 44
	Lab Number:	600655.16	600655.17	600655.18	600655.19	600655.20
Lanthanum	mg/kg as rcvd	0.39	0.63	0.48	0.43	0.22
Sample Type: Fish						
	Sample Name:	OKT 45	OKT 46	OKT 47	OKT 48	BLT 24
	Lab Number:	600655.21	600655.22	600655.23	600655.24	600655.25
Lanthanum	mg/kg as rcvd	0.68	0.38	0.22	0.40	0.17
Sample Type: Fish						
	Sample Name:	BLT 25	BLT 26	BLT 27	BLT 28	BLT 30
	Lab Number:	600655.26	600655.27	600655.28	600655.29	600655.30
Lanthanum	mg/kg as rcvd	0.17	0.47	0.42	0.34	0.12
Sample Type: Fish						
	Sample Name:	BLT 31	BLT 32	BLT 33	BLT 34	BLT 35
	Lab Number:	600655.31	600655.32	600655.33	600655.34	600655.35
Lanthanum	mg/kg as rcvd	0.25	0.34	0.35	0.21	0.19
Sample Type: Fish						
	Sample Name:	BLT 36	BLT 37	BLT 38	BLT 39	BLT 40
	Lab Number:	600655.36	600655.37	600655.38	600655.39	600655.40
Lanthanum	mg/kg as rcvd	0.087	0.16	0.16	0.11	0.27
Sample Type: Fish						
	Sample Name:	BLT 41	BLT 42	BLT 43		
	Lab Number:	600655.41	600655.42	600655.43		
Lanthanum	mg/kg as rcvd	0.10	0.23	0.15	-	-

No Analyst's Comments

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Fish

Test	Method Description	Default Detection Limit	Samples
Homogenise	Mincing, chopping, or blending of sample to form homogenous sample fraction. AOAC 17th Edition	-	1-43
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, 85°C for 1 hour.	-	1-43
Lanthanum	Biological materials digestion, ICP-MS.	0.0020 mg/kg as rcvd	1-43

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

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Hill Laboratories

ANALYSIS REPORT

TMPv1

Client:	Scion	**Lab No:**	601171
Contact:	Landman, Michael	**Date Registered:**	27-Sep-2006
	c/o Scion	**Date Reported:**	25-Oct-2006
	Private Bag 3020	**Quote No:**	30098
	ROTORUA	**Order No:**	PU037582
		Client Reference:	Trout Liver Analysis
		Submitted By:	Landman, Michael

Sample Type: Liver

	Sample Name:	BLT0644	BLT0645	BLT0646	BLT0647	BLT0648
	Lab Number:	601171.1	601171.2	601171.3	601171.4	601171.5
Lanthanum	mg/kg as rcvd	0.33	0.39	0.38	0.53	0.48

Sample Type: Liver

	Sample Name:	BLT0649	BLT0650	BLT0651	BLT0652	BLT0653
	Lab Number:	601171.6	601171.7	601171.8	601171.9	601171.10
Lanthanum	mg/kg as rcvd	0.41	0.25	0.30	0.37	0.20

Sample Type: Liver

	Sample Name:	BLT0654	BLT0655	BLT0656	BLT0657	BLT0658
	Lab Number:	601171.11	601171.12	601171.13	601171.14	601171.15
Lanthanum	mg/kg as rcvd	0.18	0.21	0.26	0.45	0.55

Sample Type: Liver

	Sample Name:	BLT0659	BLT0660	BLT0661	BLT0662	BLT0663
	Lab Number:	601171.16	601171.17	601171.18	601171.19	601171.20
Lanthanum	mg/kg as rcvd	0.36	0.29	0.20	0.27	0.43

Sample Type: Liver

	Sample Name:	OKT0649	OKT0650	OKT0651	OKT0652	OKT0653
	Lab Number:	601171.21	601171.22	601171.23	601171.24	601171.25
Lanthanum	mg/kg as rcvd	0.22	0.51	1.0	0.59	1.1

Sample Type: Liver

	Sample Name:	OKT0654	OKT0655	OKT0656	OKT0657	OKT0658
	Lab Number:	601171.26	601171.27	601171.28	601171.29	601171.30
Lanthanum	mg/kg as rcvd	0.81	0.37	1.5	0.42	0.69

Sample Type: Liver

	Sample Name:	OKT0659	OKT0660	OKT0661	OKT0662	OKT0663
	Lab Number:	601171.31	601171.32	601171.33	601171.34	601171.35
Lanthanum	mg/kg as rcvd	0.47	0.86	0.97	0.49	0.54

Sample Type: Liver

	Sample Name:	OKT0664	OKT0665	OKT0666		
	Lab Number:	601171.36	601171.37	601171.38		
Lanthanum	mg/kg as rcvd	0.97	1.6	0.94	-	-

No Analyst's Comments

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Liver

Test	Method Description	Default Detection Limit	Samples
Homogenise	Mincing, chopping, or blending of sample to form homogenous sample fraction. AOAC 17th Edition	-	1-38
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, 85°C for 1 hour. H.S. Niu and R.S. Houk. Spectrochem. Acta, part B, 1996, 51, 779.	-	1-38
Lanthanum	Biological materials digestion, ICP-MS.	0.0020 mg/kg as rcvd	1-38

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

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Roger Haslemore B.Sc., PhD
Lead Quality Assurance Auditor

APPENDIX 2 TROUT FLESH LANTHANUM REPORT

Hill Laboratories

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ANALYSIS REPORT

TMPv1

Client:	Scion	**Lab No:**	601545
Contact:	Landman, Michael c/o Scion Private Bag 3020 ROTORUA	**Date Registered:**	03-Nov-2006
		Date Reported:	24-Nov-2006
		Quote No:	30098
		Order No:	
		Client Reference:	Fish Liver Lanthanum
		Submitted By:	Landman, Michael

Sample Type: Fish

	Sample Name:	OKT 0616	OKT 0617	OKT 0618	OKT 0619	OKT 0620
	Lab Number:	601545.1	601545.2	601545.3	601545.4	601545.5
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0621	OKT 0622	OKT 0623	OKT 0624	OKT 0625
	Lab Number:	601545.6	601545.7	601545.8	601545.9	601545.10
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0626	OKT 0627	OKT 0628	OKT 0629	OKT 0630
	Lab Number:	601545.11	601545.12	601545.13	601545.14	601545.15
Lanthanum	mg/kg as rcvd	0.0053	< 0.0020	0.0048	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0631	OKT 0632	OKT 0633	OKT 0634	OKT 0635
	Lab Number:	601545.16	601545.17	601545.18	601545.19	601545.20
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0636	OKT 0637	OKT 0638	OKT 0639	OKT 0640
	Lab Number:	601545.21	601545.22	601545.23	601545.24	601545.25
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	0.0095

Sample Type: Fish

	Sample Name:	OKT 0641	OKT 0642	OKT 0643	OKT 0644	OKT 0645
	Lab Number:	601545.26	601545.27	601545.28	601545.29	601545.30
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	0.0029	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0646	OKT 0647	OKT 0648	OKT 0649	OKT 0651
	Lab Number:	601545.31	601545.32	601545.33	601545.34	601545.35
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0654	OKT 0655	OKT 0656	OKT 0657	OKT 0658
	Lab Number:	601545.36	601545.37	601545.38	601545.39	601545.40
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0659	OKT 0660	OKT 0661	OKT 0662	OKT 0663
	Lab Number:	601545.41	601545.42	601545.43	601545.44	601545.45
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish

	Sample Name:	OKT 0665	OKT 0666	BLT 0601	BLT 0602	BLT 0603
	Lab Number:	601545.46	601545.47	601545.48	601545.49	601545.50
Lanthanum	mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0604	BLT 0605	BLT 0606	BLT 0607	BLT 0608
Lab Number:	601545.51	601545.52	601545.53	601545.54	601545.55
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0609	BLT 0610	BLT 0611	BLT 0612	BLT 0613
Lab Number:	601545.56	601545.57	601545.58	601545.59	601545.60
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0614	BLT 0615	BLT 0616	BLT 0617	BLT 0618
Lab Number:	601545.61	601545.62	601545.63	601545.64	601545.65
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0619	BLT 0620	BLT 0621	BLT 0622	BLT 0623
Lab Number:	601545.66	601545.67	601545.68	601545.69	601545.70
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0624	BLT 0625	BLT 0626	BLT 0627	BLT 0628
Lab Number:	601545.71	601545.72	601545.73	601545.74	601545.75
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0630	BLT 0631	BLT 0633	BLT 0634	BLT 0635A
Lab Number:	601545.76	601545.77	601545.78	601545.79	601545.80
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0635B	BLT 0636	BLT 0637	BLT 0638	BLT 0639
Lab Number:	601545.81	601545.82	601545.83	601545.84	601545.85
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0640	BLT 0641	BLT 0642	BLT 0643	BLT 0646
Lab Number:	601545.86	601545.87	601545.88	601545.89	601545.90
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	BLT 0652	BLT 0653	BLK 0654	BLK 0656	BLK 0657
Lab Number:	601545.91	601545.92	601545.93	601545.94	601545.95
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	< 0.0020

Sample Type: Fish					
Sample Name:	OKT 0658	OKT 0659	OKT 0661	OKT 0662	
Lab Number:	601545.96	601545.97	601545.98	601545.99	
Lanthanum mg/kg as rcvd	< 0.0020	< 0.0020	< 0.0020	< 0.0020	-

No Analyst's Comments

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Fish			
Test	**Method Description**	**Default Detection Limit**	**Samples**
Homogenise	Mincing, chopping, or blending of sample to form homogenous sample fraction. AOAC 17th Edition	-	1-99
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, 85°C for 1 hour.	-	1-99
Lanthanum	Biological materials digestion, ICP-MS.	0.0020 mg/kg as rcvd	1-99

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Mark Bryant, NZCS (Chemistry)
Senior Technologist - Environmental Inorganics

APPENDIX 3 KOURA TISSUE METALS RESULTS

Table 1a. Koura hepatopancreas metals suite. Metal data are presented as **mean** ± sem (mg/kg) tissue concentrations for each sampling period and lake.

Lake	Period	n	**B 10**	**Na 23**	**Mg 24**	**Al 27**	**P 31**	**K 39**	**Ca 43**	**V 51**	**Cr 53**	**Fe 54**	**Mn 55**	**Co 59**
Okareka	June	10	**0.74**	**1867**	**182**	**2.60**	**1828**	**2293**	**236**	**0.48**	**0.17**	**57.0**	**85.3**	**0.82**
			0.16	87	21	0.79	138	145	11	0.01	0.01	11.6	18.2	0.12
	July	10	**2.79**	**1806**	**258**	**1.70**	**2032**	**2498**	**269**	**0.53**	**0.19**	**77.1**	**202.5**	**0.78**
			0.81	104	29	0.30	125	142	25	0.02	0.01	14.1	53.5	0.13
	Sept	10	**2.79**	**1679**	**276**	**3.13**	**2084**	**2492**	**228**	**0.51**	**0.17**	**107.5**	**257.1**	**0.86**
			0.80	170	32	0.74	164	203	24	0.03	0.02	29.8	49.4	0.17
Tikitapu	June	10	**1.60**	**2040**	**200**	**4.77**	**1767**	**2648**	**218**	**0.11**	**0.19**	**44.2**	**22.0**	**0.45**
			0.45	179	16	1.47	120	136	15	0.04	0.04	11.1	4.0	0.04
	July	10	**5.24**	**2320**	**244**	**8.87**	**2250**	**2576**	**359**	**0.06**	**0.10**	**45.4**	**32.5**	**0.37**
			1.29	113	24	1.80	142	165	44	0.04	0.03	6.8	7.1	0.04
	Sept	14	**2.81**	**1557**	**215**	**10.09**	**2388**	**3095**	**179**	**0.19**	**0.35**	**74.1**	**66.3**	**0.67**
			0.39	78	13	1.26	125	94	12	0.04	0.05	10.1	14.8	0.08

Table 1b. Koura hepatopancreas metals suite continued.

Lake	Period	n	**Cu 63**	**Zn 68**	**As 75**	**Se 82**	**Sr 88**	**Ag 109**	**Cd 111**	**Ba 137**	**La 139**	**Hg 202**	**Tl 205**	**Pb 206**
Okareka	June	10	**379.2**	**155.9**	**2.06**	**1.01**	**2.74**	**0.69**	**0.63**	**2.67**	**0.17**	**2.22**	**0.003**	**0.018**
			85.1	24.0	0.19	0.06	0.33	0.09	0.10	0.65	0.04	0.57	0.001	0.003
	July	10	**211.3**	**100.3**	**3.18**	**0.94**	**6.15**	**0.63**	**0.65**	**9.01**	**0.54**	**2.04**	**0.006**	**0.014**
			51.3	10.0	0.52	0.12	1.39	0.10	0.09	2.61	0.13	0.29	0.001	0.003
	Sept	10	**212.5**	**139.5**	**3.42**	**0.94**	**5.50**	**0.53**	**0.55**	**11.32**	**0.93**	**2.22**	**0.010**	**0.022**
			78.1	28.4	0.80	0.09	1.31	0.15	0.11	3.03	0.17	0.16	0.002	0.005
Tikitapu	June	10	**169.5**	**114.9**	**0.79**	**1.29**	**3.80**	**0.13**	**0.56**	**3.79**	**0.11**	**0.65**	**0.038**	**0.152**
			48.8	20.5	0.08	0.09	0.67	0.03	0.11	0.86	0.02	0.30	0.003	0.028
	July	10	**80.8**	**98.0**	**1.07**	**1.37**	**10.23**	**0.09**	**0.79**	**10.59**	**0.24**	**0.01**	**0.053**	**0.383**
			20.7	26.6	0.16	0.11	2.53	0.02	0.16	2.87	0.07	0.01	0.004	0.088
	Sept	14	**55.5**	**74.1**	**1.09**	**1.28**	**4.19**	**0.08**	**0.83**	**7.44**	**0.08**	**0.10**	**0.070**	**0.243**
			20.8	10.2	0.10	0.09	0.56	0.02	0.11	0.94	0.01	0.08	0.006	0.048

Table 2a. Koura tail flesh metals suite. Metal data are presented as **mean** ± sem (mg/kg) tissue concentrations for each sampling period and lake.

Lake	Period	n	B 10	Na 23	Mg 24	Al 27	P 31	K 39	Ca 43	V 51	Cr 53	Fe 54	Mn 55	Co 59
Okareka	June	10	**0.04**	**1065**	**203**	**0.65**	**2229**	**3000**	**136**	**0.26**	**0.27**	**1.36**	**1.48**	**0.01**
			0.02	96	9	0.15	100	170	21	0.02	0.01	0.20	0.20	0.00
	July	10	**0.03**	**1041**	**227**	**0.44**	**2534**	**3590**	**116**	**0.32**	**0.25**	**1.26**	**4.18**	**0.01**
			0.01	59	9	0.07	95	173	9	0.01	0.00	0.15	1.95	0.00
	Sept	10	**0.03**	**1068**	**225**	**0.67**	**2553**	**3349**	**152**	**0.34**	**0.25**	**1.55**	**2.65**	**0.01**
			0.00	61	12	0.12	97	141	16	0.01	0.00	0.25	1.12	0.00
Tikitapu	June	10	**0.07**	**1015**	**272**	**0.31**	**2623**	**3806**	**102**	**0.17**	**0.28**	**1.15**	**0.49**	**0.02**
			0.02	77	8	0.14	54	90	4	0.05	0.07	0.29	0.05	0.00
	July	10	**0.11**	**1297**	**283**	**0.95**	**2526**	**3610**	**159**	**0.17**	**0.43**	**1.66**	**0.75**	**0.02**
			0.03	101	10	0.22	93	106	22	0.04	0.02	0.12	0.12	0.00
	Sept	14	**0.08**	**941**	**266**	**2.59**	**2713**	**3491**	**122**	**0.25**	**0.38**	**2.50**	**0.90**	**0.02**
			0.03	37	9	0.91	67	84	9	0.03	0.04	0.59	0.13	0.00

Table 2b. Koura tail flesh metals suite continued.

Site	Period	n	Cu 63	Zn 68	As 75	Se 82	Sr 88	Ag 109	Cd 111	Ba 137	La 139	Hg 202	Tl 205	Pb 206
Okareka	June	10	**2.87**	**16.0**	**0.36**	**0.12**	**0.44**	**0.009**	**0.002**	**0.13**	**0.06**	**3.78**	**0.001**	**0.004**
			0.56	1.2	0.02	0.01	0.07	0.002	0.000	0.02	0.02	0.52	0.000	0.001
	July	10	**4.44**	**15.7**	**0.93**	**0.12**	**0.45**	**0.015**	**0.002**	**0.21**	**0.07**	**4.76**	**0.002**	**0.002**
			0.67	0.6	0.25	0.00	0.05	0.003	0.000	0.05	0.02	0.81	0.000	0.000
	Sept	10	**4.46**	**17.1**	**0.63**	**0.12**	**0.56**	**0.014**	**0.001**	**0.30**	**0.09**	**6.30**	**0.004**	**0.002**
			0.93	1.5	0.07	0.00	0.09	0.004	0.000	0.07	0.02	0.79	0.001	0.000
Tikitapu	June	10	**4.89**	**14.9**	**0.16**	**0.26**	**0.36**	**0.004**	**0.003**	**0.13**	**0.03**	**0.73**	**0.008**	**0.040**
			0.46	1.0	0.01	0.01	0.01	0.001	0.001	0.02	0.01	0.21	0.001	0.025
	July	10	**5.40**	**12.9**	**0.22**	**0.24**	**0.68**	**0.005**	**0.008**	**0.31**	**0.02**	**0.05**	**0.009**	**0.017**
			0.80	0.6	0.03	0.03	0.13	0.001	0.002	0.05	0.00	0.05	0.001	0.004
	Sept	14	**5.27**	**12.7**	**0.29**	**0.29**	**0.37**	**0.004**	**0.003**	**0.24**	**0.01**	**0.27**	**0.013**	**0.016**
			0.36	0.3	0.03	0.02	0.04	0.001	0.001	0.03	0.00	0.12	0.001	0.004