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30 October 2007

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## EXECUTIVE SUMMARY

Phoslock™ 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™ have been carried out in 2005, 2006 and 2007 as part of the Lake Okareka management plan. Fish health monitoring in Lake Okareka following the 2005 Phoslock™ application identified changes in fish health but these could not be directly attributed to Phoslock™ exposure. Two subsequent studies in 2006 and 2007 were conducted to examine fish health following repeat mineral applications in Lake Okareka. The 2006 and 2007 studies included the use of a reference lake (Tikitapu) to delineate seasonal or lake-specific changes in fish health, and also utilized a benthic invertebrate species (koura) to monitor contaminant accumulation.

For two successive years, the results demonstrate that trout and koura in Lake Okareka significantly accumulate lanthanum in the liver and hepatopancreas tissues following the mineral Phoslock™ application. Lanthanum accumulation in the flesh of these organisms is generally low and has been measured in only a small number of specimens from each sampling period after mineral application. Lanthanum accumulation is an effective marker of exposure to the Phoslock™ product in trout and crayfish. Levels prior to lake dosing are low, suggesting that the interval between applications is sufficient to allow the biota to depurate the lanthanum accumulated by the previous administration. There is little literature available on the effects of long-term lanthanum exposure or on the long-term effects of repeated sub-lethal, acute exposures in aquatic organisms.

Small yet significant changes in some physiological parameters such as energy allocation and haematology do occur over time and between lakes. Most measured changes occurred during reproductive development, and

spawning, but are generally mirrored in both lake populations. Moderate between-lake differences are observed and are suspected to be linked to variations in lake water quality and chemistry, reproductive timing and allometry.

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## 1.0 INTRODUCTION

In the Lake Okareka Catchment Management Plan (EBOP, 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™ 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™ applications have been performed in Western Australia on the Swan and Canning Rivers (Douglas et al. 1999; Robb et al. 2003) and now over three successive years 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™ 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™ application. However, the cause of this fish health change could not be resolved.

A subsequent fish health assessment of Lake Okareka and Tikitapu rainbow trout (*Oncorhynchus mykiss*) was performed in early 2006 to examine

recovery (Landman et al 2006b). Trout health was found to have improved over the period of October 2005 to April 2006. Continued monitoring took place following a second application of Phoslock™ in June 2006. This study found significant accumulation of lanthanum in Lake Okareka trout and koura (*Paranephrops planifrons*), demonstrating that lanthanum derived from the Phoslock™ application was bioavailable to these organisms (Landman and Ling 2006). Moderate changes in other parameters such as haematology and histopathology were found to be seasonally or physiologically influenced. In general, trout and common bully (*Gobiomorphus cotidianus*) from both lakes were in relatively good health throughout the 2006 monitoring cycle.

The current study is a continuation of the Lake Okareka fish health monitoring program initiated in 2005, focusing on the 2007 mineral application. In this study, rainbow trout, common bully and koura were investigated in Lake Okareka and neighboring Lake Tikitapu for the purposes of comparison.

## 2.0 METHODS

### 2.1 Fish collection

Twenty tonnes of Phoslock™ was applied to Lake Okareka between 20-23 March 2007. This was approximately three months earlier than in 2006 and five months earlier than 2005. Fish capture and sampling was timed around the current 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.

The initial sampling of trout, bully and koura was performed in the weeks (14-23 March) prior to the mineral application. All species were then collected at approximately two weeks (10-17 April) and two months (6-15 June) post-mineral application.

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 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 SCUBA divers.

### 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 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 archived at -20°C. Common bullies were immediately transported back to the laboratory after capture. Fish were first anaesthetised with MS-222 (0.1 g $L^{-1}$). Approximately 15-100 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 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 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) for trout were determined by flow cytometry at Scion. Two microlitres of whole blood was mixed with 98 µL of red cell diluting fluid and stored on ice for manual count validation. Manual bully 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).

## 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 haematoxylin-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 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

A suite of metals were measured in trout and koura 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 and MMCs were measured as percentages, data were arcsine transformed prior to analysis (Sokal and Rohlf, 1973).

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

### 3.1 General physiological parameters

General physiological parameters have been summarized and tabulated for ease of comparison in Tables 1-3.

Although trout were abundant in both lakes, March fishing in Lake Okareka was difficult due to particularly warm surface waters. In order to capture fish at this time, deep-set gill netting was necessary as fish could not be captured by setting along the shoreline. Some small, yet statistically significant changes were noted in male trout condition and other somatic indices (Table 1). However, over the course of the entire monitoring period, these parameters were relatively constant. Female condition was also constant throughout the study. More obvious changes in female liver- and spleen-somatic indices were observed. Spleen-somatic index gradually decreased over time, while LSI did not decrease until the June sampling period. Female GSI changed considerably, with an obvious increase over approximately four weeks between March and April, until fish were nearly ripe or already spawning in June.

Common bully abundance was lower in Tikitapu, typically requiring 3 fishing days per period to obtain sufficient numbers and larger specimens. Tikitapu bullies were slightly smaller and lighter per unit length, which was reflected in lower mean condition factors in this population (Table 2). In both populations there was a trend of increasing condition over the monitoring periods. Liver-somatic index increased for all bullies, although this trend was slightly delayed in Tikitapu males and Okareka females. Male and female GSI was largely unchanged throughout the study.

Large koura were abundant in Lake Okareka (Table 3). Smaller koura dominate Tikitapu and more effort was required to obtain sufficiently sized

specimens for sampling. Tikitapu koura were significantly smaller and lighter per unit length.

**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.

| | March | | April | | June | | Hypothesis | |
|---|---|---|---|---|---|---|---|---|
| | Okareka | Tikitapu | Okareka | Tikitapu | Okareka | Tikitapu | Lake | Period |
| **Males** | | | | | | | | |
| Length (mm) | 540 (20, 5) | 513 (11, 11) | 547 (10, 8) | 536 (14, 6) | 525 (12, 10) | 539 (6, 13) | | |
| Weight (g) | 1606 (112, 5) | 1494 (121, 11) | 1684 (103, 8) | 1557 (149, 6) | 1582 (90, 10) | 1804 (93, 13) | | |
| Condition (K) | 0.99 (0.05, 5) | 1.04 (0.03, 11) | 0.98 (0.02, 8) | 0.94 (0.02, 6) | 1.05 (0.01, 10) | 1.10 (0.03, 13) | | * |
| GSI | 2.37 (0.76, 5) | 2.81 (0.34, 11) | 2.90 (0.24, 8) | 4.14 (0.80, 6) | 2.74 (0.20, 9) | 3.22 (0.31, 13) | | * |
| LSI | 0.85 (0.03, 5) | 0.93 (0.04, 11) | 0.74 (0.04, 8) | 1.05 (0.08, 6) | 0.73 (0.04, 10) | 0.91 (0.05, 13) | * | |
| SSI | 0.12 (0.02, 5) | 0.18 (0.03, 11) | 0.12 (0.02, 8) | 0.15 (0.03, 6) | 0.14 (0.02, 10) | 0.17 (0.02, 13) | * | |
| MMC (% area) | 0.40 (0.26, 5) | 0.10 (0.02, 11) | 0.09 (0.02, 8) | 0.09 (0.03, 5) | 0.03 (0.01, 10) | 0.04 (0.01, 13) | Interaction | |
| **Females** | | | | | | | | |
| Length (mm) | 515 (8, 11) | 515 (13, 12) | 508 (16, 11) | 506 (15, 15) | 507 (10, 13) | 540 (9, 11) | | |
| Weight (g) | 1375 (77, 11) | 1611 (104, 12) | 1553 (111, 11) | 1549 (100, 15) | 1516 (95, 13) | 2106 (122, 11) | | |
| Condition (K) | 0.96 (0.03, 11) | 1.13 (0.08, 12) | 1.09 (0.03, 11) | 1.10 (0.03, 15) | 1.11 (0.02, 13) | 1.22 (0.05, 11) | * | * |
| GSI | 2.58 (0.48, 11) | 3.51 (0.69, 12) | 6.36 (0.99, 11) | 5.22 (0.85, 15) | 29.76 ( - , 1) | 17.83 (4.34, 5) | | * |
| LSI | 1.13 (0.03, 11) | 1.17 (0.07, 12) | 1.17 (0.04, 11) | 1.33 (0.05, 15) | 0.61 (0.04, 13) | 0.91 (0.11, 11) | Interaction | |
| SSI | 0.13 (0.03, 11) | 0.18 (0.05, 12) | 0.10 (0.02, 11) | 0.15 (0.02, 15) | 0.05 (0.01, 13) | 0.08 (0.01, 11) | * | * |
| MMC (% area) | 0.09 (0.02, 11) | 0.03 (0.01, 12) | 0.06 (0.01, 11) | 0.12 (0.03, 15) | 0.06 (0.02, 13) | 0.08 (0.02, 11) | 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.

| | March | | April | | June | | Hypothesis | |
|---|---|---|---|---|---|---|---|---|
| | Okareka | Tikitapu | Okareka | Tikitapu | Okareka | Tikitapu | Lake | Period |
| **Males** | | | | | | | | |
| Length (mm) | 65.00 (1.32, 8) | 61.64 (1.66, 11) | 61.63 (1.39, 8) | 60.22 (1.88, 9) | 74.64 (2.58, 14) | 57.9 (1.09, 10) | | |
| Weight (g) | 2.90 (0.26, 8) | 2.04 (0.21, 11) | 2.36 (0.13, 8) | 1.94 (0.28, 9) | 4.97 (0.66, 14) | 1.90 (0.08, 10) | | |
| Condition (K) | 1.02 (0.04, 8) | 0.84 (0.04, 11) | 0.98 (0.03, 8) | 0.84 (0.05, 9) | 1.08 (0.03, 14) | 0.96 (0.04, 10) | * | * |
| GSI | 0.54 (0.09, 8) | 0.46 (0.11, 11) | 0.51 (0.12, 8) | 1.10 (0.43, 9) | 0.73 (0.04, 14) | 0.79 (0.12, 10) | | |
| LSI | 1.05 (0.10, 8) | 0.97 (0.10, 11) | 1.81 (0.46, 8) | 1.11 (0.12, 9) | 1.96 (0.27, 14) | 1.78 (0.18, 10) | | * |
| **Females** | | | | | | | | |
| Length (mm) | 68.08 (1.14, 12) | 67.00 (3.11, 9) | 63.50 (1.94, 12) | 64.64 (2.46, 11) | 71.5 (2.53, 8) | 65.3 (4.31, 10) | | |
| Weight (g) | 3.04 (0.13, 12) | 2.95 (0.62, 9) | 2.69 (0.30, 12) | 2.75 (0.50, 11) | 4.37 (0.74, 8) | 3.43 (0.99, 10) | | |
| Condition (K) | 0.93 (0.03, 12) | 0.86 (0.04, 9) | 0.99 (0.03, 12) | 0.89 (0.05, 11) | 1.08 (0.06, 8) | 0.98 (0.05, 10) | * | * |
| GSI | 2.23 (0.71, 12) | 2.09 (0.52, 9) | 1.24 (0.40, 12) | 1.74 (0.28, 11) | 1.27 (0.24, 8) | 1.38 (0.22, 10) | * | |
| LSI | 1.31 (0.08, 12) | 1.69 (0.24, 9) | 1.31 (0.19, 12) | 2.14 (0.26, 11) | 2.32 (0.52, 8) | 2.08 (0.24, 10) | * | |

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

| | March | | April | | June | |
|---|---|---|---|---|---|---|
| | Okareka | Tikitapu | Okareka | Tikitapu | Okareka | Tikitapu |
| **Males** | | | | | | |
| Weight (g) | 62.91 (8.11, 6) | 30.55 (2.36, 7) | 63.89 (7.75, 9) | 21.64 (1.81, 5) | 89.18 (7.97, 5) | 10.86 (0.63, 5) |
| | *36.98 - 81.23* | *23.92 - 42.01* | *34.23 - 94.91* | *15.45 - 25.06* | *70.79 - 116.82* | *9.19 - 13.02* |
| Total length (mm) | 132.8 (5.8, 6) | 108.3 (2.9, 7) | 132.3 (5.2, 9) | 97.2 (1.8, 5) | 145.2 (4.5, 5) | 75.2 (1.6, 5) |
| | *112 - 147* | *98 - 122* | *112 - 152* | *92 - 102* | *133 - 155* | *70 - 80* |
| Carapace length (mm) | 45.5 (1.8, 6) | 35.4 (1.2, 7) | 45.9 (2.1, 9) | 32.4 (0.7, 5) | 51.2 (1.9, 5) | 24.8 (0.9, 5) |
| | *39 - 50* | *32 - 41* | *37 - 54* | *31 - 35* | *46 - 57* | *23 - 28* |
| **Females** | | | | | | |
| Weight (g) | 41.53 (4.39, 6) | 31.92 (2.41, 5) | 34.34 (5.45, 5) | 21.50 (4.2, 5) | 59.54 (5.92, 5) | 17.63 (1.38, 5) |
| | *26.72 - 55.26* | *26.05 - 38.48* | *16.75 - 45.15* | *12.09 - 34.19* | *49.41 - 79.62* | *14.74 - 21.05* |
| Total length (mm) | 120.0 (4.4, 6) | 114.8 (2.9, 4) | 112.6 (7.0, 5) | 96.6 (5.7, 5) | 131.8 (4.7, 5) | 88.4 (2.0, 5) |
| | *107 - 135* | *106 - 118* | *92 - 126* | *85 - 110* | *124 - 149* | *84 - 95* |
| Carapace length (mm) | 40.8 (2.0, 6) | 37.5 (0.6, 4) | 39.6 (3.4, 5) | 31.8 (2.5, 5) | 45.4 (1.6, 5) | 28.8 (1.0, 5) |
| | *35 - 47* | *37 - 39* | *28 - 46* | *26 - 39* | *42 - 51* | *26 - 31* |

### 3.2 Haematology

Changes in the overall haematology of both fish species were observed throughout the study (Tables 4 and 5). Although there was no change in Hct and RBC numbers for male trout between sampling periods, lower Hct was typically found in females from Lake Okareka, and a reduction in RBC numbers occurred in both populations between April and June. Whole blood haemoglobin (Hb) was variable in both sexes throughout the study, but was generally high for all sample groups. Accessory haemoglobin measures (MCH and MCHC) expectedly matched the variation in Hb.

Total and differential leukocyte counts have been shown to respond to a variety of stressors and changes in water quality in various fish species (Tierney et al. 2004). Changes in the trout leukocyte profile were observed over the 3 sampling periods in the current study. For both male and female trout, significant changes in the relative proportions of leukocyte cell types were observed in the June sampling period that were slightly more pronounced in the Okareka trout. This was similar to observations made during the spawning months in 2006 where the proportion of lymphocytes decreased while granulocyte and thrombocyte ratios increased. Changes in the differential leukocyte profile also coincided with greater total leukocytes in June. Although leukocyte changes for sampling period and between the two lakes were evident in the current study, changes in these parameters appear to be seasonal in origin and probably relate to endocrine changes and physiological stress associated with reproduction and spawning. Studies have demonstrated lymphocytopenia (depression of lymphocytes) in sexually mature brown trout (*Salmo trutta*) of both sexes (Pickering 1986). Lymphocytopenia in brown trout was later found to coincide with elevated levels of cortisol (Pickering and Pottinger 1987).

Tikitapu bullies typically had lower mean values for all haematological parameters. This observation is similar to that reported in 2006 where an allometric relationship between haematology and body size was implied.

Allometry of haematological variables has been recently documented in another species (*Basilichthys australis*; Nespolo and Rosenmann 2002). Although more emphasis was placed on obtaining similar sized bullies from each lake, Tikitapu bullies were still generally smaller (Table 2). Reductions in some parameters, such as RBCC, Hb and Hct were seen in both populations in April immediately following the mineral application in Lake Okareka. By June, these values had begun to increase again in Okareka bullies, with some parameters remaining low in the Tikitapu sample.

The haematological results from this study do not show any clear trends in the data that coincide with the mineral application in Lake Okareka. Fish haematology may significantly vary with environmental conditions, sex and season in numerous fish species (Munkittrick and Leatherland 1983; Pickering 1986; Guijarro et al. 2002). Given the complex differences and changes in measured parameters in both species, it is concluded that these responses are primarily seasonal, but may also be linked to temporal differences or allometric effects related to size.

**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.

| | March | | April | | June | | Hypothesis | |
|---|---|---|---|---|---|---|---|---|
| | Okareka | Tikitapu | Okareka | Tikitapu | Okareka | Tikitapu | Lake | Period |
| **Males** | | | | | | | | |
| Hct (%) | 46.3 (2.6, 5) | 44.0 (3.2, 11) | 49.0 (2.1, 8) | 45.7 (2.8, 6) | 43.4(2.1, 9) | 43.5 (1.5, 13) | | |
| RBCC ($x10^{12}$ cells/L) | 1.36 (0.11, 5) | 1.29 (0.09, 11) | 1.39 (0.05, 8) | 1.24 (0.08, 6) | 1.24 (0.05, 9) | 1.16 (0.03, 13) | | |
| Hb (g/L) | 95.2 (3.5, 5) | 102.5 (7.6, 11) | 112.5 (4.9, 8) | 105.8 (7.1, 6) | 135.3 (5.6, 9) | 95.1 (4.9, 13) | Interaction | |
| MCH (pg/cell) | 71.8 (6.1, 5) | 80.0 (3.7, 11) | 80.8 (1.6, 8) | 85.9 (4.8, 6) | 109.4 (4.1, 9) | 81.8 (2.9, 13) | Interaction | |
| MCHC (g/L) | 208 (11, 5) | 235 (9, 11) | 230 (6, 8) | 231 (5, 6) | 314 (11, 9) | 219 (9, 13) | Interaction | |
| MCV (fl) | 347 (27, 5) | 340 (8, 11) | 352 (10, 8) | 372 (22, 6) | 348 (6, 9) | 376 (9, 13) | | |
| WBCC ($x10^{10}$ cells/L) | 1.91 (0.28, 5) | 2.31 (0.26, 11) | 1.97 (0.24, 8) | 1.90 (0.39, 6) | 2.83 (0.11, 9) | 2.49 (0.17, 13) | | * |
| Lymphocyte (%) | 72.8 (2.2, 5) | 78.9 (2.4, 9) | 81.4 (1.6, 8) | 75.0 (1.7, 6) | 40.8 (5.5, 9) | 55.4 (2.9, 13) | Interaction | |
| Granulocyte (%) | 15.6 (2.7, 5) | 15.2 (1.9, 9) | 13.9 (1.3, 8) | 12.8 (2.1, 6) | 40.3 (4.9, 9) | 37.3 (2.9, 13) | | * |
| Thrombocyte (%) | 11.6 (1.9, 5) | 5.9 (0.9, 9) | 4.8 (1.2, 8) | 12.2 (2.9, 6) | 18.9 (5.2, 9) | 7.3 (1.6, 13) | Interaction | |
| **Females** | | | | | | | | |
| Hct (%) | 38.7 (2.7, 11) | 46.9 (1.9, 11) | 46.3 (1.5, 11) | 46.2 (1.6, 15) | 41.8 (1.7, 13) | 44.8 (1.8, 11) | * | |
| RBCC ($x10^{12}$ cells/L) | 1.20 (0.08, 11) | 1.38 (0.05, 11) | 1.36 (0.04, 10) | 1.37 (0.05, 15) | 1.22 (0.06, 13) | 1.19 (0.05, 11) | | * |
| Hb (g/L) | 79.0 (6.9, 11) | 103.2 (5.7, 11) | 107.0 (4.2, 11) | 105.1 (4.6, 15) | 126.4 (7.0, 13) | 105.3 (8.1, 11) | Interaction | |
| MCH (pg/cell) | 66.0 (3.6, 11) | 75.2 (4.0, 11) | 79.4 (3.8, 10) | 77.4 (3.2, 15) | 105.1 (4.7, 13) | 89.6 (6.5, 11) | Interaction | |
| MCHC (g/L) | 204 (9, 11) | 221 (11, 11) | 229 (5, 10) | 228 (7, 15) | 303 (12, 13) | 238 (19, 11) | Interaction | |
| MCV (fl) | 324 (12, 11) | 343 (16, 11) | 346 (14, 10) | 339 (7, 15) | 347 (8, 13) | 379 (9, 11) | | * |
| WBCC ($x10^{10}$ cells/L) | 2.72 (0.55, 11) | 1.87 (0.12, 11) | 1.81 (0.18, 10) | 1.54 (0.09, 15) | 2.62 (0.32, 13) | 2.32 (0.20, 11) | * | * |
| Lymphocyte (%) | 74.8 (3.0, 10) | 75.6 (1.8, 8) | 77.9 (1.8, 11) | 73.5 (2.4, 15) | 37.8 (4.4, 13) | 53.7 (3.8, 11) | Interaction | |
| Granulocyte (%) | 17.1 (1.9, 10) | 18.5 (2.2, 8) | 15.2 (1.4, 11) | 14.5 (1.4, 15) | 31.7 (4.4, 13) | 26.3 (3.9, 11) | | * |
| Thrombocyte (%) | 8.1 (1.9, 10) | 5.9 (0.9, 8) | 6.9 (1.5, 11) | 12.0 (2.1, 15) | 31.2 (6.1, 13) | 20.0 (4.0, 11) | | * |

**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.

| | March | | April | | June | | Hypothesis | |
|---|---|---|---|---|---|---|---|---|
| | Okareka | Tikitapu | Okareka | Tikitapu | Okareka | Tikitapu | Lake | Period |
| **Males** | | | | | | | | |
| Hct (%) | 33.6 (2.9, 7) | 29.7 (2.5, 11) | 25.5 (3.1, 8) | 20.5 (2.3, 9) | 25.1 (1.8, 14) | 23.9 (2.0, 10) | | * |
| RBCC ($x10^{12}$ cells/L) | 1.88 (0.17, 7) | 1.72 (0.09, 11) | 1.34 (0.15, 8) | 1.18 (0.12, 9) | 1.47 (0.08, 14) | 1.68 (0.09, 10) | | * |
| Hb (g/L) | 68.0 (6.3, 7) | 62.2 (4.4, 11) | 47.4 (3.2, 8) | 44.1 (5.3, 9) | 56.2 (3.0, 14) | 32.9 (3.1, 10) | Interaction | |
| MCH (pg/cell) | 42.1 (3.0, 7) | 46.8 (3.6, 11) | 43.9 (3.8, 8) | 44.7 (3.3, 9) | 43.2 (2.5, 14) | 24.9 (1.9, 10) | Interaction | |
| MCHC (g/L) | 205 (15, 7) | 215 (12, 11) | 196 (16, 8) | 222 (16, 9) | 231 (12, 14) | 141 (13, 10) | Interaction | |
| MCV (fl) | 209 (16, 7) | 222 (17, 11) | 233 (26, 8) | 210 (21, 9) | 190 (11, 14) | 181 (8, 10) | | |
| **Females** | | | | | | | | |
| Hct (%) | 34.5 (2.4, 12) | 25.8 (1.5, 9) | 19.7 (2.2, 12) | 20.6 (1.4, 11) | 21.1 (2.4, 8) | 23.9 (0.9, 10) | Interaction | |
| RBCC ($x10^{12}$ cells/L) | 2.09 (0.11, 11) | 1.54 (0.14, 9) | 1.33 (0.07, 12) | 1.12 (0.09, 11) | 1.47 (0.08, 8) | 1.78 (0.07, 10) | Interaction | |
| Hb (g/L) | 75.3 (4.9, 12) | 57.6 (3.8, 9) | 40.3 (2.9, 12) | 41.4 (4.1, 11) | 54.4 (2.9, 8) | 36.0 (4.2, 10) | Interaction | |
| MCH (pg/cell) | 41.4 (2.7, 11) | 46.0 (3.5, 9) | 34.9 (1.7, 12) | 44.8 (3.0, 11) | 40.7 (1.9, 8) | 24.2 (2.9, 10) | Interaction | |
| MCHC (g/L) | 226 (18, 12) | 225 (12, 9) | 219 (14, 12) | 205 (20, 11) | 273 (22, 8) | 149 (15, 10) | Interaction | |
| MCV (fl) | 183 (12, 11) | 207 (17, 9) | 169 (14, 12) | 236 (25, 11) | 154 (10, 8) | 160 (7, 10) | * | * |

### 3.3 Histology

The density of splenic melano-macrophage centres (MMCs) was greater in Okareka trout in March but greater in Tikitapu trout in April (Fig. 1). 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). They are also associated with natural processes such as aging, starvation, nutritional imbalance and temperature stress (Wolke 1992). Lower and consistent MMC area in both populations in April and June following the Phoslock$^{TM}$ application suggest no impact of the mineral product on this endpoint. The variability in Okareka trout MMC area in March prior to application implicates natural differences in lake water quality and/or fish physiological status in MMC development. This is further demonstrated by a similar observation in 2006 where greater splenic MMC area was found in Okareka trout prior to the mineral application.

Also consistent with observations made in 2006 was the lack of any obvious histopathological gill changes of trout from either lake over the monitoring period (Fig. 2). Clubbing at the tips of the secondary lamellae, lifting of the lamellar epithelia, lamellar fusion, hyperplasia and vascular congestion were commonly observed throughout the study. Severities of lesions were typically low to moderate with no examples showing extreme damage or change.

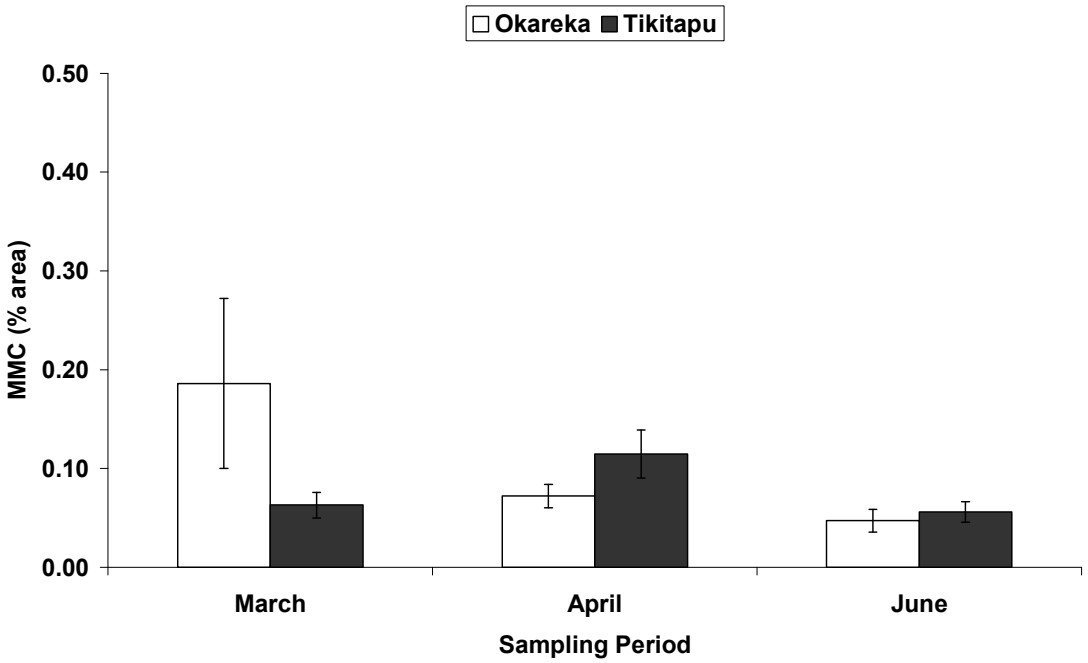


**Fig. 1.** Density of melanomacrophage centres (MMCs) in splenic tissue of rainbow trout. Greatest MMC densities were measured in March for Okareka trout and April for Tikitapu trout. Error bars indicate standard error of the mean.

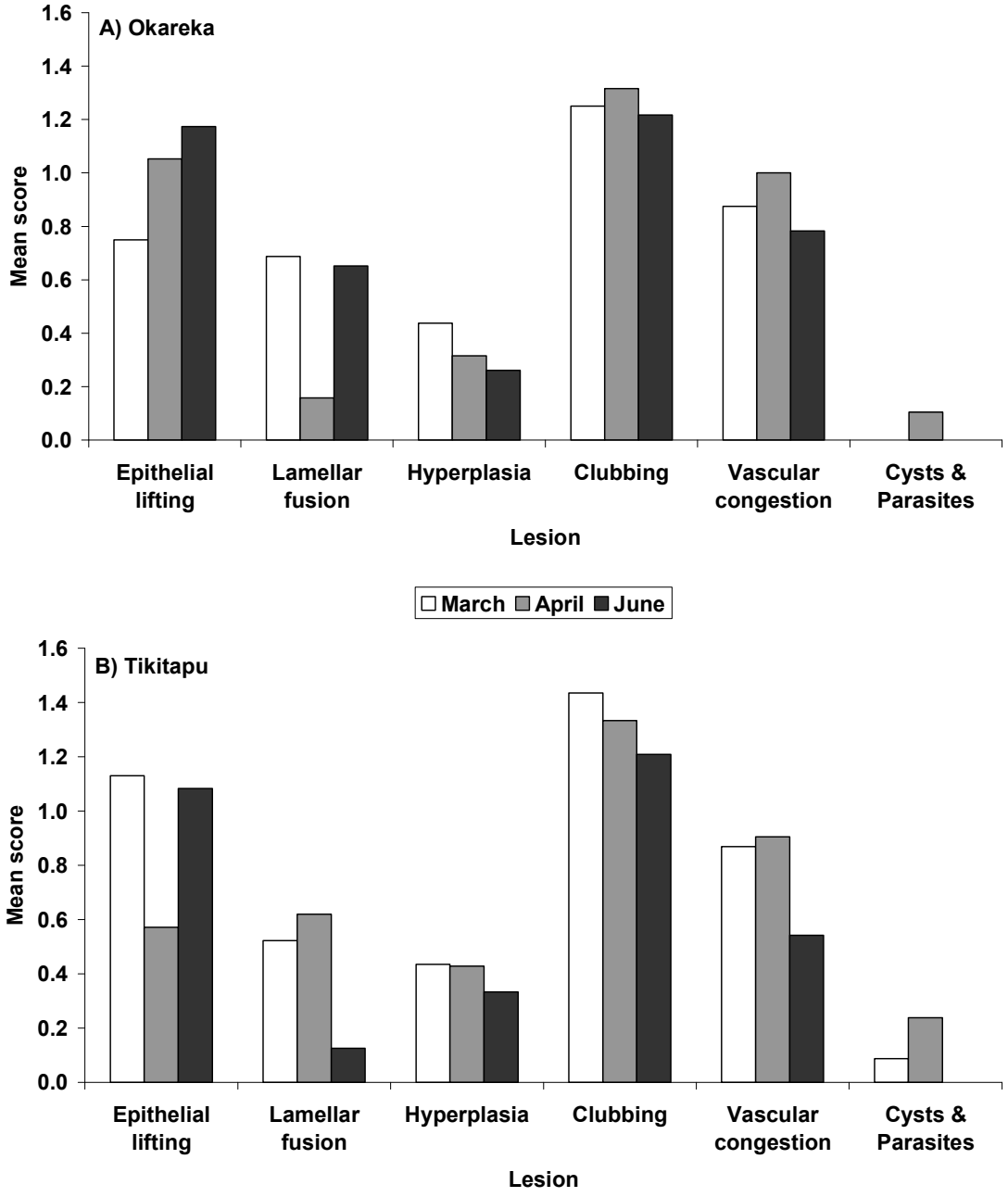


**Fig. 2.** Gill histopathological observations for rainbow trout from A) Okareka and B) Tikitapu. Frequency and severity of lesions were ranked from 0-3, corresponding to none (0), low (1), moderate (2) and severe (3). Data are presented as a mean score of the ranks for each lesion type.

### 3.4 Plasma ions

Plasma ions were measured to indicate any possible osmoregulatory disruption due to toxicant exposure. Lanthanum is a known potent blocker of chloride and calcium channels in the chloride cells of the gills (Perry 1997), and ion losses may be expected following exposure (Eddy and Bath 1979). Statistical changes in plasma sodium, potassium, chloride and calcium ions were observed during this study (Table 6). These changes were typically minor and tended to increase over the monitoring period. Strong seasonal changes, particularly for plasma chloride, were previously observed in 2006. Although similar seasonal changes are also indicated here, with the exception of potassium in male trout, the present values are more consistent throughout the monitoring. The interpretation of plasma potassium concentration is more problematic as significant amounts of potassium may be released from the muscle tissue following strenuous exercise (Holk and Lykkeboe 1998). Varied degrees of strenuous exercise would be expected in trout captured by gill-netting, in turn, affecting plasma potassium levels.

**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.

| | March | | April | | June | | Hypothesis | |
|---|---|---|---|---|---|---|---|---|
| | Okareka | Tikitapu | Okareka | Tikitapu | Okareka | Tikitapu | Lake | Period |
| **Males** | | | | | | | | |
| $Na^+$ (mM) | 142.4 (5.3, 5) | 144.6 (3.8, 11) | 145.3 (3.9, 8) | 156.5 (4.1, 6) | 160.9 (2.3, 10) | 157.2 (2.3, 13) | | * |
| $K^+$ (mM) | 5.18 (2.01, 5) | 2.73 (0.99, 11) | 1.45 (0.41, 8) | 2.86 (1.24, 6) | 0.42 (0.14, 10) | 3.41 (0.85, 13) | | Interaction |
| $Cl^-$ (mM) | 139.9 (3.9, 5) | 152.1 (3.6, 11) | 139.0 (4.8, 8) | 166.9 (11.9, 6) | 128.6 (2.2, 10) | 139.6 (4.3, 13) | * | * |
| $Mg^{2+}$ (mM) | 1.23 (0.14, 5) | 1.28 (0.06, 11) | 1.28 (0.06, 8) | 1.40 (0.07, 6) | 1.26 (0.11, 10) | 1.31 (0.05, 13) | | |
| $Ca^{2+}$ (mM) | 1.55 (0.15, 5) | 2.07 (0.12, 11) | 1.96 (0.09, 8) | 2.20 (0.13, 6) | 1.94 (0.05, 10) | 2.33 (0.07, 13) | * | * |
| **Females** | | | | | | | | |
| $Na^+$ (mM) | 145.1 (2.8, 11) | 145.2 (3.3, 11) | 142.4 (5.1, 11) | 148.5 (3.5, 15) | 162.3 (2.6, 13) | 160.4 (2.3, 11) | | * |
| $K^+$ (mM) | 3.90 (1.12, 11) | 2.28 (0.85, 11) | 2.27 (0.80, 11) | 3.19 (0.82, 15) | 1.83 (0.54, 13) | 2.42 (0.65, 11) | | |
| $Cl^-$ (mM) | 140.1 (2.3, 11) | 149.4 (3.6, 11) | 138.2 (5.2, 11) | 163.6 (5.1, 15) | 130.5 (1.5, 13) | 154.4 (6.6, 11) | * | |
| $Mg^{2+}$ (mM) | 1.31 (0.07, 11) | 1.25 (0.05, 11) | 1.37 (0.08, 11) | 1.39 (0.06, 15) | 1.41 (0.08, 13) | 1.43 (0.08, 11) | | |
| $Ca^{2+}$ (mM) | 2.13 (0.18, 11) | 2.43 (0.13, 11) | 2.71 (0.22, 11) | 2.89 (0.17, 15) | 3.01 (0.31, 13) | 2.74 (0.12, 11) | | * |

### 3.5 Tissue lanthanum accumulation

Previous monitoring showed the mineral product introduced a bioavailable source of lanthanum into Lake Okareka, as demonstrated by significant uptake in the liver and hepatopancreas tissues of trout and koura subsequent to the application. Significant accumulation was again observed in Okareka trout and koura tissues following the 2007 application (Figs 3 and 4), reconfirming the source of lanthanum in the lake. It is worthwhile noting that the interval between applications was sufficient to permit the previously accumulated lanthanum levels in trout liver and koura to return towards baseline, suggesting a biological capacity to depurate lanthanum in the Lake Okareka biota. Lanthanum was not found above the limits of detection in trout flesh or the tissues of Tikitapu koura, and was only found at low concentrations in tail flesh of 6 out of 24 koura sampled from Lake Okareka after the mineral application. Data from the two studies shows that koura rapidly accumulate lanthanum within 2 weeks following mineral application. In contrast to previous findings, male trout also rapidly accumulated lanthanum in a similar manner to koura, but to a greater extent. Female trout accumulated lanthanum more slowly, with significant increases only observed at the 2 month post-application monitoring period.

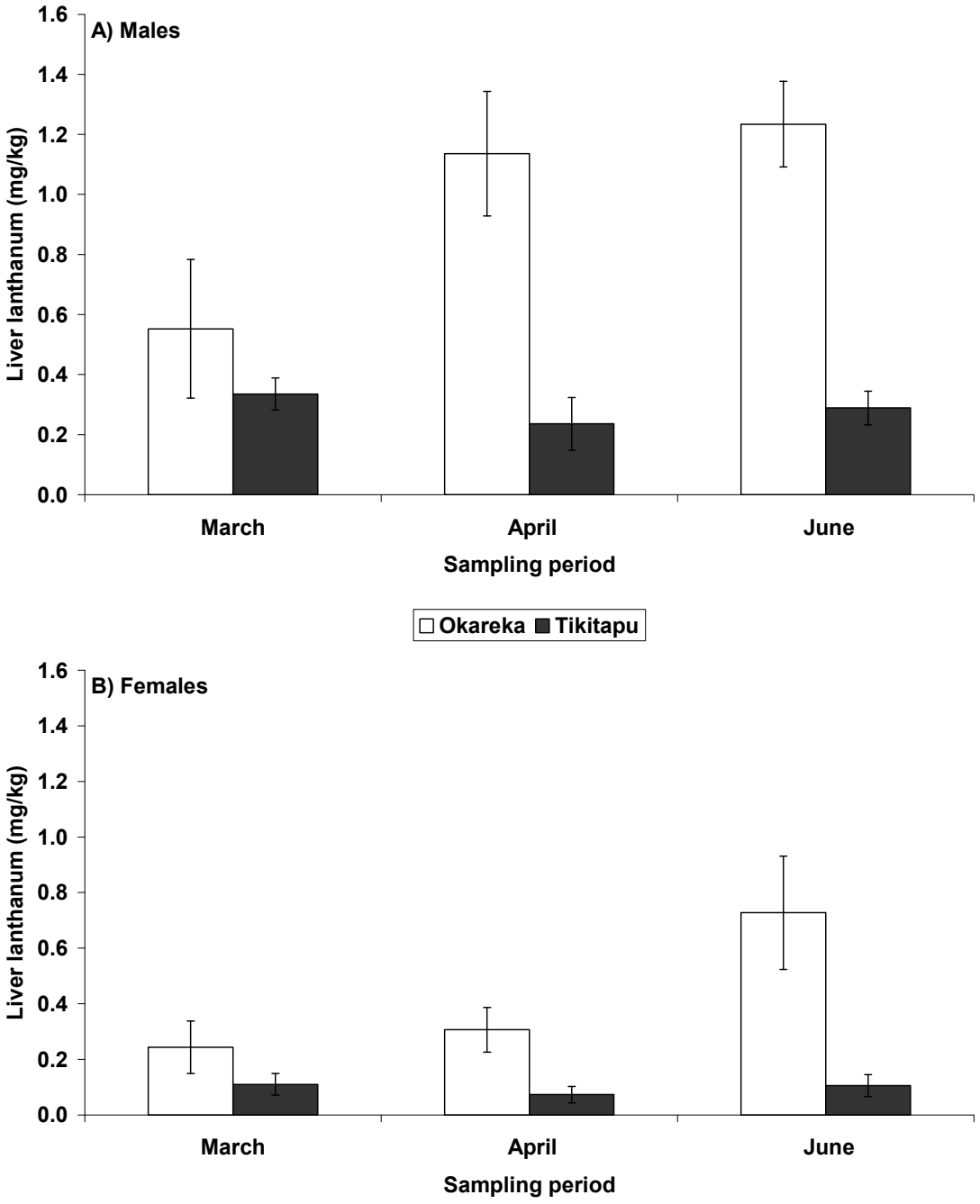


**Fig. 3.** Liver lanthanum concentration (mg/kg) in A) male and B) female rainbow trout. Error bars indicate standard error of the mean.

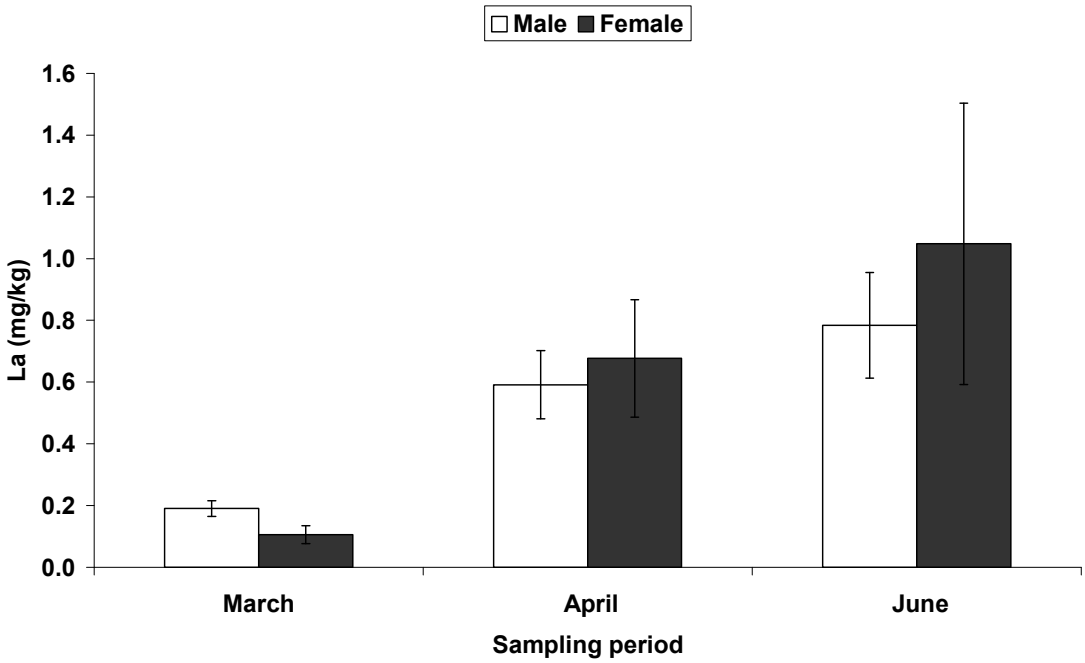


**Fig. 4.** Hepatopancreas lanthanum concentration (mg/kg) in male and female koura from Lake Okareka. Lanthanum was not found above detection limits in Tikitapu koura tissues. Error bars indicate standard error of the mean.

## 4.0 CONCLUSIONS

For two successive years, the results demonstrate that trout and koura in Lake Okareka acquire and significantly accumulate lanthanum in the liver and hepatopancreas tissues following the mineral Phoslock$^{TM}$ application. Lanthanum accumulation in the flesh of these organisms is generally low and has been measured in only a small number of specimens from each sampling period after mineral application. There is little literature available on the long-term effects of repeated sub-lethal, acute exposures in aquatic organisms.

Small yet significant changes in some physiological parameters such as energy allocation and haematology do occur over time and between lakes. Most measured changes occurred during reproductive development and spawning periods but are generally mirrored in both lake populations. Moderate between-lake differences are observed and are suspected to be linked to variations in lake conditions, reproductive timing and allometry.

## 5.0 ACKNOWLEDGEMENTS

This study was funded by Environment Bay of Plenty. The authors thank the following organizations and people for their help during this project Environment Bay of Plenty – John McIntosh; Waikato University – Dudley Bell, Annie Barker, Emma Joss and Steve Cameron; Scion – Sean Taylor, Trevor Stuthridge and Natalie Bleackley.

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