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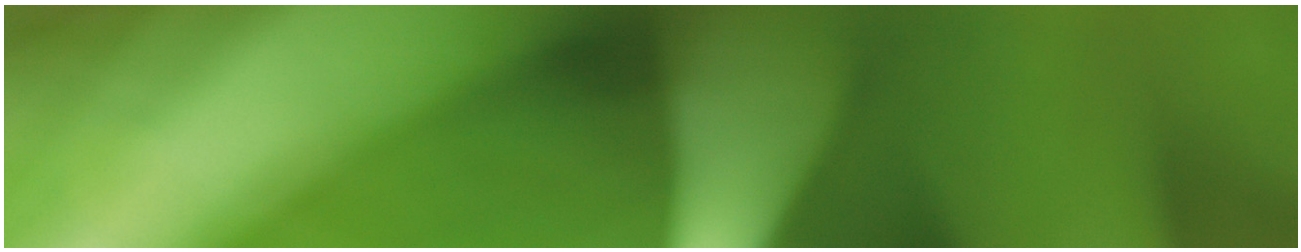

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Fish community surveys and biomonitoring in selected Rotorua Streams

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EXECUTIVE SUMMARY

As part of the catchment nutrient reduction strategy for Lake Rotorua, the Utuhina Stream has been subjected to alum (aluminium sulphate) dosing since mid-2006. Under resource consent conditions, monitoring of the fish community and in situ toxicity were required. This report summarises the results of two studies: 1) the Utuhina Stream fish community surveys and 2) a comparative stream biota monitoring study. Six community surveys have been performed over a two year period beginning prior to the initiation of the alum trial. Between November 2006 and June 2007 during the first year of alum dosing, notable changes in fish abundance and fishing effort were recorded. Although trout (*Oncorhynchus mykiss* and *Salmo trutta*) and koura (*Paranephrops planifrons*) numbers appeared to generally increase during this time, common bully (*Gobiomorphus cotidianus*) numbers were substantially reduced at all sites, leading to reduced fish density (total fish) and increased fishing effort (fewer fish caught per hour). Reduced common bully abundance in the stream coincided with highest stream concentrations of aluminium (Al) and lowest pH values, and may be linked to avoidance responses in the absence of known toxicity. Recent surveys completed in April and July of 2008 reveal recovery of the common bully population at all sites compared to similar survey periods in 2007. Due to difficulties encountered during in situ rainbow trout toxicity studies, a comparative stream study was subsequently undertaken to assess the status of selected biota in the Utuhina, Waiowhiro, Ngongotaha and Waiteti Streams. This study demonstrated that Al from alum dosing in the Utuhina Stream was bioavailable to both common bully and koura with accumulation occurring in the tissues. Although minor differences were observed between sites for other measured parameters, stream populations were generally similar in most respects. Despite the occurrence of Al bioaccumulation in Utuhina Stream biota, it is concluded that there has been no clear impact of alum on physiological endpoints after approximately 2 years of continuous exposure.

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1. INTRODUCTION

The proposed Lakes Rotorua and Rotoiti Action Plan (EBOP, 2007) aims to lower the trophic level index (TLI) of Lake Rotorua from 4.9 to 4.2 by reducing internal and catchment-derived nutrients (N and P). Catchment reduction targets of 250 tonnes N and 10 tonnes P have been established. The Utuhina Stream carries an estimated 7.6 tonnes of P into Lake Rotorua each year, of which approximately 2 tonnes is in the form of dissolved reactive phosphorous (DRP). The Action Plan proposes P-locking in up to three streams (Utuhina, Puarenga and one other) to reduce 6 tonnes of DRP entering into Lake Rotorua using continuous alum (aluminium sulphate) treatment. It has been estimated that an alum dosing rate of 1 ppm (1 g/m^3) should remove the majority of DRP (i.e. ~2 tonnes) in the Utuhina Stream. The purpose of the current study is to summarise the results of regular Utuhina Stream fish community surveys performed over the last two years and a recent comparative stream biota monitoring assessment to satisfy resource consent conditions for the discharge of alum.

2. METHODS

2.1 Utuhina Stream fish community surveys

The occurrence of fish species, approximate density and catch per unit effort (CPUE) were determined for three 50 m site reaches of the Utuhina Stream (Fig. 1). Six community surveys have been completed to date. Two surveys were conducted each year between 2006 and 2008 with the first performed in June 2006 prior to the initiation of the alum discharge in July 2006. Fish density and CPUE (fish captured per hour) were estimated using a two-pass electrofishing procedure performed during periods of low flow in the stream. A MAF Aquatronics pulsed DC mains set electrofishing machine, powered by a Honda 3kVA petrol generator, operating at 420 V and approximately 3 A with two hand-held anodes was used to enable simultaneous fishing of each stream side (Fig. 2). Two teams of three people performed the fishing while one or two people remained on the bank for operation and safety. Estimates of total fish numbers (density) in the stream could not be calculated from the two-pass removal model as variable and occasionally greater fish numbers were captured in the second fishing passes. For practical purposes, an estimate of fish density was determined by simply adding the total catch from both passes at each site. Total CPUE and CPUE for each pass at each site could be determined normally.

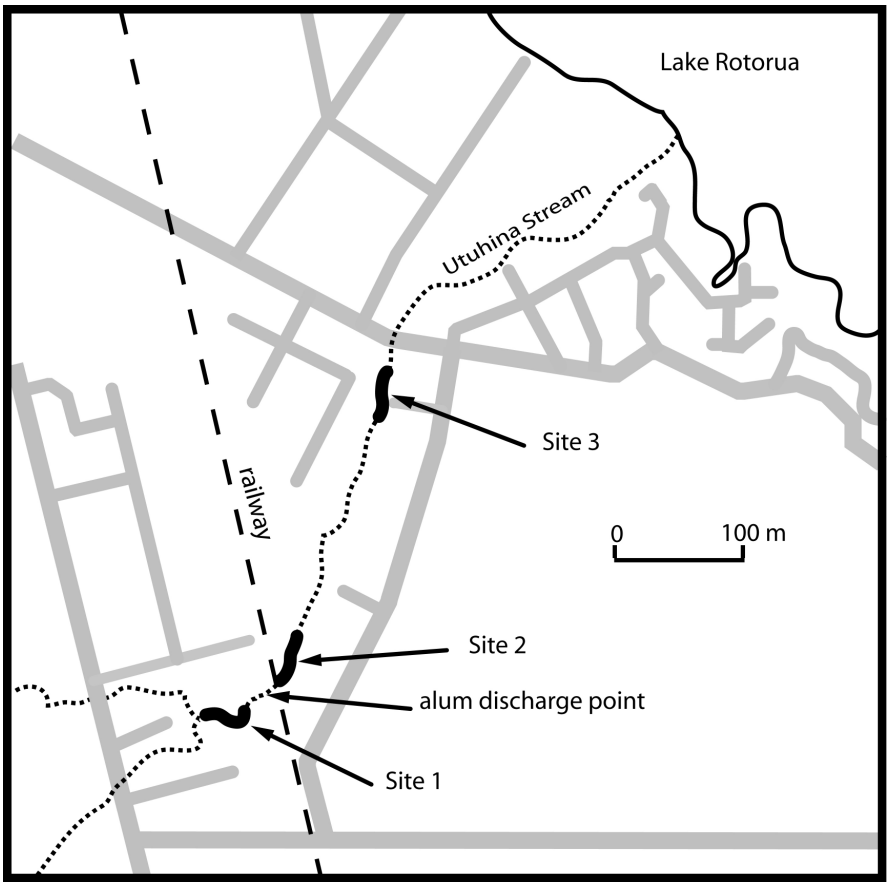

Fig. 1. The Utuhina Stream with fish community survey sites marked above the alum discharge (Site 1), in the alum mixing zone (Site 2) and upstream of Lake Rd (Site 3).

2.2 Rotorua Streams biomonitoring

2.2.1 Animals

Common bully (*Gobiomorphus cotidianus*) and koura (*Paranephrops planifrons*) were collected from selected Rotorua Streams (Fig. 3). Due to the absence or presence of small koura specimens in some streams, additional koura were collected from Lakes Rotorua, Rotoiti and Rotoma. A total of approximately 50 koura (19-51 mm ocular carapace length, 6-86 g total weight, n = 10 per site) and 80 common bully (54-87 mm total length, 1.97-9.23 g total weight, n = 20 per site) were collected. Koura were captured from the Utuhina and Waiowhiro Streams by backpack electrofishing during April and May 2008. Electrofising in the Puarenga Stream downstream of Te Ngae Road failed to capture any fish or koura. Lake koura were hand-collected during May

2008 by SCUBA divers. Common bully were captured by electrofishing during April and May 2008 from the Utuhina, Waiowhiro, Ngongotaha and Waiteti Streams.

Fig. 2. Two teams of three people simultaneously electrofishing fishing each bank in the alum mixing zone (Site 2) of the Utuhina Stream.

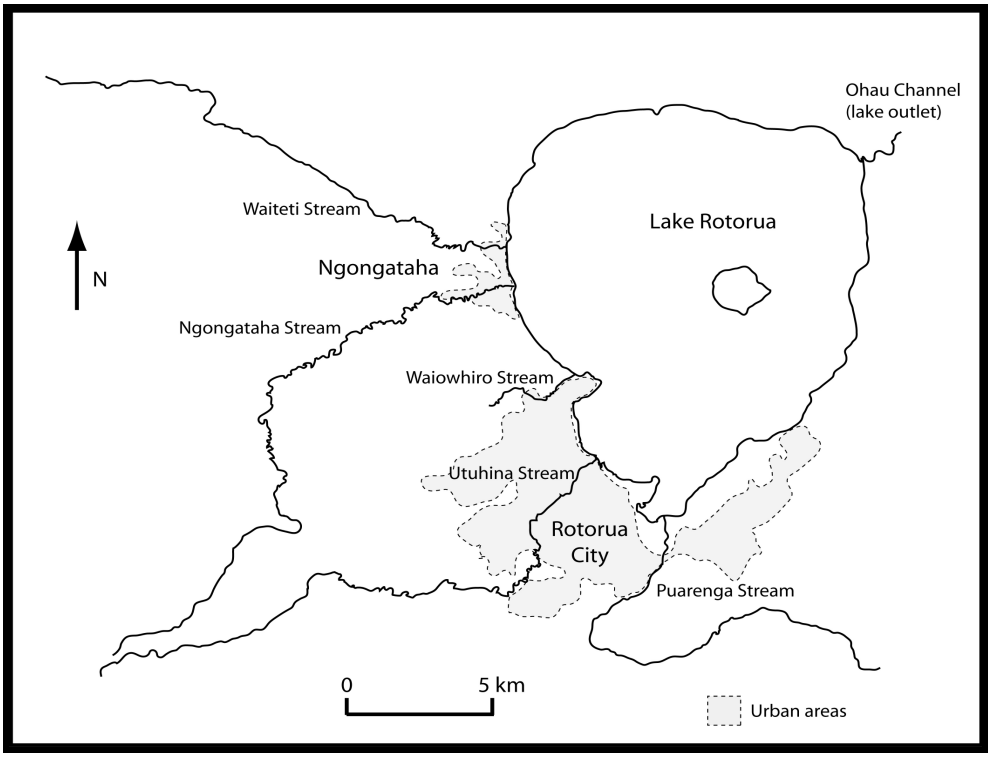

Fig. 3. Map of the Puarenga, Utuhina, Waiowhiro, Ngongotaha and Waiteti Stream biota monitoring sites.

2.2.2 Sampling procedure

Upon capture, bully and koura specimens were transported back to the laboratory in 20 L plastic pails. Fish were first anaesthetised with MS-222 (0.1 g/L). Approximately 10-30 µL of blood was taken by caudal venipuncture using 0.5 mL heparinised (20 IU NH_4-heparin) tuberculin syringes and processed immediately. Fish were sacrificed by an overdose of anaesthetic, then weighed and measured. Liver and gonads were removed and weighed. Whole bully livers were retained and frozen at -20°C for metals analysis. Koura were anaesthetised on a slurry of ice for 10 min before being weighed and measured. Haemolymph samples (100 µL) were withdrawn dorsally from the pericardial sinus between the carapace and first abdominal segment using a 0.5 mL syringe fitted with a 27-gauge needle. Syringes were preloaded with 100 µL of ice-cold haemolymph fixative solution so that samples were diluted 1:1 (v/v). Hepatopancreas (digestive gland) and tail muscle tissues were removed and frozen at -20°C for metals analysis.

2.2.3 Haematology

Blood and haemolymph cell counts were determined by flow cytometry as described in Section 2.2.4. Other haematological variables were assessed for bully blood only and included whole blood haemoglobin concentration (Hb), haematocrit (Hct), red blood cell count (RBCC), mean red cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and differential leukocyte counts (DWBC). Methods were generally based on those of Dacie and Lewis (1991) and have previously been described in detail (Landman *et al.*, 2007; Landman and Ling, 2008).

2.2.4 Flow cytometry

For flow cytometric assessments, blood and haemolymph samples were prepared according to an optimised protocol previously developed at Scion. In brief, anticoagulated whole blood (3 µL) was suspended in a 5 mL cytometry tube coated with bovine serum albumin (BSA) containing 3.893 mL phosphate buffered saline (PBS) with 0.25% BSA and 100 µL of a fluorescent counting bead suspension. Immediately after cell dispersion, 4 µL of $DiOC_6$[3] (Molecular Probes, USA) was added and the sample incubated on ice for 30 min in the dark. Haemolymph samples in collection fluid (200

µL) were transferred to pre-chilled 5 mL cytometry tubes coated with BSA and diluted to 1 mL with ice-cold PBS (696 µl) and 100 µL of a counting bead suspension. Diluted samples were incubated on ice with 4 µL of $DiOC_6$[3] for 15 min in the dark.

Flow cytometry counts were performed on a FACSVantage SE DiVa flow cytometer (BD Biosciences) equipped with a 488 nm laser, powered at 300 mW. Forward scatter (FSC), side scatter (SSC) and fluorescence were measured in the 500-560 nm wavelength range (FL1). The detector photomultiplier voltages were set at 200, 300 and 500 mV, respectively for blood samples, and 125, 300 and 250 mV for haemolymph samples. Outputs were viewed in logarithmic mode. Threshold was adjusted between 200 and 5000 as necessary to exclude debris and enhance cell population resolution at high event counts. The instrument sheath fluid was PBS. Sample flow was adjusted to yield a count rate of 1000 events/s. Data was displayed on SSC vs. FL1 (blood) and SSC vs. FSC (haemolymph) dot plots, and gates were set around the individual cell and fluorescent bead populations to define each group. A total of 100 or 250 fluorescent beads were counted for blood and haemolymph samples, respectively. Total and differential blood and haemocyte cell counts were determined using the following formula:

$$\text{Cells/ml} = \frac{\text{cells counted} \times \text{beads per tube}}{\text{beads counted} \times \text{sample volume}}$$

Individual haemocyte populations based on previously determined gates were sorted using the flow cytometer for subsequent morphological examination and identification. Sorted cells were collected in 5 mL BSA-coated cytometry tubes containing 3 mL PBS supplemented with 0.25% BSA. Collected cells were spun directly onto polylysine-coated Shandon cytoslides using a Shandon Cytospin 4 centrifuge (Thermo Electron Corporation, USA). Cells were fixed and stained with Leishman's–Giemsa. Slides were then cover-slipped and examined. Photomicrographs of representative cells were taken at 1000x magnification using an Olympus BX61 microscope with image processing using AnalySIS software (LifeScience Series, Olympus, USA).

2.2.5 Tissue metals

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

2.2.6 Statistical analyses

Common bully condition (weight per unit length), liver and gonad size data were analysed using one-way analysis of covariance (ANCOVA), with body size (length or weight) as the covariate. Haematology data were analysed by one-way analysis of variance (ANOVA). Variables were log transformed prior to analysis. Because differential counts were measured as proportions, these data were arcsine transformed (Sokal and Rohlf, 1973). All statistical tests were performed using sampling site as a factor, followed by Tukey's post-hoc tests where significant site effects were observed. Although statistical comparisons using ANCOVA were completed on body, liver and gonad 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-somatic index (LSI) was calculated in the same manner, substituting gonad weight for liver weight. Fulton's condition factor (*K*) was calculated as [(body weight – combined organ weights)/length^3] x 100. All statistical analyses were performed using STATISTICA v8.0 software. The critical level of statistical significance for all tests was $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Utuhina Stream water quality monitoring

Selected physicochemical water quality monitoring data for the Utuhina Stream (data provided by John McIntosh and Paul Scholes, Environment Bay of Plenty) have been summarised in Fig. 4. Stream dissolved oxygen (DO) levels were consistently between 8.0 and 10.5 mg/L (Fig. 4A). Temperature varied seasonally between 10.0 and 16.0°C at both upstream and downstream sites (Fig 4B). At sites above the alum discharge, pH was between 6.5 and 7.5 (Fig. 4C). Downstream pH was slightly lower on average (6.5-7.0), with brief episodes of < pH 6.5 coinciding with increased aluminium concentrations [Al] (Fig. 4D). Total [Al] at upstream sites varied between 0.01 and 0.46 g/m^3, while downstream [Al] fluctuated between 0.08 and 2.86 g/m^3 with varying stream alum dosing rates.

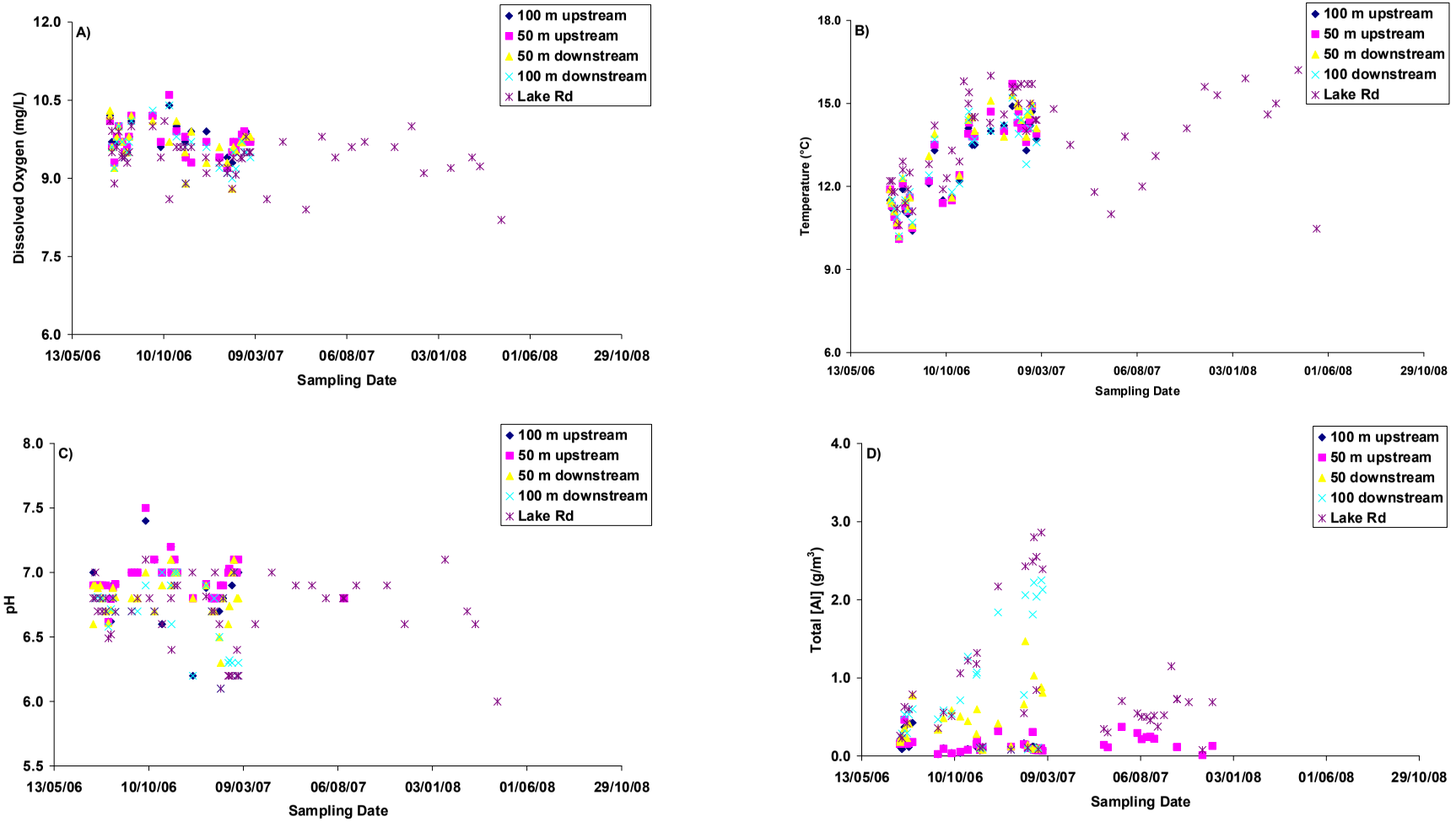

Fig. 4. Utuhina Stream A) dissolved oxygen (mg/L), B) temperature (°C), C) pH and D) total aluminium concentration [Al] (g/m^3) data recorded between 2006-2008.

3.2 Utuhina Stream community surveys

Changes in the abundance and composition of species have been observed throughout the Utuhina Stream fish community surveys (Table 1). In June 2006 prior to the commencement of alum dosing, common bully were abundant at all survey sites above and below the then proposed discharge point. Comparatively fewer trout (*Oncorhynchus mykiss* and *Salmo trutta*) and koura were also observed in the stream at that time. Between November 2006 and June 2007 during the first year of alum dosing, notable changes in estimated fish density and fishing effort were recorded (Tables 2 & 3). Although trout and koura numbers appeared to generally increase over this time, common bully numbers were substantially reduced at all sites, leading to reductions in fish density and increased fishing effort, i.e. fewer fish caught per fishing hour. The most recent community surveys performed in April and July of 2008 reveal relatively consistent koura and trout numbers in the stream, as well as an apparent recovery of the common bully population at all sites when compared to similar survey periods in 2007. The change in common bully abundance is also reflected in greater total fish densities and generally improved fishing effort.

Detectable stream [Al] was recorded in the range of 0.5-1.5 mg/L throughout most of 2006-2007, with peaks of between 2.0 and 3.0 mg/L at downstream sites in early 2007. Peaks in downstream [Al] also coincided with episodes of slightly reduced pH (i.e. < 6.5) at these sites. Toxicity in fish typically occurs at low pH (i.e. < pH 5.5) as a result of complex chemistry resulting in the formation of soluble Al species e.g. Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$ which typically exert their effect at the gill surface (Wood, 1989; Gensemer and Playle, 1999; Wood, 2001). At c. neutral pH (6.5-7.5), Al solubility is low and therefore toxicity is significantly reduced. As such, much of the literature is concentrated on the effects of Al at low pH. Although low doses of Al (36 µg/L) under acidic conditions (pH 5.2) have been shown to influence rainbow trout behaviour with associated haematological responses and some mortality (Allin and Wilson, 2000), at the recorded stream pH values, acute toxicity in the 0.5-1 mg/L Al range would be unlikely.

The possible cause(s) of changes in fish abundance may relate to avoidance behaviours even in the absence of toxicity. Avoidance of Al has been demonstrated in mixing zones (Atland and Barlaup, 1995) and laboratory studies (e.g. Atland, 1998; Allin and Wilson, 2000; Exley, 2000). Exley (2000) demonstrated that rainbow trout fry are

able to detect and avoid as little as 1 µg/L Al at pH 5.0, but also observed that the behavioural response is dependent on both pH and Al as avoidance could be removed by increasing pH. It has been shown that pre-exposure to Al may be an important factor influencing responses to subsequent exposures (Allin and Wilson, 2000). Although there is no record of Al avoidance in common bully, this species has been observed to avoid pH of ≤ 6.5 (West *et al.*, 1997), Cu (Richardson *et al.*, 2001) and complex mixtures such as pulp mill effluent (Bleackley, 2008). Reduced common bully abundance anecdotally coincided with the initial alum pulse, and periods of highest dosing and lowest pH. Furthermore, since alum dosing rates (and downstream pH) have stabilised, there is evidence of increased and consistent common bully numbers in the stream. Thus, the initial decline in common bully numbers in the Utuhina Stream may represent avoidance responses to the initial Al pulse and increased dosing rates, followed by subsequent acclimatisation and recovery of the population.

Table 1. Summary of all fish captured by species and survey period in the Utuhina Stream between June 2006 and July 2008. Alum dosing commenced in July 2006.

		Survey Period					
Species	**Site**	Jun-06	Nov-06	Apr-07	Jun-07	Apr-08	Jul-08
Common Bully	1	582	364	151	158	232	390
(*Gobiomorphus cotidianus*)	2	1056	333	128	307	630	706
	3	699	880	224	304	609	583
Trout	1	6	36	36	38	37	20
(*Oncorhynchus mykiss* &	2	36	27	40	102	33	36
Salmo trutta)	3	3	9	7	38	7	23
Koura	1	3	14	11	22	30	14
(*Paranephrops planifrons*)	2	3	15	12	10	52	9
	3	6	26	55	47	73	12
Eel	1	0	0	0	0	1	0
(*Anguilla australis*)	2	0	0	0	0	0	0
	3	0	0	0	0	1	0

For this table Site 1 Old Taupo Rd end; Site 2 Mixing zone below discharge site; Site 3 Lake Rd end.

Table 2. Fish density estimates (total fish and koura captured) in the Utuhina Stream obtained over 2006 to 2008. Alum dosing commenced in July 2006.

	Density (total fish)					
Site	Jun-06	Nov-06	Apr-07	Jun-07	Apr-08	Jul-08
1	591	414	198	218	300	424
2	1095	375	180	419	715	751
3	708	915	286	389	690	618

For this table Site 1 Old Taupo Rd end; Site 2 Mixing zone below discharge site; Site 3 Lake Rd end.

Table 3. Catch per unit effort (CPUE) for each fish survey period, determined independently for each fishing pass and averaged to provide the mean site CPUE. Alum dosing commenced in July 2006.

		CPUE (fish/h)						Mean site CPUE					
Site	**Pass**	Jun-06	Nov-06	Apr-07	Jun-07	Apr-08	Jul-08	Jun-06	Nov-06	Apr-07	Jun-07	Apr-08	Jul-08
1	1	329.0	200.5	98.2	87.6	142.1	213.9	291.3	171.9	95.6	86.1	136.5	190.4
	2	253.5	143.3	93.1	84.5	130.9	166.9						
2	1	471.4	127.0	87.0	93.2	142.8	217.2	401.0	141.2	73.5	95.4	151.9	164.7
	2	330.5	155.3	60.0	97.6	161.1	112.1						
3	1	136.2	261.0	131.1	120.0	225.1	179.4	240.2	253.3	132.0	119.1	189.2	169.4
	2	344.2	245.6	132.9	118.1	153.3	159.3						

For this table Site 1 Old Taupo Rd end; Site 2 Mixing zone below discharge site; Site 3 Lake Rd end.

3.3 Rotorua Streams biomonitoring

3.3.1 Fish size and abundance

For common bully captured in April/May 2008, significant site effects ($p < 0.05$, ANOVA) were observed in male condition and LSI (Table 4). Subsequent analysis revealed small, but significant differences in condition between sites ($p < 0.05$, Tukey's post-hoc). Here it was observed that Utuhina males had the lowest condition, while the generally larger Waiowhiro males possessed the highest condition. Increased LSI also accompanied greater condition in the Waiowhiro Stream. No statistically significant site differences were found for female common bully.

Koura size data are summarised in Table 5. Koura were abundant in both the Utuhina and Waiowhiro Streams, with very few and typically juvenile koura found in the Ngongotaha and Waiteti Streams. For this reason, additional lake koura were collected and sampled for purposes of comparison. Koura sampled from the Utuhina and Waiowhiro Streams, and Lake Rotorua were of similar size. Lake Rotoma and Rotoiti koura were substantially larger than other lake and stream populations.

Table 4. Mean (± sem) of size (weight and length), condition factor (*K*) and gonad (GSI) and liver (LSI) somatic indices for male and female common bully (*Gobiomorphus cotidianus*) in the Utuhina, Ngongotaha Waiteti and Waiowhiro Streams. Lower case letters represent statistically homogenous values (Tukey's $p > 0.05$) following determination of significant site differences ($p < 0.05$, ANOVA).

Site	Sex	n	Length (mm)	Weight (g)	Condition factor (*K*)	GSI (%)	LSI (%)
Utuhina	Male	22	65.5 (1.0)	3.31 (0.14)	1.12 (0,02)[a]	1.04 (0.07)	2.85 (0.20)[a]
	Female	14	64.9 (1.6)	3.25 (0.22)	1.13 (0.05)	1.81 (0.20)	3.00 (0.27)
Ngongotaha	Male	15	61.5 (1.6)	2.89 (0.24)	1.17 (0.02)[b]	0.87 (0.09)	2.49 (0.29)[a]
	Female	5	59.2 (1.0)	2.51 (0.15)	1.14 (0.05)	1.94 (0.14)	3.66 (0.58)
Waiteti	Male	12	62.6 (1.6)	2.99 (0.24)	1.17 (0.05)[b]	0.89 (0.11)	2.24 (0.21)[a]
	Female	8	65.3 (3.4)	3.55 (0.85)	1.09 (0.05)	2.38 (0.24)	2.89 (0.28)
Waiowhiro	Male	14	71.1 (1.3)	4.68 (0.27)	1.22 (0.03)[c]	1.15 (0.07)	4.26 (0.21)[b]
	Female	8	70.6 (2.2)	4.40 (0.43)	1.16 (0.02)	1.74 (0.23)	3.18 (0.29)

Table 5. Mean (± sem) length and weight data for koura (*Paranephrops planifrons*) from the Utuhina and Waiowhiro Streams, and Lakes Rotorua, Rotoiti and Rotoma.

Site	Sex	n	Total length (mm)	Ocular carapace length (mm)	Weight (g)
Utuhina	Male	2	69.0 (1.0)	21.5 (0.5)	8.7 (1.1)
	Female	7	68.0 (1.7)	22.3 (0.5)	7.7 (0.5)
Waiowhiro	Male	8	67.8 (2.8)	22.9 (1.0)	8.9 (1.1)
	Female	2	68.0 (7.0)	22.0 (3.0)	9.0 (3.1)
Rotorua	Male	5	75.4 (5.7)	25.0 (2.2)	13.8 (3.6)
	Female	5	77.4 (3.3)	24.0 (1.3)	12.1 (1.6)
Rotoiti	Male	5	119.4 (4.8)	42.4 (1.6)	51.6 (6.1)
	Female	5	117.4 (8.2)	38.6 (3.1)	39.8 (9.1)
Rotoma	Male	5	131.6 (2.6)	48.2 (0.6)	70.4 (5.0)
	Female	5	127.8 (5.1)	42.2 (2.3)	51.6 (6.3)

3.3.2 Aluminium accumulation

Accumulation of Al in the tissues was observed for Utuhina Stream common bully and koura specimens. Aluminium was generally not measured above the limits of detection in other streams biota, suggesting that the bioavailable Al in the Utuhina Stream was derived from the stream alum dosing. However, it should be noted that depending on the size of individuals (and consequently the weight of tissue available) the accuracy of Al measurement (i.e. the limits of detection) varied considerably between tissue types, individuals and populations. The highest levels of Al accumulation were observed in the hepatopancreas tissue of Utuhina koura (Fig. 5A), followed by the tail flesh of Utuhina and Rotoma koura (Fig. 5B). Although observed in Utuhina bully livers, Al was measured only fractionally above mean detection limits in these specimens (Fig. 6). Aluminium is typically regarded as a specific surface-active toxicant in fish (Gensemer and Playle, 1999; Wood, 2001), most often associating with the gills, followed by lesser accumulation in the internal organs (Spry and Wiener, 1991). As for fish, Al has also been shown to be more strongly associated with the gills in some freshwater crayfish species (Alexopoulos et al., 2003). However, accumulation and excretion of metals is known to vary dramatically between invertebrate taxa (Rainbow, 2002).

There is considerable literature available on metal bioaccumulation and toxicity for numerous crustacean species, yet there is little information available for the koura. Anecdotally, koura appear to be rapid and efficient accumulators of certain metal contaminants, with strong partitioning of various metals occurring in the different tissues. The current study shows that koura had approximately 5-fold greater Al in the hepatopancreas compared to the tail, while earlier studies found that koura may accumulate up to 10-fold greater lanthanum in this organ (Landman and Ling, 2006; Landman et al., 2007). Presumably, these metals are being detoxified and partitioned preferentially into the hepatopancreas via metallothioneins or metaliferous granules for later excretion, thereby reducing the metabolically available fraction that may lead to internal toxicity (Rainbow, 2002).

The risk to humans from consuming koura with elevated Al would depend largely on the tissues (i.e. hepatopancreas *vs.* flesh) and total volume consumed. However, the risks associated with dietary Al intake are considered to be small as there are no maximum acceptable concentrations in food recommended by the New Zealand Food Safety Authority. Human dietary Al intake is typically considered to be around 10

mg/day, however, the Agency for Toxic Substances and Disease Registry of the United States Centres for Disease Control has recommended a lowest observed adverse effect level of 180 mg/kg/day, corresponding to a total daily intake of around 9 g for a 70 kg human. The low levels of aluminium measured in Utuhina Stream biota do not represent any significant health risk from human consumption as one would need to consume in excess of 500 kg of combined edible koura tissues.

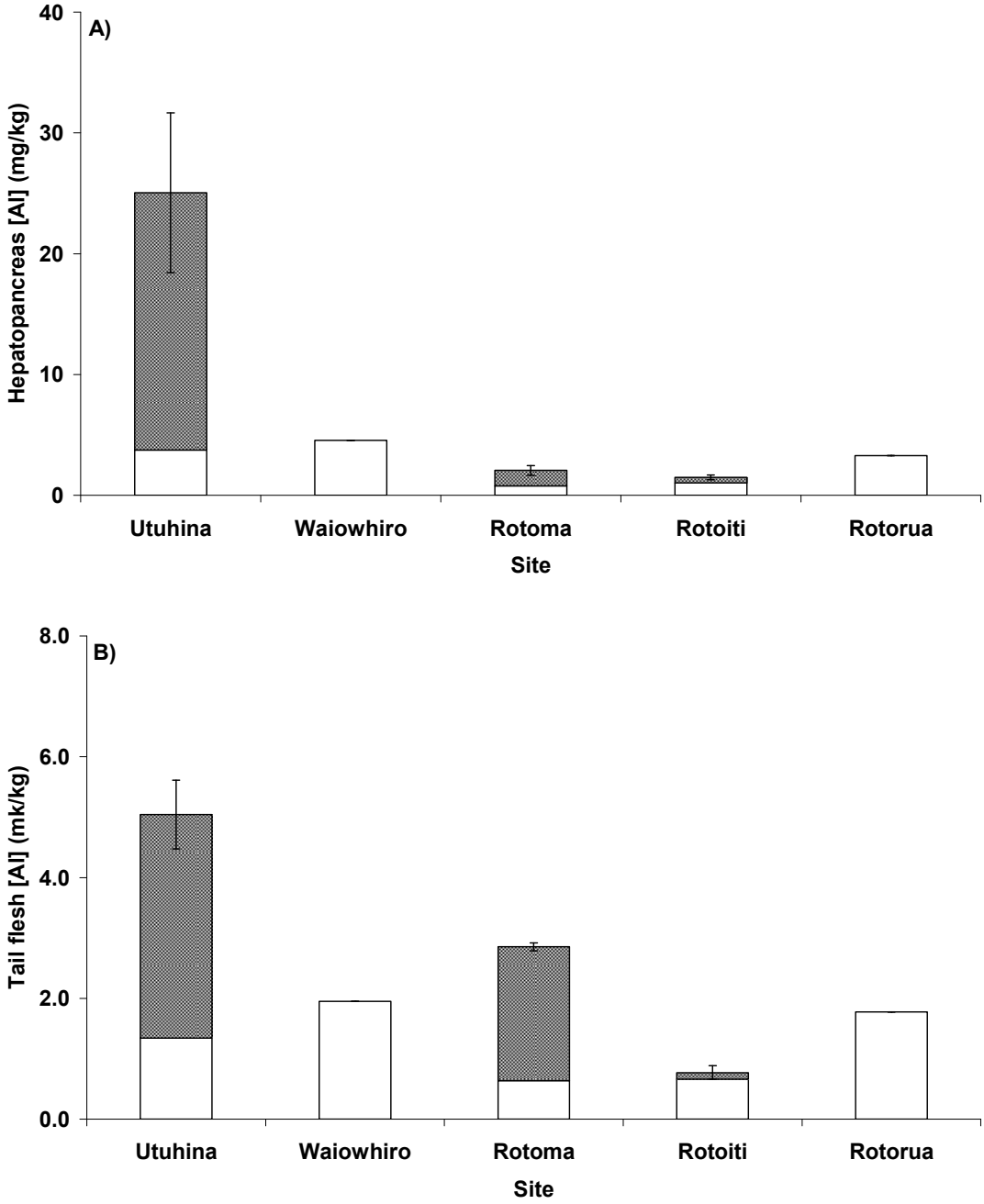

Fig. 5. Aluminium concentrations [Al] (mg/kg wet weight) in A) hepatopancreas and B) tail flesh tissues of koura (*Paranephrops planifrons*). Tissue [Al] was below detection limits in Waiowhiro Stream and Lake Rotorua koura. Open bars represent mean

detection limits for each population, while hatched bars represent mean [Al] measured above detection limits.

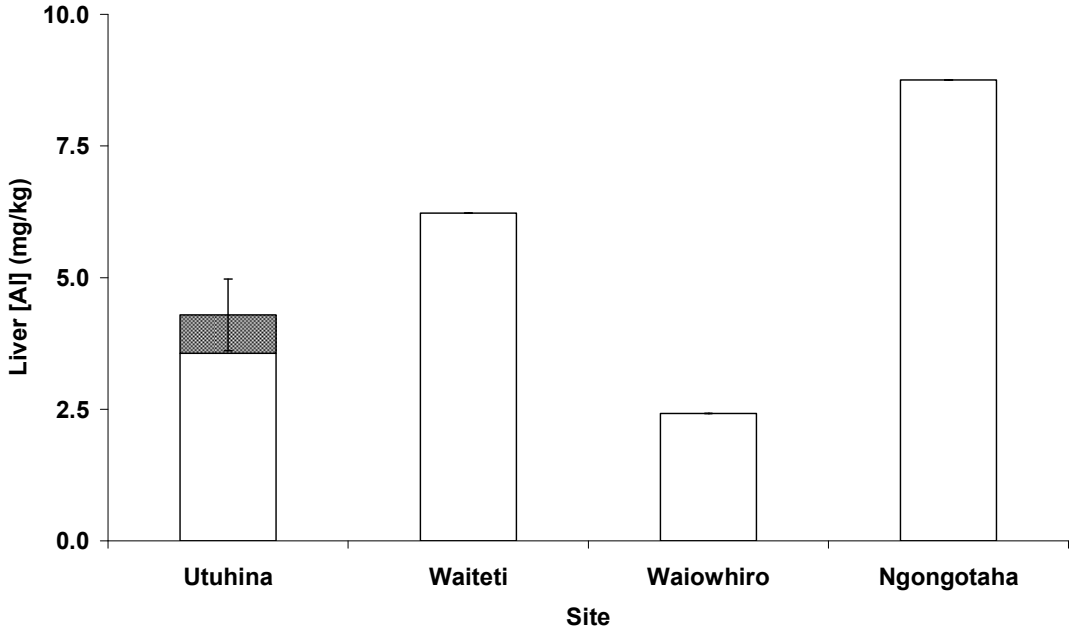

Fig. 6. Aluminium concentration [Al] (mg/kg wet weight) in common bully (*Gobiomorphus cotidianus*) livers. Liver [Al] was below detection in the Waiteti, Waiowhiro and Ngongotaha Stream bully tissues. Open bars represent mean detection limits in each population, while hatched bars represent mean [Al] measured above detection limits.

3.3.3 Haematology

Koura haemocyte counts

Contaminant bioaccumulation alone does not demonstrate an effect on animal fitness. Therefore, in an attempt to measure the possible ecological effects of long-term alum exposure, this study sought to identify, characterise and measure koura haemocytes as a potential indicator of physiological status or fitness. Invertebrate haemocytes perform a wide variety of functions such as clotting and wound repair, phagocytosis and encapsulation, carbohydrate synthesis and storage, osmoregulation, vitellogenesis and exoskeleton hardening (Bauchau, 1981). The three generally recognised haemocyte types (Hose *et al.*, 1990) have separate functional roles associated with the innate and complement invertebrate immune systems (Johansson *et al.*, 2000; Jiravanichpaisal *et al.*, 2006) and may have diagnostic enumeration value.

The flow cytometer facilitated a significant level of discrimination between koura haemocyte types based on morphology and light scatter, which was validated by microscopic examination (Fig. 7). Morphology of koura haemocytes appeared to conform to the established and generally accepted hyaline, semigranular and granular crustacean haemocyte descriptions (as reviewed by Bauchau, 1981). Analysis revealed a significant site effect ($p < 0.05$, ANOVA) on total haemocyte counts (Fig. 8A). Subsequent inter-site comparison demonstrated that only the Waiowhiro Stream and Lake Rotorua populations differed ($p < 0.05$, Tukey's). Apart from total haemocyte counts, Lake Rotorua and stream koura possessed similar proportional haemocyte counts. Waiowhiro Stream koura had a significantly greater proportion of hyaline cells compared to Lake Rotoma and Rototi populations (Fig. 8B). This observation was loosely mirrored by an opposing trend of proportionally fewer semigranular cells in both stream populations (Fig. 8C). Although a significant site effect was also observed for granular cell counts, significant differences between sites were not observed. However, near significant differences ($p \leq 0.1$) in the granular cell proportion were observed between the Waiowhiro Stream and lake populations (Fig. 8D).

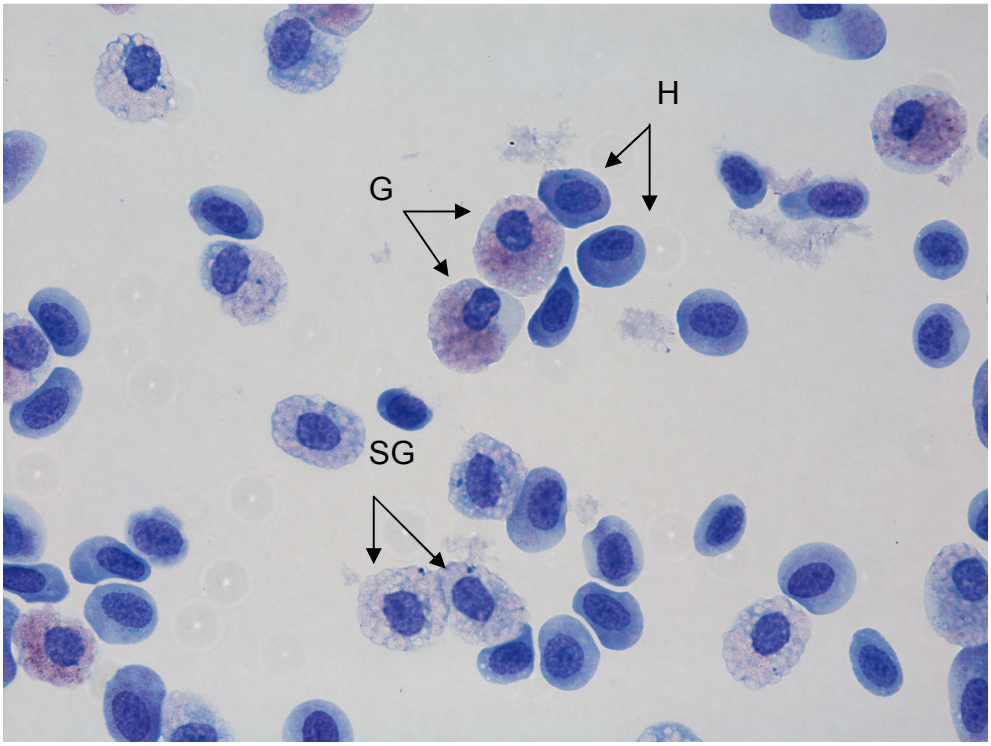

Fig. 7. Representative photo of preserved and cytospun koura (*Paranephrops planifrons*) haemolymph. Arrows denote the haemocyte types categorised as hyaline (H), semigranular (SG) and granular (G) cells.

It is not clear if haemocyte counts are generally indicative of maturity in crustacea, but elevated hyaline cell counts have been observed before and soon after moulting in shrimp (Sequeira *et al.*, 1995). Given that Lake Rotoiti and Rotoma specimens were found to be significantly larger than those in the Utuhina and Waiowhiro streams, differences in haemocyte counts here may be related to ontogeny or life history. Toxic exposure, prolonged air exposure, acute hypoxia and bacterial infection are also known to lower granular cell counts in crustacea (Soderhall and Smith, 1983; Smith *et al.*, 1995; Le Moullac *et al.*, 1998; Fotedar *et al.*, 2001), but are usually also associated with reduced total haemocyte counts. Differing haemocyte counts predominately in the Waiowhiro Stream koura in particular are suggestive of a localised or site-specific effect in this population. However, the functional significance of these differences could not be established in this study.

Aluminium is known to be toxic to other crayfish species (e.g. North American signal crayfish, *Pacifasticus leniusculus*) with the main site of action at the gill (Alexopoulos *et al.*, 2003). Ward et al. (Ward *et al.*, 2006) have also found that not only

can continuous exposure of sub-lethal levels of Al (0.5 mg/L) to crayfish initially result in an increase in THC in response to localised damage at the gill, but the animals also demonstrated immunological system recovery after >10 d exposure. Thus, aluminium may be potentially toxic to koura with possible immunological consequences. However, Utuhina Stream koura were generally comparable with at least two of the other sampled populations for any given haematological endpoint. Given that this population has been constantly exposed to Al over ~2 years, sublethal immunological effects may no longer be evident as a result of adaptation and recovery. However, koura tolerance to Al is unknown and it is equally possible that alum dosing in the Utuhina Stream may have had no sublethal effect on these haematological measures at any point in time.

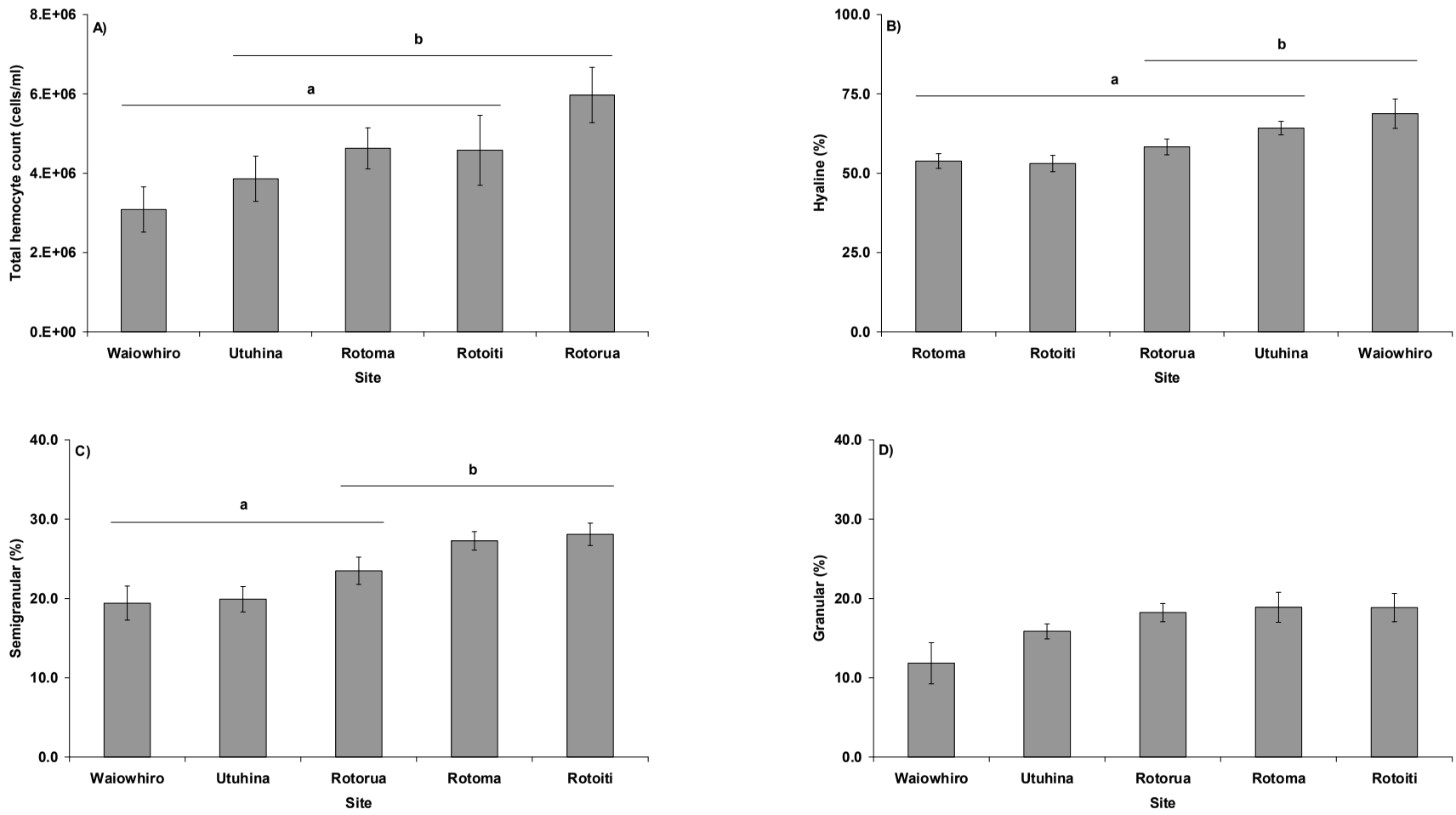

Fig. 8. Mean (± sem) A) total haemocyte count (cells/mL), and proportional (%) B) hyaline, C) semigranular and D) granular cell counts for koura (*Paranphrops planifrons*). Horizontal bars denoted by lower case letters represent homogenous ranges for cell counts (Tukey's $p > 0.05$).

Bully haematology

Considerable difficultly was encountered when sampling common bully due to generally small size restricting blood sampling. For a number of samples, significant clotting of the blood was observed which had varied affects on blood preparation and results. The effects of physiological stress associated with electrofishing and handling (e.g. Arnekleiv et al., 2004) on general haematology of common bully are also unknown. In future, capture methods such as minnow trapping may be more appropriate. Minnow trapping would presumably yield larger and less stressed fish which would enable more reliable blood sampling with lessened effects on sensitive endpoints such as haematology.

Therefore, these results are only cautiously interpreted. Nonetheless, significant site differences ($p < 0.05$, ANOVA) were observed for some haematological variables, most notably for male fish (Table 6). For female common bully, significant site effects were observed for only Hct and RBCC. In this instance, Ngongotaha bully had significantly lower Hct than all other sites, while Utuhina bully had slightly reduced total RBCC compared to the Ngongotaha and Waiowhiro. For male bully, site differences were observed for all parameters except MCV and differential leukocyte counts. These site effects were driven largely by differences in Hb content, RBCC and WBCC for Ngongotaha bullies, although some other minor differences were also observed between sites. In general, Utuhina bully results were similar to those from other sites and an obvious impact of the alum application on the haematology was not detected.

Table 6. Mean (sem) red and white blood parameters for male and female common bully (*Gobiomorphus cotidianus*) from the Utuhina, Ngongotaha, Waiteti and Waiowhiro Streams. Lower case letters represent statistically homogenous values (Tukey's $p > 0.05$) following determination of significant site differences ($p < 0.05$, ANOVA).

Haematological parameter	Utuhina	Ngongotaha	Waiteti	Waiowhiro
Males				
N	8	15	12	14
Hb (g/L)	44.4 (3.4)[a]	61.8 (2.9)[b]	48.9 (3.0)[a,c]	57.9 (2.1)[b,c]
Hct (%)	32.9 (2.0)[a,b]	25.1 (1.7)[a]	29.8 (2.4)[a,b]	34.5 (1.5)[b]
RBCC (x 1012 cells/L)	2.01 (0.14)[a]	1.42 (0.14)[b]	1.86 (0.20)[a,b]	1.93 (0.17)[a]
MCHC (pg/cell)	136.7 (10.0)[a]	255.5 (12.4)[b]	172.9 (11.1)[a]	170.0 (6.0)[a]
MCH (g/L)	22.2 (1.3)[a]	52.0 (7.1)[b]	28.6 (2.7)[a]	32.0 (2.1)[a]
MCV (fl)	166.0 (9.1)	214.4 (31.1)	168.5 (14.7)	191.8 (13.8)
WBCC (x 1010 cells/L)	5.04 (0.59)[a,b]	4.15 (0.56)[a]	7.55 (1.24)[b]	6.89 (0.74)[b]
Lymphocytes & Thrombocytes (%)	76.8 (3.5)	76.8 (1.9)	82.2 (2.8)	79.5 (1.9)
Granulocytes (%)	23.2 (3.5)	23.2 (1.9)	17.8 (2.8)	20.5 (1.9)
Females				
N	4	5	8	8
Hb (g/L)	34.7 (2.3)	56.5 (5.2)	43.2 (4.7)	60.9 (4.9)
Hct (%)	27.4 (2.5)[a]	20.6 (3.3)[b]	24.7 (3.1)[a]	37.6 (3.4)[a]
RBCC (x 1012 cells/L)	2.02 (0.22)[a]	1.68 (0.48)[b]	1.71 (0.21)[a,b]	2.09 (0.20)[b]
MCHC (pg/cell)	128.6 (9.3)	298.2 (40.1)	197.9 (42.4)	163.4 (4.1)
MCH (g/L)	17.7 (2.0)	42.4 (8.9)	26.4 (2.4)	30.0 (2.2)
MCV (fl)	137.0 (6.4)	155.4 (40.2)	146.4 (14.7)	184.6 (15.6)
WBCC (x 1010 cells/L)	7.08 (1.07)	4.91 (1.45)	8.05 (1.48)	9.04 (1.90)
Lymphocytes & Thrombocytes (%)	89.5 (1.2)	79.4 (3.6)	80.8 (3.1)	77.5 (5.7)
Granulocytes (%)	10.5 (1.2)	20.6 (3.6)	19.2 (3.1)	22.5 (5.7)

4. CONCLUSIONS

The biannual fish community surveys demonstrated changes in common bully abundance and increased fishing effort during the first year of alum dosing in the Utuhina Stream. Reduced common bully abundance in the stream coincided with highest stream concentrations of aluminium (Al) and lowest pH values. Recent surveys completed in April and July of 2008 since the alum dosing rate (and downstream pH) has stabilised show increased and consistent common bully numbers in the stream. It is suggested that the initial decline in fish abundance during the first year of alum dosing may have been linked to avoidance of exposure to increased Al, reduced pH or a combination of the two, followed by subsequent acclimatisation and recovery of the population. The comparative stream monitoring study demonstrated that Al from alum dosing in the Utuhina Stream was bioavailable to both common bully and koura with accumulation occurring in the tissues. Some differences were observed between sites for other measured parameters, such as fish condition and haematology. However, differences were typically minor and stream populations were similar in most respects. Despite the occurrence of Al bioaccumulation in Utuhina Stream biota, it is concluded that there has been no clear impact of alum on physiological endpoints after approximately 2 years of continuous exposure.

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