Hydrobiologia
DOI 10.1007/s10750-014-1805-9

PRIMARY RESEARCH PAPER

The effect of chronic exposure to phosphorus-inactivation agents on freshwater biota

Susan J. Clearwater · Christopher W. Hickey · Karen J. Thompson

Received: 23 May 2013 / Revised: 26 December 2013 / Accepted: 3 January 2014

Abstract The phosphorus (P)-inactivation agents alum or modified zeolite (Aqual-P^{TM}) are used in eutrophic lake remediation. Lake managers must evaluate the benefits of P-removal against potential adverse effects on lake biota. Laboratory mesocosms were used to determine whether a 2 month exposure to alum or Aqual-P had lethal or sublethal effects on native benthic-dwelling macroinvertebrates or fish. The P-inactivation agents were applied while the organisms were present to evaluate both acute- and longer-term effects. A gradient of doses up to 344 g alum m^{-2} (>7 mm capping layer thickness) and a single 200 g Aqual-P m^{-2} dose were applied with no detectable acute effects on survival or behaviour. After 2 months, there was no significant effect of alum or Aqual-P on the survival or growth of the crayfish, mussels or fish, but aluminium accumulation was measurable in some treatments. Fingernail clams were held in a sub-mesocosm to prevent predation, which resulted in exposure to intact capping layers. The highest alum dose significantly decreased fingernail clam survival and reburial rates, while 200 g Aqual-P m^{-2} caused highly variable survival. Our findings can be used by lake managers to assist the selection of site-specific application rates for these P-inactivation agents.

Keywords Alum · Aqual-P · Aluminium · Lake restoration · Sediment-capping · Z2G1

Introduction

Algal growth in lakes can be stimulated by nitrogen (N) and phosphorus (P) originating from external inputs (e.g. agricultural wastewater, sewage discharges, diffuse runoff) or internal inputs from sediment release during hypolimnetic deoxygenation (Jeppesen et al., 2005; Burger et al., 2008). The application of P-inactivation agents (e.g. alum or Aqual-P) to control the release of P from sediments in eutrophic lakes is aimed at reducing internal nutrient loads (Hickey & Gibbs, 2009). Phosphorus-inactivation agents can be applied as flocculating compounds that strip P from the water column as they settle, or sediment-capping agents that ideally form a thin (<2 mm) chemically active layer on the sediment that captures P released during hypolimnetic sub-oxic or anoxic events (Hickey & Gibbs, 2009). However, depending on local conditions (e.g. bioturbation, water currents), a capping layer may remain intact for only relatively short periods (days or weeks) after

Handling editor: P. Nõges

Electronic supplementary material The online version of this article (doi:10.1007/s10750-014-1805-9) contains supplementary material, which is available to authorized users.

S. J. Clearwater (✉) · C. W. Hickey · K. J. Thompson
National Institute of Water & Atmospheric Research, P.O. Box 11-115, Hamilton 3251, New Zealand
e-mail: s.clearwater@niwa.co.nz

Published online: 24 January 2014

application, particularly in shallow epilimnetic areas, before being mixed into the sediments. Such mixing will reduce exposure of infaunal species to elevated concentrations of P-inactivation agents or intact layers of these products.

A range of P-inactivation agents have been evaluated in New Zealand for efficacy and sustainability, and two aluminium-based products, alum and Aqual-P^{TM}, have been identified as suitable for local lake-scale applications to Lake Rotorua (central North Island, New Zealand) (Gibbs & Özkundacki, 2011; Gibbs et al., 2011). Lake Rotorua is a eutrophic polymictic lake that experiences hypolimnetic anoxic events on an intermittent basis (Burger et al., 2008). Alum (aluminium sulphate) requires the presence of alkalinity, which, if naturally present in the form of bicarbonate or applied as a buffering agent (e.g. $NaHCO_3$), will form an aluminium hydroxide ($Al(OH)_3$) precipitate that will settle as a colloidal, amorphous floc to coat the lake bed. Aqual-P is a proprietary formulation of aluminium-amended zeolite (formerly named Z2G1) developed in New Zealand and is applied as a phosphorus-inactivation agent in either a granular or powdered form (Gibbs & Özkundacki, 2011; Gibbs et al., 2011). Aqual-P differs in both physical and chemical properties from alum, with a higher density and a lower weight-specific P-binding capacity, which may be expected to affect dose-dependent responses of benthic organisms. Aqual-P has the added benefit of sorbing ammoniacal-N (Gibbs et al., 2011).

Benthic macrofauna could potentially be affected both chemically and physically by P-inactivation agents and indirectly via foodweb impacts. In low pH water (<6.0), toxic Al^{3+} can be released after alum application, and recent studies have indicated that sediment-capping with both alum and Aqual-P can inhibit microbial nitrification and denitrification under aerobic conditions (Gibbs & Özkundacki, 2011, Gibbs et al., 2011). Vopel et al. (2008) demonstrated that both pH and oxygen saturation decrease within 1 mm of the surface of a capping layer of either product. The low density alum floc can be moved around by currents, accumulating in layers deeper than intended when applied. Benthic fauna may in turn affect the distribution and efficacy of the P-inactivation agents by bioturbation.

Despite widespread use of alum for lake remediation in the Northern Hemisphere, there is little information regarding effects on lake biota (Welch & Cooke, 1999; Søndergaard et al., 2007). Some studies indicate a post-treatment increase in the abundance and diversity of benthic fauna; others have shown a short-term decrease in some species followed by a long-term recovery (Narf & Hine, 1985; Doke et al., 1995; Smeltzer et al., 1999; Lund et al., 2010; Steinman & Ogdahl, 2012). In New Zealand, evaluation of potential effects of P-inactivation agents on benthic fauna has generally been limited to short-term studies. A recent study showed that a 10 day laboratory exposure to 65 g alum m^{-2} had no detectable effect on survival and behaviour of adult native crayfish *Paranephrops planifrons* (Parkyn et al., 2010). A field survey of Lake Ōkaro (another Rotorua lake), after a low dose alum application applied during the suboptimal conditions of an algal bloom, found that common bully *Gobiomorphus cotidianus* densities decreased post-treatment, possibly as a result of high dissolved Al^{3+} concentrations (Quinn et al., 2004). Laboratory-based toxicity testing of Aqual-P on amphipods *Phreatogammarus helmsii* and fingernail clams *Sphaerium novaezelandiae* showed no significant lethal or sublethal effect of 350 g Aqual-P m^{-2} for 10 days, though organisms were added after application of the P-inactivation agent (Martin & Hickey, 2007). Short-term field and laboratory exposures have shown no detectable effect of $\geq$250 g Aqual-P m^{-2} on crayfish survival, behaviour and respiration (Parkyn et al., 2010). A field study of a 350 g Aqual-P m^{-2} application at Lake Ōkaro concluded that there was no obvious negative effect on either trout *Oncorhynchus mykiss* or common bullies 2 months after application (Landman & Ling, 2011).

In Rotorua, New Zealand the local stakeholders include the indigenous Māori community, who insisted that lake managers evaluate possible adverse effects of a proposed alum application on key benthic fauna. They also requested an assessment of the potential for aluminium (Al) accumulation in fish and crayfish because they are harvested for human consumption.

In the present study four native freshwater species (crayfish, mussels, common bullies, and fingernail clams), were exposed to a range of doses of either alum or a single dose of Aqual-P in a flow-through 2 month duration laboratory exposure. The P-inactivation agents were applied while the organisms were present to incorporate any potential acute effects of the

application process (e.g., physical effects of alum floc, or high initial Al^{3+} concentrations). Growth and survival was monitored for all species, while oxygen consumption and ammonia production by freshwater mussels and crayfish were measured at the conclusion of the 60 days exposure in respiration chambers. Doses were selected to encompass and exceed those likely to be used in the Rotorua lakes and we expected to be able to demonstrate effect thresholds for survival, growth, or Al content of the muscle tissues after chronic exposure and thus establish a safety factor for use of these P-inactivation agents by lake managers.

Materials and methods

Experimental design

The four species included in this study (crayfish, *P. planifrons*; mussels, *Echyridella menziesii* (previously *Hyridella menziesi*); common bullies, *G. cotidianus* and fingernail clams, *S. novaezelandiae*) are widely distributed in New Zealand in lakes, rivers and streams (Hopkins, 1970; Winterbourn & Mason, 1983; McDowall, 1990; Fenwick & Marshall, 2006), are found in lakes from the littoral to deeper zones, and are benthic-dwelling and feeding (Devcich, 1979; Stephens, 1982; James, 1985; James, 1987; Rowe, 2001; Butterworth, 2008). The fingernail clam is a small bivalve species; the adults reach a maximum shell length of 10 mm (Roa, 1997). It is thought that their depth distribution in Lake Rotorua is constrained by the upper limits of the anoxic hypolimnion; a species from the same family, the pea clam *Pisidium* sp. has been found at densities of 49–118 m^{-2} at depths of 40–115 m in Lake Taupo (Forsyth & McCallum, 1981). Both fish and crayfish are predatory and may be exposed to aluminium via foodweb accumulation; however, uncontaminated food was provided; therefore, this aspect of their environmental exposure was not evaluated in this study.

All of the organisms, except the fish, were obtained from either Lake Rotorua (38°2′.084S, 176°15′.476E), or adjoining Lake Rotoiti (38°1′.132S, 176°20′.705E). Fish were obtained from Lake Karāpiro (37°56′.870S, 175°39′.021E).

A mesocosm design was used to examine chronic effects on four species simultaneously. The fingernail clams were held in a sub-mesocosm to prevent predation by crayfish and fish.

The exposure regime incorporated a range of P-inactivation doses of alum relative to those planned for Lake Rotorua sediments (Gibbs et al., 2011) and was designed to identify potential adverse effects thresholds on fauna. This approach also enables us to evaluate the risk should a higher dose be considered for application. The nominal alum dose rates were: half the proposed dose rate termed Low; the proposed dose rate termed Medium; and three times the proposed dose rate termed High. A single Aqual-P dose was tested at the proposed application rate. In practice, the alum doses were 40% higher than the nominal design concentrations, while the Aqual-P dose was equivalent to the target dose (Table 1).

Crayfish

In autumn (May) 2011, young-of-the-year juvenile crayfish (13 ± 2 mm occipital-carapace length (OCL); 1.6 ± 0.7 g wet weight) were obtained from Lake Rotoiti (38°1′.132S, 176°20′.705E, central North Island, New Zealand) adjacent to Lake Rotorua, using tau kōura, a traditional method of crayfish harvesting (Kusabs & Quinn, 2009). Crayfish were transported to the laboratory and placed in aquaria supplied with recirculating, filtered, dechlorinated Hamilton City tapwater (DHCT), in a constant temperature room (16:8 h light:dark, 18–20°C). Short lengths of pipe (70–100 mm, 22–28 mm) were provided as shelter, and crayfish were fed daily on frozen chironomids until 6 days prior to transfer when they were provided with a sinking pelleted food (266 g ground trout food, 128 g ground fresh marine fish, 107 g gelatin). After 35 days, crayfish were transferred to the mesocosm tanks 2 days prior to addition of the alum and fed pelleted food daily at 1% body weight day^{-1} for the first 16 days of the alum exposure, then from day 17 onwards at 2.4% of their body weight three times week (i.e. equivalent to 1% body weight day^{-1}). Crayfish also feed directly on detritus such as decaying organic matter and biofilms provided by the bracken fern in the mesocosms.

Mussels and fingernail clams

Mussels (60 ± 4 mm shell length) and clams (2.4 ± 0.3 mm shell length) were obtained from the littoral zone of Hamurana Beach, Lake Rotorua at <2 m depth. In the laboratory, the mussels were placed in aquaria supplied with recirculating, filtered

Table 1 Application rates of liquid alum (as $Al_2(SO_4)_3{\cdot}16H_2O$) or Aqual-P used in the P-inactivation agent exposures. The mesocosms (0.46 m^2) contained 86 l water (0.086 m^3) once the alum or Aqual-P was added

Treatment	Application rate[a]	Units	Liquid alum (ml)	$Na_2CO_3{\cdot}10H_2O$ (g)	Al[a] (g replicate^{-1})	Al[a] (g m^{-3})	Al[a] (g m^{-2})	Capping layer thickness (mm)[b]	Relative P-binding (g P replicate^{-1})[c]
Control	0	g alum m^{-2}	0	0	0	0	0	0	0
Low	57	g alum m^{-2}	40	20	2	27	5	4.5	2.6
Medium	115	g alum m^{-2}	80	40	5	53	10	~7	5.2
High	344	g alum m^{-2}	240	120	14	159	29	>7	15.7
Aqual-P	200	g Aqual-P m^{-2}	[92 g Aqual-P]	–	2	23	4	1.7	2.0

Application rates of 80 g alum m^{-2} and 190 g Aqual-P m^{-2} are considered to be the minimum effective doses for Lake Rotorua sediments (Gibbs et al., 2011)

[a] Calculated from Al concentration measured in Orica solution (57 g Al l^{-1}) and expressed as equivalents of alum as $Al_2(SO_4)_3{\cdot}16H_2O$, and total Al concentration measured in Aqual-P by Gibbs et al. (2011)

[b] Estimated from Vopel et al. (2008)

[c] Uses 0.87 kg Al kg P^{-1} or 1.15 kg P kg Al^{-1} for alum from Hickey & Gibbs (2009) and 21.5 mg P g^{-1} Aqual-P from Gibbs et al. (2011)

DHCT in a constant temperature room (16:8 h light:-dark, 18–20°C), and the clams were placed in small tanks, with 1 l DHCT (aerated, non-renewal) and 0.02 m depth sieved sediment from the collection site. Clams were fed 0.1 g dried invertebrate feed (GORP) and 30 ml concentrated *Pseudokirchneriella subcapitata* ($\sim 1 \times 10^6$ cells ml^{-1}) three times $week^{-1}$. Mussels were fed 100 ml concentrated *P. subcapitata* ($\sim 1 \times 10^6$ cells ml^{-1}) and 100 ml invertebrate feed (liquid yeast + trout food preparation) three to five times $week^{-1}$. Following 2 weeks acclimation to laboratory conditions, mussels and clams were added to the mesocosms or sub-mesocosms (18–20 of each species) 3 days prior to addition of the P-inactivation agents. Clam lengths (1.8–3.2 mm) were measured using a micrometre under 16× magnification. Clam condition factor indices (CFI) were calculated by:

$$\mathrm{CFI} = \left(\frac{\mathrm{Wt}}{(L^3)}\right) \times 1000$$

where Wt = wet weight (mg) and L = length (mm).

Mussel CFI were calculated by:

$$\mathrm{CFI} = \left(\frac{\mathrm{DWt}}{\mathrm{SWt}}\right) \times 10$$

where DWt = dry flesh weight (g) and SWt = Dry shell weight (g).

Fish

Bullies (35 ± 4 mm length; 0.5 ± 0.2 g wet weight) were obtained from Lake Karāpiro ((37°56′.870S, 175°39′.021E), central North Island, New Zealand) by seining in the littoral zone (1.5 m depth) shortly after nightfall. In the laboratory, fish were placed in aquaria supplied with recirculating, filtered, DHCT adjusted to 5 ppt with filtered seawater (for prophylactic disease treatment) in a constant temperature room (16:8 h light:dark, 15°C). Fish were held 28 days prior to being transferred to the mesocosms 3 days prior to application of the P-inactivation agents. Fish were fed daily on frozen chironomids in the holding tanks, but upon transfer to the mesocosms consumed the sinking pelleted food intended for the crayfish. Feed rates were increased to satiate the fish and reduce competition with the crayfish which resulted in daily feed rates of 10% body weight d^{-1}. Ten days after addition of the P-inactivation agents to the mesocosms, the fish were

separated from the crayfish in open bottom mesh corrales (0.045 m^2) to reduce competition for food with the crayfish. Fish that escaped from the corrales were recaptured in small traps. Thirty-one days after application of the P-inactivation agents, the fish were removed from the mesocosms, measured and euthanised by anaesthetic overdose (0.01% clove oil).

Mesocosms

Fifteen tanks (0.83 m × 0.56 m), with a 0.03 m deep sediment layer of 20% Lake Rotorua mud (sieved to <500 μm) and 80% washed mortar sand (<1000 μm), were filled with 95 l (0.20 m deep) DHCT. Freshwater mussels, crayfish and common bullies were placed in the main body of the tank, while the sphaeriids were held in a separate enclosure (a sub-mesocosm) with 150-μm mesh walls to prevent predation by the larger organisms. Twenty individuals of each species were added to each tank, except crayfish for which only eighteen per tank were added. Shelter was provided by small tubes for the crayfish and bracken fern bundles for both fish and crayfish. Organisms were added to the mesocosms 2–3 days prior to addition of the P-inactivation agents.

Continuous water flows of 132 ml min^{-1} (a water volume replacement time of 2 × day^{-1}) entered each tank in the rear of the small enclosure and flowed through the mesh walls in a diffuse path to the main tank, exiting through an overflow drain at the water surface at the opposite end of the tank to the inflow. The header tank and all experimental tanks were continuously aerated. The experiment was contained in a constant temperature room maintained at 20°C with a 16:8 light:dark cycle. Tanks were checked, and fish were fed daily; crayfish were fed four times $week^{-1}$, while the sphaeriids in the small mesocosms were fed three times $week^{-1}$.

Application of P-inactivation agents

The P-inactivation agents were applied with the organisms present in the mesocosms at three different application rates of alum and one application rate of Aqual-P (Table 1). Gibbs & Özkundacki (2011) demonstrated in experimental cores that application rates of 350 or 700 g Aqual-P m^{-2} (either < 1 mm grain size or 1–3 mm) will effectively block P-release from Lake Ōkaro sediments under both aerobic and anoxic conditions and a related study indicated that 190 g Aqual-P m^{-2} (<1 mm grain size) was the minimum effective removal dose for Lake Rotorua sediments (Gibbs et al., 2011). All treatments were replicated three times. The alum floc stock solution was prepared as a slurry by premixing the appropriate volumes of the nominally 47% w/w alum solution (Orica Chemicals) with sodium carbonate ($Na_2CO_3 \cdot 10H_2O$) in 6 L of water and pH adjusted to 7.5 (see Table 1 for details). Aqual-P was applied as 92 g of the powdered formulation mixed to a slurry in 6 L of water. Water flows were ceased, and 15 l of water was syphoned out of each mesocosm (leaving 80 l), before the 6 l of alum stock or Aqual-P slurry was applied by hand and gently dispersed throughout the mesocosm (0.46 m^2) – 0.6 l was applied directly to each sub-mesocosm (0.048 m^2). Water flow was recommenced 24 h later once the alum or Aqual-P had settled.

The reported alum application rates are based on the measured (rather than nominal) aluminium content of the solution provided by Orica Chemicals Ltd (57 g Al l^{-1} (2.11 M Al), equivalent to 665.6 g $Al_2(SO_4)_3 \cdot 16H_2O$ l^{-1} (1.06 M alum) or 361.3 g $Al_2(SO_4)_3$ l^{-1}(anhydrous)). The Orica MSDS describes the solution as 47% w/w aluminium sulphate (specific gravity 1.32) and uses the CAS 10043-01-3 of anhydrous aluminium sulphate (1.06 M alum is equivalent to 627.4 g $Al_2(SO_4)_3 \cdot 14H_2O$ L^{-1} (63% w/v or and 48% w/w)). Alum as $Al_2(SO_4)_3 \cdot 14H_2O$ l^{-1} is often used in industrial scale preparation of alum solutions. Alum application rates in the present study are expressed as g $Al_2(SO_4)_3 \cdot 16H_2O$ m^{-2} for consistency with related publications in which solid alum as $Al_2(SO_4)_3 \cdot 16H_2O$ was used to prepare application solutions (Vopel et al., 2008; Hickey & Gibbs, 2009; Gibbs et al., 2011). Alternative expressions of the doses such as g Al m^{-2} together with the relevant treatment descriptors are provided in Table 1.

Respiration measurements of crayfish and mussels

After 59 or 60 days of exposure (it took 2 days to complete the respiration measures) to the P-inactivation agents, crayfish and mussels were placed in 1000 mL respiration chambers in groups of 6 to 10, for 2–5 h to measure oxygen consumption (μg O_2 g wet $weight^{-1}$ h^{-1}), and total ammoniacal-N production (μg NH_3-N g wet $weight^{-1}$ h^{-1}). Following these measurements, crayfish and mussels were weighed and measured, then euthanised by freezing.

Laboratory analyses

Hepatopancreas and muscle tissues were pooled (3–7 crayfish per sample) to provide sufficient tissue for analysis of aluminium concentrations ($n = 9$ per treatment). Seven mussels or 5–7 fish were pooled into one whole-body sample per tank ($n = 3$ per treatment). Tissues were dried until constant weight then microdigested (HNO_3 and HCl acid, 85°C, 1 h) and measured by ICP-MS (DL = 0.06 mg kg^{-1} dry wt) (Hill Laboratories, Hamilton, NZ, IANZ, ILAC accredited).

Dissolved aluminium (DL = 3 μg l^{-1}) concentrations in filtered (0.45 μm) and acidified (1% HNO_3) water samples were measured by ICP-MS (APHA 3125 B 21st ed, Hill Laboratories). Water samples for dissolved ammoniacal-nitrogen (NH_3-N) [filtered (Whatman® GF/C glass fibre filter, 0.45 μm filter) then frozen prior to analysis] were analysed on a Lachat Flow Injection Analyser using standard Lachat methods for freshwaters (phenol/hypochlorite colorimetery, DL = 1 μg l^{-1}).

Statistical analyses

Data were analysed by one-way (main factor; treatment), or repeated measures (crayfish length and weight) ANOVA ($\alpha = 0.05$ unless stated otherwise, SAS Version 9.3), and a Tukey's post hoc test of significantly different means. If assumptions of normality and homogeneity of variance were not met, data were transformed. Data are reported as mean ± standard deviation (s.d.) unless stated otherwise.

Results

Application of the P-inactivation agents

One hour after dosing with the alum, pH remained within a narrow range of 7.40–7.58 in all three alum treatments. The pH in the control tanks was 7.55–7.57, and one hour after dosing with Aqual-P pH was 7.02–7.04. Conductivity was much more variable between treatments: controls (183–184 μS cm^{-1}); Low alum (618–649 μS cm^{-1}); Medium alum (1048–1170 μS cm^{-1}); High alum (2.61–2.70 mS cm^{-1}); and 200 g Aqual-P m^{-2} (240–243 μS cm^{-1}). When visible, the behaviour of all fish, mussels and crayfish appeared to be normal during the application of the alum or Aqual-P. Clarity was markedly reduced in the Aqual-P treatment and the two highest alum doses. Mussels were observed with syphons out 3 hours after application of Low alum. Twenty-four hours later before flows were recommenced, at least some mussels were observed with syphons extended in the Low alum and Aqual-P treatments; although activity was lower than in the control treatment. Although clarity was reduced in many tanks, some fish and crayfish could be observed in all tanks and their behaviour appeared to be normal.

Water quality

Once flows were recommenced after the application and maintained at 132 ± 19 ml min^{-1} for the duration of the 2 month exposure, water quality remained within a narrow range across all treatments (18.5 ± 0.4°C, 8.7 ± 0.7 mg O_2 l^{-1}, pH 7.6 ± 0.2, 166 ± 54 μS cm^{-1}, hardness 82 ± 44 as mg $CaCO_3$ l^{-1}).

One day after the application of the P-inactivation agents and immediately prior to recommencing water flows, dissolved aluminium concentrations were significantly lower in the Aqual-P treatment than the control treatment and the 57 g m^{-2} alum treatment, which in turn were significantly lower than in the 115 g m^{-2} alum and the 344 g m^{-2} alum treatment ($P < 0.001$) (Fig. 1). Ten and 60 days after application, average dissolved aluminium concentrations were less than 20 μg l^{-1} in all tanks, while 30 days after application concentrations were 16–30 ± 23 μg l^{-1} in all treatments except the highest alum treatment which was 83 ± 29 μg l^{-1} ($F = 7.2$, $P = 0.0054$). Using the average dissolved Al concentrations and water flows through the tanks, we calculate that after 2 months at least 83, 87, 93 and 92% of the initial dose remained in the Low alum, Medium alum, High alum and Aqual-P treatments, respectively. If the Al concentrations in the control treatment are taken into account estimates of the percentage dose remaining increase to 93, 92, 95 and 100% in the Low alum, Medium alum, High alum and Aqual-P treatments, respectively.

Throughout the 2-month long experiment, NH_3-N concentrations measured in the tanks varied from 1 to 101 μg l^{-1} ($n = 45$) and remained well below the ANZECC chronic water quality guideline of 1470 μg l^{-1} for protection of 95% of species at pH 7.6 (ANZECC & ARMCANZ, 2000).

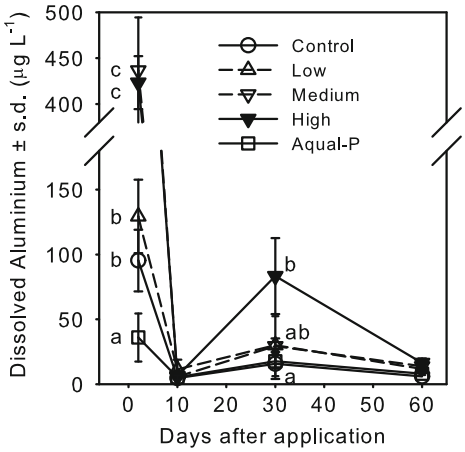

Fig. 1 "Dissolved" aluminium concentrations measured over time after application of alum or Aqual-P ($n = 3$) based on 0.45 µm filtration (see text for discussion of potential colloidal clay interference with initial measurements). *Lower case letters* indicate significantly different treatments within a day. *Circle* control; *upward triangle* Low alum; *downward open triangle* Medium alum; *downward solid triangle* High alum; 200 g Aqual-P m^{-2}. See Table 1 for details of alum and Aqual-P treatments

Freshwater crayfish

After 2 months exposure to the P-inactivation agents, crayfish survival was 92 ± 3% to 100% in all treatments. Survival in the High alum treatment (92 ± 3%) was significantly lower than in the Low alum treatment (100%) but not the control treatment (96 ± 4%) ($P < 0.05$). Growth was not significantly different between treatments after either 1 or 2 months (weight $F = 0.5$, $P = 0.74$; OCL $F = 0.8$, $P = 0.56$) (Table 2). Average lengths (OCL) and weight increased by 15% and 50%, respectively from day -2 of the exposure (13 ± 2 mm OCL; wet weight 1.6 ± 0.7 g) to day 60 (15 ± 2 mm OCL; wet weight 2.4 ± 1.0 g) (OCL, $P < 0.001$; weight, $P < 0.001$ between 1 and 2 months). There was no significant interaction between time and treatment for either length or weight ($F = 0.4$, $P = 0.84$; $F = 0.2$, $P = 0.92$, respectively). At the conclusion of the 2 month long exposure there was no significant difference in either the oxygen consumption (range 106 ± 24 to 120 ± 25 µg O_2 g^{-1} wet wt h^{-1}, $F = 0.4$, $P = 0.81$) or ammonia production rates (range 3.4 ± 0.7 to 4.0 ± 1.8 µg NH_3-N g^{-1} wet wt h^{-1}, $F = 0.4$, $P = 0.84$) of the crayfish exposed to different treatments. Aluminium concentrations in the hepatopancreas of crayfish exposed to either Low or High alum were 22 ± 12 and 36 ± 24 µg g^{-1} dry wt, respectively and significantly higher than those measured in the control treatment (4 ± 2 µg g^{-1} dry wt, $P < 0.001$) (Fig. 2a). Aluminium concentrations were 20 ± 10 and 9 ± 7 µg g^{-1} dry wt in the Medium

Table 2 Initial and final wet weight and length of crayfish, fish, mussels and clams, and condition factor index (CFI) for clams after 60 d of exposure to different application rates of alum or Aqual-P

Parameter	Treatment	Crayfish	Fish	Mussels[a]	Clams[b]	Clam CFI
Weight (g)	Control (day 0)	1.6 (0.7)	0.5 (0.2)	–	2.7 (1.5)	183.4 (31.0)
	Control	2.5 (1.2)	0.7 (0.3)	1.2 (0.4)	2.7 (1.2)	175.0 (25.0)
	Low alum	2.4 (0.9)	0.7 (0.3)	1.3 (0.3)	2.5 (0.8)	173.0 (21.0)
	Medium alum	2.4 (1.1)	0.7 (0.3)	1.3 (0.4)	2.3 (1.0)	165.4 (26.3)
	High alum	2.4 (0.9)	0.7 (0.3)	1.1 (0.4)	2.4 (1.0)	157.5 (27.7)
	Aqual-P	2.4 (1.0)	0.7 (0.4)	1.1 (0.4)	2.5 (1.8)	160.9* (23.3)
Length (mm) or SWt (g)	Control (day 0)	12.6 (1.8)	35.5 (4.1)	–	2.4 (0.3)	–
	Control	14.9 (2.4)	38.5 (5.1)	12.7 (4.2)	2.5 (0.3)	–
	Low alum	14.8 (1.9)	39.1 (4.7)	12.0 (3.6)	2.4 (0.2)	–
	Medium alum	14.6 (2.3)	39.1 (4.2)	12.3 (3.7)	2.4 (0.2)	–
	High alum	14.8 (1.9)	39.0 (4.6)	11.8 (3.8)	2.4 (0.2)	–
	Aqual-P	14.7 (2.2)	39.0 (5.6)	11.8 (3.6)	2.4 (0.3)	–

Mean (± s.d.). * indicates significantly different to control. *SWt* dry shell weight for mussels. See Table 1 for details of alum and Aqual-P treatments

[a] Mussel values are dry flesh weights, mussel dry shell weights were measured rather than shell lengths

[b] Clam wet weights are in mg

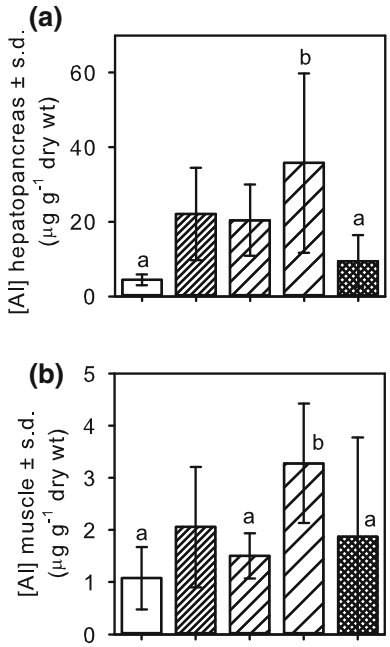

Fig. 2 Mean aluminium concentration in the crayfish **a** hepatopancreas, or **b** muscle tissues, 2 months after application of alum or Aqual-P ($n = 9$). *Letters* indicate significantly different treatments. See Table 1 for details of alum and Aqual-P treatments

alum treatment and the 200 g Aqual-P m^{-2} treatment, respectively, but neither was significantly greater than the control treatment. Aluminium also accumulated in the muscle tissues of crayfish exposed to High alum (3 ± 1 µg g^{-1} dry wt) relative to the controls and all other treatments ($1–2 \pm 2$ µg g^{-1} dry wt), except the Low alum (2 ± 1 µg g^{-1} dry wt) treatment (log-transformed, $F = 5.9$, $P < 0.001$; Fig. 2b).

Fish

Fish survival 1 month after exposure to the P-inactivation agents was 98–100% in all tanks (excluding one replicate of the High alum treatment where approximately 7 fish were accidentally killed on day 14 of the exposure) and not significantly different between treatments ($P = 0.64$). Fish weight ($P < 0.001$) and length ($P < 0.001$) increased significantly by 40% and 8%, respectively, from day -3 (0.5 ± 0.2 g, 36 ± 4 mm, $n = 50$) to day 31 (0.7 ± 0.3 g, 39 ± 5 mm, $n = 277$) (Table 2). Average final fish weight and length were not

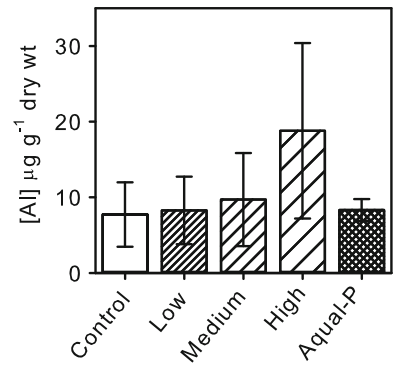

Fig. 3 Aluminium concentrations in the fish (whole body µg g^{-1} dry wt), 2 months after application of alum or Aqual-P ($n = 3$) ($F = 1.5$, $P < 0.3$). See Table 1 for details of alum and Aqual-P treatments

significantly different between treatments ($P = 0.88$, $P = 0.97$, respectively). Aluminium concentrations in the whole body of the fish ranged from 8 to 10 ± 6 µg g^{-1} dry wt in all treatments except those exposed to High alum, which were 19 ± 12 µg g^{-1} dry wt; however, these were not significantly different due to the high variability in the concentrations ($F = 1.53$, $P < 0.3$; Fig. 3).

Freshwater mussels

At day 60, mussel survival was 100% in all treatments except Aqual-P in which 1 mussel died (98%). There was no significant difference in dry flesh weights ($P = 0.53$), dry shell weights ($P = 0.63$), CFI (range 0.9 ± 0.2 to 1.0 ± 0.2, $P = 0.6$), or ammonia production rates (4.7 ± 1.6 µg NH_3-N g^{-1} dry wt h^{-1}, $P = 0.55$) of mussels exposed to different treatments (Table 2). Oxygen consumption rates of mussels exposed to Aqual-P (126 µg O_2 g^{-1} dry wt h^{-1}) were significantly lower than mussels in the control treatment (166 µg O_2 g^{-1} dry wt h^{-1}) but not compared to those exposed to alum (145–164 µg O_2 g^{-1} dry wt h^{-1}) ($P < 0.04$) (Fig. 4a). These data indicate that the alum applied at either Low, Medium or High application rates (57, 115 or 344 g alum m^{-2}) or 200 g Aqual-P m^{-2} had no marked or significant effect on mussel survival or growth, but Aqual-P may have some sublethal effect on mussel respiration. Aluminium concentrations of 127 ± 41 µg g^{-1} dry wt accumulated in the whole body of mussels exposed to High alum; this includes the contribution of gut contents. These concentrations were significantly higher than those measured in all other

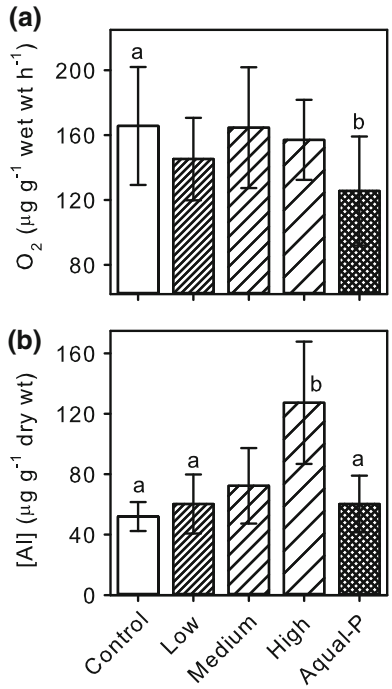

Fig. 4 Mean mussel **a** oxygen consumption ($n = 6$) or **b** whole-body aluminium accumulation (µg g^{-1} dry wt) ($n = 3$) after 2 months of exposure to the P-inactivation agents alum or Aqual-P. *Different letters* indicate significantly different treatments. See Table 1 for details of alum and Aqual-P treatments

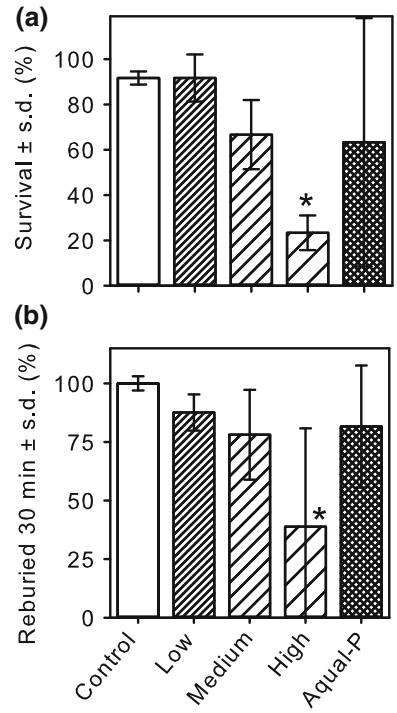

Fig. 5 Mean clam **a** survival and **b** 30 min reburial rates after 2 months exposure to the P-inactivation agents alum and Aqual-P (15 min data not shown) ($n = 3$). *Letters* indicate significantly different treatments ($P < 0.10$). See Table 1 for details of alum and Aqual-P treatments

treatments (52–60 ± 20 µg g^{-1} dry wt), except the 72 ± 25 µg g^{-1} dry wt that accumulated in mussels exposed to Medium alum ($F = 4.5, P < 0.02$; Fig. 4b).

These data indicate that the P-inactivation agents had no marked or significant effect on mussel survival, and as for crayfish and fish, there is a wide margin of safety for application rates ≤344 g alum m^{-2} (High alum) or ≤200 g Aqual-P $m^{-2.}$

Fingernail clams

Clam survival in the control treatment was 92 ± 3% and application of alum decreased survival in a dose-dependent manner (Fig. 5a). After 2 months, survival was 92 ± 10% and 67 ± 15% in the Low and Medium alum mesocosms, respectively. When alum was applied at the High dose, survival was significantly lower (23 ± 8%) than the controls ($P < 0.001$). Decreased survival in response to alum application was reflected in the 15 and 30 min reburial rates of the remaining clams which tended to decrease in a dose-dependent fashion but were only significantly lower in clams exposed to High alum ($P < 0.05$; $P < 0.10$ respectively) (Fig. 5b).

Application of 200 g m^{-2} Aqual-P resulted in 63 ± 55% survival, which, according to a post hoc Tukey's test comparing treatment means, was not significantly different to the control treatment or the three alum treatments ($P < 0.001$). Survival was highly variable in this treatment, because no live clams were found in one tank, but survival was 95% in the remaining two replicates. Reburial rates of clams exposed to Aqual-P and surviving 60 days post-application were not significantly different to those of clams in the control treatment.

At 60 days post-application, clam lengths and weight did not decrease significantly in comparison to day 0 and were not significantly different between treatments ($P = 0.67$, $P = 0.52$, respectively) (Table 2). The CFI also did not change significantly in either the control treatment or Low alum in comparison to CFI at day 0 but was significantly lower in clams exposed to either Medium or High

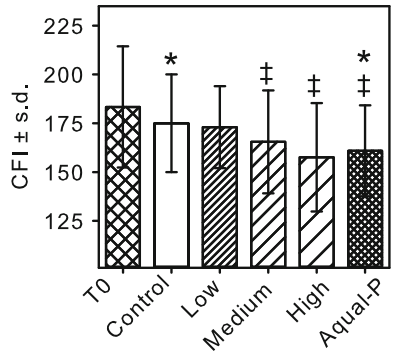

Fig. 6 Mean clam condition factor index (CFI) after 2 months of exposure to the P-inactivation agents alum and Aqual-P ($n = 3$). *Asterisk* indicates treatments significantly different to one another (day zero not included in the comparison), *double cross* indicates treatments significantly lower than day zero CFI. See Table 1 for details of alum and Aqual-P treatments

alum, or Aqual-P ($P < 0.001$) (Fig. 6). The CFI of clams exposed to Aqual-P for 60 days was significantly lower than the CFI of clams in the control treatment at 60 days ($P < 0.05$).

Discussion

Vopel et al. (2008) demonstrated that pH decreases within an intact capping layer of alum or Aqual-P which could facilitate the release of toxic Al^{3+} into the porewaters. Therefore, it is important to note that in the large mesocosm tanks where fish, crayfish and mussels were present, the bioturbation by these macroorganisms relatively rapidly mixed the phosphorus-inactivation agents into the sediment. Within approximately 10 days of the application, it was difficult to distinguish visually between the treatments. In contrast, in the sub-mesocosms containing the fingernail clams, the alum or Aqual-P formed a continuous layer that was minimally disturbed for the duration of the experiment, although multiple paired burrow holes from the clams were visible through the capping layer in all treatments within hours of the application (see Supplementary information Figs. 1 and 2).

Effect of the P-inactivation agents on water quality

In the first 24 h, near-surface application of the P-inactivation agents had some short-term effects on water quality, particularly conductivity, although pH was maintained in a circum-neutral range. The abrupt changes in conductivity would not occur in a lake environment because of dilution processes that would occur in the distance between the near-surface application of the flocs or Aqual-P and the product settling to coat the bed. In the present study, these conditions had no obvious effect on behaviour of the organisms, and no mortalities were recorded in the days following the application. Throughout the application period, the crayfish and fish were active and readily moved through the thickest alum floc (>7 mm). We consider that the initial dissolved Al concentrations (operationally defined as Al concentrations measured in a 0.45 µm filtered water sample) were elevated as a result of the resuspension of colloidal sediment-associated clays (particularly in the control treatments), disturbed by the activity of the fish and crayfish during the initial dosing procedure when water flows were ceased, and in the alum treatments by aluminium floc material which passed through the 0.45 µm filter. During the application, dissolved Al was the lowest in the Aqual-P treatment. The zeolite in Aqual-P may have adsorbed some of the dissolved Al (Gibbs et al., 2011).

Once water flows were recommenced, overall water quality (i.e., DO, pH, conductivity, ammonia) was maintained in a range not expected to cause any significant stress to the fish and invertebrates. Dissolved Al in all treatments was measured at $\leq$34 µg l^{-1} in all treatments apart from day 30 when "dissolved" Al concentrations were 68 µg l^{-1} in one replicate of the Medium alum treatment, and 51–108 µg l^{-1} in the High alum treatment (Fig. 1). The ANZECC & ARMCANZ (2000) water quality guideline is 55 µg l^{-1} for protection of 95% of species at pH > 6.0. The pH in the present study was 7.6 ± 0.2, and the water was moderately hard (82 ± 44 as mg $CaCO_3$ l^{-1}); both circumneutral water and increasing hardness decrease Al toxicity and uptake, but nonetheless concentrations above 55 µg l^{-1} may have caused some Al accumulation in the crayfish, mussels and fish (Gensemer & Playle, 1999) as discussed below.

Overall, we consider that high water quality was maintained in the flow-through mesocosms for the duration of the 60 days exposure period. The initial 24 h period of reduced water quality was necessitated by the requirement to cease water flow to allow the P-inactivation agents to settle. As such, this represents

a "worst case" condition for the organisms in terms of a rapid change in water quality, including conductivity and exposure to various quantities of alum floc.

Effects of P-inactivation agents on fish, crayfish and mussels

There was no detectable effect of the P-inactivation agents on the growth and survival of the fish, crayfish and mussels, even at the High alum application rates of 344 g m^{-2} (i.e. >7 mm capping layer thickness). Both fish and crayfish grew significantly during the 1 or 2 month exposure (by 40 and 50% by weight, respectively), providing a good basis to detect potential adverse effects. These species are mobile and may be able to continue to access smothered food items after application of alum or Aqual-P depending on the depth of the product layer. In the present study, this assumption was not tested, as uncontaminated food was supplied, whereas in a natural environment, their access to food items could be reduced by either smothering, or indirect effects on their prey species (e.g. avoidance, Al accumulation). This aspect of their exposure to P-inactivation agents (i.e., foodweb effects) should be examined in field studies (e.g. by examining gut contents of resident species, or stable isotope studies).

Mussel growth rates are relatively slow so we expected no detectable growth in 2 months, especially since no food was provided directly. We relied on addition of invertebrate feed to the internal secondary mesocosms and the activities of the other species to produce sufficient biofilm-laden waterborne particles to sustain the mussels for the 2 month exposure. The only detectable sublethal effect on the mussels was decreased oxygen consumption after exposure to Aqual-P for 2 months. The mechanism could be physical or chemical impairment of gill function by the Aqual-P; however, gill morphology was not examined. A decrease in oxygen consumption in response to Al exposure has been noted previously in fish and invertebrates (excluding aquatic insects), as a result of Al precipitation in the gill microenvironment or mucus production (Gensemer & Playle, 1999). Notably, there was no significant change or trend towards decreased respiration in the High alum treatment where mussels accumulated 2.4-fold greater whole-body Al concentrations than in the controls. This suggests that the Aqual-P response may be associated with the zeolite carrier rather than Al exposure.

Aluminium accumulation in biota

All three alum application rates caused aluminium accumulation in the crayfish hepatopancreas, which may have some metabolic cost (e.g. during detoxification and storage), although it did not affect growth rates in this 2 month exposure. The Al accumulation could have occurred via uptake of waterborne Al during the alum application; however, even during the application phase, the pH in the mesocosms was circum-neutral, and the water was moderately hard, and both conditions reduce dissolved Al bioavailability (Gensemer & Playle, 1999). The crayfish spent a large proportion of the initial 4 days of the exposure period in intimate contact with the deep floc layer of the High alum treatment where dissolved Al may have been more bioavailable relative to other treatments (Vopel et al., 2008). Given their benthic feeding habits, it is highly likely that the crayfish ingested an unknown quantity of sediment-associated aluminium throughout the exposure period, but dietborne Al is generally not very bioavailable compared to waterborne Al (Spry & Wiener, 1991; Gensemer & Playle, 1999). Two fish studies have shown minimal effect of chronic exposure to diets containing 2000 μg Al kg^{-1} wet weight as $AlCl_3 \cdot 6H_2O$ (Poston, 1991) or 10 000 μg Al g^{-1} wet wt (as $Al_2(SO_4)_3$) (Handy, 1993). Poston (1991) suggested that the uptake of dietborne Al would be inhibited by the formation of insoluble aluminium phosphate ($Al[OH]_2H_2PO_4$) compounds in the diet and during digestion. No significant Al accumulation occurred in the Aqual-P treatment relative to the controls, perhaps as a result of significantly lower dissolved Al concentrations compared to all other treatments. Landman & Ling (2011) examined both crayfish and fish tissues 2 months after an application of 350 g Aqual-P m^{-2} to Lake Ōkaro and measured lower concentrations than in the present study.

Recent studies suggest that freshwater crayfish are eaten infrequently by most human consumers in the region and that portion sizes are relatively small (I.A. Kusabs, Te Arawa Lakes Trust, 2010 Personal Communication). Assuming that 200 g is eaten in a meal and that the hepatopancreas comprises 20% of the meal, the maximum dose from the highest average concentration measured in the High alum treatment would be 0.3 mg (using wet weight concentrations). Dietary studies of human consumption of Al suggest

that average daily intakes from food for adults are 7–9 mg; however, this can be increased by thousands of mg if antacid medication or buffered aspirin is consumed (Krewski et al., 2007). Aluminium is present in many food additives so processed food can contain 400 μg Al g^{-1} wet wt. Beverages such as steeped teas can contain 2 mg Al l^{-1}. Any risk from crayfish could be markedly reduced by only eating the tail muscle portion and avoiding the hepatopancreas.

Fish are eaten more regularly by humans, and the regional recreational fishery focussed on rainbow trout is approximately 210,000 angler days per annum (Unwin, 2009). The small fish species used in this study is not consumed by humans but provides an indicator for likely effects on rainbow trout. Marked accumulation of 19 ± 12 μg Al g^{-1} dry wt was only measured in the whole body of common bullies exposed to the High alum dose, and this was not significantly different to the other treatments (8 to 10 ± 6 μg g^{-1} dry wt). Using wet weight concentrations, a 200 g meal would contain 0.9 mg Al; this overestimates dose as only the fish fillet would be consumed (not the whole fish). Common bullies are consumed by trout; therefore, there is potential for some foodweb accumulation, although Al does not biomagnify (Gensemer & Playle, 1999). Aluminium accumulation in trout or other large piscivores following application of P-inactivation agents at high dose rates should be examined in future studies.

Adult freshwater mussels are abundant in several lakes in the region and contain 10–25 g wet weight of edible flesh. Although historically they were a valued food resource by local Māori (Hiroa, 1921), in recent decades, it is reported that they are infrequently consumed due to their bland or bitter flavour (I.A. Kusabs, Te Arawa Lakes Trust, 2010 Personal Communication). In this study, significant Al accumulation relative to the control treatment was only detected in mussels exposed to the High alum dose (344 g m^{-2}). A 200 g meal comprised of the most contaminated mussels in this study would provide a dose of 1.5 mg Al, which is well below average daily intakes estimated for adult humans.

Effects of P-inactivation agents on clams

The fingernail clams were included in the study, because they are a small-bodied organism relative to the depth of the P-inactivation agents applied, and because they grow more rapidly than the long-lived *E. menziesii* and thus provide another useful metric for measuring chronic effects. Clam lengths and weight were not significantly different across the treatments, but the decreased CFI of the clams in the control treatment after 2 months indicates that conditions in the sub-mesocosm were not optimal for this species. Nonetheless, survival was 92% in the controls, and neither survival nor CFI was significantly different to the Low alum treatment suggesting minimal effect on the fingernail clams. The High alum application rate caused a marked and significant decrease in clam survival (23%) and also had sublethal effects on the surviving clams, with a mean reburial rate of only 39% after 30 min—an indicator that they would be less active and more vulnerable to predation. Application of the Medium alum dose had subtle negative effects on the clams, causing decreased CFI relative to the start of the exposure and a non-statistically significant decrease in survival rates to 67 ± 15% relative to the controls. This suggests that clam populations would survive the Medium alum dose proposed for Lake Rotorua. The highly variable survival of the clams exposed to 200 g Aqual-P m^{-2} suggests that this application rate may be close to the threshold of their tolerance. Photos of the sub-mesocosms 24 h after application of the Aqual-P show a thicker layer in the replicate with no surviving clams after 2 months—perhaps as a result of differential mixing of the slurry as it was applied. Survival was 95% in the other two replicates, and the average reburial rates and CFI of the surviving clams were not significantly different to the control treatment.

In a natural environment, it is likely that P-inactivation products will be unevenly distributed as a result of variations in the application equipment and water movement as the products settle. Burrows were observed in the sub-mesocosms of all treatments a day after the application (although fewer were observed in the Medium and High alum treatments) suggesting that the clams could maintain the irrigation of their burrows and their feeding activities for the initial exposure period. However, Vopel et al. (2008) demonstrated that deoxygenation occurred within a 200 g m^{-2} alum or 200 g m^{-2} Aqual-P capping layer. Additionally, the pH declined to pH < 5.5 in the near-surface sediments below the thick alum layer and reached pH < 6.5 below the Aqual-P, a factor which would result in the formation of potentially toxic Al^{3+}.

Both near-bed deoxygenation and increased toxicity of Al due to decreased pH within the alum would have contributed to adverse conditions for the clams. At lower doses, the presence of the alum floc or the Aqual-P powder may still have impaired their feeding and respiration, for example, by reducing the nutritional value of the particles they were feeding on, or clogging gills and feeding appendages. In a natural environment, bioturbation of a capping layer by larger organisms might reduce adverse effects on smaller organisms.

Together these results indicate that in environments where capping layers of P-inactivation agents remain intact for days to weeks after application, critical capping thicknesses should be expected for small sediment-dwelling invertebrates such as clams and infaunal amphipods. For the clam species used in this study, the critical alum capping dose of 115 g alum m^{-2} (i.e. Medium alum, equivalent to a thickness of $\sim$7 mm) resulted in approximately 35% mortality. A lower alum dose of 57 g alum m^{-2} would need to be used if this mortality was to be reduced to the no observed effect level. The effects of an intact capping layer will, however, be mitigated when factors such as physical disturbance (e.g. by wave action) and bioturbation disrupt the integrity of the layer; however, the time scales associated with such processes are not well-characterised and may require site-specific investigations.

Application of results to in-lake exposures

Gibbs et al. (2011) calculated from flow-through incubations of Lake Rotorua sediment cores that the minimum effective P-inactivation dose rates for alum and Aqual-P were 80 g alum m^{-2} and 190 g Aqual-P m^{-2}. Our data suggest that at these application rates, if P-inactivation agents were inadvertently applied to zones of a lake where macrofauna were present, that crayfish, mussels and fish would be minimally affected (assuming the treatments have no foodweb effects), and there may be a significant but not severe effect on fingernail clam populations. It must be emphasised, however, that P-inactivation agents should only be applied to the seasonally anoxic zones of lakes where minimal benthic fauna exist, and where P-release occurs. This recommendation is also made by Gibbs et al. (2011) and Hickey & Gibbs (2009) to minimise the risk of inhibition of microbial nitrification and denitrification in the littoral zones of lakes and to increase efficacy of the lake treatment.

Bioturbation of P-inactivation agents

It is possible that the lack of bioturbation by larger macroorganisms artificially reduced the survival of the fingernail clams in both the alum and Aqual-P treatments in a manner that would not occur in an in-lake situation. Our observations suggest that the crayfish were responsible for most of the bioturbation in the mesocosms; however, the fish tended to rest on the sediment surface, and their swimming movements disturbed both the alum and the Aqual-P layers. The mussels moved less frequently but produced long tracks of well-mixed surficial sediments. All four species occur in the littoral zone of Lake Rotorua, a zone where wave-movements and currents would disturb both alum and Aqual-P. In Lake Rotorua, these P-inactivation agents would be applied to the deeper zones, where it is thought that the sediments are depauperate of sedentary macrofauna as a result of anoxic periods during lake stratification, and where P-release occurs.

Conclusions

In summary, these data indicate that application of alum at rates up to 344 g alum m^{-2} (>7-mm thickness, High alum) or 200 g Aqual-P m^{-2} (1.7-mm thickness) would have no significant adverse effects on freshwater fish or crayfish growth and survival, or mussel survival over a 2 month period, assuming that all species were able to access adequate food. This aspect of their exposure to P-inactivation agents should be examined in field studies. These laboratory exposures suggest that some aluminium accumulation will occur in the crayfish and mussels, although whether this will occur in an in-lake exposure has yet to be verified. The concentrations measured were well below those that would be expected to elevate the daily intake of Al by human consumers. Our data indicate that fingernail clam survival would be adversely affected by the highest alum application rate of 344 g alum m^{-2}, but application rates of 115 g alum m^{-2} (Medium alum, $\sim$7-mm thickness) or lower would not have any detectable effect on survival rates and reburial activity. Clam survival could be affected by uneven

application of Aqual-P at 200 g m^{-2}, and respiration of freshwater mussels may be inhibited. These results should be interpreted in the context that P-inactivation agents will not usually be applied to regions of the lake bed where sedentary organisms such as freshwater mussels and clams will be present. Phosphorus-inactivation agents are applied to reduce internal recycling of P from lake sediments which occurs during periods of anoxia. Repeated periods of anoxia decrease the abundance and diversity of benthic macrofauna. It is likely that some P-inactivation agents will either drift or be inadvertently applied to regions where benthic macrofauna are present as a result of water currents and the technical difficulties of applying products to lake environments (e.g., uneven benthic topography); however, our data suggest only application rates of 344 g alum m^{-2} or greater than 200 g Aqual-P m^{-2} will have adverse effects on fingernail clams.

Acknowledgments The local stakeholders Ngati Rangiwewehi provided the stimulus for this research which was funded by Bay of Plenty Regional Council (A. Bruere) and the Te Arawa Lakes Trust granted permission to collect crayfish and mussels from the Rotorua lakes. Ian Kusabs & Associates supplied the juvenile crayfish from Okere Arm of Lake Rotoiti. The authors thank the NIWA staff who assisted with the mesocosm exposure, particularly Anathea Albert, and two anonymous reviewers for constructive comments on the manuscript. This study was performed under NIWA Animal Ethics Committee permit number 113.

References

ANZECC & ARMCANZ, 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. National Water Quality Management Strategy Paper No 4., vol 1 & 2. Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australia: 994 pp.

Burger, D. F., D. P. Hamilton & C. A. Pilditch, 2008. Modelling the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in a shallow polymictic lake. Ecological Modelling 211: 411–423.

Butterworth, J., 2008. Lake Rotokakahi: the kākahi (*Hyridella menziesi*) in a general framework of lake health. MSc Thesis, University of Waikato: 83 pp.

Devcich, A. A., 1979. An ecological study of *Paranephrops planifrons* (White) (Decapoda: Parastacidae) in Lake Rotoiti, North Island, New Zealand. PhD Thesis, University of Waikato: 247 pp.

Doke, J. L., W. H. Funk, S. T. J. Juul & B. C. Moore, 1995. Habitat availability and benthic invertebrate population changes following alum treatment and hypolimnetic oxygenation in Newman Lake, Washington. Journal of Freshwater Ecology 10: 87–102.

Fenwick, M. C. & B. A. Marshall, 2006. A new species of *Echyridella* from New Zealand, and recognition of *Echyridella lucasi* (Suter, 1905) (Mollusca: Bivalvia: Hyriidae). Molluscan Research 26: 69–76.

Forsyth, D. J. & I. D. McCallum, 1981. Benthic macroinvertebrates of Lake Taupo. New Zealand Journal of Marine and Freshwater Research 15: 41–46.

Gensemer, R. W. & R. C. Playle, 1999. The bioavailability and toxicity of aluminium in aquatic environments. Critical Reviews in Environmental Science and Technology 29: 315–450.

Gibbs, M. & D. Özkundacki, 2011. Effects of a modified zeolite on P and N processes and fluxes across the lake sediment-water interface using core incubations. Hydrobiologia 661: 21–35.

Gibbs, M. M., C. W. Hickey & D. Özkundacki, 2011. Sustainability assessment and comparison of efficacy of four P-inactivation agents for managing internal phosphorus loads in lakes: sediment incubations. Hydrobiologia 658: 253–275.

Handy, R. D., 1993. The accumulation of dietary aluminium by rainbow trout, *Oncorhynchus mykiss,* at high exposure concentrations. Journal of Fish Biology 42: 603–606.

Hickey, C. W. & M. M. Gibbs, 2009. Lake sediment phosphorus release management – decision support and risk assessment framework. New Zealand Journal of Marine and Freshwater Research 43: 819–856.

Hiroa, T. R., 1921. Maori food-supplies of Lake Rotorua, with methods of obtaining them, and usages and customs appertaining thereto. Transactions of the Royal Society of New Zealand 53: 433–451.

Hopkins, C. L., 1970. Systematics of the New Zealand freshwater crayfish *Paranephrops* (Crustacea: Decapoda: Parastacidae). New Zealand Journal of Marine and Freshwater Research 4: 278–291.

James, M. R., 1985. Distribution, biomass and production of the freshwater mussel, *Hyridella menziesi* (Gray), in Lake Taupo, New Zealand. Freshwater Biology 15: 307–314.

James, M. R., 1987. Ecology of the freshwater mussel *Hyridella menziesi* (Gray) in a small oligotrophic lake. Archives für Hydrobiologie 108: 337–348.

Jeppesen, E., M. Søndergaard, J. P. Jensen, K. E. Havens, O. Anneville, L. Carvalho, M. F. Coveney, R. Deneke, M. T. Dokulil, B. Foy, D. Gerdeaux, S. E. Hampton, S. Hilt, K. Kangur, J. Kohler, E. H. H. R. Lammens, T. L. Lauridsen, M. Manca, M. R. Miracle, B. Moss, P. Noges, G. Persson, G. Phillips, R. Portielje, S. Romo, C. L. Schelske, D. Straile, I. Tatrai, E. Willen & M. Winder, 2005. Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. Freshwater Biology 50: 1747–1771.

Krewski, D., R. A. Yokel, E. Nieboer, D. Borchelt, J. Cohen, J. Harry, S. Kacew, J. Lindsay, A. M. Mahfouz & V. Rondeau, 2007. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. Journal of Toxicology and Environmental Health, Part B 10: 1–269.

Kusabs, I. A. & J. M. Quinn, 2009. Use of a traditional Maori harvesting method, the tau kōura, for monitoring kōura (freshwater crayfish, *Paranephrops planifrons*) in Lake Rotoiti, North Island, New Zealand. New Zealand Journal of Marine and Freshwater Research 43: 713–722.

Landman, M. J. & N. Ling, 2011. Fish health changes in Lake Ōkaro, New Zealand: effects of nutrient remediation, season or eutrophication? Hydrobiologia 661: 65–79.

Lund, S. S., F. Landkildehus, M. Søndergaard, T. L. Lauridsen, S. Egemose, H. S. Jensen, F. Ø. Andersen, L. S. Johansson, M. Ventura & E. Jeppesen, 2010. Rapid changes in fish community structure and habitat distribution following the precipitation of lake phosphorus with aluminium. Freshwater Biology 55: 1036–1049.

Martin, M. L. & C. W. Hickey, 2007. Scion zeolite bioassays. NIWA Report HAM2007-030 prepared for Scion, Project SC107201, National Institute of Water & Atmospheric Research, Hamilton, New Zealand: 54 pp.

McDowall, R. M., 1990. New Zealand freshwater fishes a natural history and guide. Heinemann Reed and MAF Publishing Group, Auckland: 553.

Narf, R. P. & R. L. Hine, 1985. Impact of phosphorus reduction via metalimnetic injection in Bullhead Lake, Wisconsin. Technical Bulletin No 153, Department of Natural Resources Madison, Wisconsin, USA: 25 pp.

Parkyn, S. M., C. W. Hickey & S. J. Clearwater, 2010. Measuring sub-lethal effects on freshwater crayfish (*Paranephrops planifrons*) behaviour and physiology: laboratory and in situ exposure to modified zeolite. Hydrobiologia 661: 37–53.

Poston, H. A., 1991. Effects of dietary aluminum on growth and composition of young Atlantic salmon. Progressive Fish-Culturist 53: 7–10.

Quinn, J. M., M. Gibbs, M. De Winton & C. Hickey, 2004. Alum treatment for rehabilitation of Lake Ōkaro: ecological surveys and response of sedimentation rates to initial low alum dose trial. NIWA Report HAM2004-052 prepared for Environment Bay of Plenty, Project BOP04219, National Institute of Water and Atmospheric Research, Hamilton, New Zealand: 26 pp.

Roa, S., 1997. Studies on the pea mussel *Sphaerium novaezelandiae* (Deshayes, 1853) in Lake Rotorua. MSc Thesis, University of Waikato: 91 pp.

Rowe, D. K., 2001. Depth distribution and abundance of the common bully *Gobiomorphus cotidianus* (Eleotridae), in three oligotrophic New Zealand lakes, one of which is turbid. Environmental Biology of Fishes 61: 407–418.

Smeltzer, E., R. A. Kirn & S. Fiske, 1999. Long-term water quality and biological effects of alum treatment of Lake Morey, Vermont. Lake and Reservoir Management 15: 173–184.

Søndergaard, M., E. Jeppesen, T. L. Lauridsen, C. Skov, E. H. Van Nes, R. Roijackers, E. Lammens & R. Portielje, 2007. Lake restoration: successes, failures and long-term effects. Journal of Applied Ecology 44: 1095–1105.

Spry, D. J. & J. G. Wiener, 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environmental Pollution 71: 243–304.

Steinman, A. D. & M. E. Ogdahl, 2012. Macroinvertebrate response and internal phosphorus loading in a Michigan lake after alum treatment. Journal of Environmental Quality 41: 1540–1548.

Stephens, R. T. T., 1982. Reproduction, growth and mortality of the common bully, *Gobiomorphus cotidianus* McDowall, in a eutrophic New Zealand lake. Journal of Fish Biology 20: 259–270.

Unwin, M., 2009. Angler usage of lake and river fisheries managed by Fish & Game New Zealand: results from the 2007/08 National Angling Survey. NIWA Report CHC2009-046 prepared for Fish & Game New Zealand, Project FGC08503, National Institute of Water and Atmospheric Research, Christchurch, New Zealand: 110 pp.

Vopel, K., M. Gibbs, C. W. Hickey & J. Quinn, 2008. Modification of sediment-water solute exchange by sediment-capping materials: effects on O_2 and pH. Marine & Freshwater Research 59: 1101–1110.

Welch, E. B. & G. D. Cooke, 1999. Effectiveness and longevity of phosphorus inactivation with alum. Lake and Reservoir Management 15: 5–27.

Winterbourn, M. J. & K. Mason, 1983. Freshwater Life. Mobil New Zealand Nature Series: 76 pp.