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The Effects of Nufarm Glyphosate Gold® on Freshwater Invertebrate Communities



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CONTENTS

	Abstract	4
	Preface	5
1	Introduction	
	Aim and Context	5
	Background Knowledge	9
	Hypotheses	13
	References	16
2	Field Experiments	
	The effects of Nufarm Glyphosate Gold® on aquatic invertebrate communities; a study conducted in the field.	
	Abstract	20
	Introduction	20
	Methods	22
	Results	24
	Discussion	32
	References	35

3 Laboratory Experiments

The effects of Nufarm Glyphosate Gold® on five common aquatic invertebrate species; a laboratory study using dose response experiments.

Abstract	37
Introduction	37
Methods	38
Results	40
Discussion	43
References	46

4 Concluding Results

Field Experiments	48
Laboratory Experiments	48
Discussion	49
Conclusion	50
References	51

5 Appendices 52

ABSTRACT

The effects of the glyphosate herbicide Nufarm Glyphosate Gold® on freshwater invertebrates was examined in field and laboratory experiments. Although this agrochemical is used extensively over water in New Zealand to control emergent aquatic pest plants, its impact on aquatic invertebrates within these ecosystems has not been comprehensively examined. In a field experiment, six discrete groundwater-fed ponds were used. Aquatic invertebrate diversity and abundance within these ponds was monitored with sweep nets over nine months, with the application of the glyphosate herbicide occurring in the middle of this period. This study found that the natural variation within each pond community far exceeded any caused by the addition of the glyphosate herbicide. In a laboratory study, dose response experiments were used to determine the tolerance of five common freshwater invertebrate species. At recommended application rates of 9 l.ha⁻¹, Nufarm Glyphosate Gold® caused no mortalities within the experimental parameters in the species studied. However when the concentration was increased above the recommended dosage, mortalities did occur. The backswimmer *Anisops wakefieldi* and the snail *Physa acuta* were the most sensitive species. The most resilient species studied was the damselfly *Xanthocnemis zelandica*, requiring a concentration 1877 times the recommended rate to cause mortalities. From both studies it can be concluded that at recommended application rates, the glyphosate formulation Nufarm Glyphosate Gold® does not cause mortalities when used at recommended rates. Furthermore no changes in community structure could be attributed to the addition of the glyphosate formulation within the ponds in the field experiments. The continued use of this agrochemical can therefore be advocated.

PREFACE

This thesis has been written in four chapters. The second and third chapters, which describe the methods and results of the field and laboratory experiments, have been written as individual entities for separate publication. Because of this there may be times where text is repeated.

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1) INTRODUCTION

Aim and Context

This study investigates the effects of the glyphosate herbicide Nufarm Glyphosate Gold® on New Zealand freshwater lentic invertebrate communities. The outcomes of this study will help in the restoration and long-term management of New Zealand freshwater systems; by allowing better understanding of what effect this glyphosate formulation, the major tool in emergent plant control, has on these ecosystems.

Glyphosate herbicides (often referred to by their most common trade name; Roundup®) all contain glyphosate as their active ingredient. The specific formulation Nufarm Glyphosate Gold® (isopropylamine, ammonium and alkyl polyglucoside) is the only glyphosate herbicide certified for use over water in New Zealand and because of this is extensively used in aquatic ecosystems throughout the country. It is the most efficient and cost effective way of controlling the spread of Raupo, *Typha orientalis* (Typhaceae). Raupo, otherwise known as New Zealand Bullrush, is a native emergent rush. Raupo has historically been a very prevalent and useful plant as this recount of early Maori life (from a 1934 magazine article) shows:

*“The long reed leaves, so light and full of small air cells, made the best of thatching for his house. Easy to cut and carry, and **found nearly everywhere**, it was the material for walls and roofing. Dried and tied in thick bundles and skillfully fastened on roof and sides it made the snugest of dwellings, cool in summer and warm in winter.”* (Cowen 1934)

In modern times, instead of being a plant of many uses, Raupo is now seen as a weed to be controlled because of its rapid growth rate. Raupo has been shown to produce over 3kg of Carbon per m² per year (Pegman and Ogden 2005). This makes Raupo the second most productive plant in New Zealand, after the introduced timber generating *Pinus radiata* (Ogden 1981, Ogden and Caithness 1982). Since World War II, at which time there was a major increase in the use of agricultural fertilisers, Raupo has grown faster and spread further than ever before (Williams and Imber 1970). Wetlands are valued areas for their recreational uses, such as game bird hunting, fishing and the continued collection of food such as eels. However, Raupo is able to spread vegetatively and through its masses of airborne seeds (Gibbs 1973). This vegetative growth means encroachment by Raupo can quickly engulf even relatively large areas of open water, leaving only a dense swamp

(Cunningham et al. 1953). In the past the natural progression of lakes to swamps was also accompanied by the natural formation of new lakes. Development has turned this dynamic system into a static one, with many such landscapes now being viewed more as extensions to peoples gardens; areas to be tamed and maintained, instead of letting the dynamic processes of wetland sedimentation and formation take place. Even without this disruption in the natural cycle of wetland ecosystems, it is likely that the disproportionate growth of Raupo, due to the over fertilisation of the land, would still create a need for controlling it's growth.

To counter this excessive growth, the spread of Raupo is ordinarily controlled as part of ongoing management of lakes, wetlands and other areas with a high water table. Clearing large areas of Raupo is also often a major part of lake and wetland management and many restoration projects, halting or reversing the natural succession of these ecosystems to maintain the high amenity value of open water. To do this, there are several ways in which Raupo can be controlled. In some cases the simplest way to control it's vegetative spread for years, is to increase water depth. Raupo is unable to spread into water which is more than several meters deep (Williams and Imber 1970), and this creation of deep water can often be easily achieved when creating or restoring a wetland or lake, by excavation or blocking the out-flow with a weir. However, this technique is only possible in some areas where manipulation of the hydrology is both attainable and desired. Physically removing Raupo by cutting it out can be extremely successful in small areas, as well as having a low impact on non-target species. However, it is of course very labour and cost intensive, and is not practical for large areas. Burning has always been the most cost effective spray-free form of control over large areas (Anon 1975). However, it is not very accurate and in wetter habitats will often not destroy the large root-masses, leading to re-growth within a year. Burning was used extensively after draining wetland areas for agriculture, especially as the burning process turns the vegetative mass into nutrient rich ash. However burning large areas is rarely used now because it is non-selective and publicly unacceptable. All of these options have merits, but by far the most cost effective, precise and even selective way of controlling the spread of Raupo is by using herbicides. Herbicides can be applied from the air, quickly covering large distances. Certain herbicides, such as some glyphosate formulations, can be applied at rates that do not kill desirable species such as the wetland sedge *Carex secta*, do not bioaccumulate within the ecosystem and yet still kill the root-masses of the Raupo. The active ingredient in these herbicides, glyphosate, is also readily broken down by microbes within both terrestrial and aquatic systems (Heitkamp et al. 1992). The only formulation of

glyphosate herbicide that is certified for use over water in New Zealand is Nufarm Glyphosate Gold®.

As stated earlier there are many reasons why Raupo is often controlled in an area, but in modern times these motivations are normally driven by the desire for open water and an otherwise functioning wetland ecosystem which is able to perform its ecosystem processes. This allows use of the area for hunting, fishing, boating and other recreational activities. The functioning ecosystem also provides many services such as the filtering and storage of water and processing of organic matter, as well as a habitat for many endemic and desirable plant and animal species.

Aquatic invertebrates are a fundamental part of their ecosystems (Murkin et al. 1982), providing important roles in the breakdown of wastes and as a food source for other species such as waterfowl (Swanson et al. 1985). It is therefore important to understand the effects of Nufarm Glyphosate Gold® on invertebrates. Clearing the open water of encroaching Raupo would achieve nothing if at the same time you remove the food source of waterfowl, and the ability for the ecosystem to function as a whole. These organisms, most of which are endemic, are at high risk from many alterations to their habitat. Many of these risks stem from the fact that for most of their lives, aquatic invertebrates are unable to relocate themselves to neighbouring habitats, or remove themselves from undesirable habitats. Along with this, due to their size and physical composition, they are more at the whim of their environment than larger, vertebrate organisms. Sedimentation can detrimentally affect invertebrates by smothering them (Suren et al. 2005, Matthaei et al. 2006). The sediment also affects food supply, often eliminating periphyton and creating trophic cascades affecting many species. Nutrient and other chemical runoff or inputs can also affect both food supply and the ability of certain species to survive (Niyogi et al. 2007), and many such additions to the aquatic ecosystem can often create an environment which is anoxic and uninhabitable for many species. Reduced water flows have also been shown to have a huge impact on freshwater invertebrates (Dewson et al. 2007). The effects of these impacts show that invertebrates are in danger of being detrimentally affected by many modifications to their environment, which could include the removal of Raupo using herbicides. The addition of the chemicals in glyphosate formulations could, therefore, have the potential to adversely affect the aquatic invertebrates that inhabit our lakes and wetlands. It is clear that in order to know that such herbicides are safe to use, their impact must be understood.

Background Knowledge

Glyphosate herbicides come in many different formulations. All contain the active ingredient, glyphosate, which comes in the form of water-soluble salts, normally isopropylamine. Glyphosate herbicides are non-specific and kill plants by inhibiting the formation of three essential amino acids during photosynthesis; tyrosine, tryptophan and phenylalanine (EPA 1993). Although glyphosate is broken down naturally, it has been shown to persist for up to 60 days in pond water and up to a year in pond sediment (EPA 1993). In addition to the active ingredient glyphosate, all glyphosate formulations contain a surfactant. Surfactants function by enhancing uptake of glyphosate by decreasing water surface tension, and promoting breakdown of the cuticle. The differing surfactants are the main variation between each of the available formulations. Polyethoxylated tallow amine (POEA) is the surfactant normally used in most glyphosate formulations (Relyea 2005), including the one available to the public in New Zealand for non-commercial use and most agricultural formulations.

Although most formulations contain the surfactant POEA, these are not certified for use over water in New Zealand. The only formulation certified in New Zealand for use over water is Nufarm Glyphosate Gold®. This contains 20-30% alkyl polyglucosite as a surfactant (NufarmNZ 2009). In this formulation, the glyphosate is also supplied in the form of two soluble compounds; isopropylamine and ammonium. Most glyphosate formulations only contain the former. This addition of a second salt, referred to as Dual Salt Technology, makes Nufarm Glyphosate Gold® more effective against a wider range of plants. Although glyphosate is the active ingredient that kills the plants, it has been shown that the surfactants added are often the cause of mortalities in animals. Because of these perceived threats, the overall effects of many glyphosate formulations have been studied on many different organisms since Monsanto developed and patented the glyphosate molecule in the early 1970's.

One study showed that using recommended quantities of one glyphosate formulation killed the tadpoles of three species of frog and one toad species when used over water (Relyea 2005). Similarly, larval anuran and salamander species have been shown to be equally at risk (Relyea and Jones 2009). Many other studies also show the high risk of glyphosate formulations to all amphibians, but these do cite the surfactants used (mainly POEA) as being the most harmful component of the chemical (Mann and Bidwell 1999, Perkins et al. 2000, Wojtaszek et al. 2001, Bernal et al. 2009a, b).

Studies on fish species have yielded equivocal outcomes, with some stating that glyphosate formulations appeared to have little effect on the fish (Anton et al. 1994, Albinati et al. 2009, Becker et al. 2009, Contardo-Jara et al. 2009), and some alleging that the herbicides had a negative effect (Caffrey 1996, Tsui and Chu 2008). The more comprehensive studies of fish often included internal assessments of the organisms, and most of these found mild to moderate internal organ and gill damage after exposure (Sopinska et al. 2000, Gluszczak et al. 2006, Ramirez-Duarte et al. 2008, Lushchak et al. 2009). In these studies it is also stated that the physiological changes induced by the Roundup formulations to vital organs; the central nervous system and olfactory receptors, would reduce reproductive performance of the fish and would therefore certainly have negative repercussions at the population level. The economically significant Nile Perch has shown lower tolerance to the chemicals and is particularly susceptible to Roundup (Ayoola 2008). In this study, the Perch exhibited many signs of toxicosis after exposure, from agitation and erratic swimming, air gulping, lack of balance, excessive secretion of mucus and swimming upside-down to death. Many internal symptoms were also identified, from brain hemorrhages and lesions on liver and kidneys, to cellular infiltration of the gills.

Most studies on the effects of glyphosate formulations on invertebrates have been on terrestrial species, because of the widespread use of these herbicides in agriculture. Even so, there are many parallels that can be drawn from terrestrial invertebrates to their aquatic counterparts within the same Order. Studies on terrestrial hemipterans, of which there are many aquatic species, have clearly shown that those studied were all affected by a glyphosate herbicide in some way (Jackson and Pitre 2004). Jackson and Pitre did find that some species densities were reduced simply due to habitat loss, but as well as this there were three species that were also negatively affected by the herbicide directly, with their populations being significantly depleted, even when their habitat and food sources were not disrupted.

Certain coleopteran species also seem to be affected. In a study looking at the effects of four different herbicides on carabid beetles (Insecta: Carabidae), the glyphosate formulation was shown to be one of the two chemicals which elicited a response from the beetles, with most of them moving out of area for approximately 28 days after spraying had occurred (Brust 1990). This is of interest as the Order Coleoptera is well represented within aquatic invertebrates. The species in this order, which are highly mobile, generally vacate the area once sprayed, and stay away for a prolonged time (Brust 1990, Bergvinson and Borden 1992, Jackson and Pitre 2004). Many aquatic invertebrates (especially lentic species) would be

unable to do this, showing that if an aquatic species were negatively affected by a glyphosate formulation, the inability for many of the animals to migrate out of the area during certain life stages, would compound the effects of the chemical.

Invertebrates that live within leaf litter, other detritus and soil, appear to be less affected. One study found that abiotic factors have a much higher influence over terrestrial leaf litter invertebrate densities than glyphosate formulations at normal rates of application (Lindsay and French 2004). Even multi-year trials showed little effect on the soil biota in the field (Locke et al. 2008). It has been shown that the constituents of glyphosate herbicides bind to soil particles and are broken down quickly by microbes, therefore being inert once it reaches invertebrates below the soil surface (Baylis 2000) which explains why it has little effect on the invertebrates in these environments.

Arachnids have been found to be unaffected by glyphosate formulations. In field trials using increasing concentrations, wandering spiders were shown not to be affected, and the decrease in web-building spiders noted in the study, was clearly caused by changes in environment structure and micro-climate due to vegetation loss (Haughton et al. 1999). Widespread loss of habitat can result in the inability for invertebrates to seek refuge and find or catch food. This loss of habitat could easily affect aquatic predators such as Odonata species, as the nymphs of this Order have been shown to favour certain habitats in which to stalk their prey (Prestidge 1979). Lab trials on a common agricultural spider, *Lepthyphantes tenuis* (Linyphiidae), also found that the glyphosate formulation had no toxic effect over the 72 hours in which they were studied (Haughton et al. 2001b). However corresponding field trials on the same species found that abundance decreased, again cited as being due to the indirect decrease in available habitat (Haughton et al. 2001a). This once more amplifies the need for some predator species that have evolved specific methods for catching prey, such as web building spiders and stalking or ambushing Odonata nymphs, all requiring specific habitats that could be inadvertently lost due to the use of herbicides.

Of the freshwater invertebrates studied, the Anostraca (Fairy Shrimp) *Thamocephalus platyurus* of North America has had the most study. This species lives in seasonal ponds in the Southern High Plains, where it is subjected to many herbicides used in this intensive agricultural area. This Anostraca was negatively affected by glyphosate formulations. The chemicals were shown to reduce growth rates, which are important for this very short lived invertebrate, reducing sexual maturation and therefore population dynamics (Brausch et al. 2006). This can be seen as important as there are several freshwater Crustacea in New Zealand; freshwater shrimp, *Paratya curvirostris*, amphipods such as *Paracalliope fluviatilis*,

and the freshwater crab, *Amarinus lacustris*. There is also a species in New Zealand with a similar life cycle to *T. platyurus*, of which relatively little is known; the Tadpole Shrimp, *Lepidurus apus viridis*.

Very few studies have been completed on many aquatic invertebrate taxa. Those that have been published often investigate stream invertebrates and mainly focus on invertebrate drift, which is accepted to be a mechanism invertebrates use to remove themselves from unfavourable conditions. When an individual wants to drift, it simply moves up into the current of the stream and drifts down until the environment better suits it, at which point it drops back onto the substrate. Drift occurs at a relatively constant rate naturally. Catastrophic Drift, when significantly higher numbers of individuals drift, can be triggered by many factors including temperature increase, change in water flow and chemical exposure (Anderson and Lehmkuhl 1968). Studies published include one which found that only one genus of Ephemeroptera, *Paraleptophlebia sp.*, increased in drift due to the introduction of a glyphosate herbicide in field trials (Kreutzweiser et al. 1989). Another study using choice chambers again found that Ephemeroptera larvae of the same genus chose to move into water that did not contain the glyphosate formulation (Folmar 1978) and a further similar study found that although several genera of the Order Ephemeroptera avoided water containing glyphosate formulation, it did not negatively affect them at the concentrations studied (Folmar et al. 1979).

One study assessing the effects of a glyphosate formulation on several species of aquatic invertebrates, including a chironomid and a mollusc species, found that using recommended application rates of the chemicals did not harm the invertebrates (Henry et al. 1994). However like most studies, the glyphosate formulation used was not the same as Nufarm Glyphosate Gold®, which is used in New Zealand over aquatic ecosystems.

For many of these reviewed studies, it is stated that the surfactant, normally POEA, is the constituent of the glyphosate formulation that is detrimental to the organisms (Giesy et al. 2000, Gluszcak et al. 2006). POEA is derived from animal fatty acids and has the ability to breakdown the cuticle of plant cells. It has been hypothesised that POEA can also affect the structural integrity and function of the exoskeleton of aquatic invertebrates (Giesy et al. 2000). This is the reason only Nufarm Glyphosate Gold® is certified for use over water in New Zealand; the surfactant in this formulation is considered to be less toxic, especially in aquatic ecosystems. Even so it is clear that for animal species the surfactants are usually more toxic than the glyphosate itself.

Glyphosate formulations with especially caustic surfactants (such as POEA) can be used freely in numerous countries. Surfactants are not classed as active ingredients, and in many countries there are no regulations governing the use of such 'inert' components in agrichemicals. This means their use and their effect on organisms is not required to be considered or tested by manufactures before sale to the agricultural industry or the public. This has lead to so many glyphosate formulations being toxic to animals, and is also why most studies on these herbicides are on those which contain POEA – it is by far the most common surfactant used in glyphosate herbicides (EPA 1993).

The effects of the surfactant POEA on aquatic organisms are well documented, from causing mortalities in *Daphnia magna* (Wang et al. 2005) to one study looking at a glyphosate formulation containing POEA which found that this surfactant accounted for 86% of the chemical toxicity to protozoans and bacteria (Tsui and Chu 2003). These studies clearly show that even though surfactants are often classed as inert, their effects are often more detrimental than the glyphosate itself. Hence only the certified formulation (Nufarm Glyphosate Gold®) should be used over water.

In summary, most of the studies reviewed were carried out using different glyphosate formulations, with different surfactants, that are not certified for use over water in New Zealand. Furthermore, the conditions and species found in New Zealand are unrelated to those previously studied. These major differences mean the effects of glyphosate formulations on freshwater invertebrates in New Zealand are not clear. Such knowledge is required to be able to make informed decisions about the use of Nufarm Glyphosate Gold® over water in New Zealand, and in the management of these ecosystems.

Hypotheses

When trying to establish if, how and by how much freshwater invertebrates are affected by glyphosate formulations, the effects can be grouped into two main categories; indirect and direct.

Indirect effects are harder to monitor, especially within the limitations of this thesis. These effects could be detrimental or even beneficial to individual species. Susceptibility of a certain species could lead to trophic cascades within the community, especially if a top predator is overly susceptible. If this was to occur, the release of predation pressure would cause a temporary population increase in species further down the food chain, possibly then causing a lack of food for these species to feed on. Such trophic level effects would be most

apparent in species with the ability to reproduce quickly such as the zooplankton *Daphnia magna*. Habitat loss, due to the reduction of both Raupo and non-target plant species could affect certain invertebrate species, leaving individuals that require this habitat with little shelter from predators, or for predators themselves losing habitat needed to stalk prey. As well as the temporary loss of primary production food sources such as algae, the influx of nutrients as the killed plants decay in the water could destabilise the ecosystem, leaving little food for some species and an excess for others. Again such effects would cause trophic cascades. To determine if there are any indirect effects, and to which species, an experiment was carried out in the field, reproducing the exact conditions which invertebrate communities would be subjected to when their habitat is sprayed with Nufarm Glyphosate Gold® to control Raupo. The community composition of each sample pond in the field was monitored before and after the spraying occurred, to track changes in the abundance of individual species in both control and treatment ponds.

The most direct effect on the various species would be where the chemicals themselves cause mortalities to the invertebrates. To study this, experiments were carried out in the laboratory where abiotic variables and interaction with other species and biotic factors were limited and controlled. With this setup, the quantities of Nufarm Glyphosate Gold® added were also altered to find how susceptible each species of interest was to the different effects of this chemical.

This line of investigation with field experiments being used to track possible indirect effects, and laboratory experiments used to determine any direct mortalities, has been used before when studying the effects of glyphosate formulations on invertebrates (Haughton et al. 2001a, Haughton et al. 2001b). The field experiments are described in full in Chapter 2 of this thesis and the laboratory experiments are described in Chapter 3.

The aim of this study was to investigate the effects of Nufarm Glyphosate Gold® on freshwater invertebrate communities. Two hypotheses were derived, each answering part of the leading question and each being answered through different experiments:

- A) The spraying of Raupo with the glyphosate formulation Nufarm Glyphosate Gold® will change the composition of the aquatic invertebrate communities present, either directly or indirectly.

This hypothesis was tested with experiments in the field, where natural variability and indirect interactions influenced the invertebrate communities.

B) Different aquatic invertebrate species will show different levels of tolerance to the glyphosate formulation Nufarm Glyphosate Gold®.

This hypothesis was tested with experiments in the laboratory, where variables could be controlled to determine the direct mortalities caused by this chemical on specific invertebrate species.

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2)

The effects of Nufarm Glyphosate Gold® on aquatic invertebrate communities; a study conducted in the field.

ABSTRACT

The effects of the glyphosate herbicide Nufarm Glyphosate Gold® on freshwater invertebrate communities was examined in field experiments. Although this agrochemical is used extensively over water in New Zealand to control emergent aquatic pest plants, its impact on the invertebrates within these ecosystems has not been comprehensively examined. Six discrete groundwater-fed ponds were used in this study, three permanent and three ephemeral, with one of each type of pond sprayed. Aquatic invertebrate diversity and abundance within these ponds was monitored with sweep nets over nine months, with the application of the glyphosate herbicide occurring in the middle of this period. This study found that the natural variation within each pond community far exceeded any caused by the addition of the glyphosate herbicide. It was concluded that the application of the glyphosate formulation Nufarm Glyphosate Gold® did not cause discernable changes within the aquatic invertebrate communities present.

INTRODUCTION

This study investigated whether the glyphosate formulation Nufarm Glyphosate Gold® affects aquatic invertebrates communities in New Zealand. Glyphosate herbicides (often referred to by their most common trade name; Roundup®) all contain glyphosate as their active ingredient and Nufarm Glyphosate Gold® is the formulation that is certified for use over water within New Zealand. This formulation differs from most because of its surfactant; alkyl polyglucoside (NufarmNZ 2009). The surfactants in glyphosate herbicides are often cited as being the constituent most toxic to animal life (Giesy *et al.* 2000, Ayoola 2008), especially the most commonly used of these; Polyheptoxylated tallow amine (POEA).

Nufarm Glyphosate Gold® is used over water to control the spread of aquatic emergent plants, particularly Raupo, *Typha orientalis*, which can quickly encroach on wetlands, ponds and lakes, and occlude open water. This glyphosate herbicide is the most effective tool in use to control the spread of Raupo. Because of this, identifying any affect it has on non-

target animal species is important, especially if it is to continue being used as a tool to manage aquatic habitats. It's effects on aquatic invertebrates are of special interest as invertebrates have fundamental roles within these ecosystems, in the breakdown of organic matter and as a food source for many larger species (Murkin *et al.* 1982). Once the effect, if any, of Nufarm Glyphosate Gold® is known, aquatic environments can continue to be managed and maintained as functioning ecosystems and provide opportunities for recreation such as waterfowl shooting and eel fishing.

Although many studies have been conducted on the effects of glyphosate herbicides on animal species, aquatic and terrestrial alike, the results are often species specific (Ayoola 2008), frequently inconclusive (Kreutzweiser *et al.* 1989), and sometimes show glyphosate herbicides have no effect (Haughton *et al.* 1999). However, few studies have been conducted on aquatic invertebrates, and even fewer on the effects of the particular formulation Nufarm Glyphosate Gold®. There have been no studies of this chemical on New Zealand aquatic invertebrate species, yet despite this Nufarm Glyphosate Gold® is certified for use over water in New Zealand.

To help rectify this apparent knowledge gap, the following hypothesis was tested with experiments in the field:

The spraying of Raupo with the glyphosate herbicide Nufarm Glyphosate Gold® will change the composition of the aquatic invertebrate communities present, either directly or indirectly.

The effect of this glyphosate herbicide on the eel population present in the ponds was also monitored. This addition to the study was made after consultation with a local Iwi representative, who raised concerns over the effects of glyphosate herbicides on eel. Previously published studies have shown that some glyphosate herbicides can have detrimental effects on organs such as the gills of eel and can cause damage to DNA (Guilherme *et al.* 2009b); however, these studies did not use the formulation Nufarm Glyphosate Gold® and thus differed in chemical composition . They also didn't specifically examine the New Zealand species of eel *Anguilla dieffenbachia* and *Anguilla australis* . The effects of this glyphosate herbicide on eel is of interest as in many freshwater habitats in New Zealand where this agrochemical is used and eel are harvested for food.

METHODS

Site selection

This study was conducted at the Pukepuke Reserve, a wetland managed by the Department of Conservation, 15km north of Foxton on the southern west coast of the North Island of New Zealand (Fig. 2.1) (Latitude -40.340030, Longitude 175.271172). The reserve is used for waterfowl hunting during the May-June hunting season, but otherwise little activity or disturbance takes place there. The site is surrounded by agricultural land used mainly for dairying. Six of the smaller ponds within the reserve (marked 1 to 6 within the insert of Fig. 2.1) were used in the field trials. All six ponds, created by 1972, are groundwater fed, and are not open to any of the surrounding farm drains which flow past into the main lagoon (Fig. 2.1). Ponds 1, 2 and 3 retain water year-round to a depth of between 1.5m and 3m. Ponds 4, 5 and 6 are ephemeral and dry out during periods of low rainfall.

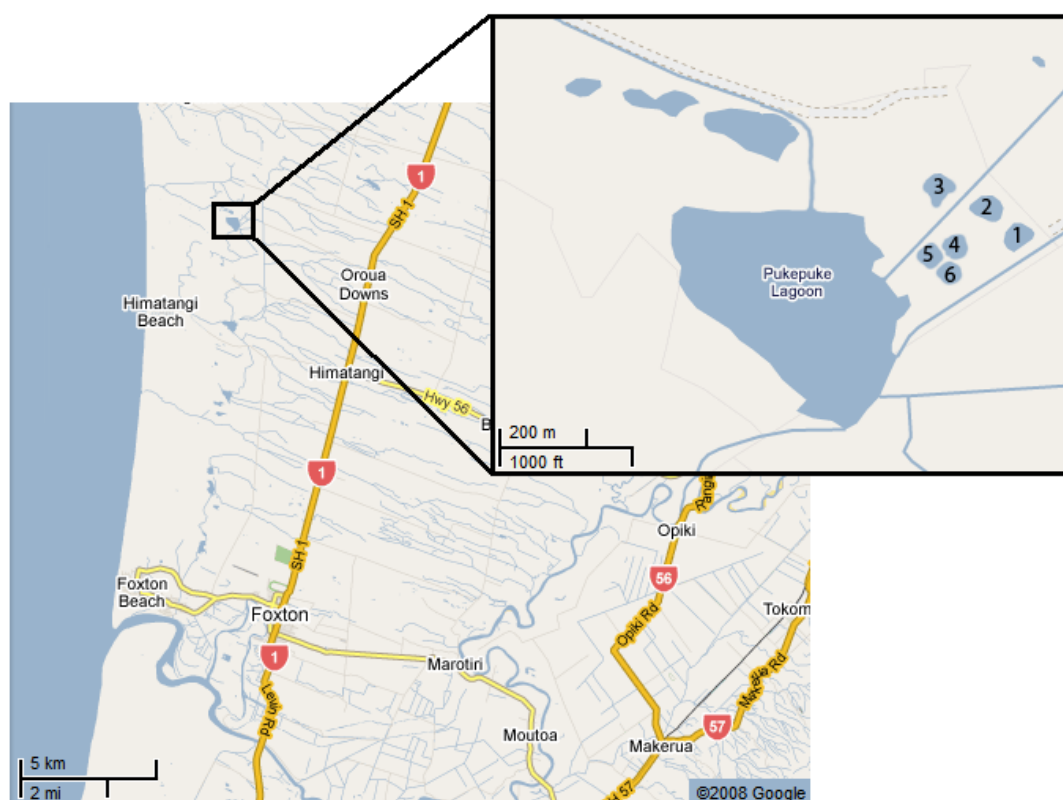


Figure 2.1: Map showing the location of the Pukepuke Reserve. '1', '2' and '3' within the insert show the three permanent ponds studied, '4', '5' and '6' show the three ephemeral ponds studied. Ponds 1 and 4 were sprayed with Nufarm Glyphosate Gold®, the remainder were used as controls.

Pukepuke reserve was a suitable field site because all six ponds are discrete with no water flow between them, they are all subjected to the same physical and environmental conditions, and the same flora is present in and around each. Although the main lagoon within the reserve was sprayed with Nufarm Glyphosate Gold® several years previously, the six study ponds have not been sprayed for several decades.

Experimental design

When spraying Raupo with Nufarm Glyphosate Gold®, it is recommended to do so in late February after the seed-head has formed and when the sap is sinking back into the rhizome, thus aiding the penetration of the chemical into the root-mass of the plant. Therefore, spraying of vegetation in the field trials occurred on February 24, 2009. Two ponds were sprayed; one permanent pond (pond 1) and one ephemeral (pond 4). The other four ponds served as controls. The spraying was conducted with a Robinson 44 helicopter, fitted with a boom with air induction nozzles. This setup achieved a fine spray yet reduced spray drift due to the design of the air induction nozzles on the boom. Spraying was carried out at 6am on the February 24, 2009. At this time the air was still, creating no spray drift from the treatment ponds to the control ponds. The recommended spray rate for Raupo control of 9 litres per hectare of Nufarm Glyphosate Gold® (concentrate) was applied over the entire area of the pre-designated ponds.

Beginning in September 2008, five months prior to spraying, monthly samples were taken from each pond using a sweep net; four samples were taken from within a stand of Raupo, and four were taken from open water without Raupo. These samplings enabled detection of differences in community structure between habitat types. Each sample was stored in 70% ethanol. The samples were then sorted in the laboratory and each organism identified. The monthly sampling continued until the spraying occurred in late February 2009. Daily samples were then collected from each pond for the next week, followed by weekly samples for the subsequent month. After this, monthly samples were collected for the following four months. This increase in sampling frequency immediately after spraying was designed to enable any rapid crash and recovery trends in the invertebrate communities to be identified.

To control the variability of the samples, several steps were taken. The same person collected all samples with the same net, using a fixed sampling effort. This fixed sampling

effort included the time taken (5 seconds for each sample) and the movement of the net. The samples were all taken between 11am- 2pm in an attempt to mitigate any pattern of diurnal activity of the animals and their positions within the ponds at the time of sampling. Eight samples were taken from each pond to minimise sampling variability. While weather conditions could not be controlled, sampling was always done on days with a maximum of forecast 50% cloud cover, using MetService forecasts. This was achievable for the monthly samples by tracking weather forecasts in the week coming up to each round of sampling, which was always completed within a four-day window.

Species were identified using the Guide to the Aquatic Insects of New Zealand by Winterbourn, Gregson and Dolphin. Community composition differences between sprayed and non-sprayed ponds was examined with Nonmetric multidimensional scaling on log (x+1) transformed data, using a Bray-Curtis dissimilarity distance measures in Primer (PRIMER-E). Univariate relationships between variables were examined with regression using SPSS (SPSS Inc.).

Eel monitoring

Fyke net samples were taken one week before and one week after spraying in two of the permanent ponds. The likelihood of eels being in the ephemeral ponds was considered to be low. Pond 1 was the treatment pond and pond 2 was the control. In each pond two large and four small fyke nets were deployed. These were set, baited with sheep heart and left over night. The nets were retrieved the following day before 12 noon.

RESULTS

Sweep Net Study

The most notable result was that the ponds, especially the permanent ponds, had very different invertebrate communities. Figure 2.2 is an ordination showing each of the communities in the 6 different ponds. In the ordination, the 3 ephemeral ponds (Pond 4,5,6) are grouped together, showing that they are relatively similar, compared with the 3 permanent ponds (Ponds 1,2,3) which are spaced further apart and are therefore more different from one another.

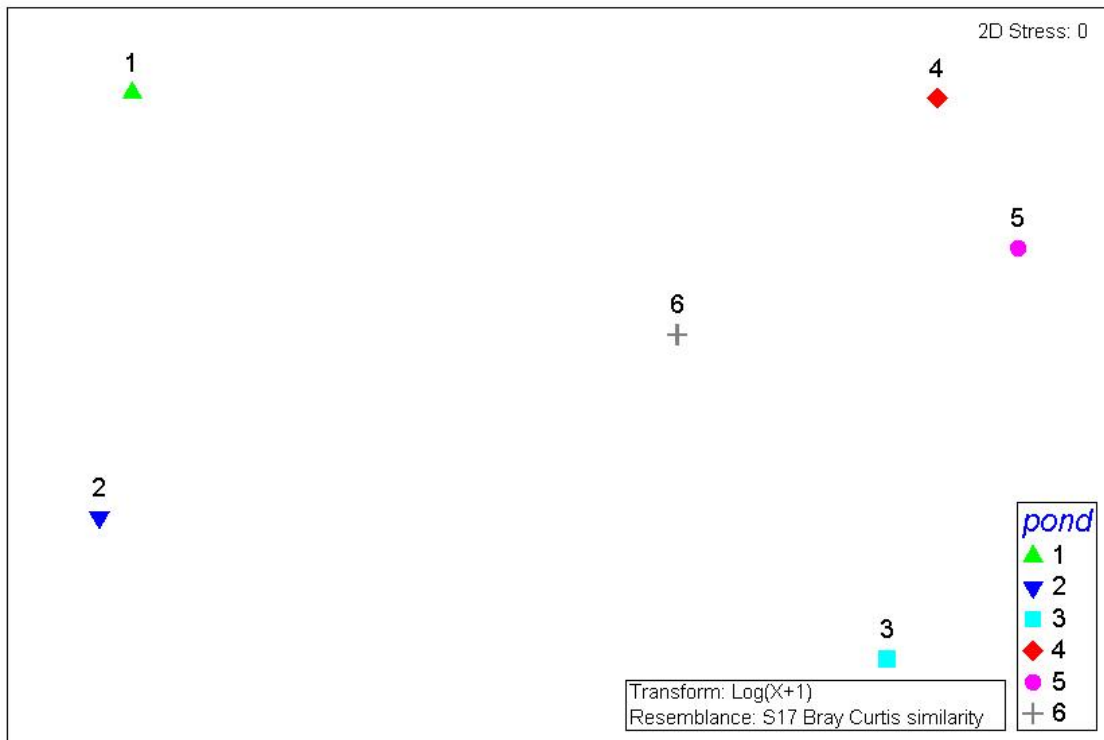


Figure 2.2: Ordination of mean invertebrate communities collected in six ponds at the Pukepuke Reserve between October 2008 and June 2009 . Communities that are closer together are more similar, communities further apart are less similar.

Figure 2.3 is an ordination showing the communities in each pond before and after spraying. For the ephemeral ponds (Ponds 4,5,6) the change in community within the treatment pond is the same as the change in the control ponds indicating the spraying with the glyphosate herbicide had no discernable affect. The change in community in the permanent treatment pond (Pond 1) was greater than the change in the permanent control ponds (Ponds 2,3).

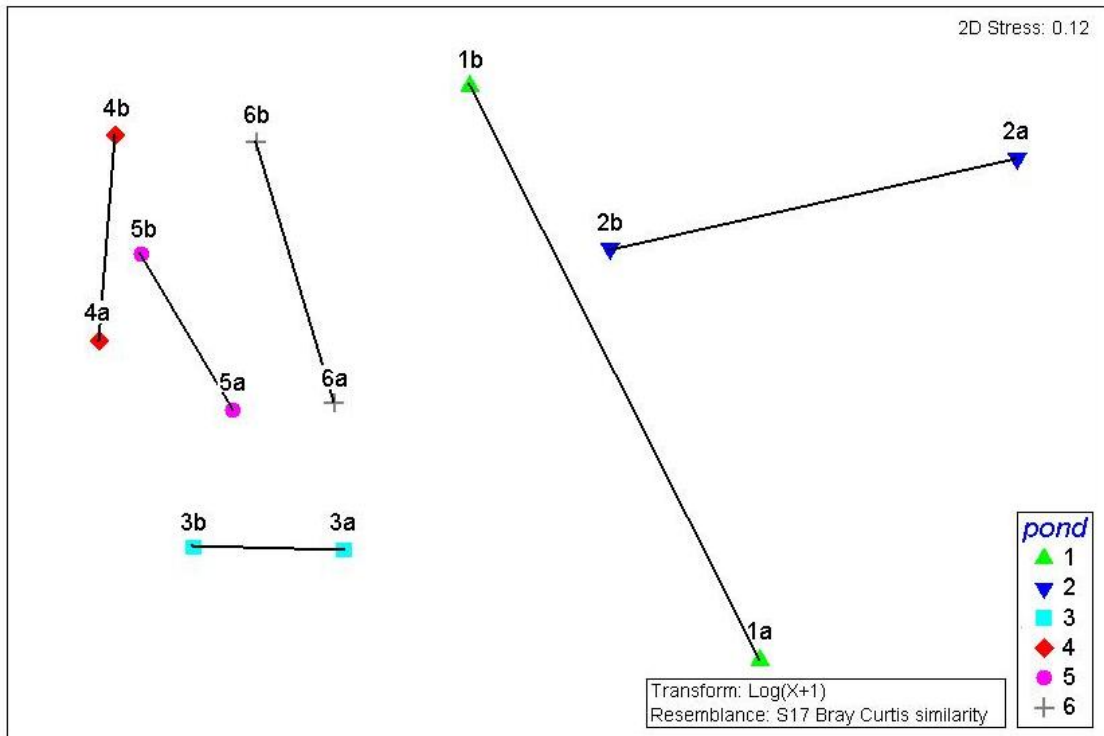


Figure 2.3: Ordination of invertebrate communities collected in six ponds at the Pukepuke Reserve, before (a) and after (b) the spraying of Nufarm Glyphosate Gold®. ‘a’ signifies each pond before spraying and ‘b’ signifies after. The line for each pair show the trajectory of change.

Figures 2.4 to 2.10 show the mean abundances for the most abundant species in each of the six ponds over the study period. Note that the amphipod, *Paracalliope fluviatilis*, was only found in Pond 1 (Fig. 2.4), and that for the other species error bars are not shown as they make interpretation difficult. Regression lines are shown on all graphs, indicating the change over time for all species in all ponds. The treatment ponds (Pond 1 and Pond 4) show similar trends to the control ponds. The values for these lines are given in Table 2.1 of the Appendix; only four of the p-values are statistically significant; the change in abundance within the samples of *Lobohalacarus subterraneusin* in Pond 2 and 3 (both of which were control ponds), the abundance of *Anisops wakefieldi* in Pond 4 which was the treatment ephemeral pond, and the abundance of *Xanthocnemis zelandica* in Pond 1, the treatment permanent pond. All data supplied within Table 2.2 in the Appendix. An overview of all of these graphs reveals two major trends: The ephemeral ponds have a higher abundance of many of the species with a drop in abundance at the end of March for all species. This reduction in abundance is not due to the glyphosate herbicide treatment as it occurs in all ponds.

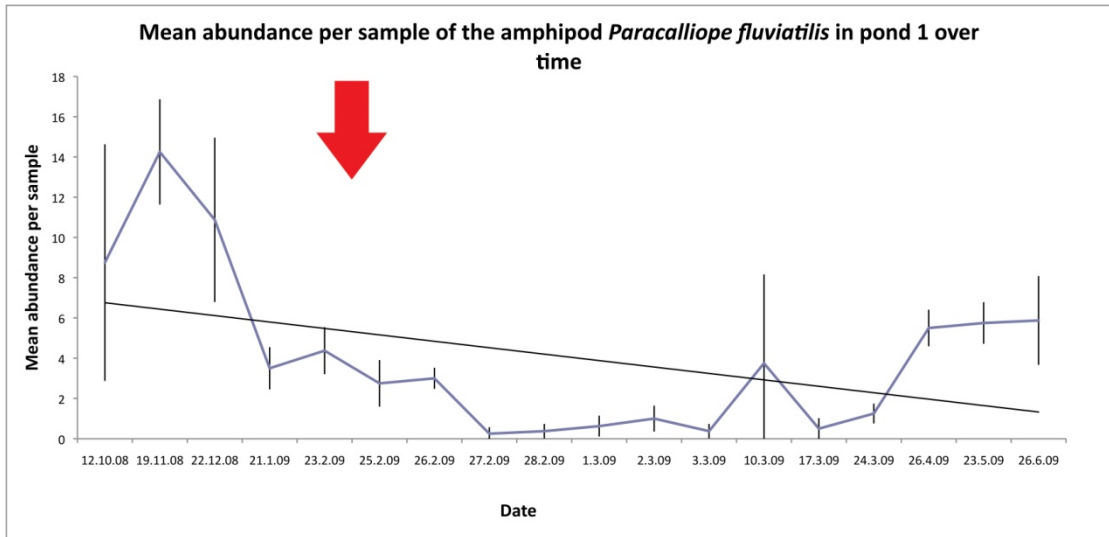


Figure 2.4: Mean abundance of *Paracalliope fluviatilis* collected in pond 1 at the Pukepuke Reserve between October 2008 and June 2009. Spraying date is indicated by the red arrow. Error bars show standard error, 95% confidence.

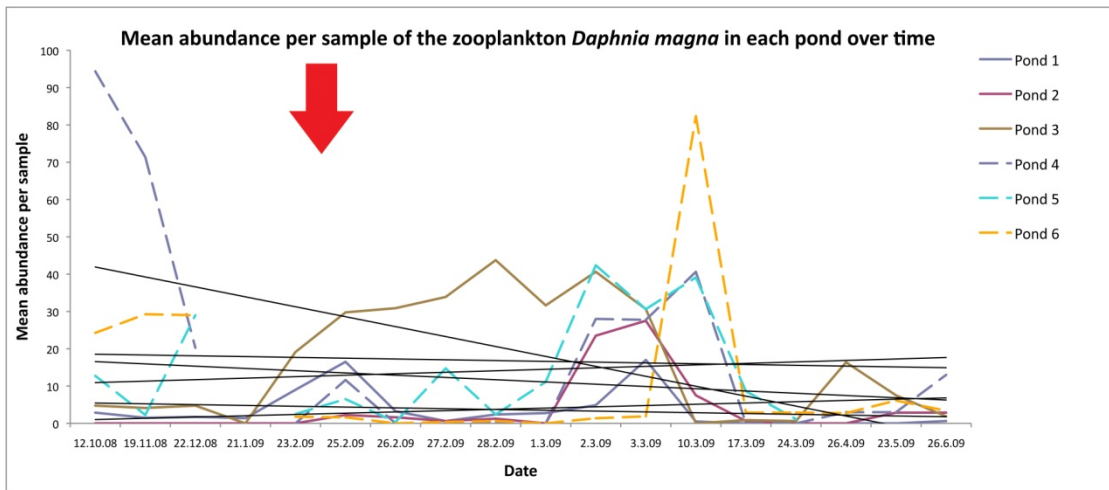


Figure 2.5: Mean abundance of *Daphnia magna* collected in the six ponds at the Pukepuke Reserve between October 2008 and June 2009. Solid lines indicate permanent ponds, dashed lines indicate ephemeral ponds. Date of spraying is indicated by a red arrow for treatment ponds 1 and 4. Gaps in lines correspond to no water in ponds.

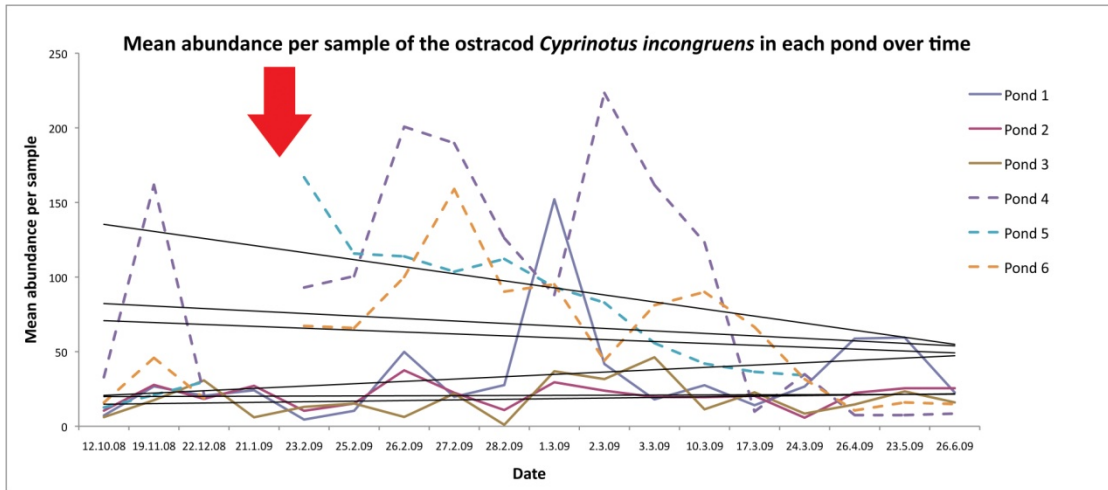


Figure 2.6: Mean abundance of *Cyprinotus incongruens* collected in the six ponds at the Pukepuke Reserve between October 2008 and June 2009 . Solid lines indicate permanent ponds, dashed lines indicate ephemeral ponds. Date of spraying is indicated by a red arrow for treatment ponds 1 and 4. Gaps in lines correspond to no water in ponds.

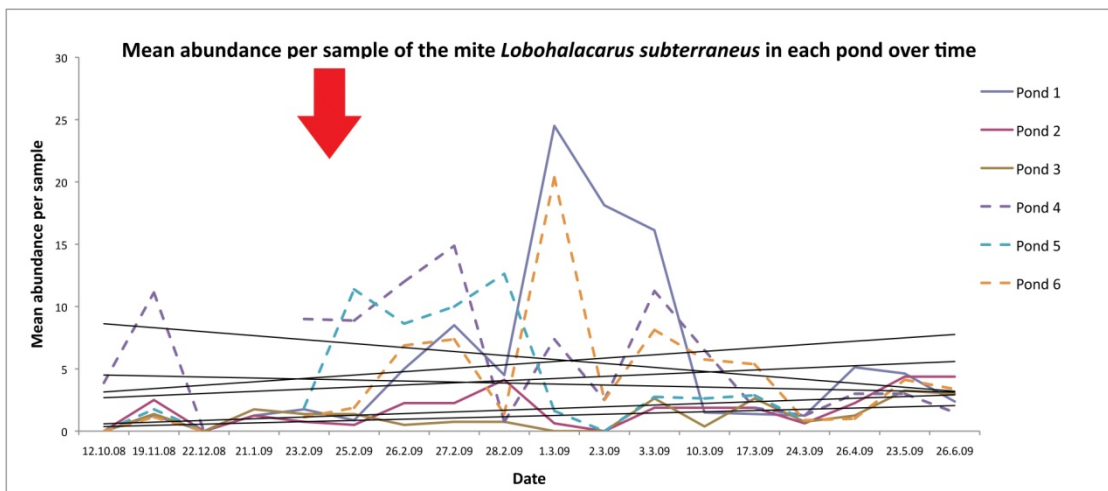


Figure 2.7: Mean abundance of *Lobohalacarus subterraneus* collected in the six ponds at the Pukepuke Reserve between October 2008 and June 2009 . Solid lines indicate permanent ponds, dashed lines indicate ephemeral ponds. Date of spraying is indicated by a red arrow for treatment ponds 1 and 4. Gaps in lines correspond to no water in ponds.

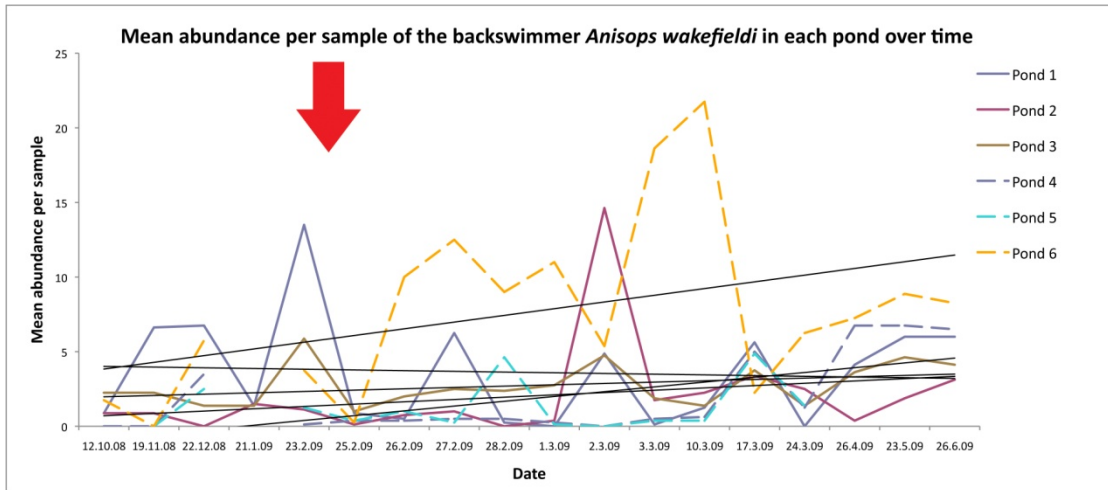


Figure 2.8: Mean abundance of *Anisops wakefieldi* collected in the six ponds at the Pukepuke Reserve between October 2008 and June 2009 . Solid lines indicate permanent ponds, dashed lines indicate ephemeral ponds. Date of spraying is indicated by a red arrow for treatment ponds 1 and 4. Gaps in lines correspond to no water in ponds.

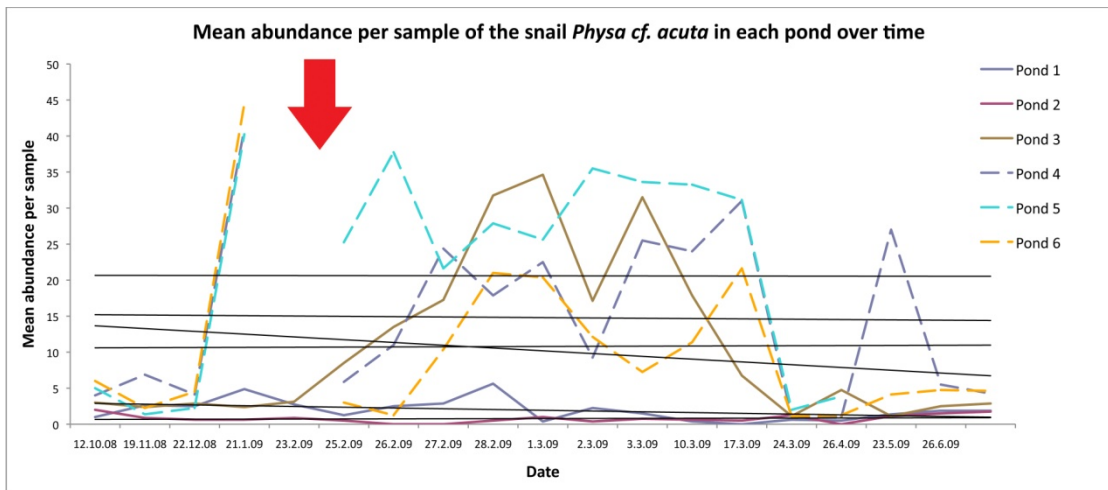


Figure 2.9: Mean abundance of *Physa cf. acuta* collected in the six ponds at the Pukepuke Reserve between October 2008 and June 2009 . Solid lines indicate permanent ponds, dashed lines indicate ephemeral ponds. Date of spraying is indicated by a red arrow for treatment ponds 1 and 4. Gaps in lines correspond to no water in ponds.

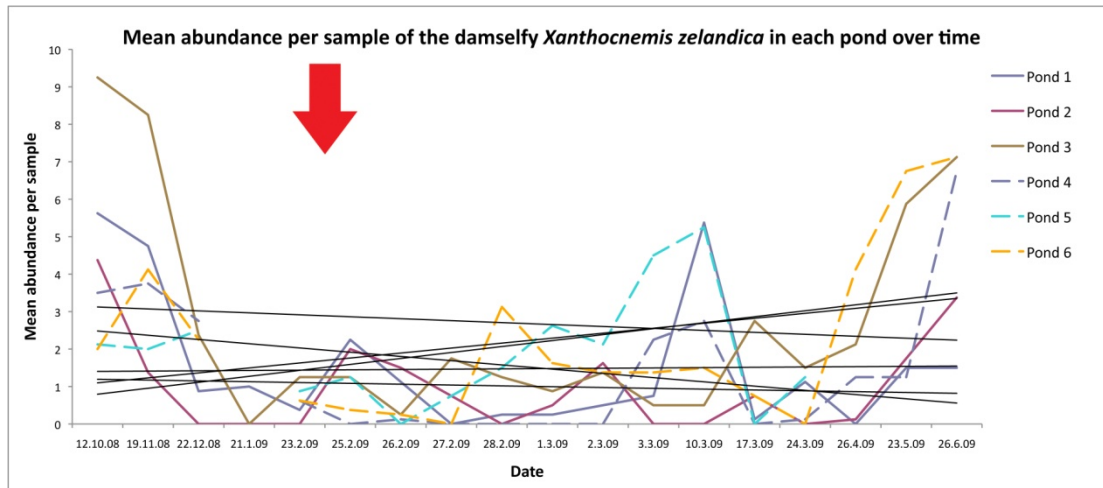


Figure 2.10: Mean abundance of *Xanthocnemis zelandica* collected in the six ponds at the Pukepuke Reserve between October 2008 and June 2009. Solid lines indicate permanent ponds, dashed lines indicate ephemeral ponds. Date of spraying is indicated by a red arrow for treatment ponds 1 and 4. Gaps in lines correspond to no water in ponds.

The results for the amphipod *Paracalliope fluviatilis* are displayed in Figure 2.4. This species only occurred in Pond 1, hence there is no control population for this species. The abundance of *P. fluviatilis* was at its peak near the start of the study, with a mean abundance of 14 individuals per sample in late November. This peak in the population during spring was followed by a decrease in abundance over Summer and Autumn. The drop in abundance began before spraying occurred. The population then slowly increased over the following four months.

Daphnia magna's populations are displayed in Figure 2.5. The abundances within each sample were very low in most ponds over the study period, although there were occasional population rises in some ponds. The ephemeral ponds show the highest stable population of *D. magna* during February, but this fails to persist beyond early March; note that as the date axis is not to scale this apparent persistence is not over a very long period. All ponds appear to experience a small population increase in early to mid March, especially the ephemeral Pond 6. None of these changes in abundance within the samples coincides with the application of the herbicide.

The Ostracod species *Cyprinotus incongruens* was, by far, the most abundant invertebrate species found in the samples. As can be seen in Figure 2.6, a count of over 100 individuals was common in each sample, especially for the ephemeral ponds (Ponds 4, 5 and 6). Pond 4 did have a higher abundance within samples of *C. incongruens* than the two control ephemeral ponds on the 2nd and 3rd March '09, 5 days after being sprayed with the glyphosate herbicide, although this increase was not statistically significant. This slight

difference in abundance then receded within a week. A similar trend was seen 2 days previously in the permanent treatment pond, Pond 1. On the 1st March the abundance of *C. incongruens* is higher than in the two permanent control ponds; 2 and 3. This higher abundance within the samples did however, only last for one day.

Figure 2.7 shows that the aquatic mite, *Lobohalacarus subterraneus*, also increased in abundance within the samples, after the glyphosate herbicide was applied. Both treatment ponds; 1 and 3, showed an increase in the abundance for this species within the samples on the 1st March. This trend was most pronounced in the permanent Pond 1, where an average of 25 individuals per sample was recorded. This fluctuation was abnormal when compared to the mean abundance within the permanent pond samples of 2.3 individuals. In both treatment ponds this increase of abundance within the samples did return to normal within two days in ephemeral Pond 3, and within a week in permanent Pond 1. Although this change in abundance within the samples is noted and could be of interest, it was not statistically significant.

The abundances within the samples of the Backswimmer *Anisops wakefieldi* can be seen in Figure 2.8. This species was most abundant in Pond 6, but the abundances in each sample fluctuated indiscriminately.

The abundance of the aquatic snail *Physa acuta* can be seen in Figure 2.9. *P. acuta* was more abundant in Pond 3 and the three ephemeral ponds during late summer and autumn than in Pond 1 and Pond 2. In these highly populated ponds the abundance did fluctuate over time, however no correlations with the application of the glyphosate herbicide are present in the data.

The abundances within the samples for the damselfly larvae *Xanthocnemis zelandica* are displayed in Figure 2.10. The abundance of this species decreased during summer and early autumn, and increased again from April onwards. There were no statistically significant changes in the abundances between the treatment and control ponds during the sampling period. The fluctuations in abundance are indiscriminate.

Eel monitoring

There were no observable differences in numbers of eel between samples collected before and after spraying, as only 3 eels were caught in total. The fyke nets did however, also catch many adults of the diving beetle *Onychohydus hookeri*. These results can be seen in Fig. 2.11 below. The glyphosate herbicide had no significant effect on the diving beetles.

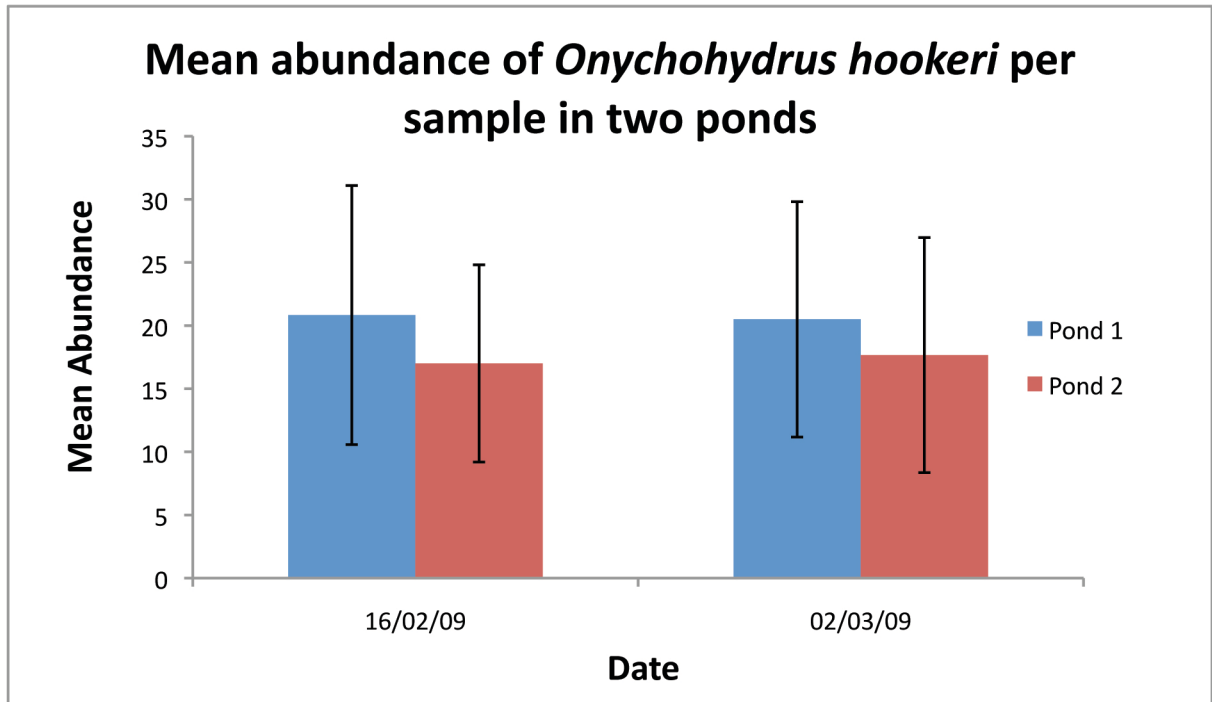


Figure 2.11: Mean abundance of *Onychohydrus hookeri* collected in fyke nets in ponds 1 and 2 at the Pukepuke Reserve before spraying of Pond 1 with Nufarm Glyphosate Gold® (16/02/09), and after spraying (02/03/09). Error bars show standard error, 95% confidence.

DISCUSSION

No statistically significant changes in community structure were observed in the treatment ponds from the use of Nufarm Glyphosate Gold®. The Sweep Net Study found that each pond had distinct communities, and that the natural fluctuations of these communities far exceeded any effects the glyphosate herbicide may have had. Apart from the community level differences between these seemingly environmentally homogenous ponds, the major finding in this study was the relative similarity between the ephemeral ponds (Chase 2007, Evanno *et al.* 2009).

The similarity between communities that undergo regular disturbances, such as the drying out of ephemeral ponds, has been well documented in recent years. One study found that permanent ponds showed much more variation than ephemeral ponds due to stochastic ecological drift within the permanent ponds (Chase 2007). This study concluded that the harsh environmental conditions within disturbed ponds limited the community drift, compared with the communities in permanent ponds, which could drift further and further apart over time. Another study suggested that the communities of such ecosystems would remain similar as the species which are able to cope with the disturbances of that

particular habitat were fewer in number than those which could inhabit undisturbed ecosystems. This smaller species pool filters out many which cannot persist, leaving the highly disturbed environments relatively homogenous (Lepori and Malmqvist 2009). The results in another study even suggest that disturbances can lead to similar selective pressures on species within ephemeral ponds, as well as community structure, leading to these communities undergoing similar evolutionary changes, to cope with similar stresses, even if they are separated (Evanno *et al.* 2009).

The differentiation between permanent and ephemeral ponds could also be driven by the presence or absence of larger predators such as fish. It has long been known that ecological interactions, and principally predation, determine the success of individual species and therefore the community structure in such ecosystems (Wellborn *et al.* 1996). The fyke net study showed that the permanent ponds contained short-finned eel, *Anguilla australis*, the common bully, *Gobiomorphus cotidianus*, and goldfish, *Carassius auratus auratus*. These species would all have an effect on the invertebrates in the permanent ponds, and their absence from the ephemeral ponds could potentially lead to a far different food chain with different ecological interactions and biotic effects.

One finding which was not statistically significant, but is still of interest and suggests further avenues of study, is the response of two species which inhabit the substrate; the aquatic mite, *Lobohalacarus subterraneus*, and the Ostracod, *Cyprinotus incongruens*. Five days after spraying these two species both increased in abundance within the samples in the treatment ponds. This increase in abundance within the samples could be due to these invertebrates rising up out of the sediment and being caught in higher numbers within the sweep net samples. In freshwater environments, glyphosate herbicides are known to bind to substrate particles (Wang *et al.* 2005, Tsui and Chu 2008, Contardo-Jara *et al.* 2009). Other research shows that benthic invertebrates are less tolerant of the effects of glyphosate herbicides than non-benthic species (Tsui and Chu 2004), with benthic species suffering substantially more fatalities. The response time (of 5 days after spraying) can also be explained, as studies using different glyphosate herbicides have shown varying rates of chemical decomposition, with half-lives found to be from 1.5 – 11.2 days (Goldsborough and Beck 1989, Goldsborough and Brown 1993). Such studies often track the presence of glyphosate itself along with its primary metabolite, aminomethylphosphonic acid (AMPA). In one study AMPA increased within sediment samples up to day 36 after glyphosate herbicide application, compared with AMPA concentration within the water column increasing up to day 14. This again suggests that the constituents of glyphosate herbicides

and its metabolites quickly bind to substrate particles. Higher pH levels and calcareous sediments have been shown to slow decomposition and accumulation within the substrate (Goldsborough and Beck 1989, Goldsborough and Brown 1993), compared with substrates which contain a higher proportion of organic matter (Feng *et al.* 1990). It is highly likely that the two species in this study, *L. subterraneus* and *C. incongruens*, displayed avoidance behavior to chemicals binding to sediment particles.

None of the other species of invertebrate sampled in the sweep net study showed any signs of being affected by the application of the herbicide.

The fyke net side study could not be used to measure any effect of the chemical on the local eel population as only 3 eels were caught. Previous studies have found some formulations of glyphosate herbicide have many sub-lethal effects on eel, including gill tissue damage (Ceron *et al.* 1996, Sancho *et al.* 1997, Sancho *et al.* 2000) and even damage to DNA (Guilherme *et al.* 2009a). Unfortunately any possible avoidance of the eels to the treatment pond could not be found due to lack of data. This avoidance was thought to be possible due to the ability of eel to move from pond to pond over land. This theory could also warrant further study, as eel are a key species of interest both as part of the ecosystem and as a food species for humans.

It can be concluded that Nufarm Glyphosate Gold® had no discernable effect on the aquatic invertebrate communities present in the treatment ponds. Although sub-lethal effects are clearly possible, this study found that natural variations in abundance far exceeded any change due to the addition of the glyphosate herbicide Nufarm Glyphosate Gold®. Therefore it's continued use can be advocated to control Raupo and other aquatic emergent plants, enabling the restoration and long-term management of many aquatic ecosystems.

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3)

The effects of Nufarm Glyphosate Gold® on five common aquatic invertebrate species; a laboratory study using dose response experiments.

ABSTRACT

The direct effects of the glyphosate herbicide Nufarm Glyphosate Gold®, on five freshwater invertebrate species was examined in laboratory dose response experiments. This agrochemical is used extensively over water in New Zealand to control emergent aquatic weeds, however it's impact on the invertebrates within these ecosystems has not been comprehensively examined. At recommended application rates of 9 l/ha, Nufarm Glyphosate Gold® caused no mortalities in the species studied. However when the concentration was increased above the recommended dosage, mortalities did occur. The backswimmer *Anisops wakefieldi* and the snail *Physa acuta* were the most sensitive. These species both use an air bubble for gas exchange, and it is likely that the surfactant in the chemical interfered with this important process causing death. The most resilient species studied was the damselfly *Xanthocnemis zelandica*, requiring a concentration 1877 times the recommended application rate to cause mortalities. It was concluded that the application of the glyphosate formulation Nufarm Glyphosate Gold® did not cause mortalities in the experiments when used at recommended rates.

INTRODUCTION

This study investigated the direct effects of the glyphosate formulation Nufarm Glyphosate Gold® on five common species of aquatic invertebrates in New Zealand. Glyphosate herbicides (often referred to by their most common trade name; Roundup®) all contain glyphosate as their active ingredient and Nufarm Glyphosate Gold® is the formulation that is certified for use over water within New Zealand. This formulation differs from most because of it's surfactant; alkyl polyglucosite (NufarmNZ 2009). The surfactants in glyphosate formulations are often cited as being the constituent most toxic to animal life (Giesy *et al.* 2000, Ayoola 2008), especially the most commonly used of these; Polyethoxylated tallow amine (POEA).

Nufarm Glyphosate Gold® is used over water to control the spread of aquatic emergent plants such as Raupo, *Typha orientalis*, which can quickly encroach on wetlands, ponds and lakes, removing open water needed by waterfowl and other species. This glyphosate formulation is the most effective tool used to control the spread of Raupo. It is therefore important to know how much of an effect it has on non-target animal species, especially if it is to continue being used as a tool to manage aquatic habitats, with a goal of enabling them to remain as functioning ecosystems. It's effect on aquatic invertebrates is of special interest as they are known to have fundamental roles in the breakdown of organic matter and as a food source for many larger species (Murkin *et al.* 1982).

Although many studies have been conducted on the effects of glyphosate formulations on animal species, aquatic and terrestrial alike, the results are often species specific (Ayoola 2008), frequently inconclusive (Kreutzweiser *et al.* 1989), and sometimes show glyphosate formulations have no effect (Haughton *et al.* 1999). However, few studies have been conducted on aquatic invertebrates, and even fewer on the effects of the particular formulation Nufarm Glyphosate Gold®. There have been no published studies of the effect of this chemical on New Zealand aquatic invertebrate species, yet despite this Nufarm Glyphosate Gold® is certified for use over water in New Zealand.

To help fill this knowledge gap, the following hypothesis was tested with laboratory experiments:

New Zealand aquatic invertebrate species will vary in their levels of tolerance to the Roundup formulation Nufarm Glyphosate Gold®.

METHODS

Species selection

Five species were selected for use in the experiments, each representing a major taxonomic group of the invertebrates present at Pukepuke Reserve in the Manawatu, New Zealand (Chapter 2). To represent molluscs, *Physa cf. acuta* were used as the representative species. This species is by far the most common aquatic snail found in the Pukepuke Reserve ponds. *Daphnia magna* were used as a representative zooplankton. This species is commonly used for similar toxicity experiments, and is also very common at the study site. To represent aquatic hemipterans, the more common of the two species of Backswimmer, *Anisops wakefieldi*, was used. The freshwater amphipod, *Paracalliope fluviatilis*, was

selected, which have also previously been used in toxicology studies (Burnet 1972, Hunt 1974, Hicky and Vickers 1994). The final species used was the Odonata *Xanthocnemis zelandica*. The response of this Damselfly is of interest as it is an abundant predator and is common throughout most New Zealand ponds, lakes and wetlands.

Experimental design

Polystyrene experimental tanks were half filled with 15 litres of water and left to acclimatise for 30 minutes. Tanks were housed within a temperature controlled laboratory and kept at 15°C with 13:11 hours of day:night. Each numbered tank was then randomly assigned as treatment or control and organisms added to the tanks. Each species was tested separately. Ten individual *P. acuta*, *A. wakefieldi*, *P. fluviatilis* and *X. zelandica*, and 100 *D. magna* were used in each tank. A 10% solution of glyphosate formulation (Nufarm Glyphosate Gold®) was prepared and added to the treatment tanks, with the same quantity of distilled water added to the control tanks. Each tank was then monitored for at least 24 hours, recording mortality levels after 30 minutes, and at hours; 1, 2, 3, 4, 6, 12, and 24 hours, and for some experiments, every 24 hours for the following 6 days. Mortalities were recorded as a percentage of the original population.

Initial concentrations of 0.33ml/l of Nufarm Glyphosate Gold® were used in the experimental tanks. These experiments will be referred to as the Fixed Concentration Experiments. In these first experiments filtered bore water was used. To examine threshold concentrations for each species, concurrent trials were carried out where the concentration was repeatedly doubled until a response was detected. This produced experiments with concentrations of 0.33, 0.66, 1.32, 2.64, 5.28, 10.56, 21.12, 42.24 and 84.48 ml/l. In these experiments untreated rainwater was used. One round of the Varying Concentration Experiments was also completed using water from the Pukepuke Reserve, to check the validity of the findings with water from a 'real world' site.

RESULTS

Fixed Concentration Experiments

The Fixed Concentration Experiments had a single treatment concentration of 50 ml of herbicide solution within the 15l tanks. This gave a concentration of 0.33 ml of Nufarm Glyphosate Gold® per litre. The results from these experiments are shown in Table 3.1 in the appendix and the *D. magna* experiments are shown below in Figure 3.1.

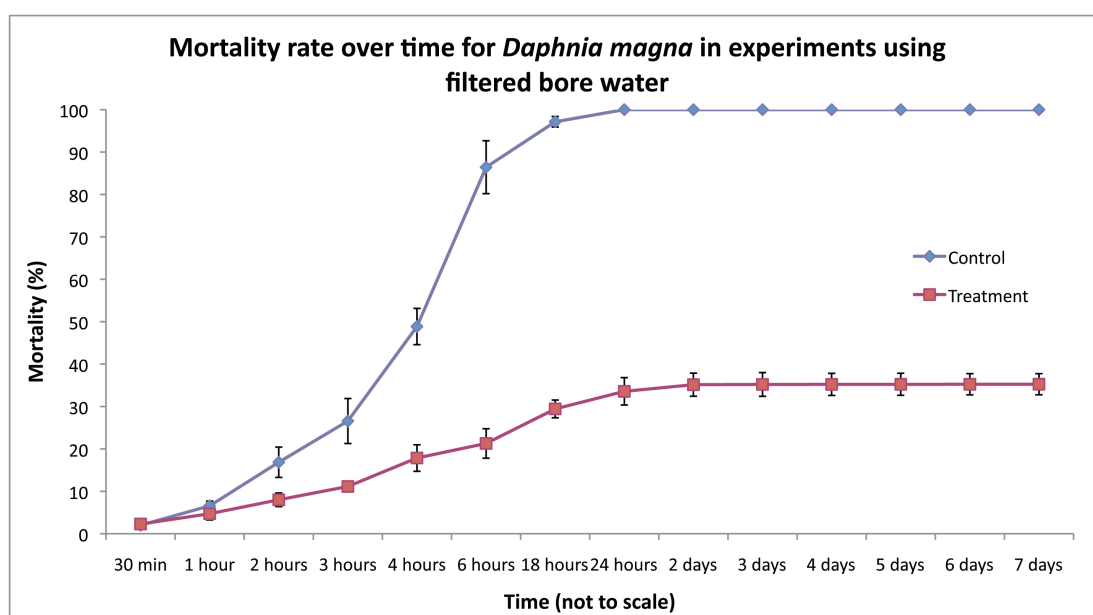


Figure 3.1: Cumulative percentage mortality of *D. magna* in tanks with (red line) and without (blue line) Nufarm Glyphosate Gold®. Error bars show 95% confidence. Results obtained from 12 runs.

Mortality was higher in the control tanks than those containing the glyphosate formulation. In these tanks the mortality attained 100% within the first 24 hours. In the Treatment tanks the mortality rate leveled out at 30% within 24 hours, and did not change significantly after this time.

There were no mortalities of any other taxa. After several preliminary tests it was found that *Daphnia* mortalities were due to the use of the filtered bore water. It was then decided that untreated rainwater, collected from concrete water butts, would be used in further experiments.

Varying Concentration Experiments

There were no mortalities in control tanks. Although none of the species showed an adverse effect to the herbicide concentrations of 5.28 ml/l or less over the 24 hour period,

above this level each species showed different levels of tolerance (Fig 3.2, 3.3, 3.4 and 3.5). It is of interest to note that for the concentrations where species were affected, most of the mortalities occurred within the first 6 hours, with the mortality rate plateauing after this initial die-off.

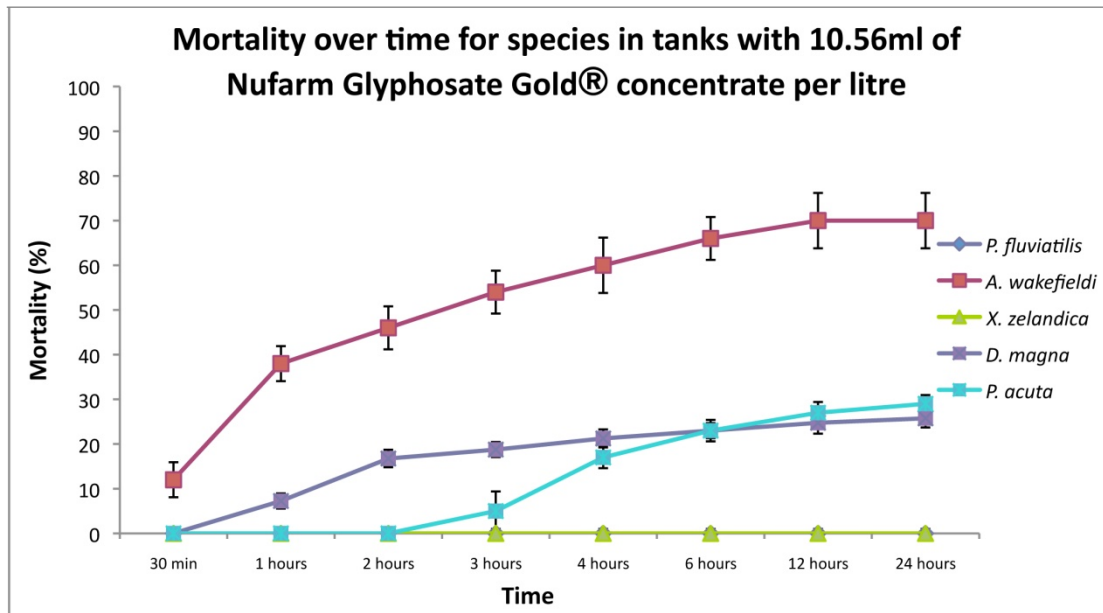


Figure 3.2: Cumulative percentage mortality over time for 5 invertebrate species at a concentration of 10.56 ml/l of Nufarm Glyphosate Gold®. Error bars show 95% confidence.

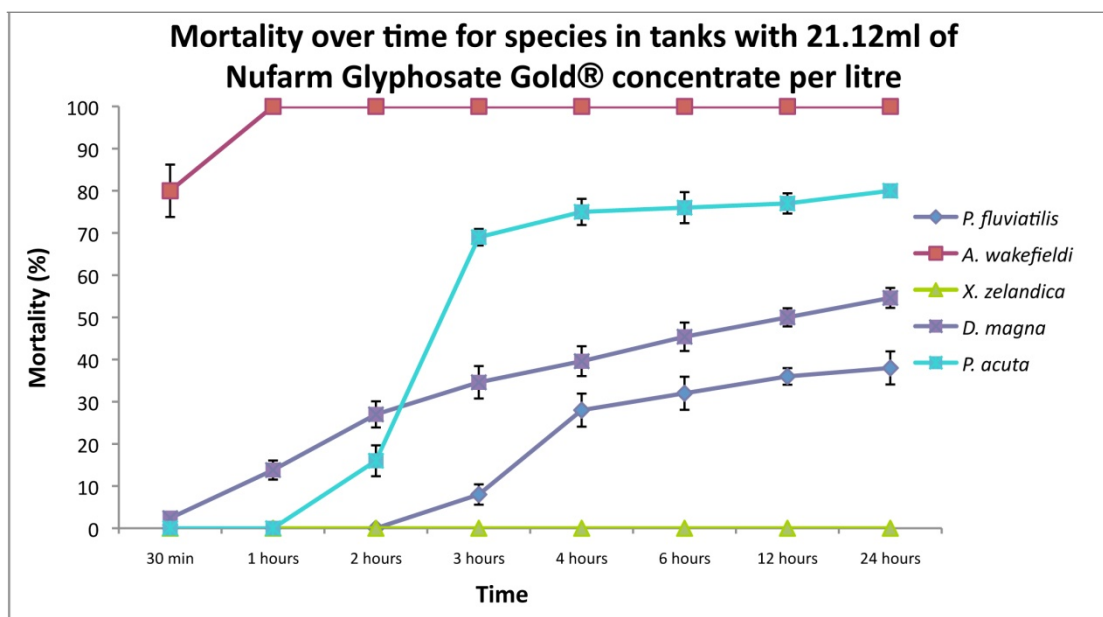


Figure 3.3: Cumulative percentage mortality over time for 5 invertebrate species at a concentration of 21.12 ml/l of Nufarm Glyphosate Gold®. Error bars show 95% confidence.

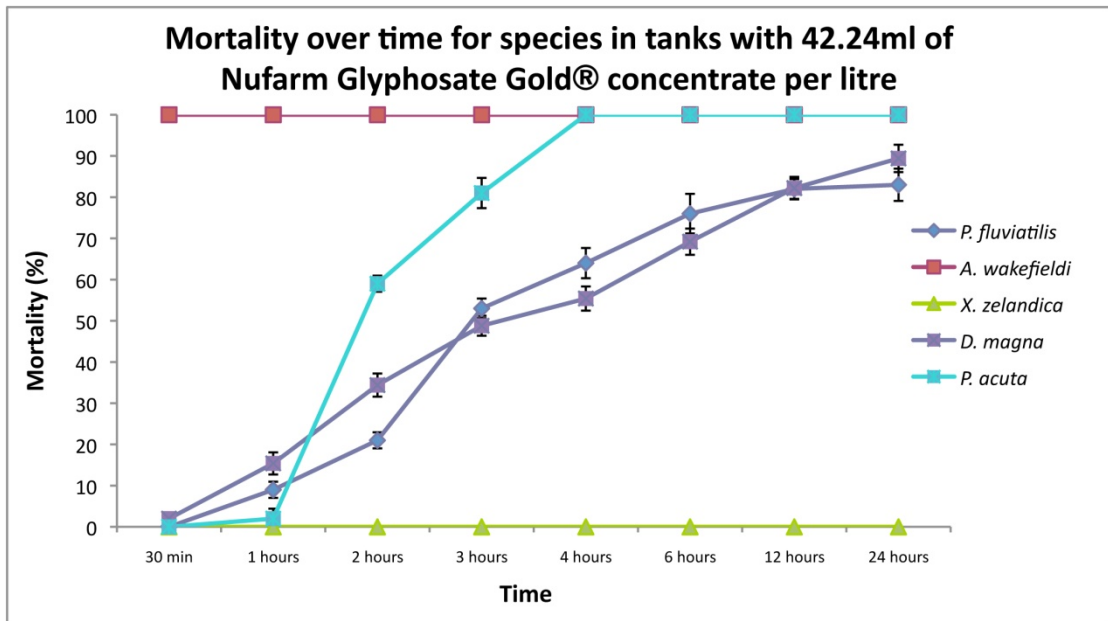


Figure 3.4: Cumulative percentage mortality over time for 5 invertebrate species at a concentration of 42.24 ml/l of Nufarm Glyphosate Gold®. Error bars show 95% confidence.

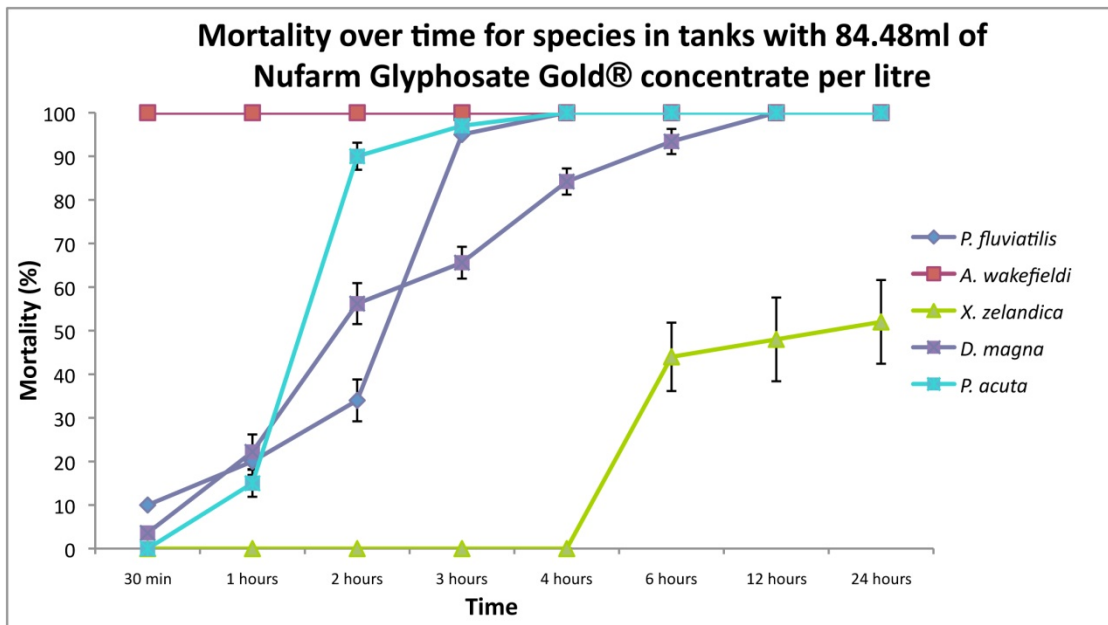


Figure 3.5: Cumulative percentage mortality over time for 5 invertebrate species at a concentration of 84.48 ml/l of Nufarm Glyphosate Gold®. Error bars show 95% confidence.

The species which was most susceptible to Nufarm Glyphosate Gold® was the Backswimmer, *A. wakefieldi*, with a concentration of 10.56ml/l, yielding 50% mortality

within 24 hours (Fig. 3.2). At higher concentrations all died (Fig. 3.3). The next species to reach its threshold, was the freshwater snail *P. acuta*. At the concentration of 10.56 ml/l, 25% mortality occurred within 24 hours (Fig 3.2). However this jumped to 80% at a 21.12 ml/l concentration (Fig. 3.3), and with a concentration of 42.24 ml/l 100% mortality occurred (Fig. 3.4). *D. magna* were similarly affected by the 10.56 ml/l of the glyphosate formulation as *P. acuta*, with a mortality of 25% after 24 hours (Fig. 3.2). In 21.12ml/l a mortality rate of over 50% occurred after a 24 hour period (Fig. 3.3), climbing to 90% in the 42.24ml/l tanks (Fig. 3.4) and 100% mortality in the 84.48 ml/l experiments (Fig. 3.5). *P. fluviatilis* first died in the tanks containing 21.12 ml/l of the glyphosate formulation, where there was over 30% mortality (Fig. 3.3). This jumped to over 80% mortality when the concentration was 42.24ml/l (Fig. 3.4), and in the 84.48 ml/l tanks 100% mortality occurred within 4 hours (Fig. 3.5). The Damselfly *X. zelandica* showed the highest resilience, with no signs of mortality for all but the highest concentration, 84.48 ml/l. At this point a mean of 52% mortality was recorded after 24 hours (Fig. 3.5).

DISCUSSION

Recommended concentrations of Nufarm Glyphosate Gold® caused no invertebrate fatalities. The recommended application rate of 9 litres per hectare would result in a maximum concentration of 0.045 ml/l if an aquatic habitat 1 hectare in area was only 2 cm deep. As a minimum concentration of 10.56 ml/l was required to cause mortalities, this shows that this glyphosate formulation must be applied at greater than 230 times the recommended rate to cause direct mortalities of any of the species studied, and 1877 times the recommended rate for the Damselfly species *X. zelandica*.

Fixed Concentration Experiments

Interestingly, although the glyphosate formulation did not directly kill any invertebrates at the concentration used (0.33 ml/l), it reduced mortality rates of *D. magna* in the filtered bore water. In the control tanks in these experiments, all *D. magna* died, compared with 30% mortality in tanks with the glyphosate formulation. This situation arose because the filtered bore water contained heavy metals. This fact was not known at the time the experiments were being set up. Numerous studies have found that many metals negatively affect *D. magna*. These effects include morphological changes such as low levels of copper causing the loss of the defensive neck teeth (Mirza and Pyle 2009), to mortality caused by

copper, lead or zinc (Cooper *et al.* 2009), cadmium (Murano *et al.* 2007) and mercury (Fjallborg *et al.* 2006). Glyphosate has been shown to bind to cadmium and other metals in the soil (Subramaniam and Hoggard 1988, Wang *et al.* 2004), reducing their bioavailability and therefore the absorption of these metals into plants and animals. This process has also been shown to occur in both the sediment (Tsui and Chu 2008) and water column within aquatic systems (Tsui *et al.* 2005). In all, glyphosate has been shown to reduce the acute toxicity of iron, silver, cadmium, copper, chromium, nickel, lead and zinc. One of these studies also found that the glyphosate significantly increased the uptake of mercury (Tsui *et al.* 2005). Glyphosate (and therefore glyphosate formulations) have the ability to mitigate the bioavailability and absorption of metals, and can buffer the effects such metals have on *D. magna* (Zalizniak and Nugegoda 2006). Interestingly, in the same way that glyphosate increases the absorption of mercury, it has also been shown to facilitate the uptake of several essential elements which are in low concentrations (Zalizniak and Nugegoda 2006). It is clear that the glyphosate in Nufarm Glyphosate Gold® was mitigating the effects of the heavy metals in the bore water, causing a much lower mortality rate than the control tanks.

Varying Concentration Experiments

The most sensitive species to various concentrations of Nufarm Glyphosate Gold® were the Backswimmer *A. wakefieldi*, and the snail *P. acuta*. These two species were the first to show symptoms of being negatively affected by the glyphosate formulation, perhaps because they both respire using a bubble of air. *A. wakefieldi*, has a plastron (air bubble) which is held underneath its elytra, and this is not only used in gas exchange, but also aides in the regulation of the invertebrates buoyancy (Matthews and Seymour 2008). In *P. acuta* the air bubble is held within the pallial lung, an organ which is possessed by all snails in the group Pulmonata (Berg and Ockelmann 1959). Both of these species rely on pressures and the surface tension of the water to maintain their air bubbles and the ability of these bubbles to aid in gas exchange. As a study on *A. wakefieldi* has shown (Schwind 1984), the fragility of such a method of breathing limits the environments where the species can survive, due to the pressures created by depth and temperature reducing the structural integrity of the plastron. This dependence on the structural integrity and stability of their air bubbles is what makes these species the most susceptible to the effects of the glyphosate formulation, as the surfactant in this chemical is designed to breakdown surface tension.

The zooplankton *D. magna* is often used in toxicity studies. It has been shown to be susceptible to many contaminants, such as heavy metals (Cooper *et al.* 2009), a broad range of pesticides (Kashian and Dodson 2002, Palma *et al.* 2009, Zvinavashe *et al.* 2009) and herbicides (Mastin and Rodgers 2000), including glyphosate formulations (Zvinavashe *et al.* 2009). In this study *D. magna* did show susceptibility to several contaminants. *P. fluviatilis*, the Amphipod, had a very similar threshold level to *D. magna*. These species are both freshwater Crustacea, which as a Subphylum have been shown to be affected by other glyphosate formulations in many studies (Chen *et al.* 2001, Brausch *et al.* 2006, Brausch and Smith 2007). These two species are also the smallest invertebrates examined. Glyphosate and surfactants are postulated to alter the structure and function of the exoskeleton (Giesy *et al.* 2000); possibly making the smaller species with a smaller mass more susceptible to the negative effects of these chemicals on their exoskeletons. The most resistant species studied was the damselfly *X. zelandica*. This species was clearly far more resilient to the effects of the chemical than any other, possibly because of its lack of reliance on air for gas exchange, and its larger overall body mass buffering against the effects of the loss of exoskeleton integrity.

Even though each species studied differs in its tolerance to the glyphosate formulation Nufarm Glyphosate Gold®, the levels required to cause fatalities within the parameters of this study were far higher than those which would be experienced in the field under normal spraying conditions with recommended rates. An application rate of 230 times the recommended rate was required to kill any invertebrates within this study, and for the most tolerant species, *X. zelandica*, a concentration 1877 times the recommended rate was required to kill 50% of the individuals.

Therefore, I conclude that within the limitations of this study, it appears that the direct effects on aquatic invertebrates of using Nufarm Glyphosate Gold® to control aquatic weeds such as Raupo are minimal. Although sub-lethal effects are clearly possible, this study found that no mortalities were caused when using Nufarm Glyphosate Gold® at recommended rates. Further long term studies should be carried out to test any long term, cumulative effects of Nufarm Glyphosate Gold®. From the experiments completed it does, however, seem that the practice of using this glyphosate formulation as a tool for the restoration and management of aquatic ecosystems can be considered relatively safe, with the knowledge that its direct effects on the invertebrate communities present are minimal.

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4) CONCLUDING RESULTS

The aim of this study was to investigate the effects of the glyphosate herbicide Nufarm Glyphosate Gold® on freshwater invertebrate communities. The outcomes from both parts of this study, field and laboratory experiments, are considered here.

FIELD EXPERIMENT RESULTS

Field experiments showed no statistically significant changes in community structure occurring in the treatment ponds, due to the use of Nufarm Glyphosate Gold®. Despite similar size and topography each pond had highly different communities, and the natural fluctuation of these communities far exceeded any affects the glyphosate formulation may have had. Apart from the community level differences between these seemingly homogenous ponds, the major finding in this study was the relative similarity between the ephemeral ponds (Evanno *et al.* 2009, Lepori and Malmqvist 2009).

LABORATORY EXPERIMENT RESULTS

Laboratory experiments found no invertebrate fatalities in concentrations equivalent to the recommended rates for Nufarm Glyphosate Gold® application. Even if an aquatic habitat was only 2 cm deep, a maximum concentration of only 0.045 ml/l would be achieved using the recommended rate of application. As a minimum concentration of 10.56ml/l was required to cause mortalities, this that the glyphosate formulation must be applied at greater than 230 times the recommended rate to cause direct fatalities to the species studied, and this is still only achievable if the water is very shallow . Even with prolonged exposure of up to a week, no mortalities occurred in the fixed concentration experiments using the recommended rate of application, showing again that only extremely high concentrations are detrimental to invertebrates. It should of course be noted that these results can only be relied upon within the limitations of this study. Further, long term studies are recommended to ensure that there are no adverse cumulative effects of Nufarm Glyphosate Gold® when used at the recommended rates.

DISCUSSION

From both the field and laboratory study there appear to be no effect of the glyphosate formulation Nufarm Glyphosate Gold® when using this agrochemical as recommended.

One limitation of the study was that the only species studied closely were the ones present in high numbers at the field site; the Pukepuke Reserve. This meant that many species that could theoretically come into contact with Nufarm Glyphosate Gold® in New Zealand were not considered. One species of interest is the native Tadpole Shrimp *Lepidurus apus viridis* (Crustacea: Notostraca). In an email in November 2009, Ian Stringer (istring@doc.govt.nz) expressed that in recent years this species has become rarer and rarer. Historically this species was found in agricultural regions, making it quite possible that its decline is due to the ever-increasing use of chemicals such as glyphosate formulations, especially if the species suffers from a similar chemically induced growth inhibition to *Thamocephalus platyurus* of North America. This proposition is made more likely with the knowledge that the life span of *L. apus viridis* is only up to 80 days (Cvetkovic-Milicic and Petrov 2000). This short life span is very similar to that of *T. platyurus*, which has been shown to be at extremely high risk from similar chemicals to Nufarm Glyphosate Gold®. Most Tadpole Shrimp species are now threatened and many even extinct across the globe. Often cited as being due to agriculture causing changes to hydrology (Angeler *et al.* 2008, Zierold *et al.* 2009), addition of agrichemicals is also seen as one of the biggest threats to these ancient invertebrates (Eder and Hodl 2002). It would therefore be of interest to carry out a study on this species to see if the application of Nufarm Glyphosate Gold® increased mortality or reduced fecundity in this species.

Another avenue of study that could be pursued is the effect of Nufarm Glyphosate Gold® on non-target plant or algae species. It was presumed that the possibility of effects on non-target species, including algae, bacteria and higher plants was fairly high. Such effects could have the potential to create trophic cascades, removing either food sources or habitat from the aquatic invertebrates. However, (Pesce *et al.* 2009) has shown that at normal rates, glyphosate formulations have no effect on algae or bacteria within the water column. This is hypothesised to be due to the constituents of the agrochemical quickly binding to organic matter with the water column, rendering them inactive (Baylis 2000). Although the specific formulation Nufarm Glyphosate Gold® has not been used in any similar studies, it can be assumed that the effects would be similar. This reduces the

likelihood of the use of glyphosate formulations over water affecting these food sources of aquatic invertebrates, which would otherwise have the potential to cause trophic cascades.

Non-target emergent plants have been shown to be affected by glyphosate formulations. However, these effects are not always necessarily negative for invertebrates. One study found that Cattail reduction, caused by the use of a glyphosate herbicide, caused an increase in many insect and gastropod species (Linz *et al.* 1999). It is clear that this plant was affected due to being above the water surface, and having direct contact with the herbicide. The loss of habitat would, for many species, be detrimental. From what is known about these ecosystems it can be presumed that loss in habitat could remove the hunting habitat required by predatory species, such as Odonata nymphs, enabling prey species to increase in number. This issue of the loss of non-target plant species in New Zealand can be mitigated by only using recommended application rates of Nufarm Glyphosate Gold®, as at this rate at which many native species such as the sedge *Carex secta* are unaffected by the agrochemical.

One of the limitations to the field study, which could not be remedied, was the lack of testing for the presence of the constituents of Nufarm Glyphosate Gold® in the ponds over time, such as its primary metabolite, aminomethylphosphonic acid (Goldsborough and Beck 1989, Goldsborough and Brown 1993). Unfortunately this process is costly (Abdullah *et al.* 1995) and was not possible for this study which had limited resources. If this study was to be repeated with resources which could permit this testing, it would be highly recommended to understand how this specific glyphosate formulation persists in the ecosystem over time, in both the water column and sediment.

CONCLUSION

In conclusion, the effects on aquatic invertebrates of using Nufarm Glyphosate Gold® to control aquatic weeds such as Raupo are minimal. Although sub-lethal effects are possible, this study found that no mortalities were caused when using Nufarm Glyphosate Gold® at recommended rates in the laboratory, and its use in the field caused no detectable changes to the invertebrate community structure. The practice of using Nufarm Glyphosate Gold® to control Raupo, and as a tool for the restoration and management of aquatic ecosystems can therefore be advocated, with the knowledge that its effects on the invertebrate communities present are negligible to non-existent.

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5) Appendices

Species	Pond	r ² Value	Deg. Of Freedom	F Statistic	p-value
<i>Paracalliope fluviatilis</i>	1	0.060	1,16	1.018	0.328
<i>Daphnia magna</i>	1	0.011	1,16	0.161	0.694
	2	0.013	1,16	0.214	0.650
	3	0.004	1,16	0.066	0.801
	4	0.509	1,15	15.538	0.001
	5	0.001	1,13	0.012	0.914
	6	0.094	1,15	1.555	0.232
<i>Cyprinotus incongruens</i>	1	0.051	1,16	0.851	0.370
	2	0.028	1,16	0.458	0.508
	3	0.011	1,16	0.182	0.676
	4	0.061	1,15	0.980	0.388
	5	0.031	1,13	0.400	0.538
	6	0.002	1,15	0.035	0.854
<i>Lobohalacarus subterraneus</i>	1	0.026	1,16	0.433	0.520
	2	0.332	1,16	7.945	0.012
	3	0.309	1,16	7.151	0.017
	4	0.047	1,15	0.740	0.403
	5	0.022	1,13	0.291	0.599
	6	0.034	1,15	0.528	0.479
<i>Anisops wakefieldi</i>	1	0.008	1,16	0.131	0.722
	2	0.025	1,16	0.408	0.532
	3	0.175	1,16	3.397	0.084
	4	0.356	1,15	9.851	0.007
	5	0.068	1,13	0.951	0.347
	6	0.113	1,15	1.919	0.186
<i>Physa acuta</i>	1	0.112	1,16	2.020	0.174
	2	0.149	1,16	2.805	0.113
	3	0.001	1,16	0.015	0.903
	4	0.008	1,15	0.119	0.735
	5	0.059	1,13	0.810	0.385
	6	0.045	1,15	0.699	0.416
<i>Xanthocnemis zelandica</i>	1	0.246	1,16	5.222	0.036
	2	0.013	1,16	0.208	0.654
	3	0.047	1,16	0.792	0.387
	4	0.012	1,15	0.007	0.933
	5	0.075	1,13	1.051	0.324
	6	0.152	1,15	2.692	0.122

Table 2.1. Values for the Regression Lines for mean abundance of each species collected in each sample over time, for six ponds at the Pukepuke Reserve. Statistically significant p-values are indicated in bold.

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesus plicatus	Gyrinus convexiusculus larvae	Liodesus plicatus larvae
12.10.08	1	1	3	3	15	2	0	4	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0
12.10.08	1	1	2	4	26	0	0	5	0	2	0	0	4	1	23	0	0	0	0	0	0	0	0
12.10.08	1	1	4	1	12	1	0	3	0	1	0	0	0	0	5	0	1	0	0	0	0	0	0
12.10.08	1	1	1	5	5	0	0	6	0	6	0	0	0	0	9	0	0	0	0	1	0	0	0
12.10.08	1	2	0	2	4	17	0	3	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0
12.10.08	1	2	0	4	5	13	0	9	0	7	0	0	1	2	3	0	0	0	0	0	0	0	0
12.10.08	1	2	1	1	1	3	0	12	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
12.10.08	1	2	2	3	2	2	0	16	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0
12.10.08	2	1	16	0	0	17	0	12	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
12.10.08	2	1	27	0	0	32	0	22	0	0	0	2	0	2	3	0	0	0	0	0	0	0	0
12.10.08	2	1	23	0	0	24	0	25	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0
12.10.08	2	1	35	0	0	12	1	8	0	0	0	0	0	0	2	0	0	0	0	3	0	0	0
12.10.08	2	2	14	0	0	42	0	6	0	1	0	0	2	0	3	0	0	0	0	1	0	0	0
12.10.08	2	2	13	0	0	13	1	3	0	1	0	0	1	0	5	0	0	0	0	0	0	3	0
12.10.08	2	2	18	0	0	27	1	3	0	3	0	0	3	1	9	0	0	0	0	2	0	2	0
12.10.08	2	2	24	0	0	25	0	5	0	0	0	0	1	1	12	0	0	0	0	4	0	0	0
12.10.08	3	1	28	3	0	5	0	7	0	1	2	0	2	0	4	0	0	0	2	4	0	0	0
12.10.08	3	1	13	1	0	4	0	4	0	4	3	1	4	1	7	0	0	0	0	8	0	0	0
12.10.08	3	1	27	0	0	7	0	9	0	2	2	0	6	3	6	1	0	0	1	4	0	0	0
12.10.08	3	1	38	6	0	9	1	12	0	8	1	0	2	1	12	0	0	0	2	3	0	0	0
12.10.08	3	2	27	6	0	0	0	3	0	0	0	0	0	0	13	0	0	0	1	2	0	0	0
12.10.08	3	2	41	8	0	2	1	4	0	1	0	1	1	0	21	0	0	0	0	1	0	0	0
12.10.08	3	2	29	12	0	6	1	7	0	3	1	0	0	1	8	0	0	0	1	5	0	0	0
12.10.08	3	2	32	2	0	1	1	3	0	0	2	0	3	1	3	0	0	0	0	1	0	0	0
12.10.08	4	1	0	5	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.10.08	4	1	4	2	0	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
12.10.08	4	1	2	31	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.10.08	4	1	7	16	0	0	1	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.10.08	4	2	23	160	0	46	3	41	4	11	0	0	0	0	4	0	0	2	0	4	0	0	0
12.10.08	4	2	57	215	0	52	0	62	13	17	0	1	0	0	7	0	0	4	0	7	0	0	0
12.10.08	4	2	41	170	0	41	1	52	9	12	0	0	0	0	6	0	0	3	1	9	0	0	0
12.10.08	4	2	46	156	0	37	0	41	5	15	1	0	0	0	11	0	0	3	1	5	0	0	0
12.10.08	5	1	2	32	0	0	2	4	0	0	0	0	1	0	3	0	0	0	0	0	0	1	0
12.10.08	5	1	8	14	0	0	1	28	0	0	0	0	2	0	1	0	0	0	0	0	0	5	0
12.10.08	5	1	13	27	0	0	0	15	0	0	0	0	2	0	2	0	0	0	0	0	0	9	0
12.10.08	5	1	4	14	0	0	1	31	0	0	0	0	0	0	1	0	0	0	0	0	0	8	0
12.10.08	5	2	24	2	0	5	0	3	0	3	1	1	3	1	2	0	0	0	0	3	3	0	0
12.10.08	5	2	18	4	0	9	0	4	0	5	0	0	2	2	4	0	0	0	0	4	0	0	0
12.10.08	5	2	33	8	0	13	0	7	0	1	0	1	3	3	1	0	0	0	0	1	1	0	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratomyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodryus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
12.10.08	5	2	37	1	0	22	2	9	0	2	0	2	1	0	3	0	0	0	0	0	0	0	0
12.10.08	6	1	6	12	0	0	3	5	0	0	0	0	1	4	0	0	0	0	0	0	0	8	0
12.10.08	6	1	12	4	0	0	1	13	0	0	0	1	1	0	0	0	0	0	0	0	0	23	0
12.10.08	6	1	4	23	0	0	0	10	0	0	0	1	3	3	0	0	0	0	0	0	0	7	0
12.10.08	6	1	9	11	0	0	0	7	0	0	0	0	0	2	0	0	0	0	0	0	0	11	0
12.10.08	6	2	16	37	0	23	1	32	0	5	0	0	1	2	3	0	0	0	0	3	0	0	0
12.10.08	6	2	15	42	0	21	0	12	0	3	0	0	1	2	7	0	0	0	0	2	0	0	0
12.10.08	6	2	24	41	0	17	1	25	0	8	0	0	4	0	2	0	0	0	0	7	0	0	0
12.10.08	6	2	34	24	0	39	1	23	0	2	0	0	3	1	4	0	0	0	0	3	0	0	0
19.11.08	1	1	2	1	12	3	0	25	2	2	0	0	12	0	2	0	0	0	0	2	0	2	0
19.11.08	1	1	1	2	17	2	1	22	1	3	0	0	5	0	8	0	1	0	0	0	0	4	0
19.11.08	1	1	0	0	14	0	1	16	0	2	0	0	6	1	5	0	0	0	0	1	0	6	0
19.11.08	1	1	0	3	21	0	1	19	3	4	0	0	8	0	6	0	0	0	0	1	0	4	0
19.11.08	1	2	0	1	15	13	0	36	2	0	0	0	5	0	4	0	0	0	0	1	0	7	0
19.11.08	1	2	1	2	13	15	0	39	1	4	0	0	2	0	6	0	0	0	0	4	1	15	0
19.11.08	1	2	0	2	8	14	1	26	1	3	0	0	7	0	3	0	0	0	0	0	1	8	0
19.11.08	1	2	1	1	14	11	0	31	0	2	0	0	8	0	4	0	0	0	0	2	0	12	0
19.11.08	2	1	2	0	0	4	0	16	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.11.08	2	1	3	0	0	1	0	19	2	1	0	0	0	0	2	0	0	0	0	0	0	0	0
19.11.08	2	1	0	0	0	2	0	28	3	1	0	1	0	0	1	0	0	0	0	2	0	0	0
19.11.08	2	1	2	0	0	6	0	33	1	0	0	0	0	2	2	0	0	0	0	0	0	0	1
19.11.08	2	2	5	0	0	2	1	24	2	1	0	0	2	0	2	0	0	0	0	1	2	0	0
19.11.08	2	2	3	0	0	2	1	29	6	1	0	0	1	0	3	0	0	0	0	0	0	0	0
19.11.08	2	2	2	0	0	4	0	34	2	1	0	1	3	1	0	0	0	0	0	0	0	0	0
19.11.08	2	2	3	0	0	2	2	39	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0
19.11.08	3	1	23	2	0	2	1	21	0	2	4	0	3	2	5	0	0	0	1	3	0	0	1
19.11.08	3	1	21	1	0	7	1	35	1	2	3	0	2	0	2	1	0	0	3	6	0	0	0
19.11.08	3	1	23	2	0	3	1	22	2	3	3	0	3	1	7	0	0	0	1	5	0	0	0
19.11.08	3	1	42	5	0	4	3	26	2	6	1	0	5	2	7	0	0	0	1	4	0	0	0
19.11.08	3	2	40	3	0	0	2	4	3	2	1	1	0	0	17	0	0	0	0	3	0	0	0
19.11.08	3	2	34	12	0	1	1	16	1	5	1	0	1	4	13	0	0	0	2	4	0	0	0
19.11.08	3	2	35	6	0	4	2	9	0	3	0	1	2	0	6	0	0	0	1	3	0	0	0
19.11.08	3	2	28	2	0	1	1	7	2	0	2	0	2	0	9	0	0	0	0	2	0	0	1
19.11.08	4	1	2	4	0	0	0	166	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.11.08	4	1	1	3	0	0	0	153	8	0	0	0	0	1	0	0	0	0	0	0	0	2	1
19.11.08	4	1	3	26	0	2	0	159	12	0	0	0	0	0	0	0	0	0	0	0	0	0	1
19.11.08	4	1	2	23	0	1	0	123	14	0	0	1	0	0	0	0	0	0	0	0	0	1	1
19.11.08	4	2	15	145	0	36	2	164	15	7	1	2	0	1	5	0	0	3	0	5	0	4	0
19.11.08	4	2	35	150	0	24	2	148	6	12	0	0	0	0	8	0	0	3	2	3	0	4	0
19.11.08	4	2	24	95	0	32	1	193	10	5	0	2	0	0	12	0	0	2	1	6	0	3	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonyidae	Ostracod	Aquatic Mite	Physo	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
23.2.09	1	2	28	9	7	0	0	8	2	1	0	0	21	0	1	0	0	0	0	0	0	2	0
23.2.09	2	1	16	0	0	0	0	14	1	0	0	0	1	2	0	0	0	0	0	0	0	0	0
23.2.09	2	1	21	0	0	0	0	4	1	0	0	2	1	5	0	0	0	0	0	0	0	0	0
23.2.09	2	1	11	0	0	0	0	11	0	0	0	1	2	4	0	0	0	0	0	0	0	0	0
23.2.09	2	1	15	0	0	0	0	9	1	1	0	0	1	2	0	0	0	0	0	0	0	0	0
23.2.09	2	2	15	0	0	0	0	9	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
23.2.09	2	2	18	0	0	0	0	13	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
23.2.09	2	2	12	0	0	0	0	15	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0
23.2.09	2	2	9	0	0	0	0	8	1	0	0	0	2	0	0	0	0	0	0	1	0	0	0
23.2.09	3	1	26	13	0	0	1	0	2	4	1	0	1	1	0	0	0	0	0	1	0	0	0
23.2.09	3	1	22	27	0	0	2	2	0	6	1	0	0	4	0	1	0	0	0	0	1	0	0
23.2.09	3	1	27	19	0	0	1	3	1	5	2	0	1	3	0	1	0	0	0	1	0	0	0
23.2.09	3	1	25	15	0	0	2	5	0	3	1	0	4	1	0	0	0	0	0	2	1	0	0
23.2.09	3	2	24	17	0	0	2	15	2	16	4	0	9	6	2	0	0	0	1	0	0	0	0
23.2.09	3	2	41	23	0	1	3	21	1	11	2	0	13	6	3	0	0	0	0	0	0	0	0
23.2.09	3	2	47	18	0	0	2	27	2	14	3	0	11	9	4	0	0	0	1	0	0	0	0
23.2.09	3	2	39	21	0	1	4	31	3	9	2	0	8	3	1	0	0	0	2	1	0	0	1
23.2.09	4	1	3	0	0	0	0	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1
23.2.09	4	1	53	0	0	1	1	53	3	5	0	0	0	0	0	0	0	0	0	0	0	2	0
23.2.09	4	1	45	0	0	1	1	47	1	3	0	0	0	0	0	1	0	0	0	0	0	1	1
23.2.09	4	1	32	0	0	2	0	62	0	2	0	0	1	0	0	0	0	0	0	0	0	2	0
23.2.09	4	2	142	0	0	4	3	196	31	14	0	0	0	0	2	0	1	0	1	3	0	4	0
23.2.09	4	2	73	0	0	1	0	89	14	6	0	0	0	0	1	0	0	0	1	2	0	3	0
23.2.09	4	2	121	0	0	2	2	153	11	8	0	0	0	0	1	0	1	0	1	2	0	4	0
23.2.09	4	2	128	0	0	1	0	139	12	6	0	0	0	0	1	0	0	0	2	3	0	2	0
23.2.09	5	1	153	2	0	1	0	176	2	32	2	1	0	0	0	1	0	0	0	5	0	3	0
23.2.09	5	1	132	3	0	1	3	185	1	26	1	2	0	0	0	0	1	0	0	2	0	2	0
23.2.09	5	1	146	1	0	1	1	179	4	23	0	0	0	0	0	0	0	0	0	4	0	1	0
23.2.09	5	1	174	4	0	2	2	154	2	29	2	1	0	0	0	0	0	0	1	9	0	3	0
23.2.09	5	2	55	0	0	3	3	95	0	14	2	1	0	0	0	0	0	0	0	1	0	3	0
23.2.09	5	2	165	3	0	1	2	231	4	38	3	3	3	0	2	0	0	0	1	0	0	0	0
23.2.09	5	2	133	2	0	3	2	167	2	19	3	2	2	0	3	0	0	0	0	1	0	2	0
23.2.09	5	2	148	5	0	1	4	148	0	21	2	0	5	0	2	0	0	0	0	0	0	0	0
23.2.09	6	1	69	3	0	0	2	68	1	3	1	1	0	0	0	0	0	0	1	0	0	1	0
23.2.09	6	1	89	2	0	1	2	72	1	5	2	0	0	1	1	1	0	0	1	2	1	0	0
23.2.09	6	1	85	0	0	0	1	59	2	1	2	1	1	0	0	1	0	0	0	2	0	2	0
23.2.09	6	1	70	1	0	0	1	66	3	6	3	0	0	4	0	0	0	0	0	0	0	1	0
23.2.09	6	2	64	2	0	2	0	73	1	3	0	1	6	0	2	0	0	0	0	0	1	0	0
23.2.09	6	2	47	1	0	1	2	81	0	4	2	2	9	2	1	0	1	0	0	1	0	0	0
23.2.09	6	2	43	4	0	1	0	67	0	0	0	0	11	0	1	0	0	0	1	2	0	1	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychohydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodes plicatus	Gyrinus convexiusculus larvae	Liodes plicatus larvae
23.2.09	6	2	51	1	0	0	1	52	1	2	1	0	3	0	0	0	0	0	1	0	0	0	0
25.2.09	1	1	13	16	1	0	0	0	0	2	0	0	0	0	2	0	2	0	0	0	0	0	0
25.2.09	1	1	21	24	3	0	0	8	0	1	0	0	4	0	1	0	0	0	0	0	0	1	0
25.2.09	1	1	16	18	2	0	0	7	2	1	0	0	1	0	2	0	0	0	0	0	0	1	0
25.2.09	1	1	12	21	1	0	0	4	1	2	0	0	2	0	2	0	0	0	0	0	0	0	0
25.2.09	1	2	35	15	3	0	0	25	0	5	0	0	0	0	2	1	1	0	0	0	0	2	0
25.2.09	1	2	28	12	6	0	0	11	1	3	0	0	0	0	4	0	1	0	0	0	1	3	1
25.2.09	1	2	26	15	4	0	0	12	2	4	0	0	0	0	3	0	0	0	0	0	0	2	1
25.2.09	1	2	31	11	2	0	0	16	1	2	0	0	0	0	2	0	0	0	0	0	1	1	0
25.2.09	2	1	34	6	0	0	0	23	2	0	0	0	0	3	1	0	1	0	0	0	0	0	0
25.2.09	2	1	12	0	0	0	0	6	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
25.2.09	2	1	11	4	0	0	0	8	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0
25.2.09	2	1	16	2	0	0	0	13	1	0	0	1	1	2	4	0	1	0	0	0	0	0	0
25.2.09	2	2	46	3	0	0	0	42	0	0	0	0	0	6	3	0	0	0	1	0	0	0	0
25.2.09	2	2	21	0	0	0	0	6	0	0	0	0	0	3	0	0	0	0	1	0	0	0	0
25.2.09	2	2	18	2	0	0	0	9	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0
25.2.09	2	2	15	1	0	0	0	15	1	0	0	0	0	3	1	0	0	0	1	0	0	0	0
25.2.09	3	1	17	23	0	0	0	9	2	4	1	0	0	0	0	1	0	0	0	0	0	0	0
25.2.09	3	1	22	36	0	0	1	3	0	5	3	0	1	1	0	0	0	0	0	0	0	0	0
25.2.09	3	1	26	28	0	0	2	13	2	4	2	0	2	2	0	1	0	0	0	0	0	0	0
25.2.09	3	1	23	31	0	0	1	16	1	3	3	0	1	3	0	0	0	0	0	0	0	0	0
25.2.09	3	2	56	42	0	0	3	12	2	32	4	0	2	4	2	0	0	0	0	0	0	0	0
25.2.09	3	2	61	33	0	0	1	23	1	25	5	0	1	1	3	1	0	0	0	0	0	0	0
25.2.09	3	2	58	16	0	0	0	21	2	19	3	0	1	5	2	0	0	0	0	0	1	0	0
25.2.09	3	2	56	29	0	0	1	25	1	16	4	0	0	3	3	0	0	0	0	0	1	0	0
25.2.09	4	1	53	3	0	4	0	24	5	5	0	0	0	0	0	0	0	0	0	0	0	1	0
25.2.09	4	1	77	14	0	2	2	211	26	4	0	0	0	0	0	0	0	0	1	0	0	5	0
25.2.09	4	1	82	11	0	3	1	174	14	8	0	0	2	1	0	0	0	0	1	0	0	3	0
25.2.09	4	1	36	16	0	2	2	186	16	4	0	0	1	0	0	0	0	0	0	0	0	1	0
25.2.09	4	2	72	12	0	3	1	53	3	27	0	0	0	0	0	0	0	0	0	0	0	1	0
25.2.09	4	2	59	13	0	3	5	42	0	13	0	0	0	0	0	0	1	0	1	3	0	1	0
25.2.09	4	2	45	15	0	2	3	53	5	10	0	0	0	0	0	0	0	0	1	0	4	0	0
25.2.09	4	2	41	9	0	3	2	62	2	17	0	0	0	0	0	0	1	0	1	2	0	2	0
25.2.09	5	1	35	12	0	4	1	44	6	23	0	0	0	0	0	1	0	0	0	3	0	0	0
25.2.09	5	1	24	3	0	4	0	131	0	44	3	3	0	0	5	0	0	0	1	1	0	1	1
25.2.09	5	1	17	4	0	3	1	127	7	36	2	1	0	0	2	0	0	0	0	2	0	1	2
25.2.09	5	1	26	2	0	1	2	114	4	41	3	2	0	1	3	1	0	0	1	1	0	1	0
25.2.09	5	2	67	6	0	1	0	121	13	36	3	0	0	0	0	1	0	0	1	2	0	1	0
25.2.09	5	2	84	12	0	0	0	144	23	42	5	0	0	0	0	0	0	0	1	3	0	2	0
25.2.09	5	2	89	8	0	1	0	132	21	48	3	0	0	0	0	0	0	0	1	2	0	0	0

date	pond	habitat	<i>Cyclops</i>	<i>Daphnia magna</i>	Amphipoda	<i>Chironomus Sp.</i>	<i>Stratiomyidae</i>	Ostracod	Aquatic Mite	Physa	<i>Gyraulid</i>	Leech	<i>Anisops wakefeldi</i>	<i>Sigara sp.</i>	<i>Xanthocnemis zelandica</i>	<i>Antipodochlora braueri</i>	<i>Aeshna brevistyla</i>	<i>Onychodrysdus hookeri</i>	<i>Gyrinus convexiusculus</i>	Hydrophilidae	<i>Liodesmus plicatus</i>	<i>Gyrinus convexiusculus larvae</i>	<i>Liodesmus plicatus larvae</i>
25.2.09	5	2	46	5	0	0	0	113	17	32	1	0	3	0	0	0	0	0	2	3	0	1	0
25.2.09	6	1	36	1	0	0	0	69	4	3	0	1	1	0	0	0	0	0	0	1	0	2	1
25.2.09	6	1	52	3	0	0	0	83	1	2	0	2	1	1	1	0	0	0	0	0	0	0	0
25.2.09	6	1	18	1	0	1	2	86	0	0	0	0	0	2	2	1	0	0	0	0	0	0	0
25.2.09	6	1	46	2	0	1	1	55	0	3	1	0	0	0	0	0	0	0	1	1	0	0	0
25.2.09	6	2	25	0	0	0	1	36	1	1	2	0	0	0	0	0	0	0	2	0	0	1	0
25.2.09	6	2	31	1	0	0	0	46	3	0	0	0	0	0	0	0	1	0	0	0	0	1	0
25.2.09	6	2	37	2	0	0	0	84	2	1	0	0	0	0	0	0	0	0	0	2	1	0	0
25.2.09	6	2	19	3	0	0	0	68	4	0	0	0	0	0	0	0	0	0	0	1	0	1	0
26.2.09	1	1	37	16	3	0	0	42	9	1	0	0	0	0	2	0	0	0	0	0	0	1	0
26.2.09	1	1	41	0	4	0	0	63	7	2	0	0	0	0	0	0	1	0	0	0	2	0	0
26.2.09	1	1	27	4	2	0	0	48	5	2	0	0	0	0	2	0	0	0	0	0	1	1	0
26.2.09	1	1	24	1	3	0	0	52	6	3	0	0	0	0	2	0	0	0	0	0	1	0	0
26.2.09	1	2	52	0	4	0	0	77	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26.2.09	1	2	25	0	2	0	0	15	3	8	0	0	2	0	2	0	0	0	0	0	0	1	0
26.2.09	1	2	37	3	3	0	0	58	2	4	0	0	1	0	1	0	0	0	0	0	0	0	0
26.2.09	1	2	41	2	3	0	0	44	3	3	0	0	1	0	0	0	0	0	0	0	0	1	0
26.2.09	2	1	59	6	0	0	1	84	6	0	0	0	0	1	3	0	0	0	2	1	0	0	0
26.2.09	2	1	36	0	0	0	2	63	2	0	0	0	1	3	2	1	0	0	0	0	0	0	0
26.2.09	2	1	45	5	0	0	1	75	4	0	0	0	1	1	0	0	0	0	1	1	0	0	0
26.2.09	2	1	38	2	0	0	2	57	4	0	0	0	0	1	2	1	0	0	1	0	0	0	0
26.2.09	2	2	114	0	0	4	1	5	1	0	0	0	0	4	2	0	0	0	0	0	0	0	0
26.2.09	2	2	35	0	0	0	0	3	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0
26.2.09	2	2	28	0	0	3	1	5	1	0	0	0	0	3	1	0	0	0	0	0	0	0	0
26.2.09	2	2	31	0	0	1	0	8	0	0	0	0	2	2	1	0	0	0	0	0	0	0	0
26.2.09	3	1	21	32	0	0	0	6	0	9	0	0	4	4	0	0	0	0	0	0	0	0	0
26.2.09	3	1	38	24	0	0	0	13	2	15	2	0	4	3	0	1	0	0	0	0	0	0	0
26.2.09	3	1	46	27	0	0	0	11	1	11	3	0	3	2	0	0	0	0	0	0	0	0	0
26.2.09	3	1	37	12	0	0	0	14	1	13	2	0	3	2	0	1	0	0	0	0	0	0	0
26.2.09	3	2	13	37	0	2	2	0	0	25	4	0	0	3	1	0	0	0	2	0	0	0	0
26.2.09	3	2	28	46	0	1	1	2	0	22	2	0	1	0	0	0	1	0	3	0	0	0	0
26.2.09	3	2	21	41	0	2	2	3	0	27	5	1	0	1	1	0	0	0	2	0	0	0	0
26.2.09	3	2	19	28	0	2	0	1	0	16	3	0	1	1	0	0	1	0	2	0	0	0	0
26.2.09	4	1	75	0	0	3	1	275	23	15	2	1	0	0	0	0	0	0	1	0	0	2	2
26.2.09	4	1	24	0	0	0	1	63	9	7	0	0	0	0	0	0	0	0	1	0	0	0	0
26.2.09	4	1	58	0	0	1	1	142	15	13	1	0	0	0	0	0	0	0	0	0	0	1	1
26.2.09	4	1	53	0	0	0	0	168	11	11	0	0	0	0	0	0	0	0	0	1	0	1	1
26.2.09	4	2	140	0	0	8	0	263	8	41	0	1	1	0	1	0	0	0	0	4	0	2	0
26.2.09	4	2	123	0	0	3	0	214	14	37	0	0	0	0	0	0	0	0	1	4	0	0	0
26.2.09	4	2	135	0	0	5	0	237	13	26	0	0	1	0	0	0	0	0	0	3	0	1	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratiomyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
26.2.09	4	2	114	0	0	3	0	244	3	45	0	0	1	1	0	0	0	0	0	0	0	0	0
26.2.09	5	1	35	0	0	0	0	116	4	6	0	0	0	2	0	0	0	0	1	1	0	0	0
26.2.09	5	1	38	0	0	0	0	143	3	8	0	0	0	0	0	0	0	0	1	0	0	1	0
26.2.09	5	1	45	0	0	0	0	123	1	16	0	0	0	1	0	0	0	0	0	1	0	2	0
26.2.09	5	1	23	0	0	0	0	95	0	13	0	1	0	0	0	0	0	0	0	3	0	0	0
26.2.09	5	2	57	0	0	0	0	152	21	25	0	0	3	0	0	0	0	0	1	5	0	3	0
26.2.09	5	2	96	2	0	0	0	131	15	47	0	0	0	0	0	0	0	0	2	1	0	0	0
26.2.09	5	2	84	1	0	0	0	69	16	31	0	0	2	0	0	0	0	0	2	0	0	1	1
26.2.09	5	2	76	0	0	0	0	82	9	27	0	0	3	0	0	0	0	0	0	1	0	0	0
26.2.09	6	1	88	0	0	0	0	184	22	6	0	0	2	0	0	0	0	0	0	0	0	0	1
26.2.09	6	1	91	0	0	1	0	157	17	14	0	0	2	0	0	1	0	0	0	0	0	1	0
26.2.09	6	1	64	0	0	3	1	144	6	34	0	0	0	0	0	0	0	0	2	0	0	3	0
26.2.09	6	1	82	0	0	2	1	94	3	25	0	0	0	0	0	0	0	0	1	0	0	2	0
26.2.09	6	2	26	0	0	2	0	44	1	3	0	0	22	4	1	0	1	0	0	0	0	0	0
26.2.09	6	2	47	0	0	0	1	57	3	0	0	2	26	7	0	0	0	0	1	0	0	0	0
26.2.09	6	2	54	0	0	1	1	84	2	1	0	0	16	2	1	0	1	0	1	0	0	0	0
26.2.09	6	2	58	0	0	0	2	36	1	0	0	1	12	1	0	0	1	0	1	0	0	0	0
27.2.09	1	1	14	0	1	0	0	13	23	4	0	0	13	0	0	0	0	0	0	0	1	6	0
27.2.09	1	1	23	0	0	0	0	58	14	6	0	0	12	0	0	0	1	0	0	0	0	8	0
27.2.09	1	1	17	0	0	0	0	43	11	8	0	0	8	0	0	0	0	0	0	0	0	7	0
27.2.09	1	1	28	0	1	0	0	37	15	7	0	0	4	0	0	0	0	0	0	1	6	0	0
27.2.09	1	2	7	0	0	0	0	0	0	7	0	0	2	0	0	0	0	0	0	0	0	12	0
27.2.09	1	2	6	3	0	0	0	2	0	2	0	0	3	0	0	0	0	0	0	0	1	14	0
27.2.09	1	2	5	1	0	0	0	1	2	6	0	0	6	0	0	0	0	0	0	0	1	13	0
27.2.09	1	2	9	1	0	0	0	4	3	5	0	0	2	0	0	0	0	0	0	0	1	9	0
27.2.09	2	1	8	0	0	0	0	0	0	0	0	0	3	1	1	0	0	0	1	0	0	0	0
27.2.09	2	1	3	0	0	0	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
27.2.09	2	1	4	0	0	0	0	8	0	0	0	0	2	2	1	0	0	0	1	0	0	0	0
27.2.09	2	1	7	0	0	0	0	3	1	0	0	0	0	1	2	0	0	0	0	0	0	0	0
27.2.09	2	2	59	3	0	0	0	77	6	1	0	1	1	1	0	0	0	0	0	1	0	0	0
27.2.09	2	2	16	0	0	0	1	32	5	0	0	0	1	2	1	0	0	0	0	1	0	0	0
27.2.09	2	2	11	2	0	0	1	31	4	1	0	0	1	3	0	0	0	0	0	2	0	0	0
27.2.09	2	2	26	0	0	0	1	27	2	2	0	1	0	1	0	0	0	0	0	0	0	0	0
27.2.09	3	1	25	5	0	0	3	35	2	16	4	0	3	2	3	1	0	0	0	0	0	0	0
27.2.09	3	1	93	45	0	0	0	3	1	21	3	0	5	2	0	1	0	0	0	0	1	0	0
27.2.09	3	1	74	37	0	0	2	5	1	20	4	0	4	1	3	2	0	0	0	0	2	0	0
27.2.09	3	1	97	8	0	0	1	15	0	17	5	0	4	2	5	1	0	0	0	0	1	0	0
27.2.09	3	2	39	46	0	0	2	12	1	34	11	0	2	1	0	0	0	0	2	0	0	0	0
27.2.09	3	2	46	62	0	5	1	37	0	52	16	0	0	3	2	1	0	0	0	0	0	0	0
27.2.09	3	2	22	44	0	3	0	42	1	45	13	0	2	2	1	1	0	0	1	0	0	0	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
1.3.09	6	2	124	0	0	0	0	131	21	7	0	1	15	1	1	0	0	0	1	1	0	1	0
2.3.09	1	1	2	14	2	0	2	32	5	4	0	0	0	0	2	0	0	0	0	0	0	2	0
2.3.09	1	1	4	8	2	0	0	25	16	3	0	0	0	0	1	1	0	0	0	0	0	4	0
2.3.09	1	1	15	11	0	0	1	30	8	3	0	0	0	0	1	0	0	0	0	0	0	6	0
2.3.09	1	1	8	6	1	0	1	35	10	1	0	0	0	0	0	1	0	0	0	0	0	4	0
2.3.09	1	2	3	0	0	0	0	67	36	0	0	0	4	0	0	0	1	0	0	0	0	7	0
2.3.09	1	2	16	0	2	0	0	43	14	0	0	0	16	0	0	0	0	0	0	0	1	15	0
2.3.09	1	2	23	0	0	0	0	48	29	1	0	0	13	0	0	0	1	0	0	0	1	8	0
2.3.09	1	2	10	0	1	0	0	54	27	0	0	0	6	0	0	0	0	0	0	0	0	12	0
2.3.09	2	1	135	83	0	0	0	21	0	0	0	0	36	0	0	0	0	0	1	0	0	0	0
2.3.09	2	1	13	16	0	0	0	16	0	2	0	0	25	0	0	0	1	0	1	0	0	0	0
2.3.09	2	1	35	23	0	0	0	18	0	1	0	0	33	0	0	0	0	0	0	0	0	0	0
2.3.09	2	1	73	54	0	0	0	31	0	1	0	0	17	0	0	0	1	0	1	0	0	0	0
2.3.09	2	2	31	4	0	0	2	27	0	0	0	0	3	4	4	0	0	0	0	0	2	0	0
2.3.09	2	2	14	2	0	0	0	21	0	1	0	1	0	0	2	0	0	0	0	0	0	0	0
2.3.09	2	2	21	5	0	0	2	30	0	1	0	1	2	1	3	0	0	0	0	0	0	0	0
2.3.09	2	2	17	1	0	0	1	27	0	0	0	0	1	2	4	0	0	0	0	0	1	0	0
2.3.09	3	1	5	7	0	0	1	8	0	9	0	0	3	1	0	0	0	0	0	0	0	0	0
2.3.09	3	1	18	15	0	0	0	16	0	23	0	0	16	4	0	1	1	0	0	0	0	0	0
2.3.09	3	1	14	19	0	0	1	18	0	22	1	0	11	3	0	0	0	0	0	0	0	0	0
2.3.09	3	1	26	11	0	0	0	13	0	15	2	0	8	2	0	1	1	0	0	0	0	0	0
2.3.09	3	2	46	74	0	0	6	42	0	56	2	0	0	0	3	1	0	0	1	1	0	0	0
2.3.09	3	2	53	68	0	0	4	56	0	42	1	0	0	0	2	0	0	0	0	0	0	0	0
2.3.09	3	2	65	75	0	0	4	38	0	51	2	0	0	0	3	0	0	0	0	1	0	0	0
2.3.09	3	2	52	56	0	0	5	62	0	34	3	0	0	0	3	0	0	0	1	0	0	0	0
2.3.09	4	1	17	2	0	3	0	230	0	5	0	0	0	0	0	0	0	0	0	2	0	0	0
2.3.09	4	1	42	4	0	0	0	187	0	23	0	0	0	0	0	1	0	0	2	0	0	2	2
2.3.09	4	1	51	3	0	1	0	173	0	16	0	0	0	0	0	0	0	0	1	1	0	0	1
2.3.09	4	1	26	1	0	2	0	241	0	11	0	0	0	0	0	0	0	0	1	1	0	1	0
2.3.09	4	2	130	24	0	1	2	210	12	45	0	0	0	0	0	0	0	0	1	0	0	4	0
2.3.09	4	2	186	67	0	0	2	260	0	37	0	2	0	0	0	0	0	0	1	3	0	4	0
2.3.09	4	2	166	72	0	1	1	255	6	35	0	2	0	0	0	0	0	0	1	1	0	3	0
2.3.09	4	2	147	51	0	0	2	231	2	32	0	0	0	0	0	0	0	0	2	2	0	5	0
2.3.09	5	1	56	21	0	0	2	32	0	24	0	2	0	0	3	1	0	0	0	0	0	0	0
2.3.09	5	1	164	122	0	0	0	204	0	34	0	0	0	0	5	0	0	0	2	0	0	0	0
2.3.09	5	1	112	97	0	0	1	145	0	19	0	2	0	0	3	0	0	0	2	0	0	0	0
2.3.09	5	1	86	86	0	0	1	187	0	27	0	1	0	0	3	0	0	0	1	0	0	0	0
2.3.09	5	2	63	5	0	0	1	32	0	41	0	0	0	0	2	0	0	0	0	3	0	0	2
2.3.09	5	2	17	2	0	0	1	14	0	32	0	0	0	0	0	0	0	0	0	2	0	0	0
2.3.09	5	2	24	4	0	0	1	21	0	52	0	0	0	0	1	0	0	0	0	3	0	0	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratomyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
2.3.09	5	2	21	2	0	0	2	28	0	40	0	0	0	0	0	0	0	0	0	4	0	0	1
2.3.09	6	1	14	6	0	0	2	32	8	3	0	0	0	0	2	0	0	0	0	0	0	0	3
2.3.09	6	1	45	2	0	0	0	24	3	23	0	0	0	1	0	0	0	0	1	1	0	0	0
2.3.09	6	1	43	2	0	0	1	17	5	16	0	0	0	0	1	1	0	0	1	0	0	0	1
2.3.09	6	1	38	1	0	0	2	35	3	11	0	0	0	1	1	0	0	0	1	2	0	0	0
2.3.09	6	2	52	0	0	0	1	43	0	2	0	2	19	8	0	0	0	0	0	0	0	0	0
2.3.09	6	2	68	0	0	0	3	72	1	1	0	0	6	2	5	1	0	0	0	2	0	2	0
2.3.09	6	2	79	0	0	0	5	64	0	1	0	0	13	5	0	0	0	0	0	1	0	0	0
2.3.09	6	2	66	0	0	0	4	66	0	1	0	1	5	7	2	1	0	0	0	2	0	1	0
3.3.09	1	1	12	7	1	0	0	8	26	0	0	0	0	0	2	1	0	0	0	0	0	2	0
3.3.09	1	1	15	21	0	0	0	12	14	2	0	0	0	0	0	0	0	0	0	0	0	0	0
3.3.09	1	1	11	24	1	0	0	15	16	0	0	0	0	0	1	0	0	0	0	0	0	1	0
3.3.09	1	1	18	18	0	0	0	14	19	0	0	0	0	0	0	1	0	0	0	0	0	0	0
3.3.09	1	2	6	12	0	0	0	32	16	1	0	0	1	0	2	0	0	0	1	0	0	1	0
3.3.09	1	2	13	18	0	0	0	21	7	0	0	0	0	0	0	0	0	0	1	0	0	1	0
3.3.09	1	2	21	15	0	0	0	23	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.3.09	1	2	30	21	1	0	0	19	17	0	0	0	0	0	1	0	0	0	1	0	0	0	0
3.3.09	2	1	83	58	0	0	0	4	0	0	0	0	2	1	0	0	0	0	0	0	0	0	1
3.3.09	2	1	96	53	0	0	0	7	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
3.3.09	2	1	53	42	0	0	0	9	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0
3.3.09	2	1	42	51	0	0	0	14	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0
3.3.09	2	2	68	4	0	0	0	27	1	0	0	0	3	3	0	0	0	0	1	0	0	0	0
3.3.09	2	2	71	3	0	0	1	36	3	1	0	0	1	5	0	0	0	0	0	0	0	0	0
3.3.09	2	2	55	6	0	0	2	41	7	2	0	0	2	6	0	0	0	0	0	0	0	0	0
3.3.09	2	2	82	3	0	0	0	19	4	1	1	0	2	4	0	0	0	0	1	0	0	0	0
3.3.09	3	1	5	5	0	0	0	3	3	5	2	0	4	1	0	0	0	0	0	0	0	0	0
3.3.09	3	1	9	11	0	0	0	11	0	12	1	0	3	1	0	0	0	0	0	0	0	0	0
3.3.09	3	1	13	8	0	0	0	15	3	8	1	0	5	0	0	1	0	0	0	0	0	0	0
3.3.09	3	1	9	4	0	0	0	17	4	5	2	0	1	0	0	0	0	0	0	0	0	0	0
3.3.09	3	2	5	58	0	0	0	9	0	42	3	0	2	0	0	0	0	0	0	0	0	0	0
3.3.09	3	2	6	46	0	0	1	96	4	37	1	0	0	2	2	0	0	0	0	0	0	0	0
3.3.09	3	2	2	51	0	0	1	87	3	26	2	0	0	1	1	0	0	0	0	0	0	0	0
3.3.09	3	2	5	62	0	0	1	132	4	8	2	0	0	0	1	0	1	0	0	0	0	0	0
3.3.09	4	1	43	10	0	1	0	122	5	9	1	0	0	0	0	0	0	0	0	0	0	0	0
3.3.09	4	1	26	5	0	0	1	174	9	6	0	0	0	0	0	0	0	0	1	0	1	0	0
3.3.09	4	1	16	9	0	0	0	135	6	12	0	0	0	0	1	0	0	0	2	0	1	0	0
3.3.09	4	1	19	11	0	0	1	105	8	5	0	0	0	0	0	0	0	0	0	0	1	0	0
3.3.09	4	2	110	42	0	0	2	211	19	56	0	0	2	0	1	0	0	0	3	0	0	4	0
3.3.09	4	2	142	48	0	0	0	232	24	37	0	0	1	0	8	0	0	0	0	1	0	3	0
3.3.09	4	2	98	51	0	0	1	226	15	34	1	0	1	0	4	0	0	0	2	1	0	4	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonomyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
3.3.09	4	2	79	46	0	0	0	89	4	33	1	0	0	0	4	0	0	0	4	1	0	6	0
3.3.09	5	1	8	57	0	0	5	37	2	18	0	0	0	0	2	1	0	0	0	0	0	0	0
3.3.09	5	1	15	58	0	0	2	48	2	17	0	0	0	0	1	0	0	0	0	0	0	1	0
3.3.09	5	1	12	53	0	0	4	42	3	23	0	0	0	0	3	0	0	0	0	1	0	0	0
3.3.09	5	1	7	47	0	0	1	36	3	21	0	0	1	0	2	1	0	0	0	0	0	2	0
3.3.09	5	2	145	4	0	0	0	92	3	42	0	2	1	1	11	0	0	0	1	5	0	0	0
3.3.09	5	2	136	9	0	0	0	43	2	48	1	0	0	2	5	0	0	0	0	0	7	1	0
3.3.09	5	2	162	11	0	0	0	76	3	41	1	1	1	1	7	0	0	0	1	2	3	1	0
3.3.09	5	2	152	6	0	0	0	72	4	56	0	2	0	2	5	0	1	0	0	2	4	1	0
3.3.09	6	1	72	4	0	0	12	88	8	15	0	0	0	0	3	0	0	0	0	0	0	0	0
3.3.09	6	1	84	3	0	0	1	114	13	23	0	1	1	0	2	0	0	0	2	0	0	0	0
3.3.09	6	1	124	0	0	0	4	93	6	19	0	1	0	0	1	1	0	0	2	1	0	0	0
3.3.09	6	1	135	2	0	0	7	98	7	26	0	0	0	0	2	0	0	0	1	0	0	0	0
3.3.09	6	2	127	1	0	0	1	74	9	3	0	0	38	8	1	1	0	0	0	0	0	0	0
3.3.09	6	2	103	2	0	0	2	53	4	2	0	0	44	5	0	0	0	0	0	0	0	0	0
3.3.09	6	2	84	1	0	0	0	71	7	2	0	0	35	5	1	0	0	0	0	0	0	0	0
3.3.09	6	2	108	2	0	0	1	58	11	1	0	0	31	4	1	0	1	0	0	0	0	0	0
10.3.09	1	1	3	0	15	0	1	24	1	0	0	1	0	0	4	0	0	0	1	0	0	0	0
10.3.09	1	1	8	4	13	0	0	41	2	0	0	0	1	0	7	1	0	0	0	1	0	0	0
10.3.09	1	1	5	0	1	0	1	56	4	0	0	0	1	0	6	0	0	0	0	1	0	0	0
10.3.09	1	1	14	0	0	0	0	33	2	0	0	2	1	0	3	1	0	0	0	0	0	0	0
10.3.09	1	2	0	0	0	0	0	23	1	0	0	0	2	1	2	0	0	0	0	0	0	1	0
10.3.09	1	2	0	0	0	0	0	12	0	0	0	0	1	0	8	0	0	0	0	2	0	0	0
10.3.09	1	2	0	0	0	0	0	14	1	0	0	0	3	1	7	0	0	0	0	1	0	1	0
10.3.09	1	2	0	0	1	0	0	17	1	0	0	0	1	1	6	0	0	0	0	2	0	0	0
10.3.09	2	1	0	0	0	0	0	3	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
10.3.09	2	1	0	0	0	0	0	8	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
10.3.09	2	1	0	0	0	0	0	9	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0
10.3.09	2	1	0	0	0	0	0	11	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
10.3.09	2	2	0	13	0	0	0	29	1	0	0	0	5	3	0	0	0	0	1	0	0	0	0
10.3.09	2	2	0	19	0	0	1	34	3	1	0	0	3	5	0	0	0	0	0	0	0	0	0
10.3.09	2	2	0	17	0	0	2	44	8	2	0	0	2	6	0	0	0	0	0	0	0	0	0
10.3.09	2	2	0	11	0	0	0	18	3	1	0	0	3	4	0	0	0	0	1	0	0	0	0
10.3.09	3	1	4	0	0	0	0	6	0	5	1	0	3	1	0	0	0	0	0	0	0	0	0
10.3.09	3	1	7	0	0	0	0	7	0	4	2	0	3	1	0	0	0	0	0	0	0	0	0
10.3.09	3	1	4	0	0	0	0	13	0	3	1	0	4	0	0	0	0	0	0	0	0	0	0
10.3.09	3	1	9	0	0	0	0	15	1	3	3	0	1	0	0	0	0	0	0	0	0	0	0
10.3.09	3	2	5	0	0	0	0	6	0	7	2	0	0	0	0	1	0	0	0	0	0	0	0
10.3.09	3	2	3	0	0	0	1	12	1	13	1	0	0	2	2	1	0	0	0	0	0	0	0
10.3.09	3	2	7	0	0	0	1	19	1	11	3	0	0	1	1	0	1	0	0	0	0	0	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
17.3.09	2	2	3	0	0	0	0	15	3	4	0	0	5	0	0	0	0	0	0	0	0	0	0
17.3.09	3	1	2	0	0	0	0	23	4	2	4	0	0	0	3	0	0	0	0	0	0	0	0
17.3.09	3	1	5	0	0	0	0	26	0	0	6	0	0	0	4	1	0	0	0	0	0	2	0
17.3.09	3	1	5	0	0	0	0	21	2	0	2	0	6	3	3	0	0	0	0	0	0	0	0
17.3.09	3	1	14	0	0	0	0	31	1	1	0	0	12	5	2	1	0	0	0	0	0	1	0
17.3.09	3	2	16	1	0	0	0	26	11	2	1	0	8	2	1	0	1	0	0	0	0	1	0
17.3.09	3	2	18	2	0	1	0	35	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0
17.3.09	3	2	13	3	0	2	0	11	2	1	6	0	0	0	5	0	1	0	0	0	0	0	0
17.3.09	3	2	21	1	0	0	0	9	1	2	0	0	4	0	4	0	0	0	0	0	0	0	0
17.3.09	4	1	15	0	0	0	0	7	0	3	0	0	8	1	0	0	0	0	0	2	0	1	0
17.3.09	4	1	13	0	0	0	0	8	0	0	0	0	14	4	0	0	0	0	0	0	0	0	0
17.3.09	4	1	11	0	0	0	0	5	2	0	0	0	11	2	0	0	0	0	0	0	0	0	0
17.3.09	4	1	9	0	0	0	0	3	2	3	0	0	7	1	0	0	0	0	0	0	0	2	0
17.3.09	4	2	6	0	0	0	0	11	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
17.3.09	4	2	11	0	0	0	0	13	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.3.09	4	2	7	0	0	0	0	18	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0
17.3.09	4	2	12	0	0	0	0	14	2	0	0	0	0	4	0	0	0	0	0	0	0	0	0
17.3.09	5	1	15	0	0	0	0	34	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
17.3.09	5	1	21	13	0	0	0	32	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0
17.3.09	5	1	24	0	0	0	0	38	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0
17.3.09	5	1	13	0	0	0	0	41	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.3.09	5	2	32	0	0	0	0	37	4	0	0	0	13	3	0	0	0	0	0	0	0	0	0
17.3.09	5	2	28	23	0	0	0	39	3	2	0	0	15	1	0	0	0	0	0	0	0	0	1
17.3.09	5	2	16	18	0	0	0	35	2	5	0	0	7	0	0	0	0	0	0	0	0	0	1
17.3.09	5	2	26	15	0	0	0	36	7	4	0	0	4	2	0	0	1	0	0	0	0	0	0
17.3.09	6	1	4	5	0	0	0	62	4	2	0	0	1	1	0	0	0	0	0	1	0	0	0
17.3.09	6	1	3	4	0	0	0	58	1	4	0	0	2	0	2	0	0	0	0	0	0	0	0
17.3.09	6	1	4	3	0	0	0	67	7	1	0	0	1	0	3	0	0	0	0	0	0	0	1
17.3.09	6	1	6	5	0	0	0	35	4	2	0	0	0	0	0	0	0	0	0	0	0	1	0
17.3.09	6	2	5	0	0	0	0	74	5	0	0	0	3	0	0	0	0	0	0	0	0	3	0
17.3.09	6	2	2	4	0	0	0	69	3	0	0	0	6	0	1	0	0	0	0	0	0	2	0
17.3.09	6	2	0	2	0	0	0	82	11	0	0	0	4	2	0	0	0	0	0	0	0	0	0
17.3.09	6	2	7	0	0	0	0	85	8	0	0	0	1	1	0	0	0	0	0	0	0	0	0
24.3.09	1	1	2	0	1	0	0	13	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
24.3.09	1	1	1	0	1	0	0	32	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0
24.3.09	1	1	1	0	2	0	0	21	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0
24.3.09	1	1	4	0	1	0	0	19	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.3.09	1	2	16	0	2	0	0	23	2	2	0	0	0	0	2	0	0	0	0	0	2	0	0
24.3.09	1	2	2	0	2	0	0	35	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0
24.3.09	1	2	7	0	1	0	0	37	1	0	0	0	0	0	4	0	0	0	0	0	1	0	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonomyidae	Ostracod	Aquatic Mite	Physa	Gyraulus	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychohydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
26.4.09	5	2																					
26.4.09	6	1	11	3	0	0	0	18	2	7	0	1	1	2	2	0	0	0	1	1	0	0	1
26.4.09	6	1	9	5	0	0	0	6	1	9	0	0	0	0	3	1	0	0	0	2	0	0	2
26.4.09	6	1	5	1	0	0	0	13	0	5	0	0	1	1	1	1	0	0	1	1	0	0	0
26.4.09	6	1	8	3	0	0	0	16	1	6	0	0	1	0	4	1	0	0	1	3	0	0	3
26.4.09	6	2	2	1	0	1	0	12	2	0	0	0	17	1	6	0	0	0	1	1	0	0	0
26.4.09	6	2	6	4	0	2	0	9	1	1	0	0	14	0	4	0	0	1	1	0	0	0	2
26.4.09	6	2	2	2	0	1	0	4	0	3	0	0	9	1	8	1	0	0	1	1	0	0	1
26.4.09	6	2	3	3	0	3	0	7	1	2	0	0	15	2	5	1	0	0	1	2	0	0	0
23.5.09	1	1	15	0	6	2	0	64	4	3	0	0	8	2	3	1	0	0	0	0	0	0	0
23.5.09	1	1	8	0	5	2	0	48	3	1	0	0	9	1	1	0	0	0	0	0	0	0	0
23.5.09	1	1	9	0	5	3	0	53	6	1	0	0	3	1	0	0	0	0	0	0	0	0	0
23.5.09	1	1	13	0	4	1	0	72	3	4	0	0	4	0	2	1	0	0	0	0	0	0	0
23.5.09	1	2	4	0	6	4	0	57	6	2	0	0	6	0	0	0	0	0	0	0	0	0	0
23.5.09	1	2	5	0	6	5	0	49	4	1	0	0	5	1	3	0	0	0	0	0	0	0	0
23.5.09	1	2	4	0	9	5	0	65	4	1	0	0	6	2	2	0	0	0	0	0	0	0	0
23.5.09	1	2	6	0	5	3	0	68	7	2	0	0	7	0	1	0	1	0	0	0	0	0	0
23.5.09	2	1	6	5	0	13	0	29	3	1	0	0	2	0	1	0	0	0	0	0	0	0	0
23.5.09	2	1	2	3	0	15	0	24	1	2	0	0	0	0	3	0	0	0	0	0	0	0	0
23.5.09	2	1	4	2	0	18	0	23	4	1	0	0	1	2	0	0	0	0	0	0	0	0	0
23.5.09	2	1	6	1	0	11	0	21	3	0	0	0	3	1	2	1	0	0	0	0	0	0	0
23.5.09	2	2	3	6	0	21	0	15	5	1	0	0	2	2	4	0	0	0	0	0	0	0	0
23.5.09	2	2	4	3	0	26	0	26	4	2	0	0	4	0	2	0	0	0	0	0	0	0	0
23.5.09	2	2	3	1	0	24	0	32	7	4	0	0	1	1	0	1	0	0	0	0	0	0	0
23.5.09	2	2	5	2	0	35	0	34	8	1	0	0	2	0	2	0	0	0	0	0	0	0	0
23.5.09	3	1	6	8	0	17	0	19	6	2	5	0	5	1	6	1	0	0	1	0	0	0	0
23.5.09	3	1	3	6	0	14	0	25	5	4	4	0	3	6	4	1	0	0	1	0	0	0	0
23.5.09	3	1	8	7	0	9	0	29	3	3	6	0	4	5	5	0	0	0	2	0	0	0	0
23.5.09	3	1	16	9	0	21	0	24	1	1	8	0	9	5	8	0	0	0	0	0	0	0	0
23.5.09	3	2	14	15	0	11	0	38	1	4	4	0	3	6	6	0	0	0	1	0	0	0	0
23.5.09	3	2	8	8	0	18	0	15	2	3	3	0	5	8	7	0	0	0	0	0	0	0	0
23.5.09	3	2	3	3	0	14	0	11	6	2	4	0	6	12	5	0	1	0	1	0	0	0	0
23.5.09	3	2	4	4	0	16	0	26	2	1	6	0	2	6	6	0	0	0	1	0	0	0	0
23.5.09	4	2	56	2	0	15	0	9	1	5	1	1	5	3	0	0	0	0	1	2	0	0	0
23.5.09	4	2	54	6	0	12	0	5	3	6	0	0	8	2	2	0	1	0	0	1	0	0	0
23.5.09	4	2	51	1	0	11	0	11	2	7	2	1	5	0	1	0	0	0	1	1	0	0	0
23.5.09	4	2	76	3	0	18	0	5	6	4	1	1	9	1	2	0	0	0	1	1	0	0	0
23.5.09	6	1	49	11	0	6	0	23	5	3	0	0	6	1	6	1	0	0	1	1	0	0	0
23.5.09	6	1	28	6	0	4	0	8	4	5	0	0	4	1	8	1	1	0	1	1	0	0	0
23.5.09	6	1	26	7	0	8	0	16	3	1	0	0	7	1	4	0	0	0	1	0	0	0	0

date	pond	habitat	Cyclops	Daphnia magna	Amphipoda	Chironomus Sp.	Stratonyidae	Ostracod	Aquatic Mite	Physa	Gyraulid	Leech	Anisops wakefeldi	Sigara sp.	Xanthocnemis zelandica	Antipodochlora braueri	Aeshna brevistyla	Onychodydrus hookeri	Gyrinus convexiusculus	Hydrophilidae	Liodesmus plicatus	Gyrinus convexiusculus larvae	Liodesmus plicatus larvae
23.5.09	6	1	18	2	0	3	0	11	7	8	0	0	8	0	12	0	0	0	0	0	0	0	0
23.5.09	6	2	34	8	0	7	0	21	6	6	0	0	5	2	6	0	0	1	1	1	0	0	0
23.5.09	6	2	16	4	0	3	0	18	1	7	0	0	11	4	5	0	0	0	1	1	0	0	0
23.5.09	6	2	25	6	0	4	0	15	2	5	0	0	16	1	7	0	0	0	0	0	0	0	0
23.5.09	6	2	11	5	0	5	0	16	5	3	0	0	14	1	6	1	0	0	1	0	0	0	0
26.6.09	1	1	18	2	9	8	0	55	2	3	0	0	8	2	3	1	0	0	0	0	0	0	0
26.6.09	1	1	15	1	11	11	0	14	4	1	0	0	9	1	1	0	0	0	0	0	0	0	0
26.6.09	1	1	11	2	6	16	0	25	3	1	0	0	3	1	0	0	0	0	0	0	0	0	0
26.6.09	1	1	16	0	8	17	0	16	1	4	0	0	4	0	2	1	0	0	0	0	0	0	0
26.6.09	1	2	8	0	4	9	0	11	1	2	0	0	6	0	0	0	0	0	0	0	0	0	0
26.6.09	1	2	11	0	2	12	0	25	2	1	0	0	5	1	3	0	0	0	0	0	0	0	0
26.6.09	1	2	13	0	3	14	0	19	4	2	0	0	6	2	2	0	0	0	0	0	0	0	0
26.6.09	1	2	7	0	4	12	0	16	2	1	0	0	7	0	1	0	0	0	0	0	0	0	0
26.6.09	2	1	6	5	0	13	0	29	3	3	0	0	1	1	2	1	0	0	0	0	0	0	0
26.6.09	2	1	2	3	0	15	0	24	1	1	0	0	6	0	3	1	0	0	0	0	0	0	0
26.6.09	2	1	4	2	0	18	0	23	4	2	0	0	1	1	2	0	0	0	0	0	0	0	0
26.6.09	2	1	6	1	0	11	0	21	3	1	0	0	5	0	2	0	0	0	0	0	0	0	0
26.6.09	2	2	3	6	0	21	0	15	5	2	0	0	4	0	6	0	0	0	0	0	0	0	0
26.6.09	2	2	4	3	0	26	0	26	4	1	0	0	3	1	5	0	0	0	0	0	0	0	0
26.6.09	2	2	3	1	0	24	0	32	7	2	0	0	2	1	4	1	0	0	0	0	0	0	0
26.6.09	2	2	5	2	0	35	0	34	8	2	0	0	3	1	3	0	0	0	0	0	0	0	0
26.6.09	3	1	5	2	0	26	0	9	2	3	5	0	2	2	7	1	0	0	0	0	0	0	0
26.6.09	3	1	5	4	0	28	0	17	4	4	5	0	4	3	6	1	0	0	1	0	0	0	0
26.6.09	3	1	12	3	0	21	0	13	3	2	4	0	3	4	3	0	0	0	1	0	0	0	0
26.6.09	3	1	9	0	0	19	0	11	1	5	6	0	7	1	8	1	0	0	1	0	0	0	0
26.6.09	3	2	8	2	0	15	0	18	6	4	2	0	5	5	12	0	0	0	1	0	0	0	0
26.6.09	3	2	23	1	0	16	0	24	5	1	4	0	4	3	4	0	0	0	0	0	0	0	0
26.6.09	3	2	25	2	0	27	0	21	2	2	6	0	2	5	9	0	0	0	0	0	0	0	0
26.6.09	3	2	26	1	0	26	0	15	1	2	2	0	6	4	8	0	1	0	1	0	0	0	0
26.6.09	4	2	21	12	0	16	0	7	2	4	2	0	8	4	6	0	0	0	1	0	0	0	0
26.6.09	4	2	23	16	0	24	0	6	2	6	1	1	7	2	7	1	0	0	1	0	0	0	0
26.6.09	4	2	19	13	0	13	0	9	1	4	1	1	6	5	8	0	0	0	2	0	0	0	0
26.6.09	4	2	26	11	0	19	0	12	1	3	1	0	5	2	6	0	0	0	1	0	0	0	0
26.6.09	5	1	31	3	0	13	0	23	1	2	0	0	2	0	4	1	0	0	0	0	0	0	0
26.6.09	5	1	24	4	0	18	0	15	0	3	0	0	1	1	3	0	0	0	1	0	0	0	0
26.6.09	5	1	26	5	0	9	0	18	1	2	0	0	2	1	8	1	0	0	1	0	0	0	0
26.6.09	5	1	15	2	0	11	0	11	1	4	0	0	7	2	6	0	0	0	0	0	0	0	0
26.6.09	5	2	13	12	0	12	0	21	0	6	0	0	4	0	8	0	0	0	0	0	0	0	0
26.6.09	5	2	15	11	0	15	0	9	2	1	0	0	6	1	4	0	0	0	1	0	0	0	0
26.6.09	5	2	26	14	0	14	0	14	1	5	0	0	5	2	5	1	0	0	1	0	0	0	0

date	pond	habitat	Cyclops	<i>Daphnia magna</i>	Amphipoda	<i>Chironomus Sp.</i>	Stratiomyidae	Ostracod	Aquatic Mite	Physa	Gyraulus	Leech	<i>Anisops wakefeldi</i>	<i>Sigara sp.</i>	<i>Xanthocnemis zelandica</i>	<i>Antipodochlora braueri</i>	<i>Aeshna brevistyla</i>	<i>Onychhydrus hookeri</i>	<i>Gyrinus convexiusculus</i>	Hydrophiliidae	<i>Liodesus plicatus</i>	<i>Gyrinus convexiusculus larvae</i>	<i>Liodesus plicatus larvae</i>
26.6.09	5	2	18	9	0	16	0	11	2	3	0	0	3	2	6	0	0	0	1	0	0	0	0
26.6.09	6	1	26	2	0	15	0	8	2	2	0	0	5	2	8	0	0	0	0	0	0	0	0
26.6.09	6	1	24	7	0	13	0	16	3	5	0	0	4	3	5	1	1	0	1	0	0	0	0
26.6.09	6	1	23	6	0	18	0	11	4	8	0	0	6	5	7	1	0	0	1	0	0	0	0
26.6.09	6	1	18	2	0	19	0	21	1	2	0	0	3	2	3	0	0	0	0	0	0	0	0
26.6.09	6	2	29	4	0	26	0	18	6	7	0	0	12	6	9	0	0	0	1	0	0	0	0
26.6.09	6	2	24	3	0	24	0	17	5	6	0	0	16	4	6	0	0	0	1	0	0	0	0
26.6.09	6	2	28	1	0	17	0	13	2	4	0	0	11	2	8	0	0	0	1	0	0	0	0
26.6.09	6	2	16	2	0	11	0	15	4	3	0	0	9	3	11	0	0	0	1	0	0	0	0

Table 2.2. Raw data showing abundance per sample of each invertebrate species collected at the Pukepuke Reserve during the field study. Eight samples were taken from each pond on each date. Gaps in the data signify drying out periods in the ephemeral ponds, when no samples could be collected. Habitat type '1' signifies samples taken from within Raupo. Habitat type '2' signifies samples taken outside of Raupo areas.

species	Con ^c added (ml)	Mortalities over time (%)							
		30 min	1 hours	2 hours	3 hours	4 hours	6 hours	12 hours	24 hours
physa	1600	0	0	0	10	20	25	30	30
physa	1600	0	0	0	10	20	25	25	30
physa	1600	0	0	0	0	15	20	25	25
physa	1600	0	0	0	5	15	25	30	30
physa	1600	0	0	0	0	15	20	25	30
physa	3200	0	0	15	70	70	70	75	80
physa	3200	0	0	10	70	80	80	80	80
physa	3200	0	0	20	65	75	75	75	80
physa	3200	0	0	20	70	75	75	75	80
physa	3200	0	0	15	70	75	80	80	80
physa	6400	0	5	60	80	100	100	100	100
physa	6400	0	5	60	85	100	100	100	100
physa	6400	0	0	60	80	100	100	100	100
physa	6400	0	0	60	85	100	100	100	100
physa	6400	0	0	55	75	100	100	100	100
physa	12800	0	10	90	95	100	100	100	100
physa	12800	0	15	90	100	100	100	100	100
physa	12800	0	15	85	95	100	100	100	100
physa	12800	0	20	95	100	100	100	100	100
physa	12800	0	15	90	95	100	100	100	100

Table 3.2. All data recorded from the Varying Concentration experiments in the Laboratory Study, showing mortalities over time (%) for 5 common aquatic invertebrate species subjected to varying concentrations of Nufarm Glyphosate Gold®.