Assessing the toxicity of biocides on the North American signal crayfish *Pacifastacus leniusculus* (Dana) to aid eradication

Sinead O' Reilly

Submitted in the fulfilment of the requirements for the Degree of Master of Science

Scottish Centre for Ecology & the Natural Environment,

Institute of Biodiversity, Animal Health and Comparative Medicine

College of Medicine, Veterinary and Life Sciences

University of Glasgow

February 2015

ABSTRACT

North American signal crayfish *Pacifastacus leniusculus* (Dana) have been introduced into much of mainland Europe over the course of the last century, primarily to satisfy a demand for human consumption. Over the last three decades the invasive signal crayfish have become a major problem throughout the UK. Control or eradicate methods have proven challenging. Since the discovery of signal crayfish in Scotland in 1995, many methodologies have been attempted to eradicate them. One of the most promising methodologies has been a natural pyrethrum known as Pyblast, a commercially available pesticide which is generally used to treat household pests. However, when used in aquatic environments, Pyblast is non-specific to crayfish and has a negative impact on non-target organisms, ranging from other aquatic invertebrates to amphibians and fish. Achieving the concentrations needed to kill crayfish means that it is also prohibitively expensive to use. Pyrethroid insecticides are well known to be highly toxic to crustaceans and the synthetic pyrethroid Deltamethrin is considered to be the most toxic of those available. Formulated products of AlphaMax (deltamethrin) and Salmosan (azamethiphos) are used to treat sea lice on farmed salmon, however the use of these chemicals as a means to eradicate signal crayfish remains unknown. This present study tested the acute lethality of these formulations on various life stages (from hatchlings to adults) of signal crayfish under laboratory conditions.

Results from this current study show early life stages most sensitive to both Pyblast and Deltamethrin. Based on the acute toxicity tests, stage I hatchlings showed significant differences in sensitivity between family populations when exposed to Pyblast with lethal concentration (LC_{50}) values ranging from 2.62 - 20.99 $\mu g/l^{-1}$ at 48h. Stage II crayfish were not significantly less sensitive than stage I, 5.23 $\mu g/l^{-1}$ and 6.43 $\mu g/l^{-1}$ respectively. Juveniles had a 48h LC_{50} of 57.95 $\mu g/l^{-1}$ and were significantly more sensitive than adults. Adult females had a 48h LC_{50} of 118.25 $\mu g/l^{-1}$ and adult males LC_{50} of 111.13 $\mu g/l^{-1}$. Adult females showed the higher tolerance than males to Pyblast at 24h exposure, however males were more affected than females after 48h exposure. Adults had an acute 48h LC_{50} of 26.49 ng/l^{-1} value for formulated Deltamethrin. The 48h LC_{50} for adult crayfish exposed to Salmosan was 15.27 mg/l^{-1}. Adult crayfish were most sensitive to Deltamethrin and least sensitive to Salmosan. The LC_{50} values obtained during the current study were 52-69% less than that previously estimated 0.2 mg/l^{-1} for field trials. Analyses of water samples taken during Pyblast toxicity trials on adult crayfish indicated that over 50% of the pyrethroid was removed from solution by rapid breakdown over 48h.

CONTENTS

List of Tables

List of Figures

List of Plates

Appendices

Acknowledgements

Firstly I would like to give my deepest gratitude to my supervisors, Prof. Colin. W. Bean from Scottish Natural Heritage and Prof. Colin. E. Adams from the University of Glasgow for this opportunity and their support, guidance and time given to me though out this study.

To IBIS, University of Glasgow and the Lough's Agency, thank you for providing the funding towards this project.

To Dr. Armin Stürm from the Institute of Aquaculture, University of Stirling, for your expertise, invaluable advice, assistance, guidance and continual patience with this project. It is true to say that without your help I would have been completely lost.

A thank you to Dr. Andy Shinn, Jane Lewis and Charlie Harrower from the Institute of Aquaculture, University of Stirling for providing me with the materials and assistance I required for the aquarium and lab work and also for making me feel so welcome in the department. I would like to thank the Institute of Aquaculture for granting me access to the research aquaria and laboratories.

To Matt Mitchell, a sincere thank you for all your help with trapping the crayfish and for all the lovely warm bowls of soup and sandwiches you gave me - you made those collection days very enjoyable and fun.

Thank you to Rona, Lindsay, Jennifer and the other staff members at SCENE for your help while I lived and worked in the field station. Many thanks to the other SCENE students, especially James, Jo, Martin, Milly, Matt, Nina, Olly and Travis for the craic, Sunday roasts, crayfish trapping and help with the dreaded stats. This work would have been a lot harder if I didn't have you all for the advice, support and when needed, welcome distractions.

And lastly a big thank you to my family and friends for their support and a special thanks to Mammy and Schira for taking care of the dogs while I was away. A sincere thank you.

Author's declaration

I declare that the material presented in this thesis is the results of research undertaken between February 2013 and January 2015 under the supervision of Professor Colin Bean, Dr. Armin Stürm, Dr. Andy Shinn and Professor Colin E. Adams. This work has not been submitted as part of any other degree and is based on individual research carried out by myself. Any published or un-published material not of my own has been acknowledged in the text.

Sinead O' Reilly

Chapter One

An introduction to the biology of the North American signal crayfish Pacifastacus leniusculus (Dana) with particular reference to assessing the toxicity of biocides to aid eradication

1.1 INVASIVE SPECIES

Alongside issues such as habitat loss and fragmentation, invasive species have been highlighted as one of the main threats to global biological diversity and ecosystem integrity (EEA, 2012). The movement of human populations and goods at a global scale has facilitated the movement of invasive species (Gallardo, 2014), threatening the conservation of biodiversity and economic interests such as agriculture, forestry and fisheries. Transport, trade and tourism are directly associated with the pathways of introduction and eventually the establishment and spread of invasive species (Hulme 2009). The programme Delivering Alien Invasive Species In Europe [DAISIE] has reported that a total of 12000 non-native species are currently present in Europe, and it is accepted that this is probably an underestimate of the real figure (DAISIE, 2009). The innate dispersal capabilities of aquatic species make freshwater systems particularly vulnerable (Vörösmarty *et al*., 2010) and crustaceans in particular, have been shown to be successful in expanding beyond their native range (Gherardi, 2007). Given the threat that these animals pose to freshwaters globally, prevention of their spread, and the control and/or eradication of established of newly arrived, non-native species is of critical importance.

Species have been moved to areas out of their native range for centuries. For example Pliny the Elder wrote, in his "*Natural History"* in 77 A.D., that the invasion of rabbits throughout the Balearic Islands was such a severe issue that assistance was needed from the Emperor Augustus and the Roman troops to help to control their numbers (EEA, 2012).

Today, examples of non-native species invasions are becoming ever increasingly frequent and widespread within the scientific and popular literature. Biodiversity loss due to the invasive species has occurred as a result of both intentional, and unintentional, introductions. Examples of vectors include: aquaculture, horticulture, recreational angling (stocking and the use of live bait), releases by aquarists, transport on recreational craft or the transport of biota in the ballast water of ships. Vié *et al*. (2009) in a global analysis of the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species, concluded

that invasive non-native species are the second greatest threat to freshwater fish species. For example, the opening of the St. Lawrence Seaway in 1959 led to the introduction of at least 182 non-indigenous species to the North American Great Lakes (Ricciardi, 2006). This equates to a new invasive species being recorded there every 28 weeks, the highest invasion rate recorded for a freshwater ecosystem. The number of ship-vectored invasive species recorded per decade is positively correlated with the intensity of vessel traffic within the basin. Ballast water release from ocean vessels is the putative vector for 65% of all invasions recorded since its opening (Ricciardi, 2006). With the number and proportion of alien species at an all-time high, some ecosystems are now dominated by non-native species (Holdich *et al*., 2009).

1.2 DEFINITIONS

Confusion exists within the scientific literature regarding terminology, with the use of the terms; non-native "alien species" and Invasive alien species" (IAS) which has led to some ambiguity in definitions. Generally, a non-native species can be defined as a species introduced by humans outside of its native range. In the EU, The European Environmental Agency (EEA) describes an alien species as *"an organism introduced outside its natural past or present distribution range by human intervention either directly or indirectly"* (EEA, 2012). Invasive non-native species (INNS) are defined by the Invasive Non-Native Species Framework Strategy for Great Britain as those species *'whose introduction and/or spread threaten biological diversity or have other unforeseen impacts'* (Defra 2008).

1.3 ECOLOGICAL IMPACTS OF INVASIVE ALIEN SPECIES

The impacts of Invasive Alien Species (IAS) can be dramatic, insidious and mostly irreversible. They can cause significant damage to biodiversity, human health, and to different economic sectors. Upon introduction, they may threaten native species through competition, predation and hybridisation and, may also alter ecosystem processes, reduce biodiversity and transmit parasites and diseases (Olden *et al*., 2004; Hector & Bagchi, 2007; Pejchar & Mooney, 2009; EEA, 2012). Amongst the thousands of invasive species currently thriving in Europe, Vilà *et al*. (2010) identified the top 10 '*worst organisms*' in terms of variety of ecological and economic impacts. The list included Sika deer (*Cervus nippon* Temminck 1838), Canada goose *Branta canadensis* (Linnaeus 1758), coypu *Myocastor coypus* (Molina 1782), Bermuda buttercup *Oxalis pes-caprae* (Linnaeus 1753), the zebra

mussel *Dreissena polymorpha* (Pallas 1771), brook trout *Salvelinus fontinalis* (Mitchill 1814), red swamp crayfish *Procambarus clarkii* (Girard 1852), the bay barnacle *Balanus improvisus* (Darwin 1854), Japanese kelp *Undaria pinnatifida* (Harvey Suringar 1873) and green sea fingers *Codium fragile* tomentosoides (P.C. Silva 1955).

1.3.1 *Biodiversity loss*

Over the last four centuries, IAS have been highlighted as one of the major factors threatening biodiversity and are regarded as the second largest cause of biodiversity loss globally through species extinction and habitat destruction (CBD, 2001; EEA, 2012). Of the 680 animal extinctions of known cause, 170 have been directly linked with the effects of species invasion (Clavero & Garcia-Berthou, 2005) A total of 54% of these extinctions have suffered the effects of IAS, and for every one out of five of these extinctions, IAS been the only cited cause of its species loss (Clavero & Garcia-Berthou, 2005). One major cause of biodiversity loss is the deliberate or inadvertent introduction of alien species which are either predators, parasites or competitors of native species. Many biologists now believe that this is the greatest of all causes of decline in freshwater biodiversity. In Europe, the rate of species invasion has increased across all taxonomic groups, leaving Europe today host to just over a staggering 12000 IAS (http://www.europe-aliens.org).

Ecosystem functioning, even in large systems may be negatively impacted through the introduction of IAS. IAS can affect biodiversity through various pathways from genetic introgression to the ecosystem level and can often influence existing biocenoses by altering their environment or facilitating the ecology of other species. Direct species interactions can also cause threats to native species through interbreeding (Rehfisch *et al*., 2010), predation, competition disease transmission (Holdich & Pöckl, 2007).

Hybridisation between IAS and native species can reduce genetic variation and erode gene pools as well as introducing maladaptive genes to wild populations, posing a serious threat to the conservation of genetic diversity (Corbet & Harris, 1991; Rehfisch *et al*., 2010). Such hybridization currently threatens several bird species with extinctions (Bird Life International, 2000). A prime example of this is hybridization between the ruddy duck *Oxyura jamaicensis* (Gmelin 1789) and the native white headed duck *Oxyura leucocephala* (Scopoli 1769). The expansion of the ruddy duck from North America across 21 European countries threaten the endangered native species through interbreeding thus may lead

directly to native species extinction (Parker *et al*., 1999; Sakai *et al*., 2001). Another example of potential species loss through hybridization include the sika deer *Cervus nippon* (Temminck 1838) and the native red deer *Cervus elaphus* (Linnaeus 1758).

IAS may also cause biodiversity loss through predation. For example, the invasive ruffe *Gymnocephalus cernuus* (Linnaeus 1758) predates on the vulnerable European whitefish *Coregonus lavaretus* (Linnaeus 1758) eggs where the two species co-exist. Ruffe, a non-native benthic feeding fish was first recorded in Loch Lomond in 1982 (Maitland *et al.*, 1983). It has been found to feed extensively on powan ova (Adams & Tippett, 1991; Etheridge *et al*., 2011). Powan are one of the rarest freshwater fish in Britain (Adams *et al.* 2014). Predation on these eggs may have a significant negative impact on powan populations as field studies would suggest (Adams & Tippett, 1991). One of the best known examples of direct impact of introduced non-native fish species is the Nile Perch *Lates niloticus* (Linnaeus 1758), introduced to Lake Victoria in 1954. By the mid 1980's it had become the dominant fish species and believed to be responsible for the extinction or loss of many of the 500+ endemic Haplochromine cichlids (Achieng 1990; Mkumbo & Marshall, 2015).

Indirect species interactions can also cause threats to native species and cause biodiversity loss. Amongst the thousands of species currently thriving in Europe, the zebra mussel *Dreissena polymorpha* (Pallas 1771) a freshwater bivalve mollusc native to the Ponto-Caspian region. It has worldwide distribution including North America, and western and northern Europe (O'Neill, 1997; Ricciardi *et al*., 1998; Strayer *et al*., 1999). This invasive species identified within the top 10 worst organisms in Europe in terms of variety of ecological and economic impacts (Vilà *et al*., 2010). The zebra mussel's introduction can also lead to significant changes in water quality of lakes altering the structure and function of entire ecosystems causing significant declines in phytoplankton and zooplankton concentrations through filter feeding (Caraco *et al*., 1997; Pace *et al*., 1998), and through the biofouling of hard structures (Elliott *et al*., 2007). In Ireland, it was accidentally introduced into the Shannon system *c.*1993-1994 (McCarthy & Fitzgerald, 1997). The mussels attach to rocky and gravelly areas, including potential pollan *Coregonus autumnalis* (Pallas 1776) spawning substrates and deposit pseudofaeces on them. This can result in a loss of usable spawning habitat and a reduction in habitat quality. It can also, in some areas, prevent access to benthic prey items. In Ireland, pollan are threatened with extinction by a range of potentially detrimental factors, including eutrophication and competition with introduced nonindigenous species such as the zebra mussel (Rosell *et al*., 2004). The zebra mussel can

be considered as a very real threat to the already reduced pollan population of Lough Ree, Lough Derg and Lower Lough Erne and has spread rapidly throughout the navigable reaches of the Shannon/Erne waterways (Minchin and Moriarty, 1998; Rosell *et al*., 1999).

Similarly, the Nile perch introduction to the Great Lakes has disrupted the ecological balance of the lake through its domination and removal of all other piscine predators. The introduction of farmed Atlantic salmon *Salmo salar* (Linnaeus 1758) or brown trout *S. trutta* (Linnaeus 1758) may have a negative impact on the genetics of native conspecifics, affecting the distribution, fitness, recruitment and structure of native salmonid fish communities and this could result in potential extinction for wild populations (Ford 2002; Youngson *et al*., 2003; McGinnity *et al*., 2009). This interaction in turn may result in non-direct ecosystem effects through species replacement resulting in a cascade effect on the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus 1758) whose life cycle is dependent on specific salmonid species (e.g. brown trout and salmon) (Williams *et al*., 2010). Gregs *et al.* (2011) found the feeding strategy of amphipods in Lake Constance, Germany was dependant on the availability of zebra mussel biodeposits. This showed temporal variation in zebra mussel density strongly impact the benthic communities and food web structure associated with hard substrates to the extent that there may be dependence on zebra mussel occurrence in such habitats.

1.3.2 *Ecosystem services*

Ecosystem Services are the benefits that ecosystems provide for human society and individuals. There are many examples of the types of benefits that humans can derive from ecosystems, at a basic level these include the provision of potable water supplies and food. IAS can also negatively impact Ecosystem Services within a range of goods and services (e.g. agriculture, industry, human health, forestry and fisheries) within the ecosystem resulting in strong socioeconomic consequences (EEA, 2012). IAS may affect ecosystem services through species composition, physical habitat components, nutrient cycling and primary production and changing ecosystem function (EEA, 2012). These impacts effect the delivery of food, freshwater, water purification, pollination, natural pest control, disease regulation, soil fertility and nutrient and water cycles. They can block waterways and obstruct navigation, reducing recreational amenity, spoil the aesthetic value of an area and damaging infrastructure and landscapes (Vilà *et al*., 2010; 2011).

Several invasive species alter disturbance regimes including fire, flooding and erosion (Mack & D'Antonio, 1998). For example, fire frequencies and intensities can be increased when grasses invade shrub land; mammal invasions can often increase soil erosion and disturbance. Aquatic macrophytes can form dense layers affecting water regulation and cause sedimentation and can cause a nuisance to boaters, anglers, divers and swimmers. The zebra mussel is well known for blocking pipes and vents (EEA, 2012). When present, this impacts greatly on power generation plants, industries and water systems inflicting huge costs for businesses and society. Lodge *et al* (2012) found the negative impacts of introduced crayfish on ecosystem services worldwide included the loss of provisioning services (e.g. reduction in edible indigenous species) interference in reproduction and hybridization of Indigenous Crayfish Species [ICS], regulatory (e.g. lethal disease spread), increased costs to agriculture and water management, supporting (e.g. large changes in ecological communities) and culture (loss of festival celebrations of ICS).

1.4 ECONOMIC IMPACTS OF INVASIVE ALIEN SPECIES

In addition to impacting humans through disruptions to Ecosystem Services, IAS can also result in direct and indirect economic costs. Direct costs include prevention, control and eradication, decreased yield and productivity, damage to infrastructure, repair damage, research, publicity and additional production costs on ecosystem services. Indirect costs, such as loss of recreational opportunity may also occur.

As IAS become more widespread, the economic impacts are expected to increase. The intrinsic value, the value people put on ensuring that an ecosystem or charismatic species may be damaged due to the presence of IAS. Endangered species and locations with high endemism, such as the Galapagos Islands, may, for example lose attractiveness as an ecotourism venue if high conservation value species are lost.

1.4.1 *Current costs to the economy*

There are various estimates of the economic impacts of IAS among countries however detail relating to the actual cost is difficult to obtain (Charles & Dukes, 2007). This means that estimates are often conservative. A comprehensive estimate of the costs of IAS at a global scale, either in terms of their impacts or the costs of prevention and control has yet to be produced. Based on their work in the United States, Pimentel *et al*. (2002, in Williams *et al*.

2010) suggest that the total loss to the world economy resulting from IAS could be in the order of 5% of annual production. Collectively, IAS related annual losses from Australia, Brazil, India, South Africa, UK and USA have been calculated to be approx. US$ 300 billion per year (Pimental *et al*., 2001, 2005). It is estimated that the economic costs due to biological invasions in Europe alone are at least EUR 12.5 billion annually (Kettunen *et al*., 2009) and the European Commission (EC) has invested over EUR 132 million over the past 15 years to combat IAS (Scalera, 2010).

The total annual cost of IAS to the British economy is estimated to be £1.3 billion to England, £0.3 billion to Scotland and £0.1 billion to Wales, totalling an estimated annual cost of £1.7 billion to Great Britain's (GB) economy (Williams *et al*., 2010, Roy *et al*., 2012). It has been conservatively estimated that research, management and river bank restoration, angling reparation caused by signal crayfish in GB is an estimated £2 million per year (Williams *et al*., 2010). This economic estimate is expected to increase annually as the species increases its distribution (Holdich *et al*., 2014). The total cost of pesticides and biocontrol agents for the control of non-native invertebrates on agricultural crops in GB is estimated £26 million, and loss of yield results in an additional cost of £129.3 million. Case studies have shown that early detection is more cost effective then long-term control of eradication of well-established IAS populations (Williams *et al*., 2010). The costs caused by IAS increases depending on the stage of invasion that a species. The cost of prevention, early detection and eradication are very low in comparison to costs incurred after the species has become established (Vander Zanden *et al*., 2010).

Another complicating factor is that, poor surveillance, particularly in aquatic and marine environments, often means IAS are not detected or obvious, until they have already become established. This makes any attempt of eradication or control extremely difficult and expensive. For example, $10 million has been spent on an electrical barrier to prevent the Asian carp entering the Great Lakes. Invasive alien crustaceans [IAC] can be hard to detect and they have a rapid dispersal.

1.4.2 *Benefits*

The impact of IAS may not always be negative. There has been several documented benefits to specific sectors of society where IAS produce economic profit and social welfare. For example, humans depend heavily on non-indigenous organisms used for agriculture, farming, fisheries, wood production, medicine, hunting and trade (EEA, 2012).

IAS can play a positive role for some elements of the natural environment through facilitating increases in basic food and cover resources (through habitat modification) and the release of major limiting factors such as the removing natural predators or competing species (EEA, 2012). They can provide positive recreation and tourism opportunities especially in the angling sector, these include brown trout and rainbow trout *Oncorhynchus mykiss* (Walbaum 1792).

Some IAS can show both a positive and negative impact on the economy. However, despite this dual impact, the high economic profit and welfare acting as a positive impact are often short lived as IAS eventually impose harmful effects on biodiversity and natural resources over time (Williams *et al.,* 2010).

1.5 CONTROL, LEGISLATION AND POLICY OF INVASIVE ALIEN SPECIES

1.5.1 *Convention on Biological Diversity*

The United Nations Convention on Biological Diversity [CBD] is an agreement to take action on issues which lead to the loss of biodiversity. This includes a wide range of topics, ranging from global climate change to, in some instances, action to prevent biodiversity loss at a local scale. The impact of IAS on biodiversity is a global issue and action is required at all levels to combat this increasing threat. The CBD addresses the introduction of alien species through both intentional and unintentional routes.

CBD has 15 guiding principles that provide an international framework for governments and other organisations to develop effective strategies to prevent introduction, mitigation, control and eradicate IAS which threaten ecosystems, habitats or species. A three-stage hierarchical approach to the management of non-indigenous species, prevention, early detection and intervention to prevent establishment and finally eradication, control and containment was adopted as the main way of dealing with invasive species. This strategy takes note of decisions from issues brought forward at conferences but sits within a European context in

particular with specific regard to the *European Strategy on Invasive Alien Species* developed in 2003 under the Bern Convention. It was agreed, by all parties to the CBD, that a significant reduction of the current rate of biodiversity loss at the global, regional and national level would be achieved by 2010. This was not achieved and the CBD targets were revised in the new Strategic Plan for Biodiversity 2011-2020. This strategy, developed at the CBD summit in Aichi commits signatories to identify pathways for the introduction of IAS, consider how priority IAS are controlled or eradicated, and put in place measures to manage invasion pathways with a view to preventing their introduction and establishment.

1.5.2 *European Strategy for Invasive Alien Species*

In 2001, the governments of the European Union member states agreed to put a stop to biodiversity loss in the EU by 2010 by tackling non-native species. National strategies were produced in 2007 and these were implemented by 2010.

The Bern Convention initiative for a *European Strategy for Invasive Alien Species* was developed in collaboration with the European Section of the IUCN Invasive Species Specialist Group in 2000 and was approved by the Bern Convention Standing Committee in 2003. The Bern Convention is a binding international legal instrument in the field of nature conservation with the aim of conserving wild flora and fauna and their natural habitats. This EU strategy on invasive species was published in 2003 and aimed to promote the development and implementation of coordinated measures and co-operative efforts within Europe to prevent or minimise adverse impacts of invasive alien species on Europe's biodiversity, as well as their economic consequences and impacts on human health. It highlights the growing problem within the EU posed by IAS and stresses the importance of early detection of potential invasive species and the need for rapid action to eradication while it is still economically and environmentally effective.

A new legislative proposal published in Oct 2013 by the European Commission on alien species aims to ensure coordinated action at EU level to reduce the impact of IAS [COM (2013) 620 final, Brussels, 2013]. This EU communication provides early warning systems, eradication and control measures. It implements a key aim of the European Union Biodiversity Strategy [COM (2011) final, Brussels, 2013] to bring EU policy in line with the CBD targets for 2020 which compels signatories: "*By 2020, Invasive Alien Species and their pathways are identified and prioritised, priority species are controlled or eradicated, and*

pathways are managed to prevent the introduction and establishment of new IAS" (COP 10 Decision X12, 2010).

Invasive Alien Species were included within the list of priorities of the sixth Environment Action Programme of the European Community [EC] for 2002-2012 and are also recognised as a key burden on biodiversity and a priority for action by the EC Biodiversity Communication [COM (2006) 216 final]. The sixth framework programme of the European Commission currently funds two IAS projects, Delivering Alien Invasive Species Inventories for Europe [DAISIE] and Assessing LArge scale Risks for biodiversity with tested Methods [ALARM].

DAISIE provides a 'one-stop-shop' for information on biological invasions in Europe and is seen as a pivotal instrument in developing a Europe-wide strategy that encompasses both the geographical scale of the problem and the study of different taxa in marine, freshwater and terrestrial environments. Data has been collated from over 248 datasets for vertebrates, invertebrates, marine and inland aquatic organisms as well as plants from up to 98 countries/regions (including islands) in the wider Europe and have identified "*100 of the worst*" invasive aliens.

ALARM was set up to develop and test methods and protocols for the assessment of large-scale environmental risks in order to minimise negative direct and indirect human impacts. In particular, risks arising from climate change, environmental chemicals, biological invasions and pollinator loss in the context of current and future European land use patterns. Research is carried out to assess and forecast changes in biodiversity, structure, function, and dynamics of ecosystems.

1.5.3 *Great Britain's Strategy for Invasive Alien Species*

The UK has entered into several commitments with regards to controlling the introduction of IAS including the Convention on the Conservation of European Wildlife and Natural Habitats [the Bern Convention] and are also a contracting party within the CBD. In 2001, a working group was set up to review invasive non-native species policy throughout Great Britain. This group was chaired by Defra and included representatives from Scotland and Wales, as well as from other government departments, statutory nature conservation bodies, Non-government organisations (NGOs) and trade interests. By 2008, the *Invasive Non-*

native Species Framework Strategy for Great Britain ("the GB Strategy") was set up. The GB Strategy was developed in close collaboration between government, industry and conservation NGOs. Its overall aim is to protect biodiversity, quality of life and economic interests, minimize the risks posed, and reduce the negative impacts caused, by invasive non-native species in GB. This is done by including prevention, early detection, mitigation and building and developing widespread awareness and understanding of the risks and adverse impacts associated with IAS and placing greater vigilance against these.

The Invasive Non-Native Species Framework Strategy for Great Britain was reviewed in 2013 with the objective of minimizing the risk of invasive non-native species entering and establishing in GB and reducing the risks associated with the movement of native species outside their native range within GB.

1.6 FRESHWATER CRAYFISH

Freshwater crayfish are globally distributed decapod crustaceans consisting of three taxonomic families. By way of a broad classification, Astacidae and Cambaridae are dominant the Northern hemisphere, whilst Parastacidae are dominant in the Southern hemisphere (Holdich, 2002). They are among the largest of the freshwater invertebrates (Souty-Grosset *et al*., 2004) and are considered to be a keystone species (Nystrom *et al*., 1996). Native crayfish densities play a key role in maintaining biodiversity in freshwater ecosystems and are an important part of freshwater ecosystems due to their size, population density and polytrophic links (Bubb *et al*., 2004). Crayfish are one of the most successful and widely distributed invasive species and of the 627 extant species worldwide (Holdich *et al*., 2014), 28 of these have been translocated from their native range and, among these, seven have been identified with having invasive potential (Gherardi, 2010). Introductions to non-native ranges have mainly been for aquacultural purposes or the aquarium trade (Chucholl, 2013). Different crayfish species vary in their growth potential, growth rate, fecundity, dispersal ability, ecological competence and dietary spectrum tolerance of a wide range of environmental conditions. This allows them to become highly successful invaders (Peay *et al*., 2006; Aquiloni *et al*., 2009; Holdich *et al*., 2014). Consequently, if introduced to or removed from a habitat, there is a potential for cascade effects within the aquatic environment (Nyström & Strand, 1996). Through physical alteration and modification of energy flow with aquatic systems, invasive crayfish have the ability to alter ecosystem

processes (Gherardi, 2007) thus impacting on the ecological health of freshwater systems (Lodge *et al*., 2012).

Impacts by Non-Indigenous Crayfish Species [NICS], come from the ability to perturb substrates, shred and consume aquatic macrophytes, increase nutrient cycling through detritus processing and consumption, predation on aquatic macrophytes and amphibian larva, predation on fish eggs and fry and competition for shelter (Holdich *et al*., 2014). Native species can then be displace through habitat deterioration, water quality degradation and competitive exclusion (Holdich & Domaniewski, 1995; Reynolds, 2011). Additionally, crayfish often act as vectors of diseases (Longshaw, 2011).

Conversely, increases in populations of animals such as predatory fish, birds and mammals that exploit NICS as a food resource may be considered positive effects. There is also the utility value of introduced crayfish populations for human food and recreational fisheries (Holdich *et al*., 2014).

1.7 CRAYFISH DISTRIBUTION

1.7.1 *Distribution in Europe*

Over the last few decades, European crayfish populations have been utilised for aquaculture. However significant declines have been observed in most EU countries (Holdich *et al*., 1999; Skurdal & Taugbøl, 2002). There are five indigenous European freshwater crayfish species [ICS], the noble crayfish *Astacus astacus* (Linnaeus 1758), the narrow clawed crayfish *A. leptodactylus* (Eschscholtz 1823), the thick clawed crayfish *A. pachypus* (Rathke 1837), the white clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) and the stone crayfish *A. torrentium* (Schrank 1803) (Holdich, 2002). All five are threatened from factors such as over fishing, poaching, habitat change, climate, NICS (particularly the North American signal crayfish *Pacifastacus leniusculus* (Dana 1852)) and the 'crayfish plague' which is caused by the oomycete parasite *Aphanomyces astaci* (Schikora, 1903) (Holdich & Sibley, 2009).

In addition to these five native species in Europe, another ten NICS have been recorded in Europe (Table 1.1) (Holdich *et al*., 2009). Of these ten species, eight have been introduced from America and a further two originated from Australia. Their introduction and distribution across Europe has been influenced by factors such as: human consumption; cultural tradition; aquarium trade; weed control; escapees from holding facilities for

research; and crayfish farming (Gherardi, 2007; Taylor *et al*., 2007; Holdich *et al*., 2014). Introductions have arisen accidentally through the transport of fish and plants, has and all but one of these are now established in the wild (Holdich *et al*., 2014). Deliberate, illegal, introductions continue to assist the easterly spread of the signal crayfish in Europe (Hudina *et al*., 2011). Crayfish introductions are not always illegal however.

Table 1.1 List of indigenous and non-indigenous crayfish species present in Europe.

ICS in Europe	**NICS in Europe**
Noble crayfish *Astacus astacus* (Linnaeus 1758)	Signal crayfish *Pacifastacus leniusculus* (Dana 1852)
Turkish crayfish *Astacus leptodactylus* (Eschscholtz 1823)	Spiny cheek crayfish *Orconectes limosus* (Rafinesque 1871)
Caspian crayfish *Astacus pachypus* (Rathke 1837)	Calico crayfish *Orconectes immunis* (Hagen 1870)
White clawed crayfish *Austropotamobius pallipes* (Lereboullet 1858)	Kentucky River Crayfish *Orconectes juvenilis* (Hagen 1870)
Stone crayfish *Austropotamobius torrentium* (Schrank 1803)	Virile crayfish *Orconectes virilis* (Hagen 1870)
	Red swamp crayfish *Procambarus clarkii* (Girard 1852)
	Marble crayfish *Procambarus fallax f. virginalis* (Hagen 1870)
	White river crayfish *Procambarus acutus* (Girard 1852)
	Common Yabby *Cherax destructor* (Clark 1936)
	Redclaw Crayfish *Cherax quadricarinatus* (Von Martens 1868)

The 19th century saw a growing demand for crayfish as a luxury food item. In Sweden where the noble crayfish consumption was particularly high, it was over-fished and also suffering loss due to crayfish plague. This led to a decline in crayfish availability. This resulted in the importation of the signal crayfish *Pacifastacus leniusculus* (Dana 1852) (Plate 1.1) to Sweden from California in 1959 to replenish and replace the depleted indigenous noble crayfish stocks (Svärdson, 1995). However the signal crayfish was also found to be a vector for the crayfish plague in 1988 (Alderman *et al*., 1990) and these introduced crayfish were followed by outbreaks of crayfish plague in populations of indigenous crayfish causing mass mortalities as it spread. Infection with crayfish plague is fatal to the five indigenous crayfish species in Europe (Souty-Grosset *et al*., 2006), causing dramatic losses and in many cases extinction or loss of populations (Holdich *et al*., 2009).

Plate 1.1 The North American signal crayfish, *Pacifastacus leniusculus* (Dana 1852).

1.7.2 *Distribution in Great Britain*

Since the 1960's, seven invasive non-native crayfish species have been deliberately introduced into Great Britain (Holdich *et al*., 2014) for various reasons (Table 1.2). Most introductions have occurred via the aquaculture industry, fish markets or the aquarium trade (Holdich *et al*., 2014).

Table 1.2 List of the seven Invasive non-native species of crayfish currently in GB.

NICS in GB
Signal crayfish *Pacifastacus leniusculus*
Turkish crayfish *Astacus leptodactylus*
Noble crayfish *Astacus astacus*
Spiny-cheek crayfish *Orconectes limosus*
Virile crayfish *Orconectes virilis*
White river crayfish *Procambarus acutus*
Red swamp crayfish *Procambarus clarkii*

The signal crayfish is known to be one of the most invasive freshwater invertebrates both globally and in GB (Stenroth & Nystrom, 2003; Creed & Reed, 2004; Zhang *et al*., 2004; Geiger *et al*., 2005; Holdich *et al*., 2009). Legally, the Australian redclaw, *Cherax quadricarinatus* (von Martens 1868) is the only crayfish species that can be imported for the

aquarium trade in England and Wales (Holdich *et al*., 2014). However, 13 other species were imported illegally there between 1996–2006 according to the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) (Peay *et al*., 2010). As recently as spring 2013 parthenogenetic marbled crayfish (*P. fallax*), a species thought to be native to North America, were recovered from several home aquaria in Scotland and northern England (Bean, *pers. comm*.). Chucholl (2013) has concluded the new exotic crayfish species entering Europe and establishing in the wild are coming from the aquarium trade, and much of this is purchased directly over the internet. Non-indigenous crayfish species now outnumber native species in Europe and are predicted to dominate the European decapod fauna in the near future (Holdich *et al*., 2009). Of these, the most widely distributed NICS is the signal crayfish (Holdich *et al*., 2009).

Crayfish are absent from the Scottish faunal list and are not considered to be native there (Gladman *et al*., 2009). However two populations of white clawed crayfish, a species native to other parts of the GB and of high conservation value, are established in two locations. These are Loch Croispol in Durness, Sutherland and White Moss Reservoir, Renfrewshire, in the lower Clyde catchment. It is unclear when these populations were established but it is likely that this occurred sometime in the last century (Thomas, 1992, Maitland *et al*., 2001). White clawed crayfish are believed to be native to GB and Ireland and are known to occur naturally across much of mainland Europe (Sibley *et al.*, 2002a; Reynolds, 2011; Holdich *et al*., 2014). Losses of the white clawed crayfish across its native range has been linked with habitat destruction, the impact of NICS and an associated spread of crayfish plague. They are listed in Annex II of the EC Habitats Directive and they are listed as a qualifying feature in ten Special Areas of Conservation spread across England, Wales and Northern Ireland. Within GB, white clawed crayfish are given strict protection through their inclusion in Schedule 5 of the Wildlife and Countryside Act 1981(as amended). This has been a contributory factor in its inclusion, under category A2ce, on the IUCN Red List as an endangered species. They are also included in Appendix III of Bern Convention (Holdich & Rogers, 1997).

Paradoxically, the rapid decline of this species in GB (Sibley *et al*., 2011), may now mean that the non-native populations in Scotland are likely to become valuable refuge populations for the conservation of the white clawed crayfish in the near future (Gladman, 2012). Loch Croispol may be particularly useful as an "*ark*", or sanctuary, site for this species since it is far removed from known signal crayfish populations elsewhere in the GB (Gladman *et al*.,

2012). The presence of signal crayfish in the River Clyde catchment has meant that the population of white clawed crayfish in White Moss Reservoir may be under greater threat than the Loch Croispol population.

1.8 THE SIGNAL CRAYFISH *Pacifastacus leniusculus* (Dana)

1.8.1 *Introduction & distribution*

Originating from western North America, the North American signal crayfish, occurs from Canada (British Columbia) in the north, central California (where it was introduced for commercial harvest) in the south and Utah in the east (Lewis, 2002). It is harvested in the western states of the USA (Washington, Oregon and Ohio) and from the introduced population in California (Shimizu & Goldman, 1983; Lewis, 2002). It is one of three most widely distributed NICS globally (Holdich *et al*., 2009). It is established in 27 territories (Souty-Grosset *et al*. 2006; Holdich *et al*., 2009). It was first introduced into Sweden in 1959 (Abrahamsson, 1973) from a translocated population in Lake Tahoe in California, following which, it was introduced to many European countries such as Finland and France (Holdich *et al.*, 1999).

Signal crayfish were imported to GB from Sweden in the 1970's, mainly for crayfish farming (Holdich & Rogers, 1992). Introductions to the wild in GB came from crayfish farms escapees which formed new populations in the wild (Holdich *et al*., 1995), some introductions may also have arisen from aquaria pet trade (Holdich *et al*., 2014), their use in fisheries management, or from use as live bait for angling (Lodge *et al*., 2000). Deliberate or intentional introductions to the wild have resulted in a wide distribution across England, Wales (Holdich & Rogers, 1997; Holdich *et al*., 2014) and several river catchments in Scotland (Gladman *et al*., 2010). To date there is no record of this species in Ireland (Holdich *et al*., 2009). Many attempts have been made to map the distribution of signal crayfish in GB on a 10 × 10 km² basis (e.g. Goddard & Hogger, 1986; Holdich & Reeve, 1991; Holdich *et al*., 1995; Sibley, 2003), and also at a finer level using geographic information system (GIS) data for England and Wales (Rogers & Watson, 2011a, 2011b), and Scotland (Sinclair, 2009). These data, collated and mapped by the National Biodiversity Network are shown in Figure 1.1. Whilst most of these records reflect actual survey data, several are based on anecdotal information only.

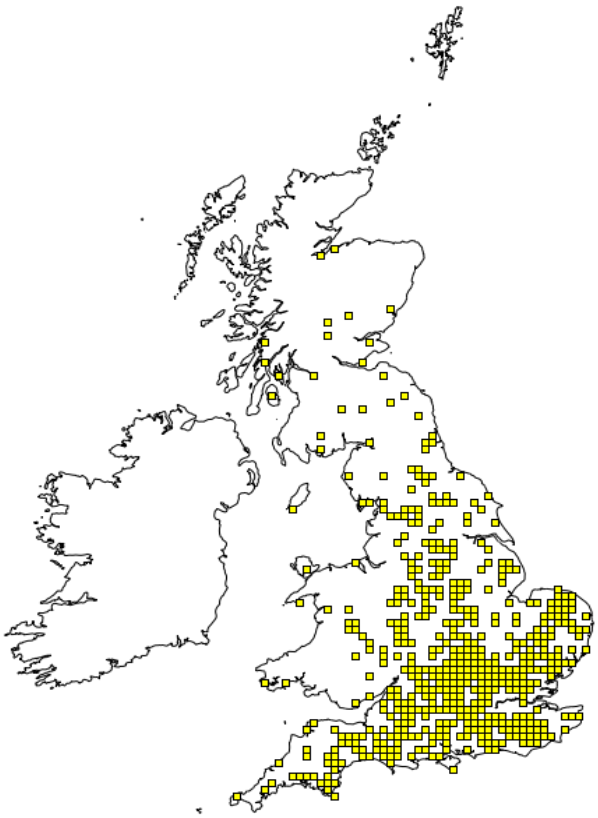


Figure 1.1 Map showing the possible distribution of signal crayfish in Great Britain on a 10km squared grid. All records up to 2014 are included. Several discrete populations may exist within each 10km square. Crown copyright and database rights 2011 Ordnance Survey [100017955].

1.8.2 *Signal crayfish in Scotland*

The signal crayfish were first identified in Scotland in 1995 (Maitland, 1996) and, so far, is the only NICS that is known to become established there (Reynolds, 2011). It is believed that they introduced to Scotland sometime in the 1980's, the source remains unknown. Following its first discovery in a stream in Kirkcudbrightshire Dee catchment, Galloway, in 1995 (Maitland, 1996), signal crayfish have become firmly established throughout that catchment (Gladman, 2012). Logic dictates that signal crayfish in Scotland probably came from previously farmed or feral populations in England. At least one population may have been introduced deliberately to stocked lakes and ponds as a means of disposing of trout carcasses in a commercial fishery. Their unintentional introduction to other waters with consignments of stocked fish cannot be discounted (Bean *et al*., 2006).

Signal crayfish are now established in many catchments in Scotland including some of the largest river systems in the country (Gladman, 2012). Since its initial discovery, the species range has extended considerably with populations found as far south as the Kirkcudbrightshire Dee to the River Nairn in the north (Maitland, 1996; Maitland *et al*., 2001; Bean *et al*., 2006; Gladman, 2012). This 'spread' between catchments has been facilitated by human introduction rather than by natural means. They have now been recorded in more than 20 sites in 15 river catchments, invading at least 173km of river length (Gladman, 2012).

In addition to their distribution within river systems, signal crayfish have also been recorded in a number of standing waters. These range in size from small ponds (including garden ponds) to large lochs. Loch Ken in Galloway for example, is thought to contain the largest population of signal crayfish in Scotland (Gladman, 2012). Once established within any water body, it is difficult to control the spread of this species and virtually impossible to eliminate once the species becomes established (Aquiloni *et al*., 2009; Holdich *et al*., 2014).

In Scotland, there is concern about the signal crayfish's potential negative impact on freshwater biota especially native species. The impact of signal crayfish on species of high conservation value was examined by Gladman (2012). Economically and culturally important species such as Atlantic salmon as well as species of high conservation value such as the freshwater pearl mussel, brook lamprey *Lampetra planeri* (Bloch 1784), river lamprey (*L. fluviatilis* (Linnaeus 1758), sea lamprey *Petromyzon marinus* (Linnaeus 1758) and benthic macrophytes could be potentially threatened by their presence (Gladman, 2012).

1.9 SIGNAL CRAYFISH LIFE HISTORY AND ECOLOGY

1.9.1 *Age and growth*

Adult crayfish have a large body size. Males typically reach a total body length of 16 cm and females can attain a length of 12 cm (Holdich *et al*., 2014). Exceptionally, they can grow as large as 28cm (Souty-Grosset *et al*., 2006). Sexual maturity usually occurs when crayfish reach 6–9 cm in total length, typically after 2–3 years and as early as one year in fast growing populations (Lewis & Horton, 1997; Westman *et al*., 1993, Holdich *et al*., 2014).

The lifespan of signal crayfish varies considerably between sites and it has been estimated that some individuals can live for at least 16 years (Belchier *et al*., 1998; Sibley, 2000).

Survival to maturity can range between 10-33% (Abrahamsson & Goldman, 1970; Abrahamsson, 1973; Schimizu & Goldman, 1983).

Juveniles feed on the nutrients of the egg sack straight after hatching (Lewis, 2002), and will undergo one to two moults in July-August before dropping off the mother and beginning to forage independently (Lewis, 2002). Within the first year the juveniles can moult 13 to 14 times (Mason, 1974). The number of moults rapidly decreases to 5- 6 in year two, 3- 4 in third year and 1- 2 in their fourth year (Lewis, 2002). Sexually mature females may only moult once a year due to overwinter with fertilised eggs (Kirjavainen & Westman, 1999, Holdich *et al*., 2014) whereas the faster growing males moult twice a year, in July-August, before the start of the breeding season (Söderback, 1995).

1.9.2 *Diet*

Signal crayfish are omnivorous (Alderman *et al*., 1990) having a broad range in diet (Holdich, 1999) and feed on algae, macrophytes (vascular and woody detritus) (Lodg*e et al*., 1994), benthic invertebrates, amphibians (Axelsson *et al*., 1997), zoobenthos, on amphibian eggs and larva (Gamradt & Kats, 1996, Axelsson *et al*., 1997), native benthic small bodied fish, fish eggs and fry (Rubin & Svensson, 1993; Guan & Wiles, 1997, 1998; Dorn & Wodjak 2004; Bubb *et al*., 2009), juvenile vertebrates and other crayfish (Nyström *et al*., 1999; Griffiths *et al*., 2004; Crawford *et al*., 2006), molluscs and macroinvertebrates (Guan & Wiles, 1998, Crawford *et al*., 2006; McCarthy *et al*., 2006; Ermgassen & Aldridge, 2011).

Studies have shown crayfish have the ability to predate on fish eggs (Dorn & Wodjak, 2004) and small fish such as bullheads *Cottus gobio* (Linnaeus 1758) and stone loach *Barbatula barbatula* (Linnaeus 1758) (Guan & Wiles, 1997).

Signal crayfish have a polytrophic feeding habit and exhibit ontogenetic diet shift with juveniles showing preference for benthic insects and adults preferring vascular detritus (Lewis, 2002). This species is also cannibalistic and cannibalism increases with size (Guan & Wiles, 1997). Signal crayfish exhibited optimal foraging behaviour when feeding on molluscs, consuming significantly more of the smaller size classes with thinner shells (Warner *et al*., 1995; Zu Ermgassen & Aldridge, 2011). Diet is strongly influenced by food availability, with individuals having the ability to adapt their diet to take advantage of available food resources, feeding on the most easily accessible food type available (Olsson

et al., 2009). As such, the presence of signal crayfish can reduce both the abundance and diversity of other macroinvertebrates, benthic fish and aquatic plants (Nyström, 2002; Crawford e*t al.*, 2006; Usio *et al*., 2009).

Crawford *et al.* (2006) showed signal can have a significant impact on the native freshwater invertebrate community. There was a 60% reduction in the total number of invertebrates impacting on community density and richness. Signal crayfish can significantly modify macroinvertebrate community structures and overall invertebrate biomass (Nystrom *et al*., 1996; Crawford *et al.*, 2006).

1.9.3 *Reproduction*

The mating season extends from late September to early October in the vast majority of populations (Holdich *et al*., 2014) and is triggered by either a sharp decrease in temperature (Reynolds *et al*., 1992) or a change in photoperiod (Lewis & Horton, 1997). Spawning occurs a few weeks after copulation where eggs are produced by the female. These eggs are attached to the female by the pleopods and carried by the female (Plate 1.2). Egg incubation ranges from 166-280 days with hatching occurring late March–July depending on latitude and temperature (Holdich *et al*., 2014). When mature, signal crayfish have a high reproductive output and high fecundity. Female reproductive output has been shown to vary from spawning three to four times during their lifespan (Mason, 1975) to spawning every second year (Westman *et al.*, 1993). There is a significant difference in the fecundity of signal crayfish, and the mean number of eggs ranges from 110 (Abrahamsson & Goldman, 1970) to 270 (Reynolds, 2002) although some females, particularly those with a large carapace length, have been reported to produce over 500 offspring (McGriff, 1983).

Plate 1.2 Berried (egg carrying) signal crayfish female collected from the River Clyde.

1.9.4 *Disease*

Crayfish often act as vectors of diseases (Longshaw, 2011), perhaps most notably crayfish plague. The signal crayfish was found to be a vector for *A. astaci* in 1988 (Alderman *et al*., 1990) and is immune to the effect of this fungal parasite unless it put under physiological stress (Cerenius & Söderhäll, 1992). *A. astaci* infection is fatal to the indigenous crayfish species in Europe (Souty-Grosset *et al*., 2006), causing dramatic losses and, in many cases, extinction or loss of populations (Holdich *et al*., 2009). Italy was the first country to encounter mass native crayfish mortalities due to this plague. Signal crayfish have also been identified as the vector of at least three species of microsporidian parasites, some or all of which may negatively impact the native white clawed crayfish (McGriff & Modin, 1983; Dunn *et al*., 2009).

1.9.5 *Predation on signal crayfish*

The signal crayfish is heavily predated upon by several fish such as grayling (*Thymallus thymallus* (Linnaeus 1758), pike *Esox lucius* (Linnaeus 1758) Neveu (2001a), perch *Perca fluviatilis* (Linnaeus 1758) (Blake & Hart, 1995; Neveu, 2001a), Zander (*Stizostedion lucioperca* (Linnaeus 1758), Atlantic salmon, bullhead *Cottus gobio* (Linnaeus 1758) (Dali, 1998), Carp *Cyprinus carpio* (Linnaeus 1758) (Rogers *et al*., 1997), Arctic charr *Savelinus*

alpinus (Linnaeus 1758), brown trout (Stenroth & Nyström, 2003), European eel *Anguilla anguilla* (Linnaeus 1758) (Blake & Hart, 1995), rainbow trout (Nyström *et al.*, 2001), chub *Leuciscus cephalus* (Linnaeus 1758), barbel *Barbus barbus* (Linnaeus 1758). In addition to fish, crayfish may also form part of the diet of mammals such as mink *Mustela vison* (Linnaeus 1758) and otter *Lutra lutra* (Brünnich 1771) (Reeve, 2001; Nyström, 2002: Ribbens & Graham, 2004). Where they are present, the introduction of signal crayfish may act as a new resource for these higher trophic level species. Despite this large list of predators, signal crayfish maintain an ability to survive and reach high population densities.

1.9.6 *Habitat*

Signal crayfish can reside in most freshwater habitats, and utilise lakes, rivers, streams, ponds and estuaries (Shimizu & Goldman, 1983), however there is no evidence of crayfish in estuarine environments in GB. They have a preference for waters temperatures below 25°C (Hogger, 1988), and optimal growth occurs when water temperatures reach 22.8°C (Firkins and Holdich, 1993; Westman *et al*., 1993). Like many invertebrates, signal crayfish are sensitive to calcium and pH levels (Kirjavainen & Westman, 1999) and despite being tolerant of low oxygen environments, they prefer well oxygenated water (Nyström, 2002). The species exhibits a number of biological adaptations which allow it to tolerate extreme environmental conditions (McMahon, 2002) such as: low temperature; high turbidity; saline waters; anoxic and dry conditions; and various pollutants (Firkins & Holdich, 1993; Holdich *et al.*, 1999). This flexibility may facilitate the displacement of ICS by invading signal crayfish (Holdich *et al*., 2014). The species can also survive out of water for up to three months in a humid atmosphere and can survive out of water for a minimum of 10 hours at 24°C (Banha & Anastàcio, 2013). The species is virtually nocturnal, with only 33% of activity taking place during diurnal periods (Lozan, 2000). This ability may help explain the great dispersal capacities of signal crayfish allowing them to move overland even in summer months and assist in passive dispersal by humans, e.g. anglers, who could transport the crayfish undetected great distances in relatively dry conditions (Banha & Anastàcio, 2013).

The size and density of signal crayfish populations is influenced by quantity and quality of habitat available, and substrate type is an important factor. Signal crayfish populations estimate densities in the America and GB habitats range from 0.9-20 individuals/m^2 (Abrahamsson & Goldman, 1970; Goldman & Rundquist, 1977; Bubb, 2004). The species shows a preference for rocky substrates (Shimizu & Goldman, 1983; Lewis & Horton, 1997),

and boulder areas (Blake & Hart, 1995) over soft or silty substrate (Goldman & Rundquist, 1977; Elser *et al*., 1994). They show ontogenetic shifts in spatial distribution (Harrison *et al*., 2006). Juveniles show a preference for shallow marginal habitat with finer substrate and cover to minimise predation by larger adults and predatory fish. Adults have a preference for deeper water with less complex substrate (Lewis & Horton, 1997) minimising vulnerability to terrestrial predators (Reynolds, 2011). Signal crayfish are extensive burrowers which can also dig interconnecting burrows (reaching up to two metres deep) in soft river banks and lakes (Lewis, 2002).

1.9.7 *Rate of spread*

Signal crayfish are mobile. Some individuals can have a range of a few metres and may stay within this for days to months at a time (Bubb *et al*., 2004). Others may spread tens to hundreds of metres within a few days (Holdich *et al*., 2014) moving either an upstream or downstream in direction within rivers (Bubb *et al*., 2005). The rate of invasion usually starts slowly, centred in the area of introduction (Peay & Bryden, 2010). However, the rate of spread is dependent on the suitability of habitat and the number and size of crayfish introduced (Peay & Bryden, 2010). In some river locations the rate of spread increases over time, and crayfish move faster in downstream direction at a rate of about 1 km y^{-1} in GB (e.g. Guan & Wiles 1996; Sinclair 2009). Flood events may displace individuals aiding their spread, may exacerbate the rate of spread (Lewis & Morris, 2008). Hiley, (2003) suggests that as few as one signal crayfish per 1000m² of riverbed can fuel a colonisation event. Detecting new populations, or tracking the rate of spread can be difficult. It can take several years for a population to be detected after introduction due to limitations with current survey methods, although the multi-method approach developed by Gladman *et al.*, (2010) offers the best chance of detection at low population size. Recent advances in the development of environmental DNA (or eDNA) detection methods (e.g. Moyer *et al.*, 2014) offer some hope that new methods of rapid monitoring may be available in coming years. With several of the catchments in Scotland already host to the signal crayfish, it is likely that this species will become increasingly prevalent.

1.9.8 *Impacts*

1.9.8.1 Biological

Signal crayfish can, as a result of their wide dietary range and ability to modify aquatic environments (see section 1.9.2), impact a range of aquatic species having a negative impact freshwater ecosystems where they have been translocated (Gherardi *et al*., 2001).

In particular, there are many ways in which the signal crayfish can impact on fish, through direct predation on fish and eggs, competition for shelter and food resources and through the modification of habitat (Reynolds, 2011). The presence of signal crayfish can make fish habitat less suitable for them due to the reduction of macrophytes that are used for spawning or shelter, sedimentation on spawning gravel beds caused by burrowing which can reduce oxygen exchange and effect egg survival (Reynolds, 2011).

Crayfish have been shown to predate on all life stages of resident fish (Ribbens & Graham, 2004) preying directly on small benthic fish sheltering fish such as Atlantic salmon (Guan & Wiles 1997). By sharing the same habitat, they out compete the Atlantic salmon for shelter, forcing them to swim in high flowing water current thus making them more expending more energy and leaving them more susceptible to predation (Guan & Wiles, 1997; Griffiths *et al*., 2004; Bubb *et al*., 2009). Finstad *et al*. (2007) found that lack of shelter reduced the growth of salmon fry and reduced survival due to predation. Together this may result in a decline in Atlantic salmon growth and stock recruitment and have detrimental effects on their population (Griffith *et al*., 2004).

Impacts on fish are particularly notable when large aggressive male crayfish congregate (Guan & Wiles, 1997). Under laboratory conditions, Atlantic salmon eggs have shown to be vulnerable to predation by signal crayfish with significant reduction in the survival of unburied salmon eggs but there is no evidence of excavation or preying upon buried eggs (Rubin & Svensson, 1993; Edmonds *et al*., 2011; Gladman *et al*., 2012; Findlay, 2014). Stenroth and Nyström (2003) set up enclosures with signal crayfish and brown trout fry in a Swedish stream and found the presence of crayfish had no effect on the survival of the fish. However, Edmonds *et al*. (2011) suggests they may pose a threat to emerging fry. Whilst impacts have been demonstrated (for fish) in controlled conditions and some field studies, population effects on fish have yet to be demonstrated. Peay *et al*. (2009) showed a significant negative relationship between resident brown trout and signal crayfish with a reduction in trout fry density in excess of 90% in a headwater stream in England.

1.9.8.2 In-stream habitats

Signal crayfish can also destroy microhabitats and alter ecosystems through habitat degradation and affect the physical environment of river systems (Newton, 2010). Crayfish grazing on aquatic macrophytes can result in the uprooting aquatic plants which are used for spawning by fish (Nystrom, 1999). By habitat modification and feeding across all tropic levels, signal crayfish have the potential to reduce taxon richness, abundance, and diversity of invertebrates available to fish and alter the flow of energy within ecosystems (Crawford *et al*., 2006, Jackson *et al*., 2014). This causes an indirect impact on fish populations.

At high population densities, their extensive interconnecting burrows can cause sediment loading in rivers and localized bank collapse (Lewis, 2002; West, 2010; Harvey *et al*., 2011). This burrowing behaviour can result in habitat degradation and bioturbidation (Sibley, 2000; Maitland *et al.,* 2001) with soil erosion, increase of water turbidity, siltation and the shallowing of streams (West, 2010) as well as through movement such as walking, fighting, feeding and tail flips (Statzer & Sagnes, 2008, Holdich *et al*., 2014). Siltation has the potential to affect the threatened pearl mussel as this species requires silt free habitat. Findlay (2014) showed a reduction of trout egg survival due to the disposition of fine sediment due to crayfish activity.

There is potential for signal crayfish to influence suspended yields of sediment thus implicating changes in morphology, physical habitat quality, the transfer of nutrients and containments, aquatic system health and water chemistry quality (Harvey *et al*., 2014). In some cases the impact of burrowing can prevent water bodies from obtaining good ecological status under the Water Framework Directive [WFD] (Holdich *et al*., 2009).

1.9.8.3 Species of conservation value

Introduced signal crayfish have led to a reduction in the number and extent of native white claw crayfish populations within their GB range. The number of populations lost has been estimated to be around 90% of the original total (Holdich & Sibley, 2009) It has been suggested that white clawed crayfish may become extinct within their natural range in the next few decades (Sibley, 2003; Rogers & Watson 2011a). The greatest impact on the white is from the crayfish plague, with outbreaks confirmed five years after signal crayfish introduction to GB (Alderman 1996). In addition, signal crayfish also have the potential to impact other species of high conservation value. The freshwater pearl mussel is one such

species, which is already in serious decline, both within the GB and globally. The impact of signal crayfish on Atlantic salmon has been described in Section 1.9.8.1. The impact on species such as brook, river and sea lamprey, and the juvenile ammocoete stage in particular, is unknown.

1.9.8.4 Economic

The physical degradation through burrowing caused by signal crayfish increases the cost of maintenance of canals, rivers and drainage canals (Peay *et al*., 2010). It has been estimated that some banks on the river Lark in Suffolk in England are being eroded at a rate of 1 m/y^{-1} because of the burrowing activity (Stancliffe-Vaughan, 2009). The cost of river restoration can range from £100-250/metre. Rivers infested with signal crayfish could get expensive especially taking into account that Scotland has over 50,000km of river watercourse length, the cost of restoration in Scotland. However it would be most extreme but it is very unlikely that the entire river length of Scotland.

Edmonds *et al.* (2011) and Gladman *et al.* (2012) suggest that signal crayfish have the potential to impact economically and culturally important salmonid (particularly Atlantic salmon) populations. The reduction of fish recruitment could be significant and carry high costs to the economy through impacts on angling tourism and recreational trout fisheries (Peay *et al*., 2009; Reynolds, 2011). The recreational value of fisheries to Scotland's economy is conservatively estimated at £113million each year with game angling accounting for 65% of this (Radford *et al*., 2004). It has been estimated that research, management and damage caused by signal crayfish in GB is £2 million per year (Williams *et al*., 2010), and this is expected to increase as the species continues to increase its distribution.

Overall, these potential impacts highlights the detrimental effect it could have on local flora, fauna, river bed characteristics, species conservation and economic value. Together these could have a profound effect on freshwater ecosystems.

1.10 CRAYFISH LEGISLATION AND LICENCING

Prior to 1981, no restrictions on the movement of exotic crayfish species to GB were in place (Hogger, 1986). Article 14 of the Wildlife & Countryside Act 1981, made it an offence to release, or to allow to escape, into the wild, any non-native organism in GB except under a licence. This included non-native crayfish. This Act has been amended since for specific crayfish species, the most recent in 2010.

In Scotland, Under The Prohibition of Keeping of Live Fish (Crayfish) (Scotland) Order 1996 banned the introduction of all non-native crayfish. This was updated to The Prohibition of Keeping or Release of Live Fish (Specified Species) (Scotland) Order 2003). In 2012 the Wildlife & Countryside Act 1981 was amended by The Wildlife and Countryside Act 1981 (Keeping and Release and Notification Requirements) (Scotland) Order 2012 to make it illegal to introduce any non-native crayfish species into Scotland.

In England and Wales, under the Wildlife & Countryside Act 1981 (as amended to suit various countries within GB), crayfish are not listed under The Prohibition of Keeping or Release of Live Fish (Specified Species) (England) Order 2014. The importation of non-native crayfish species are controlled through The Alien and Locally Absent Species in Aquaculture (England and Wales) Regulations 2011.

A trapping licence is required to trap crayfish under sections 27, 28 and 29 of the Salmon and freshwater Fisheries (consolidation) (Scotland) Act 2003. However when trapping in the border regions of Southern Scotland and Northern England in the Tweed and the Boarder Esk catchment, separate fisheries legislation applies. Crayfish would then be licensed under sections 48, 49 and 50 of the Scotland Act 1998 (River Tweed) Order 2006.

The legislative implication of the invasion of signal crayfish is of concern for Scotland through its potential to impact on its biodiversity, ecology and economy with serious implications in the future. The success of this species as an invader and the ecological consequences of this is contributing to the failure of some water bodies from reaching good ecological status as required by the WFD by 2015 (Peay & Füreder, 2011).

1.11 ERADICATION AND CONTROL

The approach to species management can be split between those aiming to control the invasive species population at low levels and those aiming to eradicate (Simberloff, 2008). Eradication is the ultimate aim of any control programme for invasive species. However this may not be achievable if the population is allowed to establish. Whilst prevention is the most cost-effective and desirable measure of IAS control, it is advantageous to eliminate invasive species as soon as possible, preferably during the early stages of establishment.

Several authors (Holdich *et al*., 1999; Hiley, 2003; Ribbens & Graham, 2004; Peay *et al*., 2006; Freeman *et al*., 2010; Stebbing *et al*., 2014) have reviewed various possible eradication or control methods for populations of invasive crayfish species throughout Europe and divided these into five broad categories:

- Legislative: local and national regulations.
- Mechanical: Hand picking, traps, seine netting, barriers, dewatering, drainage and habitat modification and electrofishing.
- Biological: introduction of disease/parasites, male sterilization and the introduction or enhancement of predatory fish (such as European eel).
- Physical: temperature, environmental manipulation.
- Chemical: biocides, biotoxins, surfactants, pheromones, repelling agents.

Currently no mechanical eradication measures or effective control measures available to prevent the spread of well-established signal crayfish populations or their ecological impacts. Some examples of mechanical, biological and biocidal methods are discussed below.

1.11.1 *Mechanical methods*

For signal crayfish, mechanical methods of control such as trapping, hand picking, seining, and electrofishing are relatively cheap and have a low impact on non-target species and their habitats (Moorhouse & MacDonald, 2010). However these have had no success in either reducing populations or in eradication (Holdich *et al.*, 1999; Peay *et al*., 2006; Freeman *et al*., 2010; Peay & Bryden, 2010, Hänfling *et al.*, 2011). These techniques are also biased

towards the removal of larger crayfish (Holdich *et al*., 1999, Gladman 2009). Crayfish have the ability to hide in small gaps between stones and gravel, very small areas in streams. Larger animals can burrow into river banks to a depth of two metres making their removal difficult to carry out and verify.

There is limited information on the effect trapping has on controlling signal crayfish populations despite the efforts used to control crayfish populations. Crayfish traps are not target species specific and other animals and fish such as otters, trout and salmon may also get trapped. Trapping is costly, time consuming, labour intensive. It is known to preferentially remove the dominant large adult males leaving juveniles almost entirely untrapped (Moorhouse & MacDonald, 2010; Gherardi *et al*., 2011). It has been suggested this reduces pressure on juveniles and give rise to higher populations and reduced competition (Gherardi *et al*., 2011).

Several long-term trapping programmes have taken place in GB. West (2011) reported that signal crayfish trapping on the River Lark, England from 2001 to present has had a 70% reduction in total catches with observed recovery of fish populations and damaged river banks.

Peay & Hiley (2001) found all attempts within England and Wales using trapping and or manual removal in-effective in the eradication or reduction of signal crayfish. In Scotland, a study of trapping a 5km stretch of the Clyde River between the years 2000-2006 noted a decrease in annual total catches however the population still spread both upstream and downstream (Sinclair, 2009). Moorhouse & MacDonald (2010) showed that trapping of signal crayfish from a river in Oxfordshire, England leads to increased movement distances of large crayfish immigration from non-removal areas into the new available space. The mean distance crayfish moved when immigrating was significantly greater at trapped removal sites (239m) than non-removal sites (187m). This showed that trapping may facilitate immigration of large individuals from neighbouring areas. This supports Moorhouse & MacDonald (2011b), suggesting trapping at the margins of a population may be sufficient in delaying colonization of new stretches by maintaining low densities and reduced movement and preferentially reduced numbers of large individuals although this approach was unsuccessful (Sibley, 2000; Wright & Williams 2000).

The lack of supporting evidence has meant that trapping has not been adopted as an active method for crayfish control. Trapping is unlikely to eradicate a population and may only work when used continuously on localised populations compared with the expansion rate of signal crayfish (Holdich *et al*., 2014).

Electrofishing has been a method used for many years in signal crayfish eradication however this method is limited to only crayfish that are present in the river channel (i.e. does not capture animals in burrows or refuges in rivers and streams). Both Westman *et al*. (1978) and Peay *et al*. (2014) noted it was an effective method for catching all class sizes of crayfish. Laurent (1988) concluded that it was more effective at night when the crayfish become active. In Scotland, electrofishing was used as a potential eradication/control method in the Skyre Burn, a tributary of the Kirkcudbrightshire Dee (Sinclair & Ribbens, 1999). Whilst the method was successfully used to remove crayfish of various sizes, it was not effective as a control or eradication. Peay *et al*. (2014) used high intensity (96kW, direct current 1600 V, 57.8A, at 7Hz) repeated shocks via electrode tapes delivered to two sections of stream as a treatment method of eradication on a signal crayfish population in a small head water in North Yorkshire High mortalities were achieved however complete eradication was not achieved.

Electrofishing is non-selective and it can impact non-target species, particularly fish. The key advantage of electrofishing is that there is no impact outside the treatment area however it is only suitable in shallow waters in summer months (Peay *et al*., 2014), but this is balanced by a lack in overall effectiveness. Electric shock treatment seems unlikely to achieve eradication of signal crayfish populations but it could be used as a control measure. Another possible method to aid eradication was dewatering ponds before a biocide treatment to reduce costs and remove crayfish from burrows. Peay *et al.* (2014) examined the behaviours of signal crayfish after dewatering and its implications for eradication treatments and management. Crayfish evacuate burrows in response to dewatering however the response time differed among crayfish with more evacuating at darkness. The trial also showed that if dewatering was to be carried out shortly before biocide treatment, there is a risk crayfish will be above water level and avoid the biocide.

1.11.2 *Biological methods*

Several suggestions have been made with regard to the use of other species to control or eradicate signal crayfish. Fish species such as eel, burbot *Lota lota* (Linnaeus 1758), perch, pike, chub, brown trout, rainbow trout, tench *Tinca tinca* (Linnaeus 1758) and carp are all recognised predators of crayfish.

The use of predatory fish such as the European eel as biological control has been documented to have a great impact on crayfish numbers since the fish prey heavily on them in the wild (Furst, 1977). Aquiloni *et al*. (2010) found that eels predating on crayfish were gape size limited on the size of animal they predated on. However they may have been the main source to the decline of crayfish populations in a study by Frutiger & Miller (2002). Neveu (2001a) showed pike to be most effective predating on all sizes while perch and zander predate on smaller sized crayfish. Studies with perch by Appelberg & Odelström (1986) have shown that predation on crayfish can interfere with their survival, activity and food consumption. Direct predation by the bullhead were found to significantly reduce signal crayfish density in Sweden (Dahl, 1998). It has been shown signal crayfish were more vulnerable to predation by perch than eel as perch are more persistent when preying on crayfish (Blake and Hart., 1995). Signal crayfish have shown anti-predator response to perch through using refuges (Söderbäck 1994) and other predator fish through mechanical and visual cues (Blake and Hart., 1995).

If it was possible to stock water bodies with predatory fish of a size range broad enough to predate all stages of the crayfish life cycle, it would be unlikely that they would eat a whole population due to their restricted gape size. Holdich & Domaniemski (1995) reported there has been no impact in lakes that have been stocked with perch, carp, and salmonids.

Although the introduction of predatory fish does control of invasive crayfish populations to some extent, there is some negative impacts to this. Fish also predate on non-target species and can also impact on the environment (e.g. carp cause turbation and may forage away for the control area (Stebbing *et al*., 2014). There are no examples of a successful eradication of crayfish population by increased fish predation (Ribbens & Graham, 2004).

Fish predation may only help control the crayfish numbers in combination with other methods (Hein *et al*., 2007). A combination of intensive trapping and predation by predatory fish may result in a substantial decrease in crayfish population densities in certain

circumstances (Rogers *et al*., 1997; Hein *et al*., 2007). A combination of trapping, manual removal and electrofishing resulted in a sharp decline in the signal crayfish population's size over a 4-year period in Spain (Dana *et al*. 2010), with 30 crayfish been caught a day in the 1st year decreasing to 10 by the 4th year. Combined approach method target multiple life stages, potentially resulting in a greater level of control than if single mechanisms were applied and are long term. Despite the effective control of populations using a combination of mechanisms, there have not been many attempts to use a multi-disciplinary approach (Stebbing *et al*., 2014) possibly due to the costs.

Once established, successful eradication of NICS are limited (Holdich *et al.,* 1999; Freeman *et al*., 2010; Gherardi *et al*., 2011). Current knowledge suggests that biological and physical methods used for eradication, control and reduction of population numbers of invasive crayfish to date have been only marginally effective. One possible mechanism for control and eradication showing some degree of success are chemical based treatments such as biocides (Peay, 2001).

1.11.3 *Biocidal methods*

Biocides are chemicals that are used for the control of noxious and invasive organisms and are used worldwide to kill unwanted pests. One of the greatest toxic effects on crayfish is when using insecticides (Ray & Stevens, 1970; Anderson, 1989; Eversole and Seller, 1997). To date, one of the most effective control agents for signal crayfish has been a commercially available insecticides (Holdich *et al*., 1999). The toxicity of the biocides used is not, however, specific to either signal crayfish or crayfish in general. These non-specific biocides, have been used in many management and eradication programmes. Morolli *et al*., (2006) suggests that biocides appear to be the only feasible way to control crayfish as they can directly affect crayfish physiology, inducing neurological symptoms such as loss of coordination of movements, paralysis and death of crayfish. The active ingredient in pyrethroids interferes with nerve membrane function, specifically targeting the sodium channels, resulting in depolarizing of the nerve endings leading to an irreversible alteration to nerve impulse transmissions and rapid death (Corbett *et al*., 1984; Baillie, 1985). Avermectins, another group of chemical biocides, increase the permeability of chloride ions at inhibitory synapses (Turner & Schaeffer, 1989) and organophosphates inhibit the enzyme acetylcholinesterase activity in the cholinergic neurons of the nervous system (Baillie, 1985).

With current knowledge suggesting that the biological and physical methods used for eradication, control and reduction of population numbers of invasive crayfish have only been marginally effective, biocides treatments in GB have been seen an a possible last resort as a mechanism for control and eradication. Many management and eradication programmes across Europe have used these non-specific biocides and they appear to be the only feasible way to control crayfish (Holdich *et al*., 1999, Morolli *et al*., 2006). Specifically, organophosphates, pyrethroid insecticides and natural pyrethrum (Pyblast), have been used in different trials as a potential means of eradicating signal crayfish and other NICS. Although it must be stated that their use in eradication and control against exotic crayfish have had variable results.

1.11.3.1 Organochlorines and organophosphorus compounds

Organochlorine and organophosphorus compounds have been used in the past for crayfish eradication (Holdich *et al*., 1999) however organochlorine compounds are now banned in many countries due to their persistence in the environment. Organophosphorus compounds are less persistent, although typical half-lives for these chemicals in freshwater still range from less than a week to more than a month (Haya *et al*., 2005). Both organophosphates and organochlorines, although reported as effective treatments for crayfish control, have been known to bioaccumulate in the food chain (Holdich *et al*., 1999) with their toxicity being associated with numerous reproductive problems in fish, having a direct disruptive effect on many aspects such as oocyte degeneration and a decline in concentration of circulating vitellogenins (Gross *et al*., 2002). Fenthion (Baytex PM 40) is an organophosphate which was used for the eradication of Spiny Cheek crayfish from Lake Geneva Lorraine in France (Laurent, 1995). In the field trials, treatment was carried out in three ponds using concentrations of 130, 83, and 60 $\mu g/l^{-1}$. Caged crayfish had 100% mortality at the highest concentration within 60 hrs of treatment and the lowest concentration lost its toxicity on the crayfish after two weeks. Total mortality was achieved after 87 h at levels as low as 60 $\mu g/l^{-1}$, however toxic levels remained in the lake for several weeks after. Some crustaceans and aquatic insects were killed but other fauna such as fish, frogs, mammals, many species of rotifera, molluscs and copepoda, were not affected. Ray and Stevens (1970) treated a fish pond in Pratt, Kansas to eradicate water nymph crayfish *Orconectes nais* (Faxon 1885) using the organophosphate insecticide Baytex PM 40 (active ingredient fenthion) was at concentrations of 22, 40, 50, 100 or 250 $\mu g/l^{-1}$. In the seven different ponds they treated concentrations of 100 $\mu g/l^{-1}$ or above had an estimated 100% mortality with four days of

treatment along with one pond treated at 50 µg/l^{-1}. This chemical has limits to its use as a biocide; the relatively long time needed for total mortality of crayfish and for the breakdown of the chemical compound means it is not ideal.

1.11.3.2 Pyrethroids

Natural pyrethrum is the oldest known botanical insecticide, produced from the extracts of the *Chrysanthemum cinerariaefolium* and *C. cineum* flower (Peay *et al*., 2006) and is one of the oldest known botanical insecticides. The active constituents of the flower extracts are referred to as pyrethrins (Davies, 1985). The pyrethrins consist of the individual compounds Cinerin I and II, Pyrethrin I and II, and Jasmolin I and II. Natural pyrethrum (trade name, Pyblast) have been used as a natural insecticide since the late 19th Century (Davis, 1985) and was first used against crustaceans in 1947 to eradicate water hog louse *Asellus aquaticus* (L.) infestations from public water mains (Hart, 1958) and is still used in this way usually at doses of 10 µg/l^{-1} (Evins, 2004). In early 1960's synthetic pyrethrins were developed and are known as pyrethroids. Both natural pyrethrum and synthetic pyrethroids (derivatives of natural pyrethrins) absorb firmly to dissolved organic carbon, sediments and plants which limit their aquatic half-life in free solution. Natural pyrethrins are very short lived in the environment, photodegrading rapidly and been susceptible to both chemical and biological degradation. It has also been shown that natural pyrethrum leaves no toxic residues, is harmless to plant material, has no bioaccumulation in the environment and the ecosystem has a quick recovery and all major flora and fauna recovering within the year of eradication process (Leahey, 1979, Holdich *et al*., 1999; Hiley, 2003; Peay *et al*., 2006). It has low environmental impact compared with other commercially available pesticides. Synthetic pyrethroids are based on the chemical structure and biological activity of natural pyrethrum but more toxic, more persistent, less degradable by light and organic matter, less expensive and more stable than natural pyrethrins (Morolli *et al.*, 2006).

Advantages of natural pyrethrum and synthetic pyrethroids have low toxicity to birds, mammals, plants and some other invertebrates which have a high tolerance to these chemicals (Coats *et al*., 1989, Hiley, 2003; Van Wijngaarden *et al*., 2006). However they are toxic to non-target aquatic species, including other crustaceans, insects and other arthropods, as well as vertebrates such as fish and amphibians (Mayer and Ellersieck, 1986; Burridge and Haya, 1997). They are highly toxic to crayfish and effective at killing crayfish at very low doses (Morolli *et al*., 2006; Paul & Simonin, 2006), especially when compared with

organophosphates insecticides (Eversole and Seller 1997). However the ecosystem recovery is slower after synthetic pyrethroid treatment when compared with natural pyrethrum.

No crayfish-specific biocide has been developed to date, and therefore the potential for non-target species to be adversely affected remains. Biocides may impact not only aquatic fauna such as fish, amphibians and molluscs, but also humans and other non-aquatic taxa (Holdich *et al.,* 1999).

Haya *et al*., 2005 reviewed the biological effects of pyrethroids in lab based studies and demonstrated that arthropods such as the water louse, *A. aquaticus* and the mysida shrimp have been shown to be very sensitive to pyrethroids while fish, echinoderms and molluscs were shown to be to be less sensitive. Recovery of synthetic pyrethroids is slower than natural pyrethrum. Kahn (1983) warns that synthetic pyrethroids such as cypermethrin can persist in the sediments for weeks and effect benthic invertebrates. However, Haya *et al*., 2005 states that synthetic pyrethroids are unlikely to be accumulated to a significant degree in fish since they are rapidly metabolised. Interestingly synthetic pyrethroids appear to have low toxicity to humans but are toxic to many invertebrates - including the white clawed crayfish (Coley, 2000).

A comprehensive review by Eversole and Seller (1997) on 35 different chemical groups found that synthetic pyrethroids most poisonous to crayfish with a 96h LC_{50} of 2.5 $\mu g/l^{-1}$ in comparison to 350 $\mu g/l^{-1}$ & 352 $\mu g/l^{-1}$ for organochlorines and organophosphates. Laboratory testing carried out by Bills and Marking (1988) also found that synthetic pyrethroids as the most selectively toxic insecticide for crayfish after results from the use of Baythroid killing rusty crayfish at concentrations as low as 0.05$\mu g/l^{-1}$ and 0.1$\mu g/l^{-1}$.

The synthetic pyrethroid BETAMAX VET® is a cypermethrin based pharmaceutical used to treat salmon louse *Lepeophtherius salmonis* (Krøyer, 1837) infestations of farmed Atlantic salmon. Cypermethrin is highly toxic to aquatic crustaceans and biodegrades with half-lives of 35-80 days in both high and low organic sediments. Laboratory toxicity testing of cypermethrin has shown high mortality levels of non-target aquatic invertebrates (McLeese *et al*., 1980; Stephenson 1982). BETAMAX VET has recently been used in a trials to remove signal crayfish in Norway (Sandodden & Johnsen, 2010). Using a double treatment method, the compound was dispersed on the surface and along the bottom of ponds. A week after the second treatment, the ponds were drained and no signal crayfish

were found. They reached 100% mortality 18hrs post treatment. After over a year of post-treatment monitoring, no surviving crayfish had been found however post-treatment monitoring is still continuing and the successful of this eradication is not possible to state.

Morolli *et al*., (2006) established that the synthetic pyrethroids Cyfluthrin, Deltamethrin (AlphaMax, Pharmaq) used as bath treatments for farmed fish and cypermethrin all share the characteristics that are effective for killing crayfish and could be used to control or eradicate Red swamp crayfish in small limited areas. All three were strong toxicants resulted in high mortality with cypermethrin shown to be the most effective. Paul and Simonin (2006) found the synthetic pyrethroids permethrin, sumithrin and resmethrin all to be highly toxic to the Calico crayfish *Orconectus immunis* (Hagen, 1870). They showed that crayfish are among the most sensitive freshwater species by 1-2 orders of magnitude to these pyrethroid insecticides. Permethrin had a 96h LC_{50} of 0.39 $\mu g/l^{-1}$ on newly hatched and 0.62 $\mu g/l^{-1}$ for juvenile red swamp crayfish *(*Jolly *et al*., 1978). Coulon (1982) stated 24h LC_{50} of 0.49 $\mu g/l^{-1}$ on red swamp crayfish. Thurston *et al*., (1985) reported a 96h LC_{50} <1.2 $\mu g/l^{-1}$ to Calico crayfish and McLeese *et al.,* (1980) determined a 96h LC_{50} of 0.76 $\mu g/l^{-1}$ on adult American lobsters.

The toxicity of Cyfluthrin was assessed by Quaglio *et al*., (2002) on red swamp crayfish and got LC_{50} of 0.13 mg/l^{-1} at 24h and 0.08 mg/l^{-1} at 48h and found it not suitable as it is much more toxic the natural pyrethroids.

Recently Pyblast was used in a preliminary biocide trial alongside ammonia, chlorine (as sodium hypochlorite) and sodium sulphite (Peay & Hiley, 2006). Four possible methods suitable for signal crayfish eradication, PH 12+, 10-100 mg/l^{-1} chlorine, 10 $\mu g/l^{-1}$ permethrin/natural pyrethrum and zero oxygen created by sodium sulphite were found.

Cecchinelli *et al*., (2012) used the natural pyrethrum Pyblast to control the red swamp crayfish in Italy. Results showed the lowest rate of concentration required to achieve the highest mortality rates in adults and juveniles at 24h LC_{50} was 0.02 mg/l^{-1} and 24h LC_{100} at 0.05 mg/l^{-1} with the highest level of mortality after only 12h (with over 90% of dead crayfish) regardless of its concentration and the age/sex of the target crayfish showing that it is effective in controlling the invasive species.

1.11.3.3 Previous biocide trials in Scotland

Attention has been given to those chemicals that are not persistent in the environment, readily available and low in costs (Gherardi *et al*., 2011). Due to its properties (high toxicity to crustaceans and rapid breakdown), natural pyrethrum (trade name, Pyblast) has been the only insecticide that has been field tested in the GB and been the biocide of choice for signal crayfish eradication in Scotland. Eradication attempts in Scotland so far have shown ambivalent results, with three of five trials unsuccessful in 100% eradication (Peay & Bryden, 2010). A study using Pyblast was conducted on North Esk catchment in Aberdeenshire and Montrose in Scotland to eradicate a small population of signal crayfish (Peay *et al*., 2006) and it proved to be effective at relatively low concentrations. Barmbyfield Reservoir in East Yorkshire was also treated in 2005 at a dosage rate of 0.18 mg/l^{-1} by spray application. By 2009 no crayfish had been recorded present however more present post treatment results are not available. A small garden pond with signal crayfish present in Perthshire was treated with a target dose of 1 mg/l^{-1}. During treatment, all test caged crayfish were killed however a crayfish was found in the garden pond two years later.

Although field studies on the toxicity of Pyblast on signal crayfish in Scotland gave the indication of the concentration to be used in the ponds for the eradication procedure with a target dose of 0.15 and 0.2 mg/l^{-1}, these trails were unsuccessful in eradication and there is no knowledge on the toxicity of this chemical under controlled (laboratory) conditions, not confounded by environmental factors.

Biocide treatment is also costly. The previous biocide treatment in five ponds was expensive (over £170,000) due to the large quantities used. A recent review (Peay & Bryden, 2010) highlighted, five catchments in Scotland as possible sites for signal crayfish eradication using biocides. However, the combination of no specific toxicant suitable for only crayfish or even crustacean-specific along with the high quantities of chemical required, will be costly. As well as the economic costs, the lack of specificity to non-target species including aquatic macroinvertebrates, fish and amphibians is ecologically costly and may entail considerable ecological damage to receptor sites.

However the cost of eradication treatment can be weighted up against the potential cumulative cost of impacts in the future if left untreated and crayfish are allowed to establish and spread. The need for an effective method for eradicating this invasive crayfish is of urgency due to the impact it has on our freshwater ecosystem and biodiversity. With many

techniques already being used to try control and eradicate the NICS including the use of a wide range of chemicals such as organophosphates, pyrethrum and synthetic pyrethroids, delaying a biocide treatment may increase the extent of treatment required, the complexity of the task and thus the financial costs.

1.**12** ECOTOXICITY OF BIOCIDES FOR USE ON SIGNAL CRAYFISH

Laboratory tests are necessary to verify the effects and the properties of biocides on target organisms as they provide important information on their toxicity in order to hypothesize field treatments for eradication of NICS (Morolli *et al*., 2006). Although Pyblast has been unsuccessful in eradication in the field, there is no knowledge on the toxicity of this chemical under controlled (laboratory) conditions, where it is not confounded by environmental factors.

Ecotoxicology gives a basic foundation for decision making on the likely impact of a chemical or effluent on an aquatic environment (Chapman 1995a). Ecotoxicology has been defined as "*the science of how chemicals, at toxic concentrations, influence basic ecological relationships and processes"* (Brown, 1986). Laboratory ecotoxicity testing has a number of valuable uses such as deriving and assessing water quality criteria, hazard assessment and screening of chemicals, establishing pre-release dilution levels of chemicals and effluents and for validating field bioindicators (Chapman 1995b). It studies the effects and impacts of manufactured chemicals and other materials (natural and anthropogenic) and their activities on organisms (Rand & Petrocelli, 1985) and the natural environment allowing a foundation for managing impacts of a chemical or effluent in aquatic environments. Ecotoxicity tests of various chemical types on a range of species and on inter-species interactions in the environment are used to analyse relationships that exist between studies of exposure and effect of chemicals or effluents under controlled laboratory conditions and a range of ecological conditions in complex field ecosystems (Chapman, 1995a).

Historically, ecotoxicology has relied on the use of toxicity tests to predict how chemical contaminants are likely to have an effect on an ecological system (Chapman 1995a). Adverse effects include both short-term and long-term lethality (expressed as mortality or survival) and sublethal effects such as changes to behaviour, growth, reproduction, development, uptake and detoxification activity (Rand & Petrocelli, 1985). Sub-organismal level effects

include induction or inhibition of enzymes and /or enzyme systems and their associated functions.

Effects at different organisational levels may be qualified by a range of criteria such as mortality (or survival) rate of organisms which is the simplest and most common end-point (OECD, 1987), their reproductive success (egg production), whole body (length and weight) or organ condition factors, induction or inhibition of enzyme activity and number and abundance of species in an ecological community (Rand & Petrocelli, 1985). Ecotoxicology studies are carried out using bioassays. A bioassay is any experiment in which a living organism is used as a test subject (Robertson and Preisler, 1992), and can be used to study the sensitivity of a target organism to pesticides and medicinal products.

Despite the increase of cost of natural pyrethrum Pyblast compared with the synthetic pyrethrins, it is the preferred choice of biocide in GB due its high toxicity to crustaceans and rapid breakdown. However there are other cheaper readily available biocides that have been used to tackle pests in GB. These including Deltamethrin which is a broad spectrum synthetic pyrethroid found in a wide variety of products used to control agricultural pests and animals ectoparasites. This product is utilized globally, to provide effective control of sea lice and to enable the introduction of an integrated pest management approach on salmon farms. The aquaculture formulation is called AlphaMax, which is applied directly to enclosed salmon sea cages to treat salmonids for the infestations of the copepod parasites, *Lepeophtheirus salmonis* and *Caligus elongatus* (sea lice) (Fairchild *et al.*, 2010). Another is Salmosan, a pesticide which is an organophosphorous compound of wettable powder also used to treat salmonids for sea lice. These could potentially be cheaper, more effective alternative pesticides, required in lower quantities compared with Pyblast. Due to the high toxicity of biocides on crustaceans and non-target species such as fish, amphibians, invertebrates and birds, it is of interest to acquire an accurate LC_{50} and EC_{50} value of pyrethrum Pyblast along with possible alternatives Deltamethrin and Salmosan on the signal crayfish.

In order to successfully eradicate the species, several bioassays need to be tested on signal crayfish to determine dose rates. This accurate dosage could then be used for crayfish eradication treatment dosage measure, reducing the likelihood of over dosing the treatment area and having adverse effects on non-target species and provide effective guidelines for use in eradication and control management.

To our knowledge, no studies have considered whether there is a difference in sensitivity at different life stages and between families of signal crayfish to these biocides. Baseline toxological data is lacking and previous field-trials have not provided the data necessary to properly assess the efficacy of Pyblast on all size classes of signal crayfish. A thorough understanding of the susceptibility of the signal crayfish population to these chemicals requires acute toxicity to be determined on several size classes that include early life stage, juveniles and mature adults of both sexes.

By carrying out a series of toxicity tests on signal crayfish in standard laboratory tests with various life stages and the biocides Pyblast, Deltamethrin and Salmosan, variation in sensitivity to these biocides between families and life stages will be investigated. Exposure to sublethal levels of these compounds and the subsequent observations for response will determine the toxicity to the signal crayfish and help determine the concentrations required for their eradication.

The recommended acute toxicity test will consist of a control and at least five concentrations of the biocide of choice, (i.e. multi concentration or definitive tests), in which the end point will be (a) an estimate of the biocide concentration which is lethal to 50% of the test organisms in the time period prescribed by the test expressed as the LC_{50} or (b) an estimate of the biocide which is sublethal expressed as EC_{50} (concentration at which 50% of the organisms exhibits some behavioural modification/immobilisation although appendage movement may still be present). The crayfish will be exposed in a static non-renewal tests where the test organisms are exposed to the same test solution for the duration of the test.

1.13 PROJECT PROPOSAL: AIMS

This study aims to use range of biocides to test their toxicity on signal crayfish, through a series of laboratory experiments. The ecotoxicity of potential biocides (Pyblast, Salmosan and Deltamethrin) for the control and eradication of signal crayfish will be undertaken through toxicity exposure and observational studies on mortality and aberrant behaviour which could be used to assist in the development of an eradication programme. The LC_{50} values obtained in these experimental tests can then be considered as a starting point for field trials to verify the real toxic effects on crayfish and the potential for eradication of unwanted populations.

The fundamental objective of this study is to obtain reliable LC_{50} concentrations of the selected biocides in a series of laboratory experiments for all life stages of signal crayfish. A full set of range and definitive tests will be carried out. Specific aims of this study are to:

1) Determine the 24h and 48h LC_{50} and EC_{50} of Pyblast to hatchlings at stage I and II, juvenile and mature adults.
2) Compare LC_{50} and EC_{50} to establish if there is variance within and between families and life stages in toxicity sensitivity.
3) Verify Pyblast exposure concentrations on the lowest and highest concentrations, both before, and after exposure. Chemical analytics will then be used to calibrate the actual exposure concentrations.
4) Explore the potential of a number of other ‘off the shelf’ biocide chemicals (sea lice chemicals Salmosan and Deltamethrin) through a range toxicity tests.

Chapter Two

Materials and methods

2.1 EXPERIMENTAL ANIMALS

2.1.1 *Collection of signal crayfish stocks*

All trapping, transport, holding and release of live signal crayfish were carried out under licences obtained from Marine Scotland (CMS-13-034). Signal crayfish juveniles and adults, including both berried (oviparous) (Plate 1.2) and non-berried females were collected between 22nd April 2013 and 27th Sept 2013 from the River Clyde, Elvanfoot, Scotland (55°25'52.55"N 3°38'57.26" W) (Plate 2.1 and 2.2).

Plate 2.1 The River Clyde, Elvanfoot, Scotland.

Plate 2.2 Map of sampling site where 30 traps were located along a 1 km stretch (trap 1-30) of the Clyde River at Elvanfoot, Scotland (55°25'52.55"N 3°38'57.26" W).

Crayfish of two life stages (juveniles, mature adult males, and mature adult females) were collected by trapping and hand collection. Trapping was undertaken using cylindrical, plastic crayfish traps (Trappy™ crayfish trap, Virserum, Sweden), 50 cm long and 20 cm in diameter with 25 x 35 mm mesh and a 51 mm diameter aperture. In total, 30 traps were set symmetrically down one side of the river bank at a spacing of 5 m along the length of the river and 5 m across the width of the river. Traps were baited with fresh fish (sardines/cod/trout/salmon) and were emptied, re-baited and reset every 48 hours until 50 females were captured. Hand collection was undertaken for juveniles and adults from shallow water along the river bank under rocks on the river bed. During the collection period, water temperature ranged between 7-12 °C. Collected animals were transported in cool boxes to the Institute of Aquaculture of the University of Stirling and kept in a secured temperature controlled room. All field equipment used in the capture of crayfish was disinfected after use with a 1% Virkon® Aquatic solution and equipment was allowed to dry before being used again.

2.1.2 *Housing and maintenance of signal crayfish stocks*

Berried females were maintained individually in 2.5 L plexiglass holding tanks with constantly aerated freshwater at ambient temperature (12°C) and a photoperiod of 16 h: 8 h light/darkness. Plastic tubes of 5-10 cm diameter were placed within each crayfish tank to provide shelter. Berried females were kept in the temperature control room (at a slightly

higher than natural temperature) to increase embryo development, hatching of eggs and release of their hatchlings for the experiments. Berried females were monitored daily and egg hatching dates recorded. To prevent cannibalism, females were removed from holding tanks once the hatchlings had left the mothers, who were then subjected to the same husbandry conditions described above. Non-berried females and male crayfish were separately housed in 80 L polyethylene holding tanks according to their life stages at a density of 30 crayfish per tank (Plate 2.4). Dechlorinated (carbon filter) tap water on a recirculation system was used for aquaria. Water quality was maintained through aeration (dissolved oxygen (DO) concentration maintained at 7.0 ± 1 mg/l^{-1}) and complete water replacement three to four times per week. Animals were fed four times per week with fish food and carrots. Plastic tubes of 5-10 cm diameter were placed within each crayfish tank to provide shelter. Animals were only used once in any of the experiments.

Plate 2.3 Berried signal crayfish female incubating eggs in 2.5L aquaria with shelter and aeration.

Plate 2.4 Holding tank (80L) for adult signal crayfish.

2.1.3 *Classification of signal crayfish life stages*

Individual crayfish were divided into two life stages (using carapace length CL) and sex of the individual. The life stages were defined as with juveniles of mixed sex (20-30 mm CL), adult females (30-40 mm CL), adult females (40-50 mm CL), adult males (30-40 mm CL) and adult males (40-50 mm CL). Carapace length was measured from the tip of the rostrum to the posterior margin of the carapace using vernier callipers to the nearest mm. Hatchlings were monitored for first ecdysis, (shedding of the exoskeleton). Hatchlings pre first ecdysis were classified as stage I hatchlings and post ecdysis were classified as stage II hatchlings. Stage I hatchlings 29-35 days old and stage II hatchlings 55-70 days old were selected for toxicity testing. The gender of these hatchlings could not be determined due to age and size.

2.2 BIOCIDES AND THEIR CHEMICAL FORMULATIONS

Below in Table 2.1 are the commercial formulations of the biocides Pyblast, Salmosan AlphaMax (Deltamethrin) that were obtained for use in toxicity assays.

Table 2.1 List of biocides used in the study and there chemical formulation.

Biocide trade name	**Formulation**
Pyblast	3% alcohol ethoxylate and piperonyl butoxide 3% w/w (30 g/l^{-1}) natural pyrethrins, 15% piperonyl butoxide
Salmosan	Organophosphate and the active ingredient an organophosphorous insecticide compound of wettable powder containing 47.5% (w/w) active ingredient azamethiphos (S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazolo [4,5-b] pyridin-3-ylmethyl)O, O-dimethyl phosphorothioate) in the formulation
AlphaMax (Deltamethrin)	Deltamethrin Liquid state $10g/l^{-1}$, 1% active ingredient (S) a-cyano-3-phenoxybenzyl(1R,3R)-3-(2,2,-dibromovinyl)-2,2 dimethylcyclopropanecarboxylate

2.3 TOXICITY TESTS – EXPERIMENTAL DESIGN

LC_{50} and EC_{50} values for the three biocides (Pyblast, Deltamethrin and Salmosan) at 24hr and 48hr were determined in static acute toxicity tests following standard procedures as outlined by the United States Environmental Protection Agency (USEPA 2002). USEPA (2002) provides information on types of tests, health and safety, quality assurance, facilities and equipment, test organisms, dilution water, acute toxicity test procedures, test data and acute toxicity data analysis. Crayfish were starved for 24 hours prior to exposure to any biocide. Prior to use in any trial, each crayfish was inspected to ensure that they were healthy and non-moulting.

To obtain an approximate indication of the biocide concentrations that should be used in the trials a series of range finding tests were conducted using widely-spaced geometric dilution series of the compounds. The concentrations for succeeding definitive tests were designed to cover the dosage range giving 0-100% mortality. The preparation of dilution series of

compounds used on the different life stages are described further in this chapter and in Appendix A.

2.3.1 *Exposure and recovery time*

The Pyblast toxicity tests carried out on hatchlings consisted of two phases, an uptake (2h exposure) and a recovery phase (46h) were hatchlings were transferred to beakers containing fresh dilution water after the exposure and incubated for 46h with full aeration. In contrast to the approach taken for Pyblast toxicity trials, hatchlings used in Deltamethrin and Salmosan trials had a 48h exposure phase with full aeration and no recovery phase. This decision was based on data contained in previous exposures studies of crustaceans to these biocides.

During the exposure phase all test organisms were exposed to various concentrations of the chosen pesticide and a separate control group were held in dilution water. After exposure, hatchlings from the Pyblast trials were removed from beakers using a fine meshed net and dipped five times into fresh dilution water (12°C) to remove any excess Pyblast before being transferred to glass jars containing 50 mls of dilution water. Jars where then placed into a temperature controlled incubator set to 12 ± 1°C and a photoperiod of 16 h: 8 h light/darkness. Each jar was aerated through a glass pipette attached to an airline.

Before trials could start on adult crayfish, information relating to the correct exposure time was required. Based on data contained in previous exposures studies, adult crustaceans had various exposure times to biocides. Two separate exposure time were chosen to investigate the best exposure time for this study. This was carried out using two Pyblast toxicity range tests using the same concentrations ranging from 0-316 $\mu g/l^{-1}$ Pyblast. It consisted of five exposure concentrations and two controls. One was a 2h exposure with 46h recovery and the other a 48h exposure with no recovery. The recovery method was the same method as used with hatchlings. This was carried out using adult females as their stock numbers were high. After the tests, it was decided to remove the 2h exposure and recovery method and just use a 48h exposure and with 48h aeration approach. This protocol was then used for juveniles and adult males/females regardless of the biocide used.

2.3.2 *Toxicant solutions*

Due to the hydrophobic nature and mixing ability of the chemical constituents of Pyblast, Deltamethrin and Salmosan, exposure solutions were prepared in either water or ethanol.

Two different preparation methods of Pyblast exposure dilutions were trialled during range finding tests on stage I hatchlings (29-35 days old). Like range testing for adult crayfish, the series of exposure concentrations used to assess which broad concentrations of Pyblast would cause mortality in these newly hatched individuals. The first stock solution dilutions were made in water only, the second stock solution involved preparing dilutions of the pesticide in ethanol, with 0.1% (v/v) of the solvent being present during crayfish exposures (Appendix A). Two controls were used during this phase of the work. The first control was untreated dilution water, and the second control was dilution water with 0.1% (v/v) ethanol added. After this trial, it was decided that all Pyblast and Deltamethrin stock solutions should be diluted with 0.1% absolute ethanol for all hatchling, juvenile and adult crayfish exposures. Salmosan dilutions were made in water because of its poor mixing quality with ethanol.

The methods described below were used in all tests with a series of dilutions carried out on each corresponding experimental concentrations. Changes to exposure concentrations were made to solutions by making increasing or decreasing the dilution with ethanol or water to correspond to the exposure concentration.

Solutions forming a dilution series of $\sqrt{10}$ were obtained in an analogous fashion by dilution of a prepared solution of 1000 µg/ml^{-1}. The dilution series were widely spaced in initial tests and the design of test concentrations were adjusted accordingly where necessary. Further details of all dilutions are given in Appendix A.

2.3.2.1 Pyblast stock solutions diluted in water

From the commercial stock solution (3% = 30 g/l^{-1}) Pyblast, a solution of 30 mg/l^{-1} was made by pipetting 250 µl of commercial stock into a 250 ml volumetric flask and topping up with dilution water to almost 250 ml. A stir bar was added and set on a stirring table for 30 min. The stir bar was then removed and the volume of the solution was brought to 250 ml with water. 8.3 ml of the 30 mg/l^{-1} solution was dispensed into a 250 ml volumetric flask and topped up to 250 ml with water to obtain a solution of 1 mg/l^{-1}.

2.3.2.2 Pyblast stock solutions diluted with absolute ethanol (0.1%) and water

From the commercial solution (3% = 30 g/l^{-1}) of Pyblast, an ethanolic solution of 1 g/l^{-1} was made by pipetting 833 µl of commercial stock into a 25 ml volumetric flask and topping up to 25 ml with absolute ethanol. The flask was then closed with stopper and shaken well to mix. To obtain the desired exposure concentrations 1000x concentrated ethanolic solutions were prepared, of which one microliter was added per ml of dilution water at the beginning of each test to obtain the desired exposure concentration.

All ethanolic solutions were prepared by first placing the desired ethanol in to the vial followed by the desired ethanolic solutions. This procedure was carried out when using ethanol as a diluent in all test experiments.

2.3.2.3 Deltamethrin stock solutions diluted with absolute ethanol (0.1%) and water

From the commercial stock solution (50% = 10 g/l^{-1}) of Deltamethrin, an ethanolic solution of 100 $\mu g/ml^{-1}$ was made by pipetting 250 µl of commercial stock into a 25 ml volumetric flask and topping up to almost 25 ml with absolute ethanol. The flask was closed with stopper and shaken well to mix. A total of 1000 µl of the 100 $\mu g/ml^{-1}$ solution was then pipetted into a 2 ml vial. The vial was then closed and shaken to mix. To obtain the desired exposure concentrations, 1000x concentrated ethanolic solutions were prepared.

2.3.2.4 Salmosan stock solutions diluted in water

From the commercial stock 20 g (50% = 10 g active), a solution of 200 mg/l^{-1} active compound was made. 100 mg powder of commercial stock was weighted and placed into a 250 ml volumetric flask and topped up to almost 250 ml water. A stir bar was added and set on a stirring table for 30 min. It was then removed and topped up to 250 ml. A solution of 1 mg/l^{-1} active compound, 1.25 ml of the 200 mg/l^{-1} solution was dispensed into a 250 ml volumetric flask and topped up to 250 ml with water. The chosen exposure concentrations were obtained as dilutions of this solution.

2.3.3 *Hatchling exposure to biocides*

Hatchlings aged 29-35 days old were selected for toxicity testing at stage I and broods aged 55-70 days for stage II hatchlings. To investigate if there was inter-family differences in crayfish susceptibility, stage I hatchlings from five large families (*n*=150) were selected and each family was exposed to one replicate of Pyblast toxicity concentrations, each replicate belonging to one family of hatchlings from each female. A lack of hatchlings of large families meant that it was not possible to investigate the same level of sensitivity between stage I hatchling families when exposed to Deltamethrin and Salmosan concentrations. For the Salmosan and Deltamethrin toxicity trials, hatchlings of smaller families of the same age were mixed.

Stage II hatchling families were also mixed throughout the concentrations as there were not any families of 80+ hatchlings to expose to a complete series of concentrations. A lack of hatchlings meant that it was not possible to expose stage II individuals to the full range of Deltamethrin and Salmosan concentrations. Because the primary aim of the project was to examine the toxicity of Pyblast on crayfish, a decision was made to use all of the available animals for this element of the study.

All chemical tests on hatchlings were conducted at 12 ± 1°C within the constant temperature incubator. The toxicity tests were performed in open 250 ml glass jars containing 50ml toxicant solution. Five hatchlings were used per beaker to reduce the potential for stress, aggression and cannibalism. The number of hatchlings used in toxicity tests varied due to the number of exposure concentrations used per test and the availability of animals.

Before the trial started and the chemical solutions were made, the selected brood of hatchlings were pipetted into a small plastic trays (*n*=5) using a wide mouthed Pasteur plastic pipette (Plate 2.5). Excess water was pipetted off each tray after which 50 ml of dilution water was placed in each beaker followed by each group of hatchlings randomly assigned to each glass beaker. To achieve the appropriate concentration of the relevant toxicant, 50 µl of the appropriate exposure concentration was pipetted to each glass jar and stirred with a glass rod, starting from the control upwards to the highest concentration to get the desired final concentration of the pesticide (detail of exposure solutions are provided in Appendix A).

Plate 2.5 Signal crayfish stage I hatchlings ready for the biocide exposure.

Two replicate 50 ml beakers (*n*=5) per toxicant concentration or control were used during the Pyblast exposure trial and one 50ml beaker per toxicant concentration or control for the Salmosan and Deltamethrin trials. Controls were separated into untreated controls (50 ml water 2x50 ml) and solvent controls with ethanol (0.1%) of which 50 µl ethanol was placed in 50 ml water per beaker. The glass beakers were then placed inside a temperature controlled incubator at 12 ± 1°C for the appropriate exposure time (Plate 2.6).

Plate 2.6 Stage I Signal crayfish hatchlings in recovery phase after biocide exposure.

The concentrations (0-40 µg/l^{-1} Pyblast, 0-316 ng/l^{-1} Deltamethrin) used in the definitive tests were derived from the interval of the dosage range in the range tests which were carried out on stage I hatchlings (Appendix A, Table A.3 and A.9). In the definitive tests, stage I hatchlings (29 days old) were exposed to five exposure concentrations and two controls (water with ethanol, water without ethanol) 0, 0 (solvent), 2.5, 5, 10, 20, 40 µg/l^{-1} of Pyblast and 0, 0(solvent), 0.00316, 0.01, 0.0316, 0.1, 0.316 µg/l^{-1} Deltamethrin. These were obtained as serial dilutions and exposed to the hatchlings.

Salmosan range finding test carried out on stage I hatchlings (aged 29 days). These hatchlings were exposed to five concentrations and one control (water without ethanol), 0, 0.316, 1.0, 3.16, 10, 31.6 µg/l^{-1}. Each trial was carried out with five hatchlings per beaker (see Appendix A, Table A.4 for dilution series). All hatchlings survived an exposure of 48 hours. Unfortunately this part of the study was terminated because insufficient stage I hatchlings were available to repeat the trial at higher concentrations.

Stage II hatchlings were exposed to the same five concentrations that were used for stage I conspecifics, plus one additional concentration of 80 µg/l^{-1} Pyblast in addition to the five exposure concentrations and two controls (water with ethanol, water without ethanol) that were exposed to stage I hatchlings.

2.3.4 *Juvenile and adult exposure to biocides*

Pyblast toxicity trials were carried out on juvenile of 20-30mm CL. For the toxicity trials on adult crayfish, male and female of 40-50mm CL were used for Pyblast toxicity experimental procedures. Salmosan and Deltamethrin toxicity experimental procedures were carried out with adult male and female signal crayfish measuring 30-40 mm CL. Their size is ≤10 mm CL compared with adults used in Pyblast trials due to shortage of stock of 40-50 mm for a full trial. Only Pyblast toxicity tests were carried out on juveniles due to lack of animals to test using Deltamethrin and Salmosan. Also due to lack of animals, it was not possible to expose both sexes to complete a full set of replicates of Deltamethrin and Salmosan concentrations or juveniles with Pyblast and compare differences in sex. Because the primary aim of the project was to examine the toxicity of Pyblast on crayfish, a decision was made to priorities adult males and females sex comparisons toxicity to Pyblast for to be made. Juveniles exposed to Pyblast and adults exposed to Salmosan and Deltamethrin had mixed sexes in each toxicity trials.

Prior to being used in tests, juvenile and adult crayfish were held in the laboratory for a minimum of two weeks to allow for acclimatization to laboratory conditions. Crayfish of known sex were randomly selected for each toxicity test and concentrations. All tests were conducted at 12 ± 1°C in a temperature controlled room due to the size of the test arenas and the number of animals required for the trials. The toxicity tests were performed in open 2-L *Fisher* laboratory glass beakers containing 500 ml aerated dilution water and one crayfish (Plate 2.7). This reduced stress, aggression and cannibalism between crayfish.

Plate 2.7 Adult signal crayfish in 500ml of solvent control dilution during a definitive test.

The number of replicate beakers per toxicant concentration or control used in toxicity tests varied due to the number of exposure concentrations used per test and the availability of animals. After each crayfish were randomly assigned to their glass beakers containing 500ml of dilution water, the crayfish were allowed to recover for one hour. The protocol for juvenile and adults was carried out the same way as the ethanolic and non-ethanolic solutions on the hatchlings when using Pyblast, Deltamethrin and Salmosan. However the test solution was scaled up to accommodate the increase of exposure solution volume to 500 ml in the 2-L test arenas. To achieve the appropriate concentration of the relevant toxicant, 500 µl of the appropriate exposure concentration was added to the beaker containing 500 ml dilution water, to get the desired final concentration of the pesticide. This method remained for all toxicity tests in both juveniles and adult male and females. Unless stated otherwise, five crayfish were used for each concentration and control.

In the juvenile toxicity trials, the Pyblast concentrations (0-100 µg/l^{-1}) used in the definitive tests were derived from the interval of the dosage range (0-400 µg/l^{-1}) within the range tests carried out at the start of the trials. For the first definitive test with juveniles, six concentrations and two controls were selected, 0, 0 (solvent), 12.5, 25, 50, 100, 200, 400 µg/l^{-1} were used. This was then changed to 0, 0 (solvent), 6.25, 12.5, 25, 50, 70, 100 µg/l^{-1} used for the second, third and fourth definitive test.

The Pyblast concentrations used in the adult definitive tests were derived from the interval of the dosage range (0-1000 µg/l^{-1}) in the range tests. For the first definitive test with the adult males and females, six concentrations and two controls were selected, 0, 0 (solvent), 12.5, 25, 50, 100, 200, 400 µg/l^{-1} were used and this was replicated three times with females and once with males. This was then changed to 0, 0 (solvent), 6.25, 12.5, 25, 50, 70, 100, 141, 200 µg/l^{-1} and this was replicated twice with female crayfish and three times with males (Plate 2.8).

Plate 2.8 Series of Pyblast concentrations and adult signal crayfish. Note the change in colour of the solution, and crayfish posture, as concentrations increase from left to right.

The Salmosan concentrations (0-100 µg/l^{-1}) in the definitive tests were derived from the interval of the dosage range (0-200 µg/l^{-1}) in the range tests that were carried out for this biocide giving 0-100% mortality (see Appendix A, Table A.8). For the definitive test with

the adult male and female crayfish, five concentrations and one control were used selected 0, 2, 6.32, 20, 63.2, 200 µg/l^{-1}. This was replicated three times.

The Deltamethrin concentrations (0-1.78 µg/l^{-1}) in the definitive tests were derived from the interval of the dosage range (0-1 µg/l^{-1}) in the range tests (see Appendix A). The first definitive test concentrations were 0(solvent), 0.0316, 0.056, 0.178, 0.316, 0.562 µg/l^{-1} and carried out on male and female crayfish. The lower concentrations were removed from this after the trial and additional higher concentrations were added for the second trial. The second definitive test concentrations were 0 (solvent), 0.1, 0.316, 0.562, 1.0, 1.78 µg/l^{-1}. Based on the results from second definitive test, the method and concentrations remained and this was replicated three times and the sexes were again mixed.

2.3.5 *Behavioural observations*

Behavioural observations were carried out on crayfish exposed to biocides and controls at 1, 3, 6, 12, 24 and 48h intervals following the commencement of the tests. The response of individual animals to a challenge (animals turned onto their back, and gently prodded on the abdomen and tail five times using a glass rod) was recorded. The behavioural responses provided an index of the degree of impairment of normal behaviour and was evaluated at each observation period (Plate 2.9 - 2.11). Mortality (animals defined as dead) and the basic condition of each animal at each exposure concentration were assessed based on the behaviour characteristics exhibited. Animals were considered dead if they failed to respond to abdomen prodding. All dead crayfish were removed during observation times and recorded during both exposure (and recovery). After trials had terminated, all surviving crayfish were humanely euthanized in 100% alcohol and incinerated. A summary of the behavioural response criteria used during this study is provided in Table 2.2.

Table 2.2 Response criteria of crayfish during observations.

Response	Description of behaviour
Self-righting	Animal looks normal and has ability to move back onto legs after been turned on back and prodded.
Not self-Righting	Animal may look normal, but fails to reposition itself onto legs to a normal upright position after being turned over and prodded. Showing signs of loss of motor control activity (inability to control appendages and loss of balance). However has movement of appendages, walking legs and swimmerets.
Immobile	Almost dead, no response to prodding or any movement of appendages or walking legs but slight movement of swimmerets; however respiratory movements can still be observed.
Dead	No visual sign of extremities movement or respiratory movement, no response to body or eye prod. Body immotile and limp.

Plate 2.9 Adult female signal crayfish showing behavioural response of autotomy to Pyblast exposure with cheliped dropping off.

Plate 2.10 Female immobile and unable to self-right during acute 48h Pyblast exposure.

Plate 2.11 A juvenile signal crayfish showing behavioural effects (immobile) during a Pyblast exposure trial.

2.4 QUALITY CONTROL

2.4.1 *Dilution water*

Treated tap water was used as diluent/control for all tests. Prior to the commencement of the experiments, a 500 ml water sample taken by University of Stirling, Institute of Aquaculture before toxicity trials began to establish chemical properties of the dilution water, analysed for metals and other water parameters including Nitrate $NO3^{-1}$, total ammonia HN3, total alkalinity titration with 0.01 HCL, conductivity and total hardness CaCO3 (Table 2.3). Hardness was tested using an established method (Golterman. H. L, 1975). Ammonia, nitrite and nitrate were analysed using a Bran Luebbe autoanalyser 3.

Table 2.3 Water quality parameters determined from a 500ml sample of the dilution water.

Properties of water	**Unit**
Hardness	17.2 ppm
Alkalinity	0.25 mg/l^{-1}
Ammonia	0.00 mg/l^{-1}
pH	5.85
Conductivity	92.3 µS/cm
DO	9.3 mg/l^{-1}

A comprehensive suite of chemical analysis was undertaken for B, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, Cd, Ba, Pb and Bi (Table 3.4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a Thermo Scientific Series 2 instrument. Dilution water was stored in a 200 L plastic tank at 12 ± 1°C and aerated for 24h before each experiment to remove any remaining chlorine.

Table 2.4 Metal analysis from a 500 ml sample of the dilution water.

Metal	**ppm**	**Metal**	**ppm**
Boron	3.838	Iron	11.44
Sodium	3684	Cobalt	0.013
Magnesium	1062	Copper	6.907
Aluminium	6.853	Zinc	4.814
Potassium	359.7	Cadmium	0.031
Calcium	4635	Barium	5.133
Chromium	0.135	Lead	0.049
Manganese	10.85	Bismouth	0.305

2.4.2 *Measurement of experimental water quality*

Water quality variables, Dissolved Oxygen (DO) mg/l^{-1}, pH and temperature of test solutions (highest concentration) and controls were monitored throughout the duration of each toxicity test (at observation times: 1, 3, 6, 12, 24, 48h) to ensure the water quality was within acceptable limits (Table 2.5). Measurements were taken from the control initially followed by the highest concentration. Probes were rinsed with dilution water to prevent cross contamination. pH measurements were taken with Hanna HI 991300 portable pH/EC/TDS/Temperature meter. DO measurements were taken using Hanna HI 9142 Portable DO meter.

Table 2.5 Water parameter ranges from controls and test solutions during all definitive tests.

Test arena	pH	DO mg/l^{-1}	Temperature °C
Biocide beakers	6.07 - 7.20	8.2 - 9.4	11.7 - 12.3
Control beakers	6.10 - 7.10	8.1 - 9.8	11.7 - 12.3

2.4.3 *Decontamination of glass and plastic ware*

All equipment used in the trials was washed by soaking in Decon detergent (Decon Laboratories Limited, East Sussex, England), washed for 24h and then rinsed twice and left to dry for 24h prior to re-use.

2.5 CHEMICAL ANALYSIS OF PYBLAST

To verify that the Pyblast exposures carried out were as accurate as possible, a Pyblast concentration check was made by fortifying the Pyblast. For this analysis, an aliquot of the Pyblast stock was removed to investigate whether the concentration of biocide matched that which was cited by the supplier. Discrepancies could arise, for example, from a chemical breakdown. To estimate Pyblast exposures, desorption of Pyblast from crayfish exposure and natural breakdown in water were measured.

Each sample was analysed for the following active ingredients that are present in the formulation of Pyblast by LC-MS/MS by adapting an existing CEMAS standard operating procedure: Cinerin II, Pyrethrin II, Piperonyl Butoxide, Jasmolin II, Cinerin I, Pyrethrin I, Jasmolin I, Cis-permethrin and Trans-permethrin.

The analysis measures a concentration of all of these active compounds together. Water samples were taken from the adult male definitive tests. Nominal concentrations of 50 µg/l^{-1}and 200 µg/l^{-1}exposure treatments were selected as well as controls (to show they are free from Pyblast). The sample volumes were adjusted according to the exposure concentrations, with 150 ml sampled from the control, 150 ml from 50 µg/l^{-1} exposure concentrations and 37.5 ml from the 200 µg/l^{-1}exposure concentrations. The samples for each concentration were pooled from two beakers as removing such volume all from one beaker would leave a crayfish under exposed.

From the tests samples, 7.5 µg of total pyrethrins in aqueous solutions were loaded into Isolute C18 SPE (Solid Phase Extraction) cartridges (with 200 mg sorbent mass, 10 ml XL reservoir volume) (on which each test solution has been extracted) at a flow rate of 2 ml/min^{-1} using Supelco Visiprep™ DL (Plate 2.12). Samples were taken at the start of exposure (0h), mid experiment (24h) and end of the experiment (48h). This procedure was repeated again during another definitive test to give 18 column samples. These were then stored at -20°C until all samples were collected and ready for shipment to CEM Analytical Services (CEMAS) for analysis. Once received, the cartridges were then eluted in acetone and reconstituted in SOP dilute prior to analyses taking place. Samples were quantified against a single point standard solution at an appropriate concentration.

Plate 2.12 Pyblast exposure test samples loaded into Isolute C18 SPE cartridges placed onto the Supelco Visiprep™ DL.

2.6 STATISTICAL ANALYSIS

Quantal response data from concentration response analysis were processed and statistically analysed using a non-linear analysis regression model with a binomial distribution. This (log) logistic model has been implemented in the extension package *drc* R (Ritz and Streibig, 2005) for the software environment (R Development Care Team, 2011). The drc package in R provides non-linear regression analysis of multiple concentration response curves and comparison of parameters. Mortality and effected observation data from replicate tests were combined. A Non-linear regression analysis model was run on the binomially distributed data collected in order to derive median LC_{50} (lethal concentration where mortality is 50% of the test organism) and EC_{50} (where 50% of the organism's show effected behaviours) concentration response values and 95% confidence intervals for various concentrations of the pyrethroids.

The effect of pyrethroids can be described by dose response curves similar to those developed by Streibig *et al*. (2005) for herbicides. The two-parameter log logistic model is given by the formula below.

$$y = C + \frac{D}{1+\exp\{b(\log(x)-\log(e))\}}$$

where y is the response variable (mortality or effected crayfish) and x is the concentration in µg/l. *D* is the upper limit of the curve which is fixed at 1 and *C* is the lower limit which is fixed at 0. The parameter *e* is the equivalent of lethal concentration (LC_{50}) which is the dose required to achieve 50% mortality or when looking at effected concentration (EC_{50}) which is the dose required to achieve 50% effective.

Parameter *b* describes the slope of the curve around the inflection point (*e*). The above two parameter log logistic model (LL.2) was chosen as it provides the two-parameter log-logistic function where the lower limit is fixed at 0 and the upper limit is fixed at 1 (0 equal to zero mortality and 1 equal to 100% mortality), mostly suitable for binomial responses. The model is based on the assumption that two or more curves have different *e* parameters and thus LC_{50}/EC_{50} values.

Effective dosage (ED) is commonly used to compare different dose response curves. ED is a function of the parameter ED*y*, which is defined as the dose that yields a response that is (100-*y)* % of the maximum response D (a reduction of the *y* %). ED*y* can be expressed by the means of the parameters *b* and *e* in the two parameter log logistic model:

$$\mathrm{ED}y = e(y(100 - y))^{1/b}$$

The package *drm* in R provides functions to compute ED*y* values. The ED*y* values represent the LC_{50} (lethal concentration where mortality is 50% of the test organisms) and EC_{50} (where 50% of the organisms show effected behaviours). This was applied to the observation data obtained in this study where concentrations were used as an indication if the intensity of the exposure from combined replicated tests using various concentrations of the pyrethroids. Estimates of ED_{50} and their standard errors for all curves were calculated with the values shown in the results section. A One-Way ANOVA was used to test whether there were differences in the ED_{50} between exposure times, life stages and chemicals at the 5% significance level. If *e* parameter for each curve were similar (based on the standard error) this showed that the curves were identical in all parameters except the LC_{50}/EC_{50}.

The response variable was the proportion of dead crayfish, calculated as the number of dead crayfish at the end of the experimental time period divided by the total number of individuals used at the start of the experiment. The response variable was plotted against concentration to create a dose response curve and calculate the LC_{50} values. This was repeated using the proportion of effected crayfish as the response variable plotted against concentration to create a dose response curve and calculate the EC_{50}.

The LL.2 two parameter log logistic model has the lower limit is fixed at 0 and the upper limit is fixed at 1. The two parameters estimated were the relative slope and the LC_{50}/EC_{50} values. The log logistic model (LL.2) was fitted to the data and a dose response analysis was carried out to estimate the parameters of the model and the associated standard errors.

Data consisted of two or more curves obtained at either an observation time where life stages (e.g. stage I hatchlings versus stage II) were the variable compared at that time (e.g. 48h), or where time (e.g. 24h versus 48h) was the variable compared at a life stage (stage I). These multiple concentration response curves were fitted for comparison assuming different individual parameters for each curve.

A lack of fit test was carried out on the model to compare its effectiveness against using a One way ANOVA. The model's slope parameters were allowed to vary between curves (fitted with unrestricted parameters). If the slope parameters for each curve in this model were similar (based on the standard error) showing curves to be identical in all parameters except LC_{50}/EC_{50} values, a second model, (fitted with restricted slope parameters) was used in the analysis. This model selection was confirmed using a Likelihood Ratio Test (LRT) (a measure of the distance between the two models) with a null hypothesis that there is no difference between the more complex (Model 1) and simpler model (Model 2), so therefore if the *p*-value is greater than 0.05 (derived from the X^2 distribution), it is accepted that there is no difference between the models and the simpler model, Model 2 is used in the analysis.

Using the selected model, the LC_{50}/EC_{50} was then compared between the concentration response curves to estimate if they were significantly different from each other using a *t*-test. All values in the text are expressed as an estimated LC_{50} or EC_{50} value and all pyrethroid concentrations are in $\mu g/l^{-1}$ unless stated otherwise. View Appendix I for X^2 and *p* values and graphical check of the data for the appropriateness of the model used for each test.

Chapter Three

Results of acute toxicity of signal crayfish to biocides

3.1 WATER QUALITY PARAMETERS

For all toxicity tests and the analytic study, water quality parameters were measured at each observation time and were as follows (ranges given as ±) pH 7 ± 2, DO 9 ± 2, temperature 12 ± 2°C. The quality of the exposure water during toxicity tests was maintained within the ranges acceptable for crayfish survival.

3.2 CONTROLS

For all definitive acute toxicity tests, fewer than 10% mortality or sublethal effects were observed among crayfish exposed to the dilution water/solvent control. These data demonstrated that the holding facilities and handling techniques were acceptable for conducting these trials. This is supported by the view that a mean survival ≥90% should be achieved as required in the standard USEPA 2002. All analysis was calculated after 24h and 48h after exposure started. Families of stage I hatchlings were numbered and compared, to examine whether there was inter-family differences in crayfish susceptibility to the biocide.

3.3 ACUTE TOXICITY TESTS

3.3.1 *Crayfish behavioural response*

During Pyblast exposure, hatchlings, juveniles and adults initially displayed hyperactivity after exposure to the higher concentrations. This tended to cease after 5-10 minutes. In some cases, juveniles and adults crayfish began to vomit in reaction to the ingestion of the pesticide and one or both chelipeds fell off (Plate 2.9). During observation, crayfish displayed various behaviours in response to abdomen prods after been turned onto their backs. Usually in control crayfish would tail flap and self-right. From the lowest concentrations upwards, behaviours changed. Crayfish in low concentrations responded to prods similar to controls. As the concentrations increased crayfish could not self-right and remained on their backs with their appendages remained mobile (Plate 2.10). Crayfish exposed to higher concentrations quickly displayed immobility and the only visible movement was that of the respiratory organs. This was quickly followed by death.

3.3.2 *Stage I hatchling families exposed to Pyblast*

The purpose of this experiment was to investigate differences in sensitivity between different families of hatchlings of the same age and life cycle when exposed to Pyblast. Separate families of stage I hatchlings at 29 days old were exposed to the same levels of concentration of Pyblast and their mortality rates were compared. Results from both the LC_{50} and EC_{50} estimates indicate that within stage I crayfish significant differences in response exist between families at both 24h and 48h (Figure 3.1).

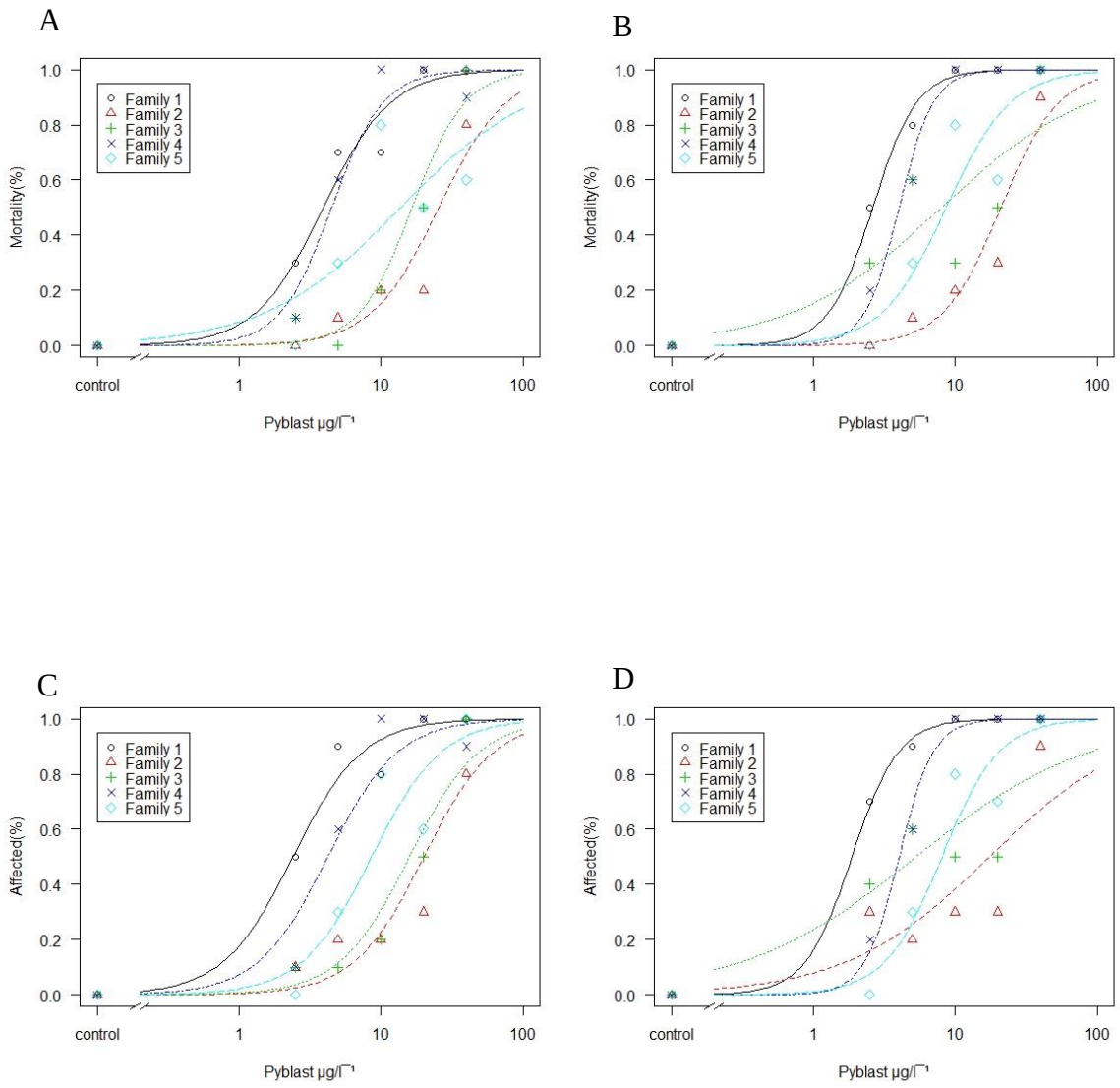


Figure 3.1 Dose-response curves for the total Pyblast lethal concentration at 24h (A) and 48h (B) and the effective dose at 24h (C) and 48h (D) to stage I signal crayfish hatchlings of different families (1-5) after 2h exposure and 46h recovery. The lower asymptote is the control. Each data point corresponds to % hatchlings dead (A/B) or affected (C/D) per beaker, per concentration with *n*=5 animals per beaker.

Results of the LC_{50} values show there is a significance difference between families in seven of the ten family comparisons at 24h ($p<0.05$) (Appendix D, Table D.3) and six out of ten family comparisons at 48h ($p<0.05$) (Appendix D, Table D. 4). The differences of LC_{50} values between families are large. The range of LC_{50} at 24h was between 3.81 ± 0.89 $\mu g/l^{-1}$ and 26.24 ± 6.16 $\mu g/l^{-1}$, indicating the highest LC_{50} as being 6.5 times greater than the lowest (Appendix D, Table D.1). The LC_{50} decreases at 48h in all families with significant differences still observed between families (Appendix D, Table D.4). The range of LC_{50} at 48h is between 2.62 ± 0.54 $\mu g/l^{-1}$ and 20.99 ± 4.14 $\mu g/l^{-1}$ (Appendix D, Table D.1), indicating the highest LC_{50} being eight times greater than the lowest.

Results from the EC_{50} values also show significant difference between families of hatchlings at both 24h and 48h with a large range between families. At 24hr there are significant differences between families in eight of the ten family comparisons ($p<0.01$) (Appendix D, Table D.5) and four of the ten family comparisons at 48h ($p<0.05$) (Appendix D, Table D.6). At 24h the EC_{50} values range from is 2.39 ± 0.63 $\mu g/l^{-1}$ to 20.08 ± 4.35 $\mu g/l^{-1}$ indicating a tenfold difference between family sensitivity (Appendix D, Table D.2). At 48hr there is a drop in EC_{50} values ranging from 1.87 ± 0.66 $\mu g/l^{-1}$ to 17.34 ± 6.87 $\mu g/l^{-1}$, the highest been nine times greater than the lowest (Appendix D, Table D.2).

3.3.3 *Stage I and II hatchlings exposed to Pyblast*

The acute toxicity of stage I and II hatchlings exposed to Pyblast was observed over a 48h period. The results obtained at 24h and 48h exposure end points for each life stage were used to calculate a LC_{50} and EC_{50}. These included the same data obtained from stage I hatchlings that was used for the data analysis of sensitivity between families. The LC_{50} and EC_{50} dose response curves at 24h were then compared with those at 48h to investigate whether there was a difference over time in their response within each life stage.

Stage I hatchlings have an LC_{50} of 9.71 ± 1.06 $\mu g/l^{-1}$ at 24h which decreases at 48h to 6.43 ± 0.72 $\mu g/l^{-1}$ (Figure 3.2). The LC_{50} values of Pyblast in stage I hatchlings were significantly greater at 24h than at 48h (t-test, $t=2.56$, $p<0.01$), by approximately 1.5 times the concentration. The EC_{50} obtained at 24h was 7.88 ± 0.88 $\mu g/l^{-1}$ which also decreases at 48h to 5.05 ± 0.61 $\mu g/l^{-1}$. The difference here was also significantly different between the two observation periods (t-test, $t=2.66$, $p<0.01$), by approximately 1.5 times the concentration.

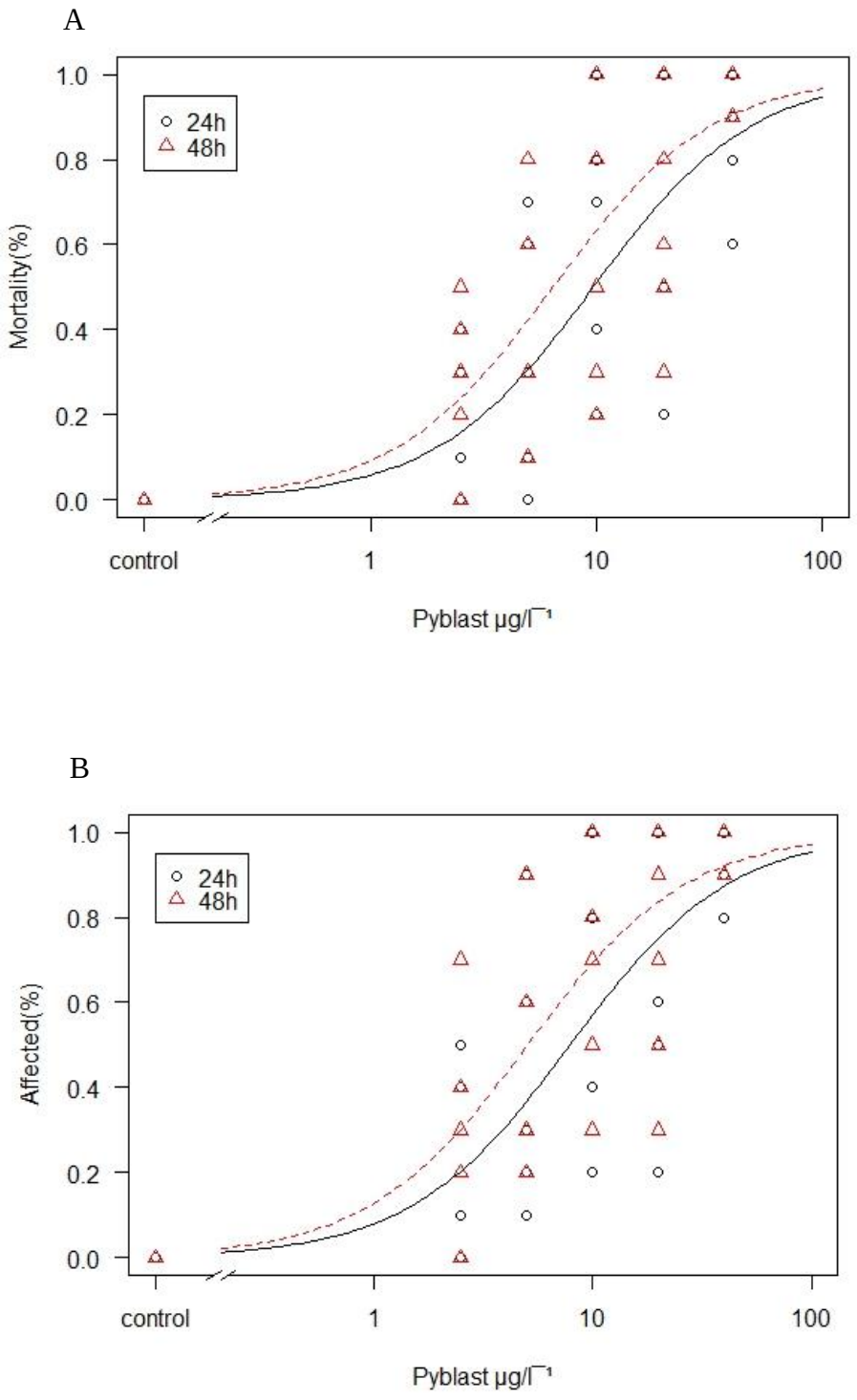


Figure 3.2 The 24h and 48h dose-response curves for the total Pyblast lethal concentration (A) and effective dose (B) on stage I signal crayfish hatchlings after 2h exposure and 46h recovery. The lower asymptote is the control. Each data point corresponds to % hatchlings dead (A) or effected (B) per beaker per concentration with *n*=5 animals per beaker.

Stage II hatchlings have an LC_{50} of 7.67 ± 1.22 µg/l^{-1} at 24h which decreases at 48h to 5.23 ± 0.89 µg/l^{-1}. The LC_{50} values of Pyblast in stage II hatchlings shows there was no significant difference over the two observation periods (t-test, t=1.66, p=0.09). The EC_{50} obtained at 24h was 5.44 ± 0.76 µg/l^{-1} which also decreases at 48h to 4.73± 0.68 µg/l^{-1}. The data indicate that there is no significant difference (t-test, t=0.72, p=0.46) in concentration between the two observation periods times (Figure 3.3).

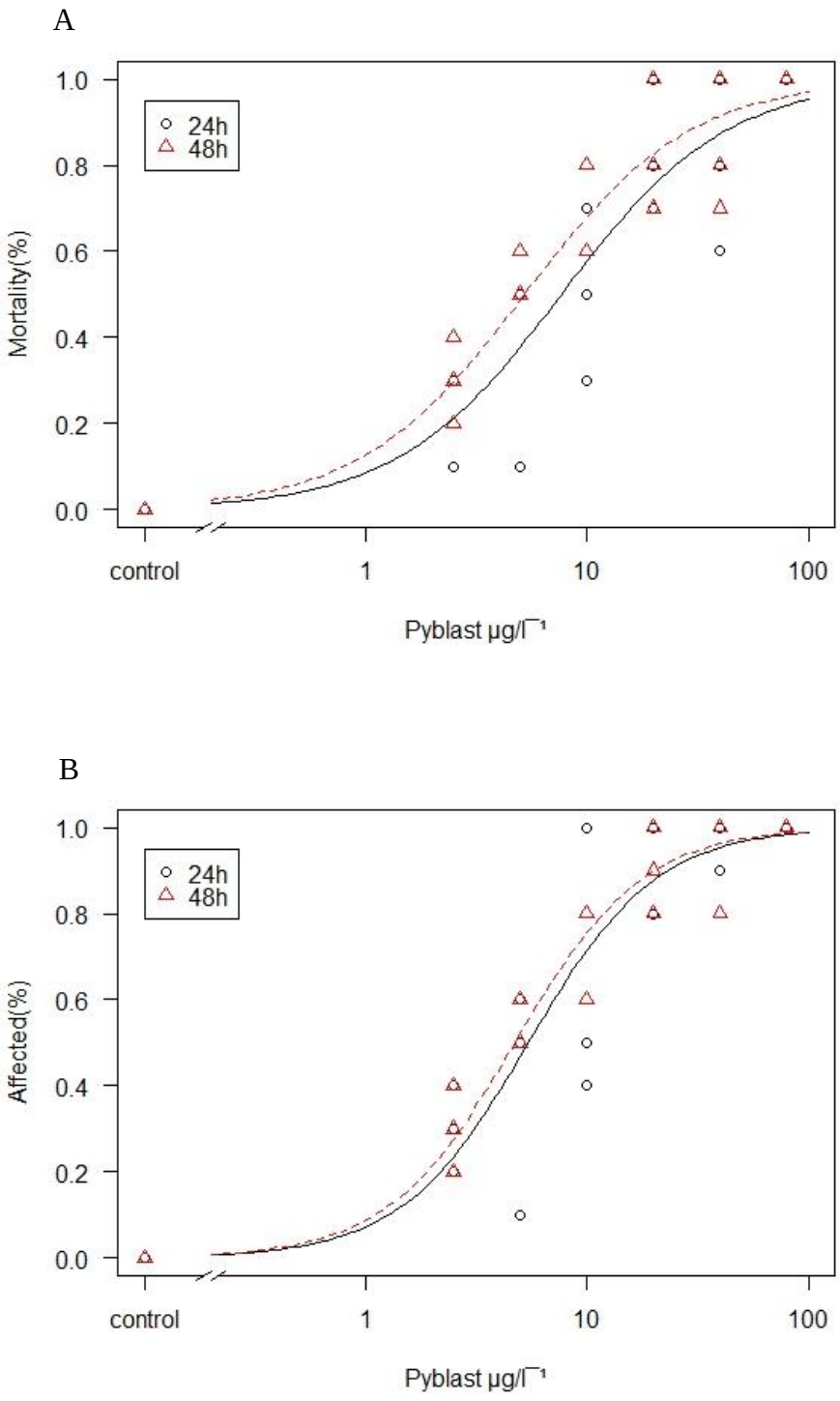


Figure 3.3 The 24h and 48h dose-response curves for the total Pyblast lethal concentration (A) and effective dose (B) on stage II signal crayfish hatchlings after 2h exposure and 46h recovery. The lower asymptote is the control. Each data point corresponds to % hatchlings dead (A) or effected (B) per beaker per concentration with *n*=5 animals per beaker.

The LC_{50} and EC_{50} dose response curves both at 24h and 48h were then compared between stages I and II using same data to investigate the effect of hatchling tolerance between these early life stages on the LC_{50} and EC_{50} dose response curves to Pyblast.

Results (Appendix D, Table D.10 and D.11) show that the LC_{50} and EC_{50} values indicate stage II hatchlings have a lower tolerance than stage I hatchlings at both 24h and 48h. Comparison of stage I and Stage II LC_{50} and EC_{50} values at different observation (Appendix D, Table D.12) show the LC_{50} values obtained at 24h were not significantly different between stage I LC_{50} of 9.71 ± 1.06 $\mu g/l^{-1}$ and stage II LC_{50} of 7.74 ± 1.20 $\mu g/l^{-1}$ (t-test, t=1.22, p=0.219). The 24h EC_{50} values were found to be significantly different between stage I EC_{50} of 7.89 ± 0.81 $\mu g/l^{-1}$ compared with stage II EC_{50} 5.24 ± 0.78 $\mu g/l^{-1}$ (t-test, t=2.37, p<0.05). At 48h stage I hatchlings LC_{50} values of 6.40 ± 0.73 $\mu g/l^{-1}$ compared with stage II LC_{50} values of 5.30 ± 0.87 $\mu g/l^{-1}$ show no significant difference (t-test, t=0.99, p=0.322). Stage I hatchlings EC_{50} values of values of 5.13 ± 0.72 $\mu g/l^{-1}$ compared with stage II EC_{50} values of 4.36 ± 0.72 $\mu g/l^{-1}$ also show no significant difference (t-test, t=0.86, p=0.388).

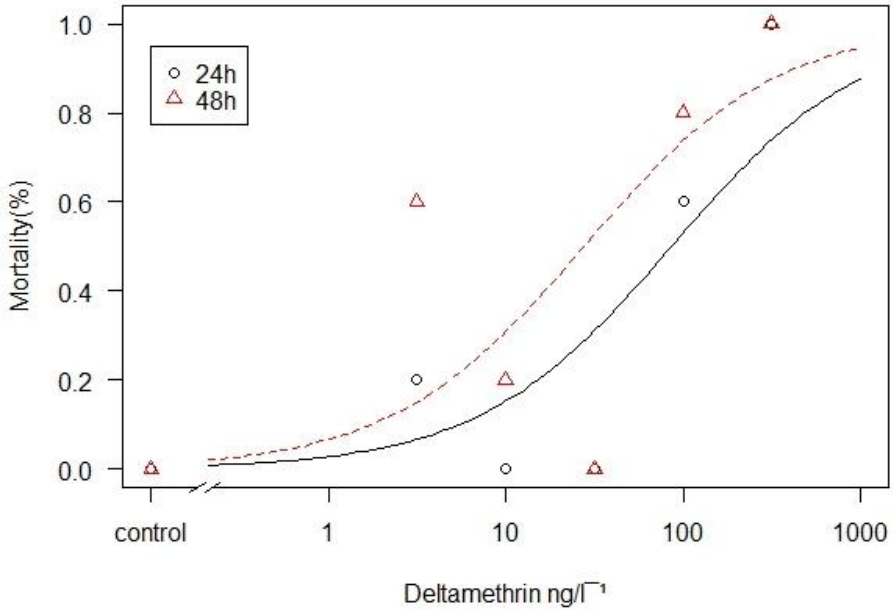
3.3.4 *Stage I hatchlings exposed to Salmosan*

From the concentrations used (0-31.6 $\mu g/l^{-1}$), LC_{50} or EC_{50} values could not be calculated as all hatchlings survived after 96h exposure and the trial was terminated.

3.3.5 *Stage I hatchlings exposed to Deltamethrin*

Results from stage I hatchlings exposed to Deltamethrin (Appendix D, Table D.13 and D.14) show an LC_{50} value at 24h of 85.76 ± 53.67 ng/l^{-1} and 48h value of 27.47 ± 16.28 ng/l^{-1}. It indicates that there is no significant difference between these two LC_{50} values (t-test, t=1.03, p=0.299). The EC_{50} results obtained at 24h is 4.82 ± 4.43 ng/l^{-1} and 6.82 ± 5.78 ng/l^{-1} at 48h. It also states that there is no significant difference between these dose curves (t-test, t=-0.31, p=0.755). However a large standard error present and this is due to the small sample size used in the experiments. There is no clear concentration-response relationship for most of the data sets as shown in Figure 3.4. Therefore the results were not suitable to derive an accurate LC_{50} or EC_{50} value.

A



B

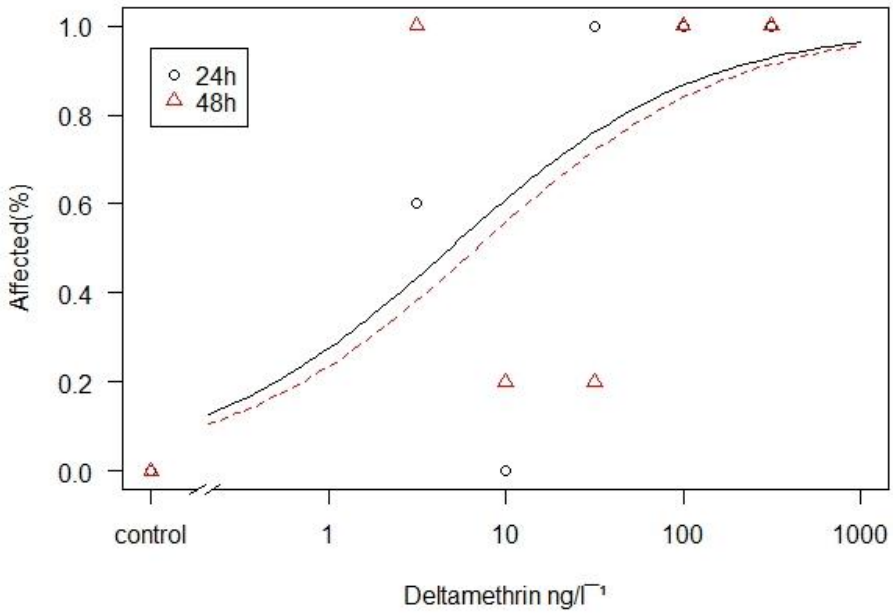


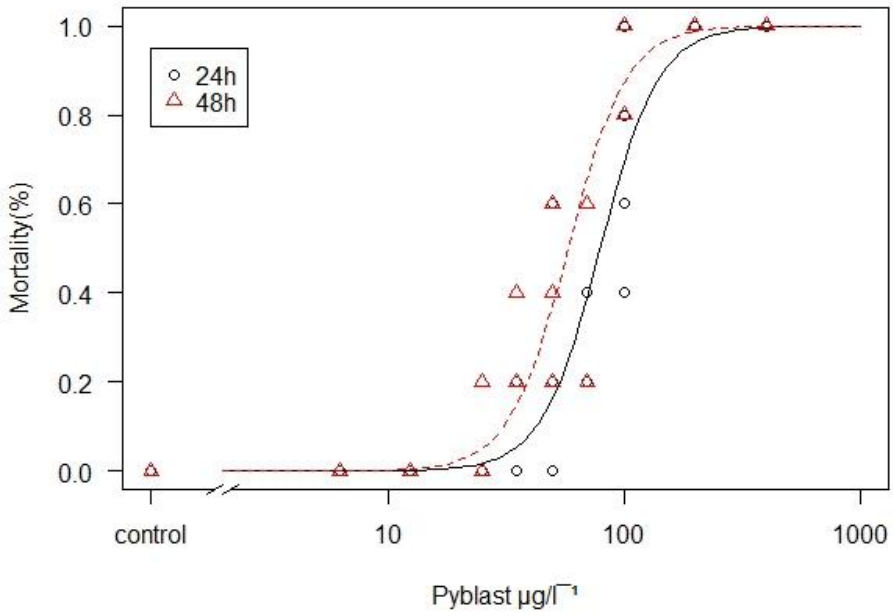
Figure 3.4 The 24h and 48h dose-response curves for the total Deltamethrin lethal concentration (A) and effective dose (B) on stage I signal crayfish hatchlings after 48h exposure and 46h recovery. The lower asymptote is the control. Each data point corresponds to % hatchlings dead (A) or effected (B) per beaker per concentration with *n*=5 animals per beaker.

3.3.6 *Juvenile signal crayfish (20-30mm) exposed to Pyblast*

The acute toxicity of juvenile crayfish of mixed sex ranging in size between 20-30mm CL exposed to Pyblast was observed over a 48h period. The results obtained at 24h and 48h exposure end points were used to calculate a LC_{50} and EC_{50}. The LC_{50} and EC_{50} dose response curves at 24h were then compared with those obtained for 48h exposure to investigate if there was a difference over time in their response (Figure 3.5).

Results from juveniles exposed to Pyblast (Appendix D, Table D.15 and D.16) have a 24h LC_{50} of 79.34 ± 6.71 $\mu g/l^{-1}$ and a 48h LC_{50} of 57.95 ± 4.54 $\mu g/l^{-1}$. From these results, it shows that the LC_{50} values of Pyblast in juveniles were significantly greater at 24h than at 48h (t-test, t=2.66, p<0.01) by approximately 1.3 times the concentration. The 24h EC_{50} is 6.01 ± 0.88 $\mu g/l^{-1}$ which slightly increases over time to a 48h EC_{50} of 6.35 ± 0.89 $\mu g/l^{-1}$. Results show that this increase is not significant over the two observation periods (t-test, t=-0.29, p=0.76).

A



B

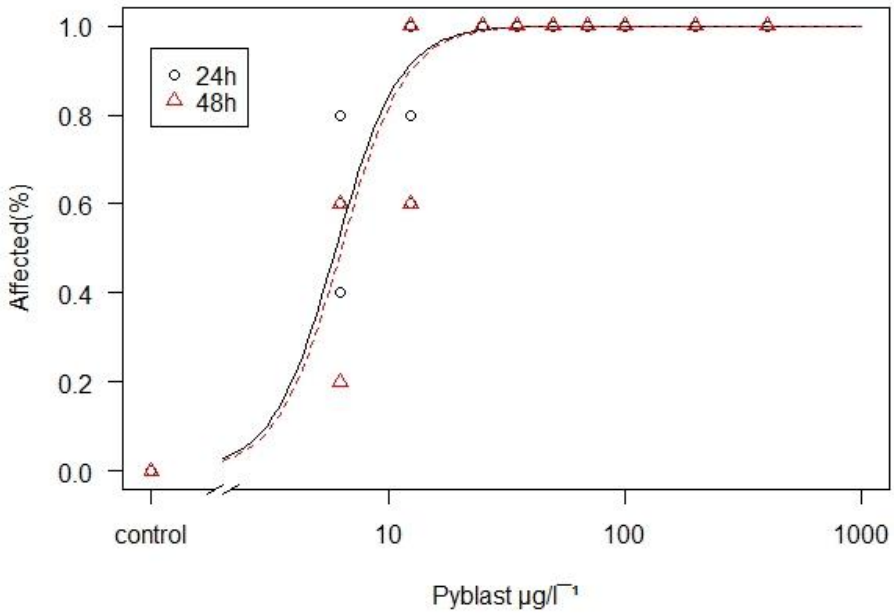


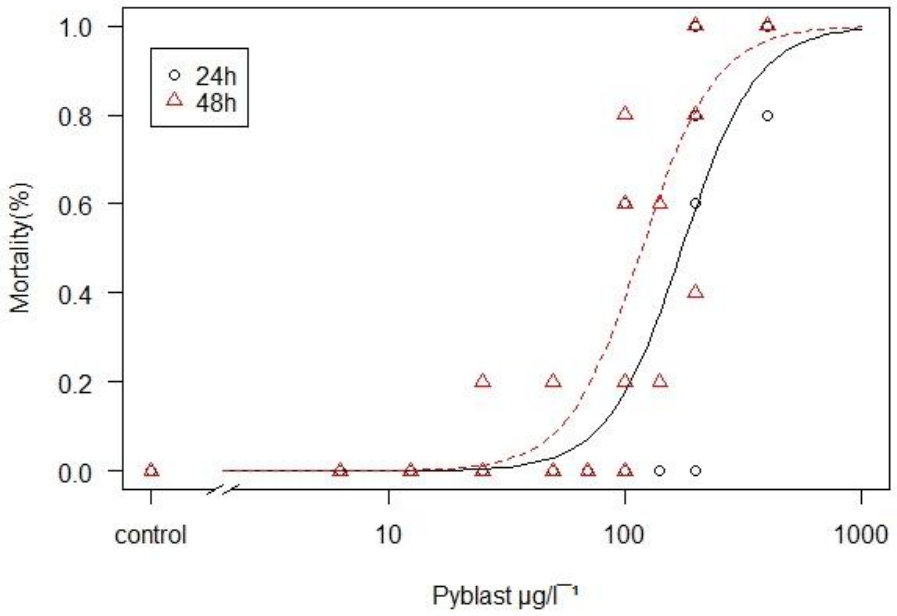
Figure 3.5 The 24h and 48h dose-response curves for the total Pyblast lethal concentration (A) and effective dose (B) on juveniles signal crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A) or affected (B) per concentration with *n*=1 animals per beaker.

3.3.7 *Adult female signal crayfish (40-50mm) exposed to Pyblast*

The acute toxicity of adult female crayfish ranging in size between 40-50mm CL exposed to Pyblast was observed over a 48h period. The results obtained at 24h and 48h exposure end points were used to calculate a LC_{50} and EC_{50}. The LC_{50} and EC_{50} dose response curves at 24h were then compared with 48h to investigate if there was a difference over time in their response (Figure 3.6).

Results from female crayfish exposed to Pyblast (Appendix D, Table D.17 and D.18) have a 24h LC_{50} of 174.66 ± 16.75 $\mu g/l^{-1}$ which decreases significantly to a 48h LC_{50} of 118.25 ± 10.54 $\mu g/l^{-1}$. From these results, it shows that the LC_{50} values of Pyblast in adult females were significantly greater at 24h than at 48h (t-test, t=2.87, p<0.01) by approximately 1.47 times the concentration. The 24h EC_{50} is 10.48 ± 4.96 $\mu g/l^{-1}$ which slightly increases over time to 48h EC_{50} of 12.08 ± 2.10$\mu g/l^{-1}$. Results show that this increase is not significant over the two observation periods (t-test, t=-0.29, p=0.76).

A



B

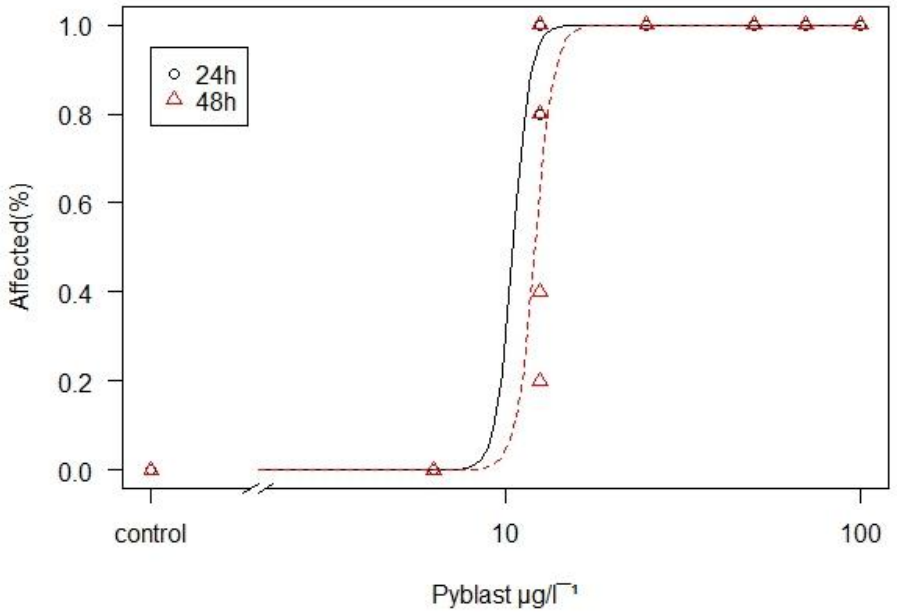


Figure 3.6 The 24h and 48h dose-response curves for the total Pyblast lethal concentration (A) and effective dose (B) on adult female signal crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A) or affected (B) per concentration with *n*=1 animals per beaker.

3.3.8 *Adult male signal crayfish (40-50mm) exposed to Pyblast*

The acute toxicity of adult male crayfish, ranging in size between 40-50mm CL, exposed to Pyblast was observed over a 48h period. The results obtained at 24h and 48h exposure end points were used to calculate a LC_{50} and EC_{50}. The LC_{50} and EC_{50} dose response curves at 24h were then compared with 48h to investigate if there was a difference over time in their response (Figure 3.7).

Results from male crayfish exposed to Pyblast (Appendix D, Table D.19 and D.20) have a 24h LC_{50} of 150.90 ± 10.57 $\mu g/l^{-1}$ which significantly decreases to a 48h LC_{50} of 111.13 ± 7.34 $\mu g/l^{-1}$. These results indicate that the LC_{50} values of Pyblast in adult males were significantly greater at 24h than at 48h (t-test, t=3.10, p=0.01) by approximately 1.35 times the concentration. The 24h EC_{50} 9.78 ± 0.79 $\mu g/l^{-1}$ slightly increases over time to a 48h EC_{50} of 10.40 ± 0.81 $\mu g/l^{-1}$. Results show that this increase is not significant over the two observation periods (t-test, t=-0.55, p=0.58).

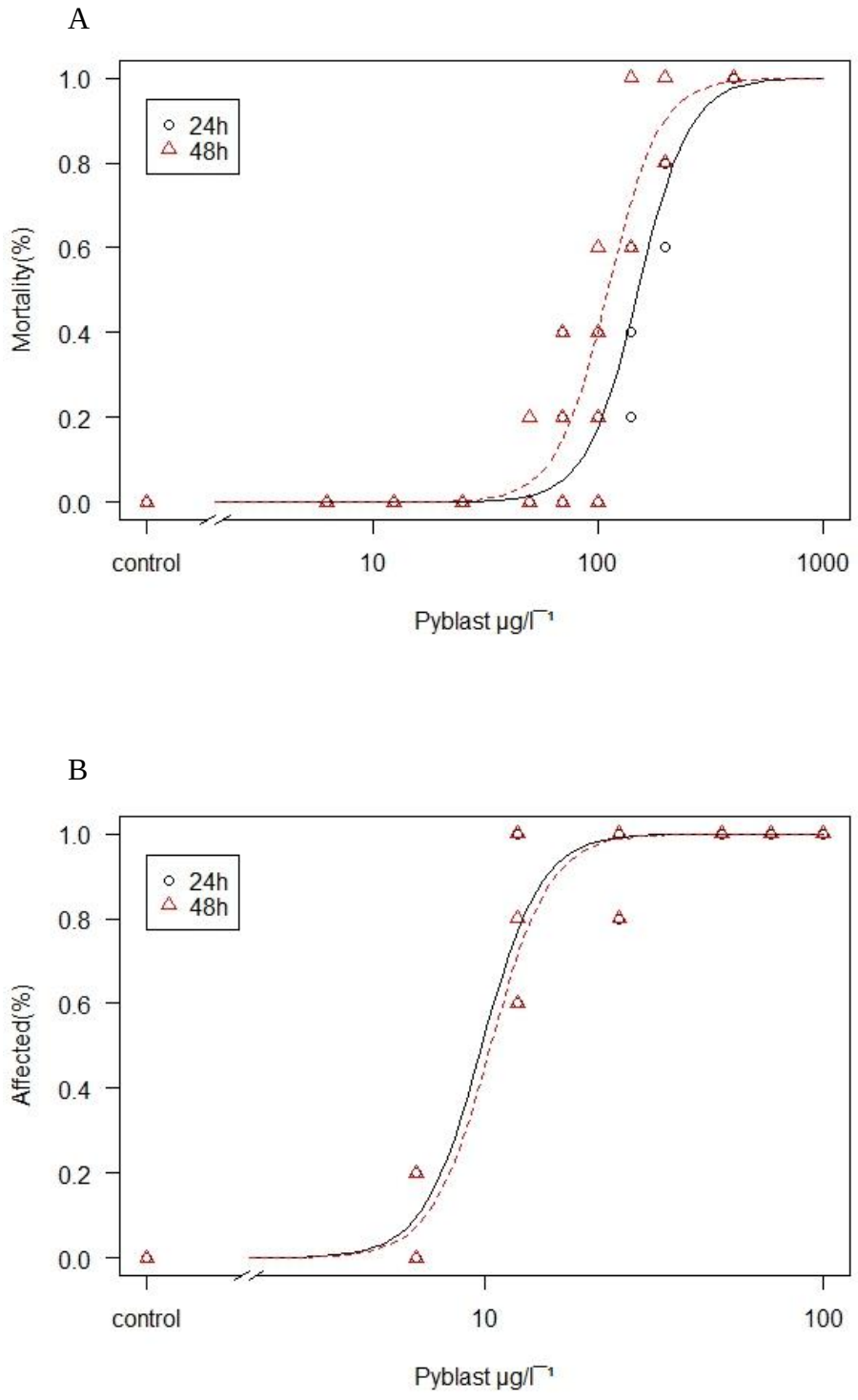


Figure 3.7 The 24h and 48h dose-response curves for the total Pyblast lethal concentration (A) and effective dose (B) on adult male signal crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A) or affected (B) per concentration with *n*=1 animals per beaker.

3.3.9 *Acute toxicity comparison between juvenile and adult signal crayfish exposed to Pyblast*

The LC_{50} and EC_{50} dose response curves both at 24h and 48h were then compared between juveniles and adult males and females using same data to investigate if there was a difference in dose response between the different life stages, and sexes, to Pyblast. (Figure 3.8).

Results of the LC_{50} and EC_{50} values (Appendix D, Table D.21 and D.23) show that juvenile crayfish have a lower tolerance to Pyblast than adult males and females at both 24h and 48h. Females have the highest tolerance to Pyblast at 24h LC_{50}. However males have a higher tolerance to Pyblast than either juveniles or adult female crayfish at 48h. At 24h the LC_{50} values were not found to be significantly different (Appendix D, Table D.22) between males LC_{50} of 150.90 ± 12.10 µg/l^{-1} and females LC_{50} of 174.66 ± 14.81 µg/l^{-1} (t-test, t=0.90, p=0.36). There is significant difference between the LC_{50} values of juveniles LC_{50} of 80.39 ± 7.18 µg/l^{-1} and adult males (t-test, t= -5.33, p<0.01) and females (t-test, t=5.57, p<0.01). At 48h the LC_{50} values were found to be significantly different between juvenile crayfish LC_{50} value of 57.92 ± 4.51µg/l^{-1} and the LC_{50} values of adult male (111.37 ± 7.64 µg/l^{-1}) (t-test, t= -6.03, p<0.01) and females (94.49 ± 8.46µg/l^{-1}) (t-test, t=3.81, p<0.01). There is no significant difference between the adult male and female crayfish 48h LC_{50} values (t-test, t= -1.48, p=0.13).

Females have the highest EC_{50} value at both 24h and 48h. The 24h EC_{50} values were found to be significantly different (Appendix D, Table D.24) between juveniles EC_{50} of 5.72 ± 1.10 µg/l^{-1} compared with adult males EC_{50} 9.81 ± 0.79µg/l^{-1} (t-test, t=3.02, p=0.01). There was no significant difference found between adult females 10.25 ± 3.13µg/l^{-1} and adult males (t-test, t=-0.13, p=0.89) and females and juveniles (t-test, t=1.36, p=0.17). The juvenile crayfish 48h EC_{50} value of 6.57 ± 0.84 µg/l^{-1} was significantly different to the EC_{50} values of obtained for adult males 10.37 ± 0.84 µg/l^{-1} (t-test, t=3.21, p<0.01) and for females 12.13 ± 1.03 µg/l^{-1} (t-test, t=4.18, p<0.01). There was no significant difference found between the EC_{50} calculated for adult males and females (t-test, t= -1.32, p=0.18).

The 24h and 48h LC_{50} results obtained suggest that there are differences in tolerance between the different size classes and for different sexes. The greatest differences in 24h LC_{50} levels are seen between adult female crayfish and juvenile conspecifics (90.66 ± 16.26 µg/l^{-1}). At 48h the LC_{50} values reduce remarkably in both juveniles and adult crayfish. The highest

difference in LC_{50} levels exists between adult male and juvenile crayfish (53.45 ± 8.85 $\mu g/l^{-1}$).

The 24h and 48h EC_{50} results also vary between sexes and life stages. At 24h the greatest difference between values occurs between adult female crayfish and juveniles (4.53 ± 3.32 $\mu g/l^{-1}$). In contrast to the LC_{50} data, the 48h EC_{50} values increase over time. The greatest differences in EC_{50} values is between adult female crayfish and juveniles (5.57 ± 1.33 $\mu g/l^{-1}$).

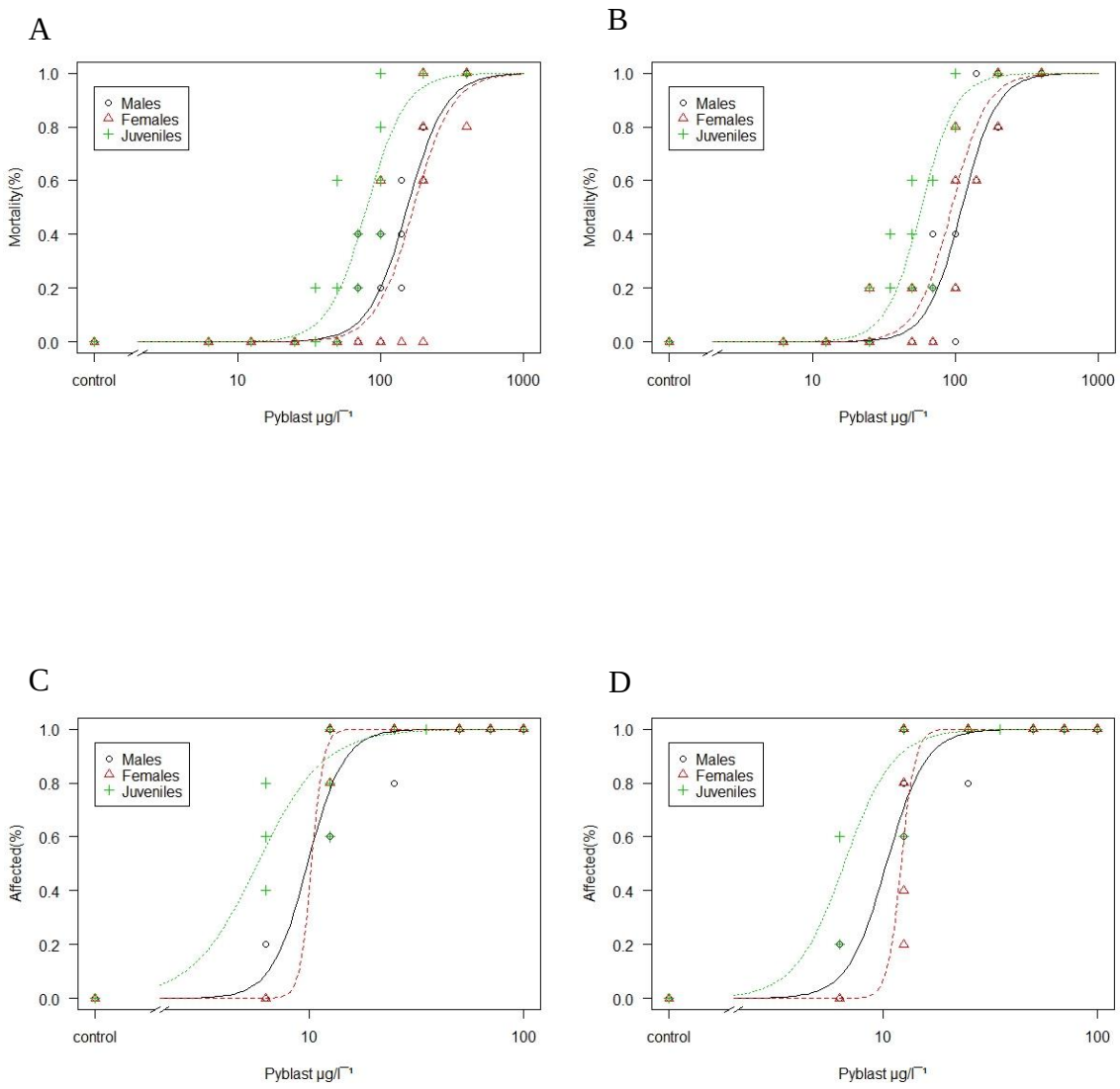


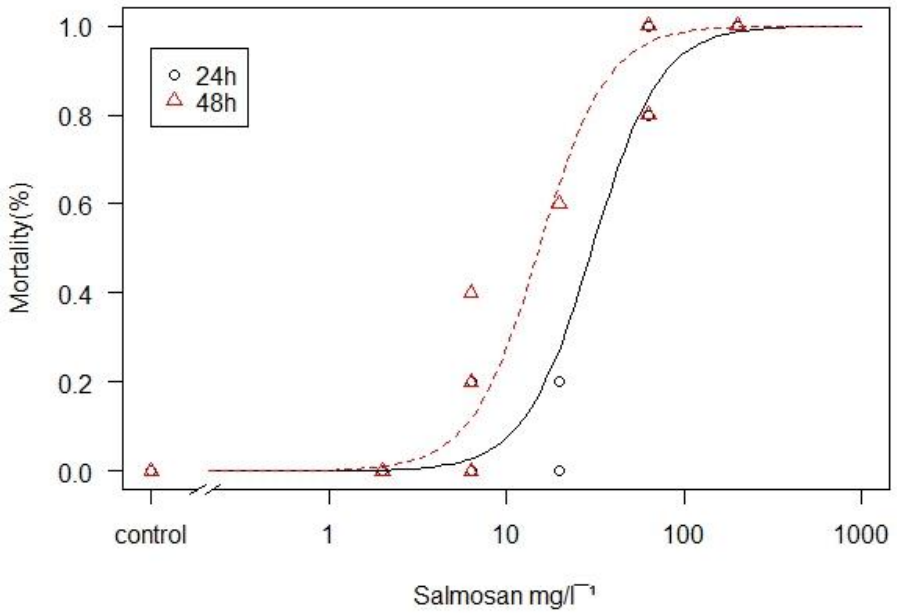
Figure 3.8 Dose-response curves for the total Pyblast lethal concentration at 24h (A) and 48h (B) and the effective dose at 24h (C) and 48h (D) to juvenile and adult male and female crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A/B) or affected (C/D) per concentration with *n*=1 animals per beaker.

3.3.10 *Adult signal crayfish (30-40mm) exposed to Salmosan*

The acute toxicity of adult crayfish of mixed sex, ranging in size between 30-40mm CL to Salmosan was observed over a 48h period. The results obtained at 24h and 48h exposure end points were used to calculate an estimated LC_{50} and EC_{50}. The LC_{50} and EC_{50} dose response curves at 24h were then compared with 48h to investigate if there was a difference over time in their response (Figure 3.9).

Results show adult signal crayfish exposed to Salmosan (Appendix D, Table D.25 and D.26) have a 24h LC_{50} of 30.46 ± 5.64 mg/l^{-1} which decreases to 15.33 ± 2.82 mg/l^{-1} after 48h. These results show that the LC_{50} values of Salmosan were significantly greater at 24h than at 48h (t-test, t=2.40, p<0.05) by approximately 1.99 times the concentration. Similarly, the estimated 24h EC_{50} 6.24 ± 0.52 mg/l^{-1} slightly decreases over time to a 48h EC_{50} of 5.20 ± 0.93 mg/l^{-1}. This decrease is not significant over the two observation periods (t-test, t=0.97, p=0.33).

A



B

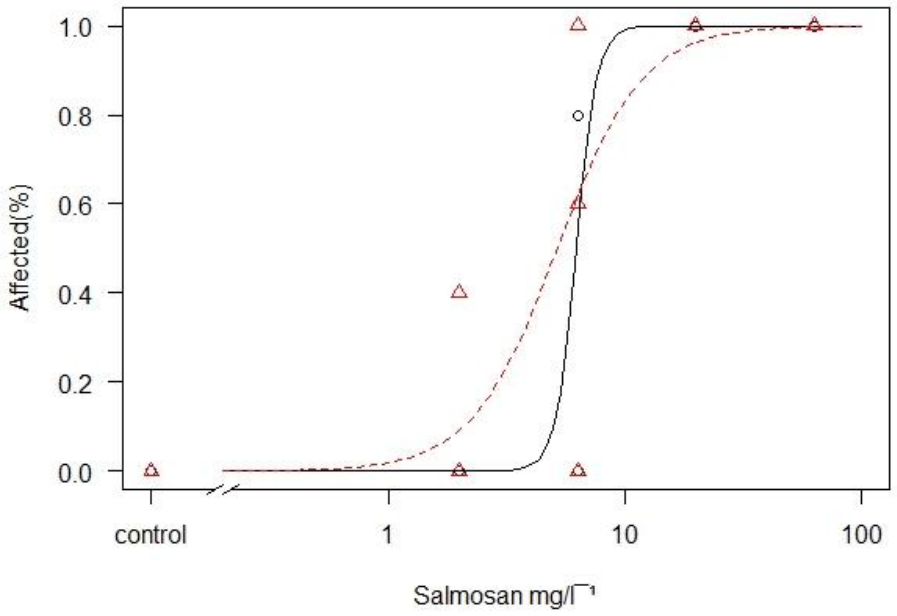


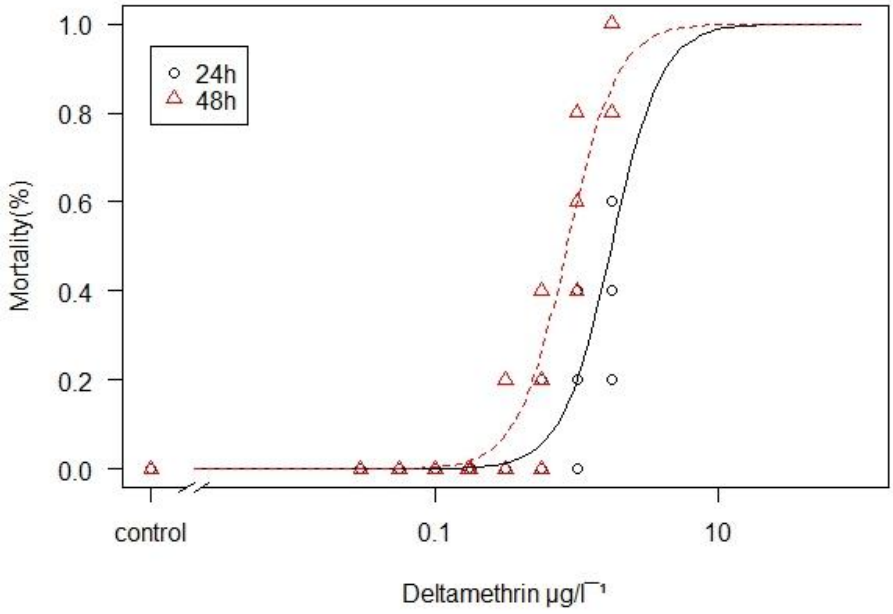
Figure 3.9 The 24h and 48h dose-response curves for the total Salmosan lethal concentration (A) and effective dose (B) on adult signal crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A) or affected (B) per concentration with *n*=1 animals per beaker.

3.3.11 *Adult signal crayfish (30-40mm) exposed to Deltamethrin*

Adult crayfish of mixed sex and size (30-40mm CL) were exposed to Deltamethrin and observed over a 48h period. The data obtained at the 24h and 48h exposure end points were used to calculate an LC_{50} and EC_{50}. The LC_{50} and EC_{50} dose response curves at 24h were then compared with 48h to investigate if there was a difference over time in their response (Figure 3.10).

Results show adult signal crayfish exposed to Deltamethrin (Appendix D, Table D.27 and D.28) have a 24h LC_{50} of 1.76 ± 0.28 $\mu g/l^{-1}$ which significantly decreases to 0.86 ± 0.11 $\mu g/l^{-1}$ after 48h. These results show that the LC_{50} value of Deltamethrin was significantly greater at 24h than at 48h (t-test, t=3.08, p<0.01) by approximately twice the concentration. The 24h EC_{50} 0.07 ± 0.01 $\mu g/l^{-1}$ increases slightly up to 0.09 ± 0.01 $\mu g/l^{-1}$ after 48h exposure. This increase is not significant over the two observation periods (t-test, t=-0.97, p<0.331).

A



B

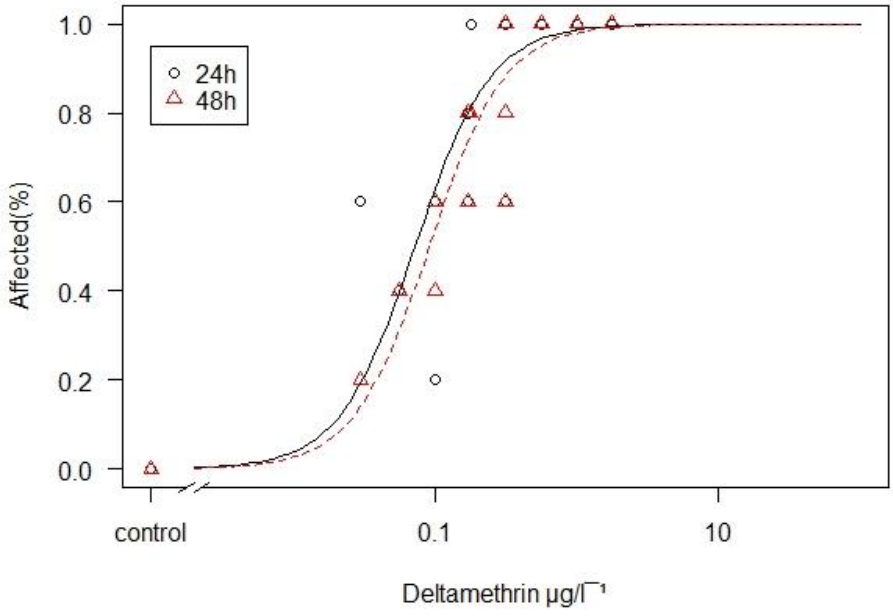


Figure 3.10 The 24h and 48h dose-response curves for total Deltamethrin lethal concentration (A) and effective dose (B) on adult signal crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A) or affected (B) per concentration with *n*=1 animals per beaker.

3.3.12 *Comparison of acute toxicity of adult signal crayfish exposed to the three biocides*

The calculated LC_{50} and EC_{50} were then compared between biocides on adult crayfish to compare response differences to the different biocides. This was done using same data obtained from the LC_{50} and EC_{50} dose response curves both at 24h and 48h results from each individual biocide toxicity data. To enable a meaningful comparison the unit of measurement for Salmosan was converted from mg/l^{-1} to $\mu g/l^{-1}$. Data obtained from both the adult males and adult females exposed to Pyblast were combined for biocide comparison since the Deltamethrin and Salmosan toxicity trails were used on adult crayfish of mixed sex.

LC_{50} and EC_{50} values of adult crayfish (Figure 3.11) show that the crayfish have a lowest tolerance to Deltamethrin and highest tolerance to Salmosan at both 24h and 48h. There is a significant difference between the LC_{50} and EC_{50} values (Appendix D, Table D.30 and D.32) found between all three biocides at both 24h and 48h. There is a reduction in LC_{50} values in all three pyrethroids from 24h to 48h (Appendix D, Table D.29). At 48h there is an increase of EC_{50} concentration of Pyblast and Deltamethrin (Appendix D, Table D.31). This pattern is not maintained for Salmosan which has an EC_{50} which decreases over time. The greatest differences in LC_{50} values is between the pyrethroids Deltamethrin and Salmosan (3028.58 ± 514.17 $\mu g/l^{-1}$) (t-test, t= -5.89, p<0.01) at 24h and Deltamethrin and Salmosan (1526 ± 305.45 $\mu g/l^{-1}$) (t-test, t= -4.99, p<0.01) at 48h. The greatest differences in EC_{50} values is also between the pyrethroids Deltamethrin and Salmosan (624.28 ± 68.03 $\mu g/l^{-1}$) (t-test, t= -9.17, p<0.01) at 24h and Deltamethrin and Salmosan (519.72 ± 93.42 $\mu g/l^{-1}$) (t-test, t= -5.56, p<0.01) at 48h.

The least differences in LC_{50} values is between the pyrethroids Deltamethrin and Pyblast (164.06 ± 11.04 $\mu g/l^{-1}$) (t-test, t= -14.85, p<0.01) at 24h and Deltamethrin and Pyblast (103.97 ± 5.66 $\mu g/l^{-1}$) (t-test, t= -18.36, p<0.01) at 48h. The least differences in EC_{50} values is also between the pyrethroids Deltamethrin and Pyblast (9.36 ± 0.56 $\mu g/l^{-1}$) (t-test, t= 16.63, p<0.01) at 24h and Deltamethrin and Pyblast (10.78 ± 0.65 $\mu g/l^{-1}$) (t-test, t= 16.53, p<0.01) at 48h.

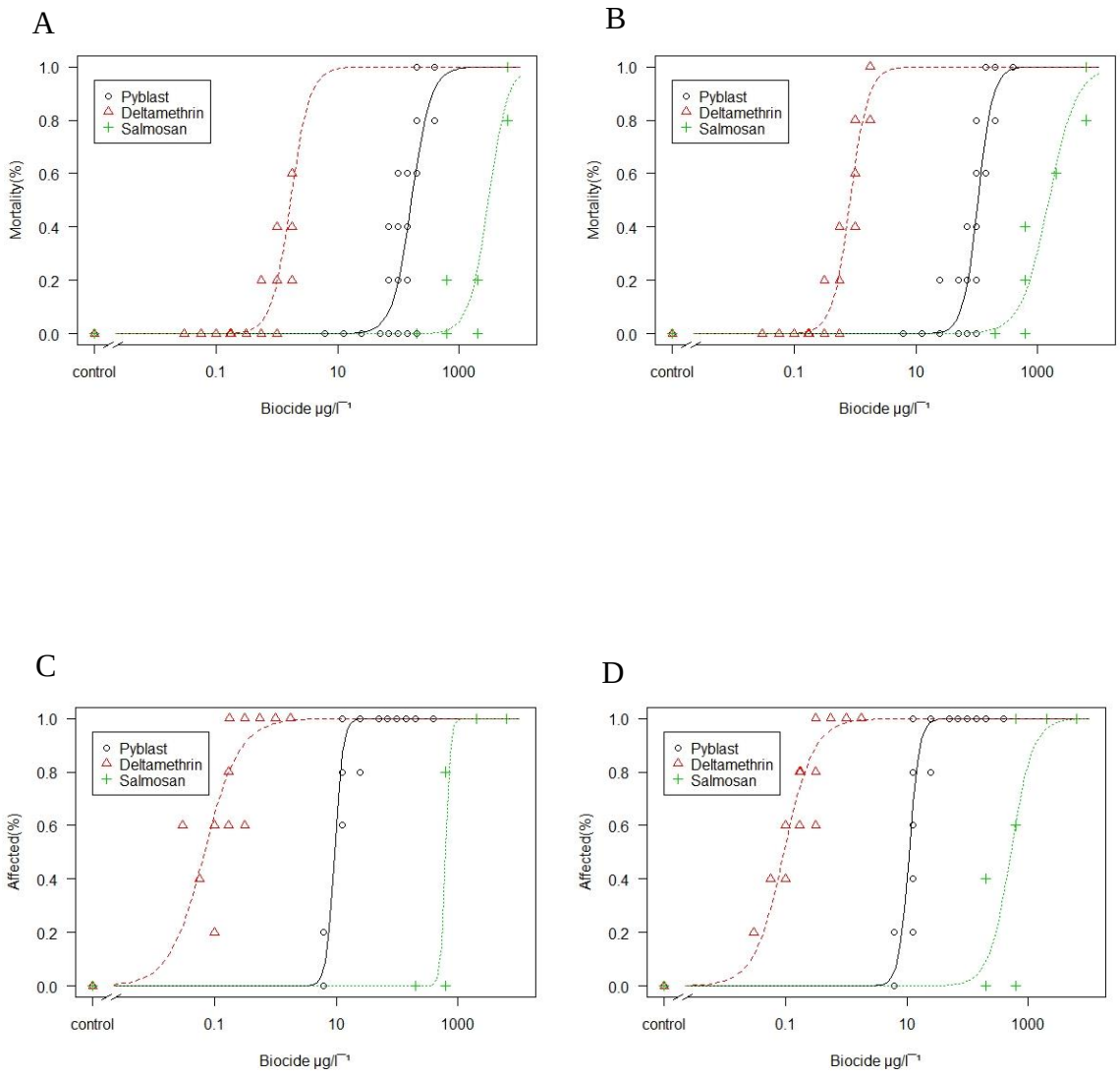


Figure 3.11 Dose-response curves for the total biocide lethal concentration at 24h (A) and 48h (B) and the effective dose at 24h (C) and 48h (D) to adult signal crayfish after 48h exposure. The lower asymptote is the control. Each data point corresponds to % crayfish dead (A/B) or effected (C/D) per concentration with *n*=1 animals per beaker.

3.4 ANALYTICAL STUDY

Pyblast concentration from exposure solutions was measured independently to estimate concentration efficiencies (see Appendix E). Analyses of water samples from the beakers of Pyblast exposed to crayfish indicate that all six compounds were present in the tests (Appendix E). The percentage of Pyblast available in dilution water during the two 48hr exposure tests on male crayfish is shown in Table 3.1. Unsurprisingly, there were no total pyrethrins present in the controls (0 $\mu g/l^{-1}$) at any stage over the 48h exposure. The loss of Pyblast from the dilution water during the 48h toxicity tests is shown in Figure 3.12. Extraction and analysis of the water samples collected from the exposures showed exposure concentrations (50 and 200 $\mu g/l^{-1}$) were below the expected levels of total pyrethrins available at 0h.

The analytics suggest that between 84.45% and 70.74% of the biocide concentration is actually available at the start of the toxicity trials (0h). This suggests that there is actually a 15-30% under estimation of the biocide concentration available. Based on both concentration samples, the average percentage of Pyblast concentrations available to the crayfish was 50% after 24h exposure and 30% after 48h exposure.

The concentration of Pyblast rapidly decreased during both toxicity tests. Average Pyblast availability, at each of the two concentration solutions, to the crayfish at 24h exposure at 50 $\mu g/l^{-1}$ was 50.21% and at 200 $\mu g/l^{-1}$ was 51.44%. The remaining concentration declined over the next 24hrs with only 23.33% present in the 50 $\mu g/l^{-1}$ and 36.81% in the 200 $\mu g/l^{-1}$ concentration at 48hrs. However there was no real decline from 24h sample of 102.88 $\mu g/l^{-1}$ to 48h sample of 92.48 $\mu g/l^{-1}$ in the 200ppb treatment in male test 3 experiment. Overall the 50 $\mu g/l^{-1}$ concentration loses an average of 54.01% over the 48hrs while the 200 $\mu g/l^{-1}$ loses an average of 42.07%.

The reason for the higher measured total pyrethrins (308.22 $\mu g/l^{-1}$) from the 200 $\mu g/l^{-1}$ exposure concentration after 24hrs in the second test is unknown and may be down to experimental error. This sample was removed from analysis.

Table 3.1 The percentage of Pyblast calculated from the total pyrethrins (nominal concentrations 50 µg/l^{-1}and 200 µg/l^{-1} exposure treatments) analysed by CEMAS against total pyrethrins (measured) available in dilution water during the two definitive 48hr exposure tests on male crayfish. Note an error in the analysis at 200 µg/l^{-1} exposure concentration after 24hrs *.

Test	Total pyrethrins (µg/l^{-1}nominal)	Exposure time (hrs)	Total pyrethrins (µg/l^{-1} measured)	% available to crayfish
3	Control 0	0	0	0
3	50	0	35.37	70.74
3	200	0	154.68	77.34
3	Control 0	24	0.7	0
3	50	24	26.33	52.66
3	200	24	102.88	51.44
3	Control 0	48	0.56	0
3	50	48	15.74	31.48
3	200	48	92.48	46.24
5	Control 0	0	0	0
5	50	0	41.97	83.94
5	200	0	168.9	84.45
5	Control 0	24	0.12	0
5	50	24	23.88	47.76
5	200	24	308.22	154.11*
5	Control 0	48	0	0
5	50	48	7.59	15.18
5	200	48	54.79	27.39

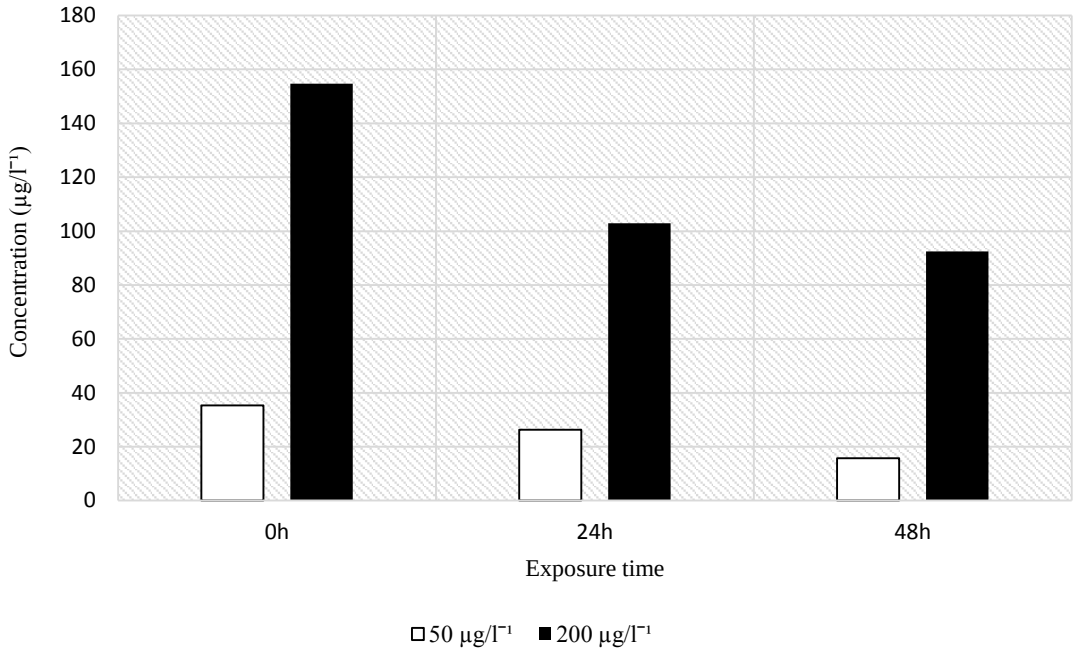


Figure 3.12 Graph showing decrease in Pyblast availability to crayfish from samples taken during the third 48h exposure definitive tests on males.

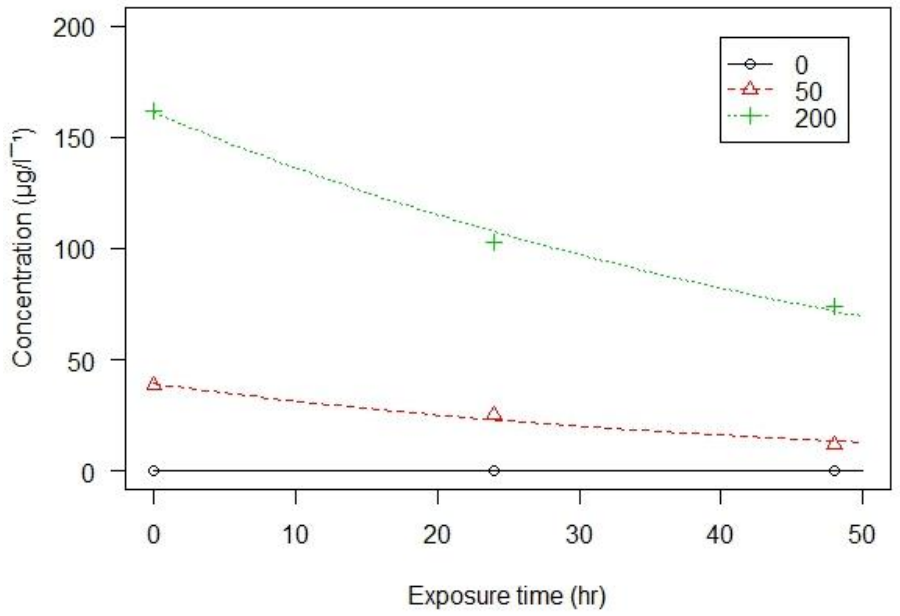


Figure 3.13 The exponential decay curves of Pyblast during toxicity tests with adult male signal crayfish. This graph is over the 48hr exposure time.

Chapter Four

Discussion

4.1 DISCUSSION

Freshwater crustaceans are generally more sensitive to pyrethroids than fish (Smith & Stratton, 1986). Among the pyrethroids, Deltamethrin is often the most toxic to crustaceans and fish in comparative tests due to the presence of the dibromovinyl group in the molecule (Haya, 1989). Of the three biocide formulations used in this study, only Pyblast has been used in field treatments against signal crayfish (e.g. Peay *et al*., 2006). These trials were carried out in the absence of detailed, laboratory-based, ecotoxicological data relating to the lethal concentrations required to kill crayfish of all sizes.

In the present study, signal crayfish of various life stages were exposed to three biocides, Pyblast, Alphamax (active ingredient deltamethrin), Salmosan (active ingredient azamethiphos). One of the key objectives of this research was to determine an accurate lethal dose of these three biocides to signal crayfish. By carrying out acute toxicity experiments on signal crayfish in laboratory conditions, accurate LC_{50} and EC_{50} values of the three biocides were obtained for several life stages. Our results confirm not only the extreme toxicity of both natural and synthetic pyrethroids and organophosphorus compounds to signal crayfish, but also demonstrate that toxicity levels may be influenced by crayfish life stage (size) and sex.

4.2 ACUTE TOXICITY OF HATCHLINGS

There are no published data relating to the LC_{50} and EC_{50} concentrations of the natural pyrethrum (Pyblast), with the synthetic pyrethroid Deltamethrin, or the organophosphorus compounds Salmosan on hatchling (stage I and stage II) signal crayfish. No other toxicity data for the early life stages of crayfish and the pyrethrum Pyblast was found in the literature for comparison; however there are a few examples in the literature of studies on the relative sensitivity of hatchlings or larvae of crustaceans to pesticides.

4.2.1 *Pyblast*

The acute toxicity of Pyblast to hatchlings was approximately 4.8% at 24h and 3.2% at 48h of the previous 0.2 mg/l^{-1} dosage applied in the field by Peay *et al*. (2006). When the results of individual hatchling family tests were compared with each other, statistically significant differences in the lethality between families at early life stages of signal crayfish was observed.

When the LC_{50} data were compared for the two hatchling life stages (I and II) of signal crayfish, the study showed that there were no differences in survival between the two stages at 24h and 48h. The ecotoxicity data demonstrate that Pyblast is most effective against stage II hatchling crayfish when they are exposed to a concentration of 5.23 µg/l^{-1}. The difficulty in applying Pyblast, at this concentration, in the field is discussed in section 4.13.

4.2.2 *Deltamethrin*

Results obtained from stage I hatchlings show no clear concentration–response relationship for hatchlings exposed to the Deltamethrin to derive an LC_{50} due to some mortalities rates been greater at lower concentrations than higher concentrations. With no suitable results to derive an accurate LC_{50} or EC_{50} value, the toxicity of Deltamethrin to stage I hatchlings cannot be compared with the toxicity of other pyrethroids on crayfish.

4.2.3 *Salmosan*

Trial exposures for stage I hatchlings to Salmosan resulted in no mortalities at concentrations ranging between 0-31.6 µg/l^{-1} after 48h exposure. Unfortunately, a lack of hatchlings meant this experiment could not be repeated at higher doses. The high survivorship of stage I hatchlings suggests that hatchlings were are more tolerant to this biocide than they are to Pyblast. Using 31.6 µg/l^{-1} as a bench mark, these data show that stage I hatchlings are five times more tolerant to Salmosan when comparing with the 48h LC_{50} results of Pyblast.

4.2.4 *Sensitivity variance between early life stages*

Results from the lethality responses show that the hatchling stages demonstrate between-family variability in response to the presence of Pyblast. Reasons for this difference in susceptibility of the hatchling families are not clearly understood. However this variability

may be a reflection of natural variation. It is possible that differences in susceptibility may be due to a range of factors, including maternal effects, the condition of hatchling families or, as in the case of pyrethroid resistance in sea lice *L. salmonis* (e.g. Fallang *et al*., 2005), genetically based differences in the way that biocide can activate the sodium channel.

The variability of response by hatchlings was not unexpected. However several studies (e.g. Wells & Sprague, 1976; Capuzzo *et al*., 1984; Young-Lai *et al.,* 1991) have described differences in sensitivity among species and life stages when exposed to biocides. Few however report the chronic and/or sublethal toxicity of pyrethroids to freshwater crustaceans. Each biocide has a specific mode of action and each larval stage may be more (or less) susceptible to this compound depending on its developmental and physiological status (Burridge *et al*., 1999).

Deltamethrin is known to be extremely toxic to crustaceans especially early life stages (Burridge *et al*., 2014). For example, it is effective against all attached stages of sea lice (Haya *et al*., 2005; Burridge *et al*., 2010). The toxicity of the American lobster *Homarus americanus* (H. Milne-Edwards 1837) reported difference of sensitivity between larval stages I, II, IV and adults to Deltamethrin (Fairchild *et al*., 2010; Burridge *et al*., 2014) as well as Salmosan (Burridge *et al*., 1999).

McLeese *et al*. (1980) carried out a lethality test on American lobsters using the synthetic pyrethroid Excis and found no difference between larval stages. This is consistent with the results from this study showing no significant difference in sensitivity between the two earliest life stages of signal crayfish exposed to Pyblast. Fisher *et al.* (1976) also found no differences in sensitivity between larval stages exposed to malachite green. However, Burridge & Haya, (1997) suggest early larval lobster stages are less sensitive to pyrethrins than post-larval stages or adults with differences in sensitivity of larval stages and the earliest stage most tolerant (Burridge *et al*., 2000). These findings support the earlier observations of Wells (1972), and Wells & Sprague (1976) who reported that of the toxicity of crude oil to American lobster larvae increases with larval stage. The higher tolerance of younger life stages is not universal however as shown in this study.

4.3 ACUTE TOXICITY TO JUVENILES AND ADULTS

A decrease in sensitivity for the adults (40-50mm) relative to the juveniles (20-30mm) and hatchlings to the biocides was expected due to the relationship between size class response and toxicity dose. This would suggest larger organisms to be more tolerant. The apparent higher sensitivity the smaller classes may partly be attributed to size, which may have increased uptake of the chemical into younger animals due to their higher surface area to volume ratio (Sprague, 1995). Although differences in results between hatchling and adult studies may have been affected by variations in experimental protocol with hatchlings were exposed for 2h with a 46h recovery and adults were exposed for 48h, this was not considered to be significant.

4.3.1 *Pyblast*

Results from the laboratory experiments carried out in this study showed 24h LC_{50} ranged from 7.67 $\mu g/l^{-1}$-174.66 $\mu g/l^{-1}$ from the earliest life stage of a crayfish to fully mature adults. The 48h LC_{50} toxicity values ranged from 5.23 $\mu g/l^{-1}$-118.25 $\mu g/l^{-1}$ across the same size gradient. Laboratory trials also indicated 24h EC_{50} values ranged from 5.44 $\mu g/l^{-1}$-10.48 $\mu g/l^{-1}$ and a 48h EC_{50} of 4.73 $\mu g/l^{-1}$-12.08 $\mu g/l^{-1}$ from the earliest life stage of a crayfish to fully mature adults. This indicates the very large range in toxicity variance through life stages, with a higher tolerance with each life stage.

Differences in sensitivity can also found between other species of crayfish and biocides. Juvenile signal crayfish had a 24h LC_{50} of 79.34 $\mu g/l^{-1}$ compared with adult males (154.90 $\mu g/l^{-1}$) and females (174.66 $\mu g/l^{-1}$). This is comparable to similar studies of other crayfish species exposed to Pyblast. Acute toxicity tests using Pyblast to control red swamp crayfish in Italy (Cecchinelli *et al*., 2012) reported higher mortality by juveniles followed by adult males then females when exposed to a Pyblast concentration of 0.02 mg/l^{-1}. It also shows that the 24h LC_{50} values were 18% more than those calculated in the current study for signal crayfish. This may indicate that signal crayfish are less tolerant then the red swamp crayfish to Pyblast.

4.3.2 *Deltamethrin*

Adults exposed to Deltamethrin show high sensitivity. Results from this study reported Deltamethrin to have a 24h LC_{50} of 1.76 $\mu g/l^{-1}$ which rapidly decreased to 0.86 $\mu g/l^{-1}$ at 48h to adult signal crayfish. Morolli *et al.* (2006) reported a 24h LC_{50} of 0.22 $\mu g/l^{-1}$ for the red swamp crayfish 6-9.5 cm (TL) when exposed to Deltamethrin and although found no great decrease from the 24h LC_{50} to 48h LC_{50}. Adult signal crayfish 30-40 mm (CL) from this study show a higher tolerance than red swamp crayfish of 6-9.5 cm (TL) when exposed to Deltamethrin.

4.3.3 *Salmosan*

It is well accepted that the sensitivity of crustaceans to organophosphates varies with development stage (Lignot *et al*., 1998) although, unfortunately, a lack of data mean that no conclusions could be made in relation to Salmosan toxicity and the survival of different crayfish life stages during the present study.

4.3.4 *Variance of acute toxicity across life stages*

This is the first study to provide evidence that LC_{50} and EC_{50} values can vary within populations and all life stages of signal crayfish when using the natural pyrethrum, Pyblast. The acute toxicity of Pyblast to signal crayfish in the laboratory clearly indicate that early life stages are much more sensitive than adults at low concentrations of this biocide. With the exception of stage I and II hatchling crayfish the levels of toxicity within families in stage I and between signal crayfish life stages were significantly different when exposed to Pyblast. Hatchlings were found to be the most sensitive of the life stages, over 22 times more sensitive than adults. Adult males are the most tolerant at 48h exposure, indicating a difference in biocide sensitivity between sexes.

Larval stages are generally considered to be more sensitive to biocides and changes in environmental conditions than adults (Johnson & Gentile, 1979; Young- Lai *et al.*, 1991, Haya *et al*., 2005). This also appears to be strongly dependant on the developmental stage of exposed individuals (Pauli *et al*., 1999, Greulich *et al*., 2002). The red swamp crayfish exposed to the synthetic pyrethroid Permethrin had a 96h LC_{50} ranging from the 0.44-1.3 $\mu g/l^{-1}$ with toxicity was correlated with crayfish size (Jarboe & Romaire, 1991). Jolly *et al*. (1978), also using Permethrin, determined a 96hr LC_{50} of 0.39 $\mu g/l^{-1}$ for newly hatched red

swamp crayfish and 0.62 µg/l^{-1} for juvenile conspecifics. A similar (LC_{50}) relationship was observed in red swamp crayfish exposed other pyrethroids, Resmethrin, Permethrin and Sumithrin (Paul & Simonin, 2006). The toxicity of the American lobster *Homarus americanus* (H. Milne-Edwards 1837) reported difference of sensitivity between larval stages and adults to Deltamethrin (Fairchild *et al*., 2010; Burridge *et al*., 2014) which is consistent with this study on signal crayfish.

It is likely that the concentrations used to kill crayfish must be able to kill those life stages which are the most tolerant. So whilst it is important to have data on the toxicity to adults, this study has shown that it is essential that toxicity data is obtained for all life stages so that we can be sure that all life stages are treated.

Mechanisms of differential uptake, absorption and excretion of Pyblast, Deltamethrin and Salmosan probably vary with crayfish size, life stage and the stage of sexual maturity as they do in other aquatic species. The variability may also be a reflection of the crayfish molt stage and endocrine status at the time of exposure, factors that are known to alter response to stressful and toxic conditions. It may also be a reflection of the natural variation that exists at these early stages (Burridge *et al*., 2000). This could explain the differences in both the LC_{50} and EC_{50} values obtained between the life stages and sex in this study.

Differences in sensitivity can also found between other species exposed to similar biocides. Amphibians have been known to exhibit differences in susceptibility at various life stages to biocide exposures (Greulich, 2003). There is a variance in sensitivity seen in marine invertebrates as well as their life stage in response when exposure to Deltamethrin (Cold and Forbes, 2004; Fairchild *et al*., 2010; Burridge *et al*., 2014; Van Geest *et al*., 2014). Rettich (1980) studied the effects of Deltamethrin and found that cladocera were eliminated by Deltamethrin application of 0.2-0.5 µg/l^{-1}but 20 µg/l^{-1} was required for copepods to be affected both of which are concentrations higher than the Deltamethrin toxicity results on adult signal crayfish from this study. There was also differences in organism sensitivity to Salmosan as shown for example between the marine invertebrates, *Mysid. Spp.*, sand shrimp and the American lobster (Burridge *et al*., 2014). Some marine invertebrates also have extremely lower tolerance when compared with adult signal crayfish in this study (Burridge *et al*., 2014; Ernst *et al*., 2014).

4.3.5 *Variance of acute biocide toxicity*

Among the pyrethroids, Deltamethrin is considered to be the most toxic to non-target organisms (Haya, 1989). A comprehensive review by Eversole & Seller (1997) on 35 different chemical groups using crayfish in laboratory 96h LC_{50} challenges found that synthetic pyrethroids were more poisonous to crayfish compared with organochlorines and organophosphates respectively. Results from the current study support these earlier findings and show the synthetic pyrethroid Deltamethrin (with a 48h LC_{50} of 0.86 $\mu g/l^{-1}$) to be more toxic to crayfish than the organophosphate Salmosan (48h LC_{50} of 1533 $\mu g/l^{-1}$).

Paul & Simonin (2006) found that other synthetic pyrethroids (Permethrin, Sumithrin and Resmethrin) were all highly toxic to the Calico crayfish *Orconectes immunis* (Hagen). Their study demonstrated that crayfish are among the most sensitive freshwater invertebrate species by 1-2 orders of magnitude to these pyrethroid insecticides. Quaglio *et al*. (2000) also observed the high levels of sensitivity by red swamp crayfish to the synthetic pyrethroid Cyfluthrin (24h LC_{50} of 0.13 $\mu g/l^{-1}$). The results of the present study show that both Deltamethrin and Pyblast are highly toxic to signal crayfish at very low concentrations (Deltamethrin: LC_{50} of 0.83 $\mu g/l^{-1}$ for adults (30-40mm); Pyblast: LC_{50} 104.8 $\mu g/l^{-1}$ for adults (40-50mm)). Adult crayfish showed the least sensitivity to Salmosan (48h LC_{50} of 1527 $\mu g/l^{-1}$).

The results obtained in this study are consistent with acute toxicity data from the literature showing that Deltamethrin is one of the most toxic to crayfish and as well as other crustaceans. Although it is not possible to directly compare all these studies because of different test conditions, results from the current study have shown that Pyblast, Salmosan and Deltamethrin vary in their toxicity to signal crayfish in the order of lethal threshold with Deltamethrin more toxic than Pyblast, which is more toxic than Salmosan. This finding is consistent with the data presented by Burridge *et al*. (2014) who, when comparing toxicity to non-target marine species and found Deltamethrin to be more toxic than Salmosan.

4.4 IMMOBILITY AND DELAYED EFFECTS

Death and immobilization is a major endpoint in almost all acute toxicity studies. As an end point, immobility can be the equivalent to ecological death because it immobilizes that organism making it unable to feed, seek shelter or avoid predation (Van Geest *et al*., 2014). From this study there greatest difference is seen between adult crayfish exposed to Deltamethrin where the 24h EC_{50} value is 25 times lower than the associated LC_{50}. The least difference was seen between 48h values in stage II hatchlings exposed to Pyblast with only 1.1 fold in the difference.

The lowest EC_{50} value from this study is 2.68ng/l^{-1} at 48h reported for stage I hatchlings exposed to Deltamethrin. Again Deltamethrin showing to be the most toxic to signal crayfish with regards LC_{50} and EC_{50} thresholds. Other studies (e.g. Fairchild *et al*., 2010; Van Geest *et al*., 2014) have found irreversible immobility to be a sensitive endpoint.

4.5 SUB-LETHAL EFFECTS

Any decrease in an organism's ability to obtain adequate nutrition is likely to have a detrimental effect on its growth, survival and reproduction capabilities. Crayfish exert energy on detoxification when exposed to pesticides in order to regain physiological balance (Greulich, 2003). This may cause delays in growth, development and mobility and lead to reduction in physiological fitness (Allran & Karasov, 2000; Bridges, 1997; Diana *et al*., 2000) but no data is available to allow an assessment of this for signal crayfish.

Induced aberrant behaviour such as loss of motor control activity (inability to control appendages) and loss of balance were present in the test and were present in crayfish that could not self-right and those that were immobile. During this study it was observed that the claws of the hatchlings and adults had become physically separated from the main body during exposure and recovery to Pyblast. While no information was found in the literature regarding the chronic effects of these chemicals on signal crayfish, similar events have been reported in lobsters. Fisher *et al.* (1976) reported that the appendages of lobster larvae were lost during short term exposure to high concentrations of malachite green. Mc Henery *et al.* (1991) also reported occasional loss of claws in larvae of the European lobster exposed to Dichlorvos. This phenomenon was observed by Burridge & Haya (1997) in American lobster larvae during the first 2h of exposure to pyrethrins at high concentrations. In the current study mortality did not appear to be associated with claw loss. Some crayfish survived after

losing the claws and mortality was seen in hatchlings which were both with, and without, claws. There is no published information on crayfish relating to the loss of appendages, as a side effect to pyrethroids.

Sub-lethal concentrations for these pyrethroids all elicited distinct behavioural abnormalities in the crayfish, ranging from self-righting at very low dosage to immobility and paralysis followed by death. Such observations may be useful under field conditions to identify the onset of crayfish toxicity.

Studies by Fornstrom *et al.* (1997) shown that juvenile red swamp crayfish lost the ability to continue feeding due to the loss of control of chelae when exposed to non-lethal doses of Terbufos, an organophosphorous insecticide. This loss of equilibrium and motor control activity may cause crayfish to be more susceptible to predation losing the ability to fight, escape or hide from predators, increasing mortality. It may also reduce their ability to feed, affect their hunting ability, food grasping or territory defence from other crayfish. Loss of major chelae in natural populations may decrease defences ability or foraging and feeding. Males will lose the ability to grasp females when mating and females will lose the ability to burrow and care for their young.

4.6 SEASONAL DIFFERENCES

Studies on American lobsters (Burridge *et al*., 2005) demonstrated significant seasonal differences in the toxicity of Salmosan. This suggests that some decapods are most sensitive during the spawning and moulting season. There may be a time of highest sensitivity of female sensitivity before spawning or moulting. This would indicate that further studies of this nature should be considered with Pyblast and any biocide considered suitable for use as a crayfish eradication tool.

The hatchling of eggs and development of hatchlings is temperature dependant, usually occur in May and June each year, a time when this most sensitive life stage is most abundant. Importantly, it indicates that targeting the most sensitive life stages signal crayfish (either hatchlings or moulting/berried female crayfish) may optimal results.

4.7 CHEMICAL ANALYSIS

Delivery of the appropriate concentration of biocide to the target organism requires a knowledge of the behaviour of that compound in the environment. An exposure assessment provides the essential information for establishing dose effect relationships that determine chemical toxicity to an organism (Landrum *et al.,* 1992). One purpose of this study was to determine the exposure bioavailability of Pyblast in water from estimated concentrations that were used to assess the toxicity of the biocide. Analyses of water samples from the beakers of indicated that all six compounds of Pyblast were present. Concentration at 0h is only 81% of the target concentration. A decrease in Pyblast concentrations exposure was to be expected. It revealed that over 50% of the pyrethroid was broken down over the course of the 48h. This decrease may be down to the breakdown of the pyrethroid or it binding to the glass walls of the test arena due to its hydrophobic nature or ingestion by the crayfish. This implies that the Pyblast concentrations decreased exponentially during the lethality tests. Further studies would be required to see whether the composition of the extract is consistent.

It can be inferred that the concentration of Pyblast is effectively reduced or removed from all three treatment concentrations from the very start of the exposure. However at 0h it can be seen the chemical concentration doesn't correspond to the desired calculated concentrations that were made for the tests with a 15-30% loss in concentration.

The levels of toxicity recorded within this study may be underestimated due to pyrethroids been lipophilic chemicals that are known to absorb to the walls of glass bioassay containers (Shariom & Soloman, 1981). While these data have no consequence for establishing lethal thresholds, these data are essential in terms of informing our understanding of how to deliver target concentrations in the environment from commercially obtained Pyblast. Similar results were observed in Deltamethrin (Burridge *et al.*, 2014). Pyblast never reached the target concentration, even in laboratory conditions, and the reason for this is unknown. This questions whether the desired concentrations were ever available at the start of any of the experiments. This may suggests that the estimated LC_{50}'s from this study and all other Pyblast toxicity studies may have been higher than intended. Further, this suggests that crayfish may be even more sensitive than the estimated LC_{50} values observed during this study. This must be taken into account when proposing which biocide concentration should be applied in the field.

4.8 ENVIRONMENTAL IMPACTS

The eradication trial programme for signal crayfish in Barmbyfield Reservoir using a treatment concentration of 0.18 mg/l^{-1} of Pyblast was found to be effective (Peay & Bryden, 2010). A similar study in the North Esk catchment (Peay *et al*. 2006) used a target dose of 0.15 mg/l^{-1} at 9°C and 0.2 mg/l^{-1} at 4°C to kill caged sentinal signal crayfish prior to wider application in the field. The acute toxicity of Pyblast from this study was approximately 82% at 24h and 52% at 48h of the 0.2 mg/l^{-1} dosage applied in the field. The concentration of Pyblast used in these studies was high when compared with the toxicity data obtained during the present study. If delivery of the biocide at an appropriate concentration could be achieved, this may ultimately lead to lower costs and less environmental impact for the same result.

While the LC_{50} values obtained in this study are valid indicators for potential eradication concentrations, a number of environmental factors such as pH (of the receiving water), temperature, sediment interaction mixing, presence of organic material, photolysis, isomerization and degradation sediment, air temperature, crayfish size and life stage would all be able to influence the toxicity of the pyrethroid.

There is evidence that physical factors can affect toxicity. Temperature can be a significant factor. It appears that exposure at lower temperatures result in greater toxicity with pyrethroids (NRCC, 1986, Hiley and Peay, 2003). Mayer & Ellersieck (1986) suggest that an increase in temperature of 10°C decreases the toxicity of most toxicants to fish by a factor of three (Eversole & Seller, 1997) suggest that this may not apply to crayfish.

Temperature for all experiments in this study were set to 12 ±1°c as this was the temperature of the water from the Clyde River where they were collected from during May and June 2013. Further work could be carried out to investigate temperature as a factor on the impact of Pyblast toxicity to crayfish. This would enable those responsible for applying the biocide to do so at a time of highest toxicity. This may allow lower volumes of the toxicant to be used, and result in significant cost and environmental benefits.

4.9 DILUTION AND ACHIEVING THE CORRECT DOSAGE

A consideration of use of these pesticides, and their direct application to water, provokes two questions. The first of these relates to the degradation characteristics of the active ingredients, and how local hydrographic conditions affect the dispersion and dilution of the pesticide? The second relates to the presence of sensitive or high conservation value species and the impact that the biocide may have on them?

The high cost of Pyblast is a significant issue and achieving the target dose requires a very good accurate estimate of the volume of water present. This, depending on the bathymetry of the receptor site, can be imprecise. Acquiring the correct dosage for any eradication treatment is crucial, not only is it economically expensive but it is ecologically damaging if the dosage is over estimated. If the dosage is underestimated, then there is the possibility that repeated applications may be required. If overestimated, non-target species are at a greater risk. The resource requirements and practical difficulties of an eradication treatment should not be taken lightly and sufficient time must go into treatment preparation, control and monitoring during treatment. It should also be remembered that post-treatment monitoring should be carried out for at least five years to establish that all crayfish have been eliminated.

4.10 APPLICATION OF THE BIOCIDE

Pesticide dispersal and partition into bottom sediments can impact benthic species. Non-target benthic organisms may be exposed to these pyrethroids via a number of breakdown phases including water, sediment, or ingestion of contaminated organic particles. Many biocide applications is through the use of sprayers over the surface of ponds and other standing waters. During previous trials, Pyblast was applied directly to the water via knapsacks sprayers (Peay and Hiley 2006). Peay and Hiley (2006) found spray treatment using knapsacks on the surface waters of ponds and shorelines most practical and economical delivery method.

Pyblast has also been applied using boat-mounted sprayers and these appear to have been effective during a recent eradication attempt in Ballachulish (Bean *pers. comm*.). Delivery of the biocide to the deeper areas of ponds and small lakes is much more difficult and no standard methodology exists. Peay and Hiley (2006) used bathymetric surveys of the treatment ponds to inform their estimate of the volume of biocide required. Using a plumb-

line at 100 sample points, the data was then used to divide the pond into zones of equal volume to ensure all parts of the pond attained Pyblast concentrations of at least 0.3 mg/l^{-1}. Generally, the treatment areas have been small waterbodies <3m in depth, meaning that the use of hydroacoustics to obtain accurate soundings is not appropriate.

Mixing through the water column was encouraged by using a boat equipped with an outboard motor to mixing up the pond after application and by the use of three shore-mounted pumps. The deepest was areas were reached by spraying Pyblast down 6m rigid hoses. The dispersal of Pyblast in the standing water may be effected if there are macrophytes present, preventing the Pyblast from reaching the bottom of the water column. Peay and Hiley (2006) suggest that prior removal of vegetation improves penetration of the surface spray. They also recommend that crayfish refuges should be flooded during treatment, meaning that careful attention should be given to maintaining water levels within the treatment site. It is still unclear whether Pyblast can diffuse into burrows at levels which remain toxic to crayfish within them. Nor is it known the rate at which the biocide binds to clays or sediments and becomes inactive.

4.11 DISPERSAL

The use of these formulations of pesticides dependents on many factors including site specific hydrographic conditions. Models of pesticide dispersion are currently been developed and used to predict the movement of the formulation and to predict the spatial and temporal distributions, including concentration profile. The break down rate and dispersal of Pyblast concentration within standing waters and the depth at which this occurs is unknown.

Ernst *et al.*, 2014 conducted a study on the dispersion of Salmosan and Deltamethrin from bath treatments. A dye, sodium fluorescein was added to the treatment solutions and a fluorometer was used to track the dispersal. Ernst *et al*., 2014 conclude that the measurement of dye in plume dispersion is a cost-effective. Pyblast use is restricted to standing waters. Testing Pyblast dispersal from the top down using florescent dye in a mesocosm or flume may be useful in determining how it spreads/disperses when applied to ponds and small lakes. It can also be a useful way of trialling delivery methods in this.

The residue left from biocides must be considered before using it to treat crayfish as it many enter food webs through biomagnification. The active molecules may persist in the environment and kill non-target species. As seen in this study, over 50% of the pyrethroid was broken down over course of the 48h exposure. The effect of Pyblast decreases over time and has been reported to breakdown after 48h of application in the field (Peay & Hiley 2006; Cecchinelli *et al*., 2012). The adsorption of pyrethroids on suspended solids can produce dramatic effects. For example, decrease in toxicity of cypermethrin when observed when exposed to rainbow trout containing suspended solids (Shires, 1983; Hill, 1985). Few studies have examined the toxicity of these pesticides in sediments to freshwater organisms despite sediment been a potential factor on their toxicity with the exception of (Muir *et al*., 1985). Observations by Ernst *et al*., 2014 found Salmosan was in aqueous phase by several orders and was consistent with the relatively low absorption characteristics of organophosphorus chemicals generally. Ernst *et al*., 2014 has suggested that aqueous toxicity is undoubtedly the highest risk for the pesticide Salmosan and sediment sequestration is probably not an important route of disappearance from the water column. This indicated that the exposure to treatments is primarily in the water column. Other dispersion studies have also suggested that Salmosan is unlikely to accumulate in sediment (SEPA 2005). The physical-chemicals characteristics of Salmosan suggest that it is unlikely that Salmosan would persist in water after just one treatment and non-target species would be exposed via water for a short period of time (Burridge *et al*., 2014).

Due to its high toxicity and rapid action, Deltamethrin could cause significant harm to ecosystems after direct application. Ernst *et al*. 2014 discovered it was three times higher in concentration in the particles phase then the water phase and also a reduced toxicity by 20 fold. A study by Westin *et al*., 2004 showed high toxicity of deltamethrin in freshwater sediment in toxicity tests with amphipods. This may represent a greater risk to particle ingesting organisms.

The half-life of Deltamethrin varies with between studies from hours to days in the water (Muir *et al*. 1985; Erstfeld, 1999). Burridge *et al*., 2014 found through the use of florescent dye, Deltamethrin has the potential to persist in aquatic environments while Salmosan remains in solution for up to 24h with little degradation or loss. This suggests that sediment-specific factors may also influence the differences in relative sensitivity observed between studies, but without measured concentrations in the other sediment studies it is difficult to speculate on this further.

4.12 NON-TARGET SPECIES

Deltamethrin is the most lipophilic active ingredients of the pesticide formulations tested in this study. This may suggest it could remain in the freshwater environment for some time. This coupled with the low lethal threshold suggests that this formulation could pose the greatest risk when used as a pesticide in particular where non-target are present (Burridge *et al*., 2014). Further studies of Pyblast, Deltamethrin and Salmosan incorporated into freshwater sediments are needed to determine the risk of these pesticides to non-target species and sediment dwellers.

Holdich *et al*. (1999) states that ecosystems can recover fairly rapidly from the toxic effects of pyrethroids and recolonisation of still waters and short stretches of river by natural flora and fauna can be expected. O' Brien *et al*. (2013), for example, found that common toad tadpoles and both larval and adult palmate newt *Lissotriton helveticus* (L.) re-established in a Pyblast-treated quarry pond after a period of four months. Similar re-colonisation events have been observed in relation to aquatic invertebrates (e.g. Peay *et al*., 2006).

The LC_{50} values of signal crayfish, as determined by the present study, may be higher than some non-target species. This means that it is inevitable that some non-target species will be impacted by the biocide in the event of further eradication exercises. In the case of rare or vulnerable species, there may be an opportunity to rescue at least some of the population likely to be affected. This is, however, dependent on the taxa involved and may not be achievable in all instances.

4.13 ALTERNATIVE CHEMICALS

The results from the current study suggest that lower concentrations of Pyblast may be suitable to eradicate or control signal crayfish in small standing waterbodies. Pyblast is more expensive than synthetic pyrethroids but has low mammalian and avian toxicity, breaks down quickly in sunlight and does not leave toxic residues (Peay *et al.* 2006). In areas where the risk of damage to non-target species is not an issue, and the water is not being used for another purpose (such as a potable water supply), then cheaper alternative biocides such as Deltamethrin (or even Salmosan) could be used.

If this was a possible option, data obtained from the present and published studies suggest that Deltamethrin would be the biocide of choice because synthetic pyrethroids have the greatest likelihood of eradicating crayfish (Holdich *et al*., 1999, Morolli *et al*., 2006). Bills & Marking (1988) found that synthetic pyrethroids are the most selective toxic biocide for crayfish. Both Salmosan and Deltamethrin are used as anti-sea lice chemotherapeutants in marine Atlantic salmon aquaculture units. Much is known about the toxicity of these biocides on invertebrates and their persistence in the environment. Previous studies on have shown the recommended dosage for use of pyrethroids on target invertebrates has a low impact on non-target species.

4.14 RECOMMENDATIONS/FUTURE WORK

The results obtained in this study confirm that the concentration found to be lethal to early life stages is well below that which has so far been used in field-based signal crayfish eradication attempts.

Further work with microcosm and mesocosm and/or field level studies would be desirable component of toxicity testing for these pyrethroids to access the ecosystem community level effects where additional stressor and biological interaction may alter a toxic effect not observed at the organism level found in the laboratory. It would also allow a complete evaluation of the short and long-term impact that these biocides have on crayfish treatment sites and the environment. Such data would maybe provide the means of developing more effective eradication programmes.

Although all of the biocides tested during this study were highly toxic to adult crayfish, environmental toxicity data is limited with relation to the impact of exposures within the sediments of freshwater habitats. Pyblast is the only biocide to be used in GB to eradicate signal crayfish and an accurate LC_{50} has now been established, additional laboratory testing should be carried out with a diverse assortment of non-target species with emphasis on the effects of these low concentrations with short-term and long-term exposures.

Eversole & Seller (1997) commented on problems of transferring laboratory derived acute toxicity value to the field, where conditions produce more unpredictable effects. They noted however that "*Acute LC_{50} values continue to be a mainstay in toxicity testing".* The outdoor toxicity tests with local water and substrates are valuable intermediate step between

laboratory tests in clean conditions and full-scale treatment. Factors operate in combination in the field, including the water temperature; the substrate, which vary among sites; the presence of vegetation; topography of habitat; the depth of water, the day length and light intensity during and after treatment. These all play a part in influencing the exposure of crayfish to the toxicant, its effectiveness and its persistence.

The LC_{50} values obtained from this study may be considered as a starting point for field trials to verify the real toxic effects on signal crayfish and the potential eradication of these invasive species.

References

Abrahamsson, S. A. A. (1973). Methods for restoration of crayfish waters in Europe – the development of an industry for production of young *Pacifastacus leniusculus*. *Freshwater Crayfish* **1**: 203–210.

Abrahamsson, S. A. A. and Goldman, C. R. (1970). Distribution, density and production of the crayfish *Pacifastacus leniusculus* Dana in Lake Tahoe, California-Nevada. *Oikos* **21**: 83– 91.

Achieng, A. P. (1990). The impact of the introduction of Nile perch, Lates niloticus (L.) on the fisheries of Lake Victoria. *Journal of Fish Biology* **37**: 17–23.

Adams, C. E., Lyle, A. A., Dodd, J. A., Bean, C. W., Winfield, I. J., Gowans, A. R. D., Stephen, A. and Maitland, P. S. (2014). Translocation as a conservation tool: case studies from rare freshwater fishes in Scotland. *The Glasgow Naturalist* **26**: 17-24.

Alderman, D. J. (1996) Geographical spread of bacterial and fungal diseases of crustaceans. In: Preventing the spread of aquatic animal diseases. Scientific and Technical Review, 15, June 1996. *Office International des Epizooties, Paris, France*, pp 603–632.

Alderman, D. J., Holdich D. M. and Reeve, I. (1990). Signal crayfish as vectors in crayfish plague in Britain. *Aquaculture* **86**: 3–6.

Allran, J. W., Karasov, W. H., (2000). Effects of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae exposed in the laboratory from post-hatch through metamorphosis. *Environmental Toxicology and Chemistry* **1**: 2850–2855.

Anderson, R. L. (1989). Toxicity of synthetic pyrethroids to freshwater invertebrates. *Environmental Toxicology and Chemistry* **8**: 403-401.

Appelberg M. & Odelström T. (1986). Habitat distribution, growth and abundance of the crayfish. *Astacus Astacus* L. in the littoral zone of four neutralized lakes. In: *The crayfish Astacus astacus L. in acid and neutralized environments* (Ed. M. Appelberg), pp. 1–35. D.Phil Thesis, Uppsala University, Sweden.

Aquiloni, L., Becciolini, A., Berti, R., Porciani, S., Trunfio, C. and Gherardi, F. (2009). Managing invasive crayfish: use of X-ray sterilisation of males. *Freshwater Biology* **54**: 1510–1519.

Aquiloni, L., Brusconi, S., Cecchinelli, E., Tricarico, E., Mazza, D., Paglianti, A. and Gherardi, F. (2010). Biological control of invasive populations of crayfish: the European eel (*Anguilla anguilla*) as a predator of Procambarus clarkii. *Biological Invasions* **12**: 3817–3824 DOI 10.1007/s10530-010-9774-z.

Axelsson, E., Nystrom, P., Sidenmark, J. and Bronmark, C. (1997). Crayfish predation on amphibian eggs and larvae. *Amphibia Reptilia* **18**: 217–228.

Baillie AC. (1985). The biochemical mode of action of insecticides. *Recent Advances in the Chemistry of Insect Control* **53**: 1–23.

Banha, F. and Anastácio P. M. (2013). Desiccation survival capacities of two invasive crayfish. *Knowledge and Management of Aquatic Ecosystems* **413**: 01.

Bean, C. W., Maitland, P. S. and Collen, P. (2006). Crayfish in Scotland: A Review of Current Status and Legislative Control. *Freshwater Crayfish* **15**: 220-228.

Belchier, M., Edsman, L., Sheehy, M. R. J. and Shelton, P. M. J. (1998). Estimating age and growth in long-lived temperate freshwater crayfish using lipofuschin. *Freshwater Biology* **39**: 439-446.

BirdLife International. (2000). Threatened Birds of the world. Lynx Editions and BirdLife International, Barcelona, Spain, and Cambridge, UK.

Bills, T. D. & Marking, L. (1988). Control of nuisance populations of Crayfish with traps and toxicants. *Progressive Fish Culturist* **50 (2)**: 103-106.

Blake, M. A. and Hart, P. J. B. (1995). The vulnerability of juvenile signal crayfish to perch and eel predation. *Freshwater Biology* **33**: 233–244.

Bridges, C. M., (1997). Tadpole swimming performance and activity affected by acute exposure to sublethal levels of carbaryl. *Environmental Toxicology and Chemistry* **16**: 1935–1939.

Brown V. M. (1986). Development of water quality criteria from toxicological data. In*: Water Quality Management: Ereshwater Ecotoxicology in Australia* (ed. B. T Hart) pp. 107-17. Water Studies Centre, Chisholm Institute of Technology, Melbourne.

Bubb, D. H., Thom T. J. and Lucas, M. C. (2004). Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwater Biology* **49**: 357–368.

Bubb, D. H., Thom, T. J. and Lucas, M. C. (2005). The within catchment invasion of the non-indigenous signal crayfish (Dana) in upland rivers. *Bulletin Francais de Peche et de Pisciculture* **376–377**: 665–673.

Bubb, D. H., O'Malley, O. J., Gooderham, A. C. and Lucas, M. C. (2009). Relative impacts of native and non- native crayfish on shelter use by an indigenous benthic fish. *Aquatic Conservation* **19**: 448–455.

Burridge, L. E. and Haya, K. (1997). The Lethality of pyrethrins to larvae and post-larvae of the American lobster (*Homarus americanus*). *Ecotoxicology and Environmental Safety* **38 (2)**: 150–154.

Burridge, L. E., Haya, K., Zitko, V. and Waddy, S. (1999). The lethality of Salmosan (azamethiphos) to American lobster (*Homarus americanus*) larvae, post-larvae, and adults. *Ecotoxicology and Environmental Safety* **43**: 165–169.

Burridge, L. E., Haya, K. and Waddy, S. L. (2005). Seasonal lethality of the organophosphate pesticide, azamethiphos to female American lobster (*Homarus americanus*). *Ecotoxicology and Environmental Safety* **60**: 277–281.

Burridge, L. E., Weis, J., Cabello, F., Pizarro, J. and Bostick, K. (2010). Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. *Aquaculture* **306**: 1–23.

Burridge, L. E., Lyons, M. C., Wong, D. K. H., MacKeigan, K. and VanGeest, J. L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax, Salmosan, and Interox Paramove™50 to lobster and shrimp. *Aquaculture* **420–421**:180–186.

Caraco, N. F., Cole, J. J., Raymond, P. A., Strayer, D. L., Pace, M. L., Findlay, S. E. G. and Fischer, D. T. (1997). Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. *Ecology* **78**: 588–602.

Capuzzo, J. M., Lancaster, B. A., and Sasaki, G. C. (1984). The effects of petroleum hydrocarbons on lipid metabolism and energetics of larval development and metamorphosis in the American lobster (*Homarus americanus* Milne Edwards). Marine Environmental Research **14**: 201–228.

CBD. (2002). Decision VI/23* of the Conference of the Parties to the Convention on Biological Diversity. Annex, footnote to the introduction.

Cecchinelli, E., Aquiloni, L., Maltagliati, G., Orioli, G., Tricarico, C. and Gherardi, F. (2012). Use of natural pyrethrum to control the red swamp crayfish *Procambarus clarkia* in a rural district in Italy. *Pest Management Science* **68**: 839-844.

Cerenius, L. and Söderhäll, K. (1992). Crayfish diseases and crayfish as vectors for important disease. *Finnish Fisheries Research* **14**: 125–133.

Chapman, J. C. (1995a). The role of ecotoxicity testing in assessing water quality. *Australian Journal of Ecology* **20**: 20–27.

Chapman P. M. (1995b). Bioassay testing for Australia as part of water quality assessment programmes. *Australian Journal of Ecology* **20**: 7–19.

Charles, H. and Dukes, J. S. (2007). Impact of Invasive Species on Ecosystems. *Biological Invasions, Ecology Studies* **193**: 217-237.

Chucholl, C. (2013). Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. *Biological Invasions* **15**: 125–141.

Clavero, M. and Garcia-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution*. **20**: 110.

Coats, J. R., Symonik, D. M., Bradbury, S. P., Dyer, S. D., Timson, L. K. and Atchison, G. J. (1989). Toxicology of Synthetic Pyrethroids in Aquatic Organisms: an Overview. *Environmental Toxicology and Chemistry* **8 (8)**: 671.

Cold, A. and Forbes, V. E. (2004). Consequences of a short pulse of pesticide exposure for survival and reproduction of *Gammarus pulex*. Aquatic Toxicology **67**: 287–299.

Corbett, J.R, Wright, K. W, Baillie, A. C. (1984). The Biochemical Mode of Action of Pesticides. 2nd edition. *Academic Press*: London, New York, Tokyo.

Crawford, L., Yeomans, W. E., and Adams, C. E. (2006). The impact of introduced signal crayfish *Pacifastacus leniusculus* on stream invertebrate communities. *Aquatic Conservation Marine and Freshwater Ecosystems* **16**: 611–621.

Creed, R. P. Jr. and Reed, J. M. (2004). Ecosystem engineering by crayfish in a headwater stream community. *Journal of the North American Benthological Society* **23**: 244–236.

DAISIE, 2009. Handbook of Alien Species in Europe, Springer, Dordrecht.

Dahl, J. (1998). The impact of vertebrate and invertebrate predators on a stream benthic community. *Oecologia* **117**: 217–226.

Dana, E. D., López-Santiago, J., García-De-Lomas J., García-Ocaña D.vM., Gámez V. and Ortega, F. (2010). Long-term management of the invasive *Pacifastacus leniusculus* (Dana, 1852) in a small mountain stream. *Aquatic Invasions* **5**: 317–322. doi:10.3391/ai.2010.5.3.10.

Davies, J. H. (1985). The pyrethroids: An historical introduction. In J.P. Leahey, ed., *The Pyrethroid Insecticides*. Taylor and Francis, London, U.K., pp. 1-41.

Defra (2008). The Invasive Non-Native Species Framework Strategy for Great Britain. Defra, London.

Diana, S.c G., Resetarits Jr, W. J., Schaeffer, D. J., Beckmen, K. B. and Beasley, V. R. (2000). Effects of atrazine on amphibian growth and survival in artificial aquatic communities. Environmental Toxicology and Chemistry **19**: 2961–2967.

Dorn, N. J. and Wojdak, J. M. (2004). The role of omnivorous crayfish in littoral communities. *Oecologia* **140**: 150–159.

Dunn, J. C., McClymont, H. E., Christmas, M. and Dunn, A. M. (2009). Competition and parasitism in the native white-clawed crayfish *Austropotamobius pallipes* (Lereboullet) and the invasive signal crayfish *Pacifastacus leniusculus* (Dana) in the UK. *Biological Invasions* **11**: 2, 315–324.

Edmonds, N. J., Riley, W. D. and Maxwell, D. L. (2011). Predation by *Pacifastacus leniusculus* on the intra-gravel embryos and emerging fry of *Salmo salar*. *Fisheries Management and Ecology* **18**: 521–524.

EEA, 2012. Invasive alien species indicators in Europe. A review of streamlining European biodiversity (SEBI) Indicator 10, EEA Technical report No 15/2012, European Environment Agency, Authors: Rabitsch, W., Essl, F., Genovesi, P., and Scalera, R.

Elser, J. J, Junge, C. and Goldman, C. R. (1994). Population structure and ecological effects of the crayfish *Pacifastacus leniusculus* in Castle Lake, California. *Great Basin Naturalist* **54**: 162-169.

Elliott, P., Aldridge, D. C., Moggridge, G. D. and Chipps, M. (2007). The increasing effects of zebra mussels on water installations in England. *Water and Environment Journal* **19**: 367–375.

Ernst, W., Doe, K., Cook, A., Burridge, L., Lalonde, B., Jackman, P., Aubé, J. G. and Page, F. (2014). Dispersion and toxicity to non-target crustaceans of azamethiphos and deltamethrin after sea lice treatments on farmed salmon, *Salmo salar*. *Aquaculture*. http://dx.doi. org/10.1016/j.aquaculture.2013.12.017.

Ermgassen, P. and Aldridge, D. (2011). Predation by the invasive American signal crayfish, *Pacifastacus leniusculus* Dana, on the invasive zebra mussel, *Dreissena polymorpha* Pallas: the potential for control and facilitation. *Hydrobiologia* **658**: 303-315.

Erstfeld, K. M. (1999). Environmental fate of synthetic pyrethroids during spray drift & field runoff treatments in aquatic microcosms. *Chemosphere* **39**: 1737-1769.

Etheridge, E. C., Bean, C. W. and Adams, C. E. (2011). Substrate specific vulnerability of Scottish powan (*Coregonus lavaretus*) ova to predation by invasive ruffe (*Gymnocephalus cernuus*). *Ecology of Freshwater Fish* **20**: 299-307.

Eversole, A. G. and Seller, B. C. (1997). Comparison of relative crayfish toxicity values. *Freshwater Crayfish* 11: 274-285.

Evins, C. (2004). Small animals in drinking-water distribution systems. In: Ainsworth, R., World Health Organisation (Ed.); *Safe Pipe Water: Managing Microbial Water Quality in Piped Distribution Systems*. IWA Publishing, London, 101-120.

Fairchild, W. L., Doe, K. D., Jackman, P. M., Arsenault, J. T., Aube, J. G., Losier, M. and Cook, A. M. (2010). Acute and chronic toxicity of two formulations of the pyrethroid pesticide deltamethrin to an amphipod, sand shrimp and lobster larvae. Canadian Technical Report of Fisheries and Aquatic Sciences **2876** (vi + 34 pp.).

Fallang, A., Denholm, I., Horsberg, T. E. and Williamson, M. S. (2005). Novel point mutation in the sodium channel gene of pyrethroid-resistant sea lice *Lepeophtheirus salmonis* (Crustacea: Copepoda). *Diseases of Aquatic Organisms* **65:** 129–136.

Finstad, A.G., Einum, S., Forseth, T. and Ugedal, O. (2007). Shelter availability affects behaviour, size-dependent and mean growth of juvenile Atlantic salmon. *Freshwater Biology* **52**: 1710–1718. doi:10.1111/j.1365-2427.2007.01799.x.

Findlay, J. D. S., Riley, W. D. and Lucas, M. C. (2014). Signal crayfish (*Pacifastacus leniusculus)* predation upon Atlantic salmon (*Salmo salar*) eggs. *Aquatic conservation: Marine and freshwater ecosystems*. DOI: 10.1002/aqc.2480.

Firkins, I. and Holdich, D. M. (1993) Thermal studies on three species of freshwater crayfish. *Freshwater crayfish* **9**: 241-248.

Fisher, W. S., Rosemark, T. R., and Shleser, R. A. (1976). Toxicity of malachite green to cultured American lobster larvae. *Aquaculture* **8**: 151-156.

Ford, M. J. (2002) Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology* **16**: 815–825.

Fornstrom, C. B., Landrum, P. F., Weisskopf, C. P. and La Point T. W. (1997). Effects of terbufos on juvenile red swamp crayfish (*Procambarus clarkii*): differential routes of exposure. *Environmental Toxicology and Chemistry* **16 (12)**: 2514-2520.

Freeman, M. A., Turnbull, J. F., Yeomans, W. E., Bean, C. W. (2010). Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. *Aquatic Conservation Marine Freshwater Ecosystem* **20**: 211–223.

Furst, M. (1977). Introduction of *Pacifastacus leniusculus* (Dana) into Sweden: methods, results and management. *Freshwater Crayfish* **3**: 229–247.

Gallardo, B. (2014). Europe's top10 invasive species: relative importance of climatic, habitat and socio-economic factors. *Ethology Ecology & Evolution* **26**, Nos. 2–3, 130–151.

Gamradt, S. C. and Kats, L. B. (1996). Effect of introduced crayfish and mosquitofish on California newts. *Conservation Biology* **10**: 1155–1162.

Geiger, W., Alcorlo, P., Baltanás, A. and Montes, C. (2005). Impact of an introduced crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions* **7**: 49–73.

Gergs, R., Grey, J. and Rothhaupt, K. O. (2011). Temporal variation in zebra mussel (*Dreissena polymorpha)* density structure the benthic food web and community composition on hard substrates in Lake Constance, Germany. *Biological Invasions* **13**, (12): 2727-2738.

Gherardi, F., Renai, B. and Corti, C. (2001). Crayfish predation on tadpoles: a comparison between a native (*Austropotamobius pallipes*) and an alien species (*Procambarus clarkii*). *Bulletin Francais de la Peche et de la Pisciculture* **361**: 659–668.

Gherardi, F. (2007). Understanding the impact of invasive crayfish, pp. 507–542. In: Gherardi F., Ed. Biological invaders in inland waters: profiles, distribution, and threats. A.A. Dordrecht, The Netherlands: *Springer*.

Gherardi, F. (2010). Invasive crayfish and freshwater fishes of the world. *Revue Scientifique et Technique* (International Office of Epizootics) **29** (2): 241–254.

Gherardi, F., Aquiloni, L., Diéguez-Uribeondo J. and Tricarico E. (2011). Managing invasive crayfish: is there a hope? *Aquatic Sciences* **73**: 185–200. doi:10.1007/s00027-011-0181-z.

Gladman, Z.F. (2012). Crayfish in Scotland. Unpublished PhD Thesis, University of Glasgow.

Gladman, Z., Adams, C. E., Bean, C. W., Sinclair, C. and Yeomans, W. E. (2009). Signal crayfish in Scotland, pp. 43–48. In: Brickland J. *et al*., Eds. Crayfish conservation in the British Isles. Proceedings of a conference held on 25th March 2009 in Leeds, UK. Leeds; British Waterways.

Gladman, Z. F., Yeomans, W. E, Adams, C. E., Bean C. W., McColl, D, Olszewska, J. P., McGillivray, C. W. and McCluskey, R. (2010). Detecting North American signal crayfish (*Pacifastacus leniusculus*) in riffles. *Aquatic Conservation: Marine and Freshwater Ecosystems* **20**: 588–594.

Gladman, Z. F., Adams, C. E., Bean, C. W., Long, J. and Yeomans, W. E. (2012) Investigating the threat of non-native North American signal crayfish (*Pacifastacus*

leniusculus) to salmonid redds. *Aquatic Conservation: Marine & Freshwater Ecosystems* **22**: 134-137.

Goddard, J. S. and Hogger, J. B. (1986). 'The current status and distribution of freshwater crayfish in Britain', *Field Studies* **6**: 383-396.

Goldman, C. R. and Rundquist, J. C. (1977). A comparative ecological study of the California crayfish, *Pacifastacus leniusculus* (Dana), from two subalpine lakes (Lake Tahoe and Lake Donner). *Freshwater Crayfish* **3**: 51–80.

Golterman, H. L. (1975). Physiological Limnology – An Approach to the Physiology of Lake Ecosystems. – Development in Water Science 23–489pp. Amsterdam-Oxford-New York: Elsevier Scientific Publishing Company.

Greulich1, K. and Pflugmacher, S. (2003). Differences in susceptibility of various life stages of amphibians to pesticide exposure. *Aquatic Toxicology* **65**: 329–336.

Greulich, K., Hoque, E. and Pflugmacher, S., (2002). Uptake, metabolism, and effects on detoxication enzymes of isoproturon in spawn and tadpoles of amphibians. *Ecotoxicology and Environmental Safety* **52**: 256–266.

Griffiths, S. W., Collen, P. and Armstrong, J. D. (2004). Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology* **65**: 436-447.

Gross, T. S., Arnold, B. S., Sepú lveda, M. S. and McDonald, K., (2002). Endocrine disrupting chemicals and endocrine active agents. Wildlife toxicology of organophosphorous and carbamate pesticides. *In*: Hoffman, D. J., Rattner, B. A., Burton, Jr., G. A., Cairns, Jr., J. (Eds.), *Handbook of Ecotoxicology*, second ed. Lewis Publishers, Boca Raton, FL, pp. 1033–1098 (Chapter 39).

Guan, R. Z. and Wiles P. R. (1996). Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a British lowland river. *Aquatic Living Resources* **9**: 265–272.

Guan, R. Z. and Wiles, P. R. (1997). Ecological impact of introduced crayfish on benthic fishes in a British lowland river. *Conservation Biology* **11** (3): 641-647.

Guan, R. Z. and Wiles, P. R. (1998). Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. *Aquaculture* **169**: 177-193.

Harrison, M. L., Hoover, T. M. and Richardson, J.S. (2006). Agonistic behaviours and movement in the signal crayfish *Pacifastacus leniusculus*: can dominance interactions influence crayfish size-class distributions in streams? *Canadian Journal of Zoology* **84**: 1495–1504.

Hänfling, B., Edwards, F., Gherardi F. (2011). Invasive alien Crustacea: dispersal, establishment, impact and control. *BioContro*l 56:573–595 DOI 10.1007/s10526-011-9380-8.

Hart, K. M. (1958). Pyrethrum in the disinfection of public water mains. *Pyrethrum Post* **4**: 8–12.

Harvey, G. L., Moorhouse, T. P., Clifford, N. J., Henshaw, A. J., Johnson, M. F., Macdonald, D. W., Reid, I. and Rice, S. P. (2011). Evaluating the role of invasive aquatic species as drivers of fine sediment-related river management problems: the case of the signal crayfish (*Pacifastacus leniusculus*). *Progress in Physical Geography* **35**: 517–533.

Harvey, G. L., Henshaw, A. J., Moorhouse, T. P., Clifford, N. J., Holah, H., Grey, J. and Macdonald, D. W. (2014). Invasive crayfish as drivers of fine sediment dynamics in rivers: field and laboratory evidence. Earth Surface Process and Landforms **39**: 259–271 DOI: 10.1002/esp.3486.

Haya, K. (1989). Toxicity of pyrethroid insecticides to fish. Environmental Toxicology and Chemistry **8**: 381–391.

Haya, K., Burridge, L. E., Davies, I. M. and Ervik, A. (2005). A review and assessment of environ- mental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. In: Hargrave, B. (Ed.), *Handbook of Environmental Chemistry*: Water Pollution, Part M **5**: 305–341.

Hector, A. and Bagchi, R. (2007). Biodiversity and ecosystem multifunctionality. *Nature* **448**: 188–190.

Hein, C. L, Vander Zanden, M. J, Magnuson, J. J. (2007). Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. *Freshwater Biology* **52**: 1134–1146.

Hiley, P. D. (2003). The slow quiet invasion of signal crayfish (*Pacifastacus leniusculus*) in England — prospects for the white-clawed crayfish (*Austropotamobius pallipes*). In Management and conservation of crayfish: Proceedings of a conference held in Nottingham on 7 November 2002, Holdich, D. M., Sibley, P. J. (eds). Environment Agency: Bristol; 127-138.

Hiley, P. D. and Peay. S. (2003). Signal Crayfish Eradication – Biocides Trial. Environment Agency Technical Report EIT34-01-022, Environment Agency, Bristol. 25 pp.

Hill, E. F. (2002). Wildlife toxicology of organophosphorous and carbamate pesticides. In: Hoffman, D. J., Rattner, B. A., Burton, Jr., G. A., Cairns, Jr., J. (Eds.), *Handbook of Ecotoxicology*, second ed. Lewis Publishers, Boca Raton, FL, pp. 281–339 (Chapter 12).

Hogger, J. B. (1986). Aspects of the introduction of signal crayfish *Pacifastacus leniusculus* (Dana), into the Southern United-Kingdom .1. Growth and Survival. *Aquaculture* **58**: 27-44.

Hogger, J. B. (1988). Ecology, population biology and behaviour. In: Holdich, D. M. and Lowery, R. S. (eds). *Freshwater crayfish: biology, management and exploitation*. Croom Helm, London, 114–144.

Holdich, D. M. (2002). Distribution of crayfish in Europe and some adjoining countries. *Bulletin Français de la Pêche et de la Pisciculture* **367**: 611-650.

Holdich, D. M. and Reeve, I. D. (1991).The distribution of freshwater crayfish in the British Isles with particular reference to crayfish plague, alien introductions and water quality. *Aquatic Conservation* **1, 2**:139–158.

Holdich, D. M. and Rogers, W. D. (1992). Crayfish populations in the British Isles, farming, legislation, conservation and management. *Finnish Fisheries Research* **14**: 23–32.

Holdich, D. M. and Domaniewski, J. C. J. (1995). Studies on a mixed population of the crayfish *Austropotamobius pallipes* and *Pacifastacus leniusculus* in England. *Freshwater Crayfish* **10**: 37–45.

Holdich, D. M. and Rogers, W.D. (1997). The white-clawed crayfish, *Austropotamobius pallipes*, in Great Britain and Ireland with particular reference to its conservation in Great Britain. *Bulletin Français de la Pêche et de la Pisciculture*, **347 (4)**: 597-616.

Holdich, D.M., and Pöckl, M. (2007). Invasive crustaceans in European inland waters. In: Gherardi, F.(ed.), Biological invaders in inland waters: profiles, distribution and threats, *Springer*, The Netherlands, 29–75.

Holdich D. M. and Sibley P. J. (2009). ICS and NICS in Britain in the 2000s, pp. 13–33. In: Brickland J. *et al*., Eds. Crayfish conservation in the British Isles. Proceedings of a conference held on 25th March 2009 in Leeds, UK.

Holdich, D. M., Rogers W. D. and Reader J. P. (1995). Crayfish conservation, Project Record 378/10/N&Y, National Rivers Authority, Bristol.

Holdich, D. M., Gydemo, R. and Rogers, W. D. (1999). A review of possible methods for controlling alien crayfish populations. In*:* Gherardi, F. and Holdich D. M. (eds.), Crayfish in Europe as alien species. How to make the best of a bad situation? A. A. Balkema, Rotterdam, 245-270.

Holdich D. M., Rogers W. D. and Reynolds J. D. (1999). Native and alien crayfish in the British Isles. In: Gherardi F. and Holdich D. M. (eds.), Crayfish in Europe as alien species, how to make the best of a bad situation? A. A. Balkema, Rotterdam, 221–235.

Holdich, D. M., Reynolds, J. D., Souty-Grosset, C. and Sibley, P. J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* **11**: 394-395.

Holdich, D. M., James, J., Jackson C. and Peay, S. (2014). The North American signal crayfish, with particular reference to its success as an invasive species in Great Britain. *Ethology Ecology & Evolution* **26**, 232-262.

Hudina, S., Galíc, N., Roessink, I. and Hock, K. (2011). Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. *Biological* Invasions **13**, 1791–1803.

Hulme, P. E. (2009) Trade, transport and trouble: managing invasive species pathways in the era of globalization. *Journal of Applied Ecology* **46**: 10–18.

Jackson, M. C., Jones, T., Milligan, M., Sheath, D., Taylor J., Ellis, A., England J. and Grey, J. (2014). Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshwater Biology* **59**: 1123–1135.

Jarboe, J. H., and Romaire, R. P. (1995). Responses of procambarid crayfish populations to permethrin applications in earthen ponds. *Bulletin of Environmental Contamination and Toxicology* **55**: 58- 64.

Johnson, M. W., Gentile, J. H. (1979). Acute toxicity of cadmium, copper, and mercury to larval American lobster *Homarus americanus*. *Bulletin of Environmental Contamination and Toxicology* **22**: 258–264.

Jolly, A. L., Avault J. W., Koonce, K. L. and Graves, J. B. (1978). Impact of permethrin to several aquatic animals. *Transactions of the American Fisheries Society*. **107(6)**: 825-827.

Kahn, N.Y., (1983). In: Miyamoto, J., Kearney, P.C. (Eds.), *Pesticide chemistry: human welfare and the environment.* Proceedings of the Fifth International Congress of Pesticide Chemistry, Kyoto, Japan, 1982. Permagon Press, Oxford, pp. 437–450.

Kettunen, M., Genovesi, P., Gollasch, S., Pagad, S., Starfinger, U., ten Brink, P. and Shine, C. (2009). Technical support to EU strategy on invasive species (IAS) — Assessment of the impacts of IAS in Europe and the EU, Final report for the European Commission, Institute for European Environmental Policy (IEEP), Brussels, Belgium.

Kirjavainen, J. and Westman, K. (1999). Natural history and development of the introduced signal crayfish, *Pacifastacus leniusculus*, in a small, isolated Finnish lake, from 1968 to 1993. *Aquatic Living Resources* **12**: 387–401.

Landrum, P. F., Lee II, H. and Lydy, M. J. (1992). Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environmental Toxicology and Chemistry* **11**: 1709–1725.

Laurent, P. J. (1988). *Austropotamobius pallipes* and *A. torrentium*, with observations on their interaction with other species in Europe. In: Holdich DM, Lowery RS (eds) Freshwater crayfish: biology, management and exploitation. Croom Helm (Chapman & Hall), London, pp 341–364.

Laurent, P. J. (1995). Eradication of unwanted crayfish species for astacological management purposes. *Freshwater crayfish*, 8, 121-133.

Leahey, J. P. (1979). The metabolism and environmental degradation of the pyrethroid insecticides. *Outlook on Agriculture* **10**: 135-142.

Lewis, S. D. (2002). *Pacifastacus.* In Holdich, D. M. (ed.), Biology of Freshwater Crayfish. Blackwell Science, Oxford: 511–540.

Lewis, S. D. and Horton, H. F. (1997). Life history and population dynamics of the signal crayfish, *Pacifastacus leniusculus*, in Lake Billy Chinook, Oregon. *Freshwater Crayfish* **11**: 34-53.

Lewis, A. and Morris, F. (2008). Report on a Major Stranding of Crayfish at Meldon, River Wansbeck, UK. *Crayfish News* **30 (4)**: 7-8.

Lignot, J. H., Charmantier, G. and Cochard, J. C. (1998). Effect of an organophosphate insecticide, fenitrothion, on survival, osmoregulation, and acetylccholinesterase activity in different life stages of two penaeid shrimps: *Penaeus stylirostris* and *Penaeus vannamei* (Crustacea, Decapoda). *Journal of Shellfish Research* **17**: 1251–1258.

Lindqvist, O.V. and Huner, J. V. (1999). Life history characteristics of crayfish: What makes them good colonizers? In: Gherardi, F. and Holdich, D. M. (eds.), Crayfish in Europe as alien species – how to make the best of a bad situation?, A.A. Balkema, Rotterdam, 23–30.

Lodge, D. M., Kershner, M. W., Aloi, J. E. and Covich, A. P. (1994). Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology* **75**: 1265–1281.

Lodge, D. M., Taylor, C. A., Holdich, D. M. and Skurdal, J. (2000). Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. *Fisheries* **25 (8)**: 7–20.

Lodge, D., Turner C., Jerde, C., Barnes, M., Chadderton, L, Egan, S. P, Feder, J. L, Mahon, A. R. and Pfrender, M. E. (2012) Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology* **11**: 2555–2558.

Longshaw, M. (2011). Diseases of crayfish: a review. *Journal of Invertebrate Pathology* **106**: 54–70.

Lozán, J. L. (2000). On the threat to the European crayfish: a contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). *Limnologica - Ecology and Management of Inland Waters* **30**: 156–161. doi:10.1016/S0075-9511(00)80010-9.

Mack, M. C. and D'Antonio, C. M. (1998). Impacts of biological invasions on disturbance regimes. *Trends In Ecology & Evolution* **13**: 195–198.

Maitland, P. S. (1996). The North American signal crayfish, *Pacifastacus leniusculus* (Dana), established in the wild in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems* **6(2)**: 107-110.

Maitland, P. S., East, K. and Morris, K. H. (1983). Ruffe *Gymnocephalus cernua* (L.), new to Scotland, in Loch Lomond. *Scottish Naturalist* **1983**: 7–9.

Maitland, P. S., Sinclair, C. and Doughty, C. R. 2001. The status of Freshwater Crayfish in Scotland in the year 2000. *Glasgow Naturalist,* **23 (6)**: 26-32.

Mason, J. C. (1974). Aquaculture potential of the freshwater crayfish, *(Pacifastacus*). I. studies during 1970. *Fisheries Research Board of Canada. Technical Report* 440.

Mason, J. C. (1975). Crayfish production in a small woodland stream. *Freshwater Crayfish* **2**: 449-479.

Mayer, F. L. and Ellersieck. M. R. (1986). *Manual of acute toxicity: interpretation and database for 410 chemicals and 88 species of freshwater animals*. U.S. Department of Interior Fish and Wildlife Resources Publication 160. Washington, D.C. 576 p.

McCarthy, T. K. and Fitzgerald, J. (1997). The occurrence of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) an introduced biofouling bivalve in Ireland. *Irish Naturalists' Journal* **25**: 413-16.

McCarthy, J. M., Hein, C. L., Olden, J. D. and Vander Zanden, M. J. (2006). Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology* **51**: 224-235.

McGinnity, P., Jennings, E., DeEyto, E., Allott, N., Samuelsson, P., Rogan, G., Whelan, K. and Cross, T. (2009). 'Impact of naturally spawning captive-bred Atlantic salmon on wild populations: depressed recruitment and increased risk of climate-mediated extinction'. *Proceedings of The Royal Society B-Biological Sciences* **276**: 3601-3610.

McGriff, D. (1983). The commercial fishery for *Pacifastacus leniusculus* in the Sacramento River-San Joaquin delta. *Freshwater Crayfish* **5**: 403-417.

McGriff, D. and Modin, J. (1983). *Thelohania contejeani* parasitism of the crayfish *Pacifastacus leniusculus* in California. *California Fish and Game* **69**: 178-183.

McHenry, J. G., Saward, D., and Seaton, D. D. (1991). Lethal and sublethal effects of the salmon de-lousing agent dichlorvos on the larvae of the lobster (*Homarus gammarus* L.) and herring (*Clupea harangus* L.). *Aquaculture* **98**, 331–347.

McLeese, D. W., Mecalfe, C. D., Zitko, V. (1980). Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp. *Bulletin of Environmental Contamination and Toxicology* **25**: 950–955.

McMahon, B. R. (2002). Physiological adaptations to environment. In: Holdich, D. M. (ed) *Biology of freshwater crayfish*. Blackwell Science Ltd. Oxford, United Kingdom pp 327-376.

Mkumbo, O. C. and Marshall, B. E. (2015). The Nile perch fishery of Lake Victoria: current status and management challenges. *Fisheries Management and Ecology*. DOI: 10.1111/fme.12084.

Minchin, D. and Moriaty, C. (1998). Distribution of the zebra mussel *Dreissena polymorpha* (Pallas) in Ireland. *Irish Naturalists' Journal* **26**: 38-42.

Moorhouse, T. P, Macdonald, D. W. (2010). Immigration rates of signal crayfish (*Pacifastacus leniusculus*) in response to manual control measures. *Freshwater Biology*. doi:10.1111/j.1365-2427.2010.02528.x

Moorhouse. T. P. and Macdonald, D. W. (2011b). The effect of removal by trapping on body condition in populations of signal crayfish. *Biological Conservation* **144**: 1826–1831.

Morolli, C., Quaglio, F., Della Rocca, G., Malvisi, J and Di Salvo, A. (2006). Evaluation of the toxicity of synthetic pyrethroids to red swamp crayfish (*Procambarus clarkii*, Girard 1852) and common carp (*Cyprinus carpio*, L. 1758). *Bulletin Français de la Pêche et de la Pisciculture* **380-381**: 1381-1394.

Moyer, G. R, Díaz-Ferguson, E., Hill, J. E. and Shea, C. (2014). Assessing Environmental DNA Detection in Controlled Lentic Systems. *PLoS ONE* **9(7)**: e103767. doi:10. 1371/journal.pone.0103767.

Muir, D. C., Rawn, G. P., Grift, N. P. (1985). Fate of the pyrethroid insecticide deltamethrin in small ponds: a mass balance study. *Journal of Agriculture and Food Chemistry* **33**: 603–609.

National Research Council of Canada. 1986. Pyrethroids: Their effects on aquatic and terrestrial eco-systems. NRCC/CNRC 24376. Ottawa, Ontario, Canada.

Newton, A. (2010). Geomorphology: Crayfish at work. *Nature Geoscience* **3**: 592.

Neveu, A. (2001a). Les poissons carnassiers locaux peuvent-ils contenir l'expansion des écrevisses étrangères introduites? Efficacité de 3espèces de poissons face a` 2 espèces d'écrevisses dans des conditions expérimentales. *Bulletin Français de la Pêche et de la Pisciculture* **361**: 683–704.

Nyström, P. (2002). Ecology. In: Holdich, D. M (ed) Biology of freshwater crayfish. Blackwell Science Ltd. Oxford, United Kingdom pp 192-23.

Nyström, P. and Strand, J. A. (1996). Grazing by a native and an exotic crayfish on aquatic macrophytes, *Freshwater Biology* **36**: 673–682.

Nyström, P. (1999). Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. In: Gherardi, F. and Holdich, D. M. (eds.), Crayfish in Europe as Alien Species: how to make the best of a bad situation, A.A. Balkema, Rotterdam, 63–85.

Nyström, P., Brönmark, C. and Granéli, W. (1999). Influence of an exotic and native crayfish species on a littoral benthic community. *Oikos* **85**: 545–53.

Nyström, P., Svensson, O., Lardner, B., Brönmark, C. and Granéli, W. (2001). The influence of multiple introduced predators on a littoral pond community. *Ecology* **82**: 1023–1039.

OECD (Organisation for Economic Co-operation and Development) Connolly (1987). *Guidelines for Testing of Chemicals.*

Olden, J. D., Leroy Poff, N., Douglas, M. R., Douglas, M. E. and Fausch, K. D. (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution* **19**: 18–24.

Olsson, K., Stenroth, P., Nystrom, P. and Graneli, W. (2009). Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology* **54**: 1731–1740.

O'Brien, D. C, Hall, J. E., O'Brien, C. T., Baum, D. and Ballantyne, L. (2013). Impact of a natural pyrethrin biocide on two amphibians, common toad *Bufo bufo* and palmate newt *Lissotriton helveticus*, in Highland, UK. *Conservation Evidence* **10**: 70-72.

O'Neill, C. R. J. (1997). Economic impact of zebra mussels – results of the 1995 National Zebra Mussel Information Clearinghouse study. *Great Lakes Research Review* **3**: 35–44.

Pace, M. L., Findlay, S. E. G. & Fischer, D. (1998). Effects of an invasive bivalve on the zooplankton community of the Hudson River. *Freshwater Biology* **39**: 103–116.

Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., Von Holle, B., Moyle, P. B., Byers, J. E. and Goldwasser, L. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* **1**: 3–19.

Paul, E. A. and Simonin, H. A (2006). Toxicity of Three Mosquito Insecticides to Crayfish. *Bulletin of Environmental Contamination and Toxicology* **76**: 614–621.

Pauli, B. D., Coulson, D. R. and Berrill, M. (1999). Sensitivity of amphibian embryos and tadpoles to MIMIC® 240LV insecticide following single or double exposures. *Environmental Toxicology and Chemistry* **18**: 2538–2544.

Pejchar, L. and Mooney, H. A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution* **24**: 497–504.

Peay, S. and Hiley, P. D. (2001). Eradication of alien crayfish. Phase II. Environment Agency Technical Report W1–037/TR1. Bristol: Environment Agency, 118 pp.

Peay, S. and Hiley, P. D. (2006) Biocide trial to eradicate signal crayfish in the North Esk catchment. Scottish Natural Heritage Commissioned Report No. 0122.

Peay, S. and Bryden, P. D. (2010). A Review of Crayfish Eradication or Control – Cost Implications. *Scottish Natural Heritage Commissioned Report*.

Peay, S. and Füreder, L. (2011). Two indigenous European crayfish under threat – how can we retain them in aquatic ecosystems for the future? *Knowledge and Management of Aquatic Ecosystems* **401**: 33. DOI: 10.1051/kmae/2011062.

Peay, S., Hiley, P. D., Collen, P. and Martin, I. (2006). Biocide treatment of ponds in Scotland to eradicate signal crayfish. *Bulletin Français de la Pêche et de la Pisciculture* **380-381**: 1363–1379.

Peay, S., Guthrie, N., Spees, J., Nilsson, E. and Bradley, P. (2009). The impact of signal crayfish (*Pacifastacus leniusculus*) on the recruitment of salmonid fish in a headwater stream in Yorkshire, England. *Knowledge and Management of Aquatic Ecosystems* **12**: 394-395.

Peay, S., Holdich, D. M. and Brickland, J. (2010). Risk assessments of non-indigenous crayfish in Great Britain. *Freshwater Crayfish* **17**: 109–122.

Peay, S., Dunn, A. M., Kunin, W. E., McKimm, R. and Harrod, C. (2014). A method test of the use of electric shock treatment to control invasive signal crayfish in streams. *Aquatic Conservation: Marine and Freshwater Ecosystems*. DOI: 10.1002/aqc.2541.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. and Tsomondo, T. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment* **84**: 1–20.

Pimentel, D., Zuniga, R. and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**: 273–288.

Quaglio, F., Malvisi, J., Maxia, M., Morolli, C., Della Rocca, G. and Di Salvo, A. Toxicity of synthetic pyrethroid Cyfluthrin to the red swamp crayfish (*Procambarus clarkii*). *Freshwater Crayfish* **13**: 431-436.

Ray, J. and Stevens, V. (1970). Using Baytex to control crayfish in ponds. *Progressive Fish-Culturist* **32**: 58-60.

Radford, A., Riddington, G., Anderson, J. and Gibson, H. (2004). The economic impact of game and coarse angling in Scotland. *Report commissioned by the Scottish Executive Environment and Rural Affairs Department*. 70 pp.

Rand. G. and Petrocelli S. (1995). *Fundamentals of Aquatic Toxicology*. Hemisphere, New York.

Rehfisch, M., Allan, J. and Austin, G. (2010). The effect on the environment of Great Britain's naturalized Greater Canada *Branta canadensis* and Egyptian Geese *Alopochen aegyptiacus. BOU Proceedings–The Impacts of Non-native Species.* http://www.bou.org.uk/bouproc-net/ non-natives/rehfisch-etal.pdf.

Reeve, I. D. (2001). The removal of the North American signal crayfish (*Pacifastacus leniusculus*) from the River Clyde. *Scottish Natural Heritage Commissioned Report* No. 020 (ROAME No. F00LI12).

Reynolds, J. D. (2002). Growth and reproduction. In: Holdich, D. M. (Ed.), *Biology of Freshwater Crayfish*. Blackwell Science Ltd., Oxford, pp. 152–191.

Reynolds, J. D., Demers, A. and Marnell, F. (2002). Managing an abundant crayfish resource for conservation – *A. pallipes* in Ireland. *Bulletin Français de la Pêche et de la Pisciculture* **367**: 823–832.

Reynolds, J. D. (2011). A review of ecological interactions between crayfish and fish, indigenous and introduced. *Knowledge and Management of Aquatic Ecosystems* **401**: 10.

Ribbens, J. C. H and Graham, J. L. (2004). Strategy for the containment and possible eradication of American signal crayfish (*Pacifastacus leniusculus*) in the River Dee catchment and Skyre Burn catchment, Dumfries and Galloway. *Scottish Natural Heritage, Commissioned Report* No. 014. (ROAME No. F02LK05).

Ricciardi, A. (2006). Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions* **12**: 425–433.

Ricciardi, A., Neves, R. J. and Rasmussen, J. B. (1998). Impending extinctions of North American freshwater mussels (*Unionida*) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology* **67**: 613–619.

Rettich, F. (1980). Field evaluation of permethrin and decamethrin against mosquito larvae and pupae (Diptera: Culicidae). Acta Entomologica Bohemoslovaca **77**:89-96.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ritz, C. and Streibig, J. C. (2005). Bioassay Analysis using R. *Journal of Statistical*. Software, **12**: 1-22.

Robertson, J. L. and Preisler, H. K. (1992). Pesticide Bioassays with Arthropods. *CRC Press*, Boca Raton, 127 pp.

Rosell, R. S., Maguire, C. M. and McCarthy, T. K. (1999). First reported settlement of zebra mussels *Dreissena polymorpha* in the Erne System, Co. Fermanagh, Northern Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy* **98B**: 191-3.

Rosell, R., Harrod, C., Griffiths, D. and McCarthy, T. K. (2004). Conservation of the Irish populations of the Pollan *Coregonus Autumnalis. Biology & Environment: Proceedings of the Royal Irish Academy* **104:** 67-72.

Rogers, W. D., Holdich, D.M., and Carter, E. (1997). Crayfish Eradication. Report for English Nature, Peterborough.

Rogers, D. and Watson, E. (2011a). Distribution database for crayfish in England and Wales, pp. 14–22. In: Rees, M., *et al*., Eds. Species Survival: securing white-clawed crayfish in a changing environment. Proceedings of a conference held on 16th and 17th November 2010 in Bristol, UK. Bristol: *Bristol Conservation & Science Foundation*.

Rogers, D. and Watson, E. (2011b). Captive breeding and reintroduction of white-clawed crayfish on the River Lathkill, Derbyshire, pp. 141–152. In: Rees, M. *et al*., Eds. Species

survival: securing white-clawed crayfish in a changing environment. Proceedings of a conference held on 16th and 17th November 2010 in Bristol, UK. Bristol: *Bristol Conservation & Science Foundation.*

Roy, H., Bacon J., Beckmann B., Harrower C. A., Hill M. O., Isaac N. J., Preston, C. D., Rathod, B., Rorke, S. L. (2012). *Non-Native Species in Great Britain: establishment, detection and reporting to inform effective decision making,* 110 pp. NERC Centre for Ecology and Hydrology, Wallingford, UK.

Rubin, J. F. and Svensson, M. (1993). Predation by the noble crayfish, *Astacus astacus* (L.), on emerging fry of sea trout, *Salmo trutta* (L.). *Nordic Journal of Freshwater Research* **68**: 100–104.

Sandodden, R. and Johnsen, S. I. (2010). Eradication of introduced signal crayfish *Pacifastacus leniusculus* using the pharmaceutical BETAMAX VET. *Aquatic Invasions* **5**: 75–81.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., Cauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N. and Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology, Evolution, and Systematics* **32**: 305–332.

Turner, M. J. and Schaeffer, J. M. (1989). Mode of action of ivermectin. In *Ivermectin and Abamectin*, Campell W (ed.). Springer- Verlag: Berlin; 73–88.

Scalera, R., (2010). How much is Europe spending on invasive alien species? *Biological Invasions* **12**: 173–177.

Scalera, R., Genovesi, P., Essl, F. and Rabitsch, W. (2012). The impact of invasive species in Europe. *EEA technical report* 2012 No 16/2012, European Environmental Agency.

Scottish Environmental Protection Agency (SEPA), 2005. See attachments in: Fish Farm Manual.http://www.sepa.org.uk/water/water_regulation/regimes/aquaculture/ marine_aquaculture/fish_farm_manual.aspx (Accessed Oct 28, 2014).

Sharom, M. S. and Solomon, K. R. (1981). Adsorption and desorption of permethrin and other pesticides on glass and plastic materials used in bioassay procedures. *Canadian Journal of Fisheries and Aquatic Sciences* **38**: 199-204.

Shires, S. W, (1983). The use of small enclosures to assess the toxic effects of Cypermethrin in fish under field conditions. *Pesticide Science* **14**: 475-480.

Shimizu, S. J. and Goldman, C. R. (1983). *Pacifastacus leniusculus* (Dana) production in the Sacramento River. *Freshwater Crayfish* **5**: 210-228.

Sibley, P. J. (2000). Signal crayfish management in the River Wreake catchment. In: Crayfish Conference Leeds, (eds Rogers, D. and Brickland, J.): pp. 95-107. Environment Agency, Leeds.

Sibley, P. J. (2003). The distribution of crayfish in Britain. In: Management and Conservation of Crayfish. Proceedings of a conference held on 7th November, 2002 (Holdich, D. M. and Sibley, P.J. Eds.), 64-72. Environment Agency, Bristol.

Sibley, P. J., Brickland, J. H. and Bywater, J. A. (2002a). Monitoring the distribution of crayfish in England and Wales. In: Knowledge-based mangement of European native crayfish. Crayfish special (eds Souty-Grosset, C. and Grandjean, F.). *Bulletin Français de la Pêche et de la Pisciculture* **367(4):** 833-844.

Sibley, P. J., Holdich, D. M. and Richman, N. (2011). Monitoring the global status of crayfish, with particular reference to the white-clawed crayfish, pp. 42–52. In: Rees, M. *et al*., Eds. Species survival: securing white-clawed crayfish in a changing environment. Proceedings of a conference held on 16th and 17th November 2010 in Bristol, UK. Bristol: Bristol Conservation & Science.

Sinclair, C. and Ribbens, J. (1999). The distribution of American signal crayfish (*Pacifastacus leniusculus*) in the Kirkcudbrightshire Dee (Dumfries and Galloway) and an assessment of the use of electrofishing as an eradication technique for crayfish populations. Scottish Natural Heritage, Commissioned Report F99AC601.

Sinclair, C. A. (2009). Fine scale mapping of signal crayfish distribution in Scotland. *Scottish Natural Heritage Commissioned Report*, Project 26686.

Simberloff, D., Martin J. L., Genovesi, P., Maris, V., Wardle, D. A, Aronson, J., Courchamp, F., Galil, B, García-Berthou, E., Pascal, M., Pyšek, P., Sousa. R., Tabacchi, E. and Montserrat Vilà. (2013). Impacts of biological invasions: what's what and the way forward. doi.org/10.1016/j.tree.2012.07.013 *Trends in Ecology & Evolution* Vol. 28, No. 1.

Skurdal, J. and Taugbøl, T., (2002). Astacus. In: *Biology of Freshwater Crayfish*. Ed.: Holdich, D. M. Blackwell Science Ltd, Oxford, 467-510.

Smith, T. M. and Stratton, G. W. (1986). Effects of synthetic pyrethroid insecticides on nontarget organisms. *Residue Rev* **97**: 93–120.

Souty-Grosset, C., Grandjean, F. and Gouin, N. (2004). Conservation and management of native crayfish populations. *Freshwater Crayfish* **14**: 1–20.

Söderbäck, B. (1994). Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia* **100**: 229-235.

Söderbäck, B. (1995). Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. *Freshwater Biology* **33**: 291–304.

Sprague, J. (1995). Factors that modify toxicity, In: Rand, G. (Ed.), *Fundamentals of Aquatic Toxicology*. Effects, Environmental Fate and Risk Assessment, 2nd ed. Taylor and Francis, London, pp. 1012–1013.

Stancliffe-Vaughan, A. (2009). Non-native crayfish – a community research and trapping initiative on the River Lark, Suffolk. *Crayfish News: IAA Newsletter* **31, 2**: 5–7.

Statzner, B. and Sagnes, P. (2008). Crayfish and fish as bioturbators of streambed sediments: Assessing joint effects of species with different mechanistic abilities. *Geomorphology* **93**: 267–287.

Strayer, D. L., Caraco, N F., Cole, J. J., Findlay, S. and Pace, M. L. (1999). Transformation of freshwater ecosystems by bivalves – a case study of zebra mussels in the Hudson River. *Bioscience* **49**: 19–27.

Stebbing, P., Longshaw, M. and Scott, A. (2014). Review of methods for the management of non-indigenous crayfish, with particular reference to Great Britain, *Ethology Ecology & Evolution*, **26 (2-3)**: 204-231, DOI: 10.1080/03949370.2014.908326.

Stenroth, P. and Nyström, P. (2003). Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. *Freshwater Biology* **48**: 466–475.

Stephenson, R. R. (1982). WL 85871 and cypermethrin: a comparison of their acute toxicity to *Salmo gaidneri*, *Daphnia magna* and *Selenastrum capricornutum*. Sittingbourne, *Shell Research* (SBGR 81.277).

Svärdson, G. (1995). The early history of signal crayfish introduction into Europe. *Freshwater Crayfish* **8**: 68-77.

Taylor, C. A., Schuster, G. A., Cooper, J. E., DiStephano, R. J., Eversole, A. G., Hamr, P., Hobbs, H. H. Jr., Robinson, H. W., Skelton, C.E. and Thoma, R. F. (2007). A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries* **32 (8)**: 372–389.

Thomas, W. J. (1992). A note on the Crayfish of Loch Criospol. *Glasgow Naturalist* **22**: 102-109.

US EPA (2002). Short-term methods for estimating chronic toxicity of effluents and receiving water to freshwater organisms. 4th edn, EPA-821-R-02-013, US Environmental Protection Agency, Office of Water, Washington DC.

Usio, N., Kamiyama, R., Saji, A. and Takamua, N. (2009). Size-dependent impacts of invasive alien crayfish on a littoral marsh community. Biological Conservation **142 (7)**: 1480–1490.

Van Geest, J. L., Burridge, L. E., Kidd, K. A. (2014). Toxicity of two pyrethroid-based anti-sea lice pesticides, AlphaMax and Excis®, to a marine amphipod in aqueous and sediment exposures. *Aquaculture* **434**: 233–240.

Van Wijngaarden, R. P. A., Brock, T. C. M., Van den Brink, P. J. (2005) Threshold levels for effects of insecticides in freshwater ecosystems: A review. *Ecotoxicology* **14**: 355-380.

Vander Zanden, M. J., Hansen, G. J. A., Higgins, S. N., Kornis, M. S. (2010). A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research* **36**: 199–205.

Vié, J.C., Hilton-Taylor, C. and Stuart, S. N. (2009). Wildlife in a changing world: An analysis of the 2008 IUCN Red List Of Threaten Species.

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D., Hulme, P.E. and DAISIE partners (2010). 'How well do we understand the impacts of alien species on ecosystem services? A pan-European cross-taxa assessment?' *Frontiers in Ecology and the Environment* **8**: 135–144.

Vila, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarosik, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y. and Pyšek, P. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* **14**: 702–708.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Reidy Liermann, C. and Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature* **467**: 555-561.

Warner, G. F., Wood, J. C. and Orr-Ewing, R. H. (1995). Signal crayfish (*Pacifastacus leniusculus*) feeding on pond snails: optimal foraging? *Freshwater Crayfish* **8**: 352–359.

Warner, G. F. (1996). Factors affecting the selection of pond snail prey by signal crayfish. *Freshwater Crayfish* **11**: 194–202.

Wells, P. G. (1972). Influence of Venezuelan crude oil on lobster larvae. *Marine Pollution Bulletin* **3**: 105-106.

Wells, P. G. and Sprague, J. B. (1976). Effects of crude oil on American lobster (*Homarus americanus*) larvae in the laboratory. *Journal of the Fisheries Research Board of* Canada **33**: 1604–1614.

West, R. J. (2010). Non-native crayfish: a new approach to trapping, a review of signal crayfish trapping on the River Lark at Barton Mills, Suffolk 2001–2009, 14 pp. In: West R.J., Ed. Lark angling and preservation society reports. Suffolk: Lark Angling and Preservation Society.

West, R. J. (2011). A review of signal crayfish trapping on the River Lark at Barton Mills, Suffolk, from 2001 to 2009. Suffolk UK: Lark Angling & Preservation Society.

Westman, K., Sumari, O. and Pursiainen, M. (1978). Electric fishing in sampling crayfish. *Freshwater Crayfish* **4**: 251–255.

Westman, K., Savolainen, R. and Pursianen, M. (1993). A comparative study on the reproduction of the noble crayfish *Astacus astacus* L and the signal crayfish *Pacifastacus leniusculus* (Dana) in a small forest lake in southern Finland. *Freshwater Crayfish* **9**: 466-476.

Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R. H., Varia, S., Lamontagne-Godwin, J., Thomas, S. E. and Murphy, S. T. (2010). The economic cost of invasive non-native species to Great Britain, CABI. org CABI Project No. VM10066.

Young-Lai, W. W., Charmantier-Daures, M., and Charmantier, G. (1991). Effect of ammonia on survival and osmoregulation in different life stages of the lobster *Homarus americanus*. Marine Biology **110**: 293–300.

Youngson, A., Jordan, W. C., Verspoor, E., McGinnity, P., Cross, T. and Ferguson, A. (2003). Management of salmonid fisheries in the British Isles: towards a practical approach based on population genetics. *Fisheries Research* **62**: 193-209.

Zu Ermgassen, P. S. E. and Aldridge, D. C. (2011). Predation by the invasive American signal crayfish, *Pacifastacus leniusculus* (Dana), on the invasive zebra mussel, *Dreissena polymorpha* (Pallas): the potential for control and facilitation. *Hydrobiologia* **658**: 303–315.

Zhang, Y., Richardson, J. S. and Negishi, J. N. (2004). Detritus processing, ecosystem engineering and benthic diversity: a test of predator-omnivore interference. *Journal of Animal Ecology* **73**: 756–766.

http://www.europe-aliens.org

https://data.nbn.org.uk/Taxa/NHMSYS0000377494/Grid_Map

Appendix A

Sequence of dilutions carried out to obtain test solutions for signal crayfish and lists of toxicity range tests

Below is the sequence of dilutions carried out to obtain test solutions for hatchlings and adults and a table showing a list of range finding tests carried out on hatchlings stage I and the type of treatments used.

The dissolution series of Pyblast in water on stage I hatchlings is shown in the table A.1 below. From the commercial stock solution (3% = 30 g/l^{-1}) Pyblast, a solution of 30 mg/l^{-1} was made by pipetting 250 µl of commercial stock into a 250 ml volumetric flask and topping up with dilution water to almost 250 ml. A stir bar was added and set on a stirring table for 30 min. The stir bar was then removed and the volume of the solution was brought to 250 ml with water. 8.3 ml of the 30 mg/l^{-1} solution was dispensed into a 250 ml volumetric flask and topped up to 250 ml with water to obtain a solution of 1 mg/l^{-1}. The exposure concentrations (1000, 316, 100, 31.6, 10, 3.16, 0, 0(solvent) $\mu g/l^{-1}$) were obtained as dilutions of this solution, as explained in the below table A.1. Of each dilution, 100 ml were prepared in order to provide exposure solutions for duplicate 50 ml beakers. Untreated controls, receiving 50 ml of dilution water, and solvent control, receiving 50 µl ethanol per 50 ml of dilution water, were also run in duplicate.

Table A.1 Dilution series protocol for Pyblast in water used in first range test on stage I hatchlings.

Exposure concentration	1 mg/l^{-1} solution (ml)	Water (ml)
1000 $\mu g/l^{-1}$	100	0
316 $\mu g/l^{-1}$	34.7	75.3 (110 ml in total with 10% to be used on lower conc)
100 $\mu g/l^{-1}$	11	99 (110 ml in total with 10% to be used on lower conc)
31.6 $\mu g/l^{-1}$	10 ml of 316 mg/l^{-1} solution	90
10 $\mu g/l^{-1}$	10 ml of 100 mg/l^{-1} solution	90
3.16 $\mu g/l^{-1}$	10 ml of 31.6 mg/l^{-1} solution	90

The dissolution series of Pyblast in ethanol on stage I hatchlings (ethanol present in exposure experiments at 0.1%) is shown in the table below. From the commercial solution (3% = 30 g/l^{-1}), an ethanolic solution of 1 g/l^{-1} was made by pipetting 833 µl of commercial stock into a 25 ml volumetric flask and topping up to 25 ml with absolute ethanol. The flask was then closed with stopper and shaken well to mix. To obtain the desired exposure concentrations (1000, 316, 100, 31.6, 10, 3.16 $\mu g/l^{-1}$), 1000x concentrated ethanolic solutions were prepared.

Table A.2 Dilution series protocol for ethanolic solution of Pyblast used in the first range test.

Exposure concentration	**Concentration of 1000x ethanolic solution**	**µl ethanolic solution (1g/l⁻¹ = 1000µg/ml⁻¹)**	**µl ethanol**
1000 $\mu g/l^{-1}$	1000 $\mu g/ml^{-1}$	1000	0
316 $\mu g/l^{-1}$	316 $\mu g/ml^{-1}$	316	684
100 $\mu g/l^{-1}$	100 $\mu g/ml^{-1}$	100 µl ethanolic solution of 1000 $\mu g/ml^{-1}$	900
31.6 $\mu g/l^{-1}$	31.6 $\mu g/ml^{-1}$	100 µl ethanolic solution of 316 $\mu g/ml^{-1}$	900
10 $\mu g/l^{-1}$	10 $\mu g/ml^{-1}$	20 µl ethanolic solution of 1000 $\mu g/ml^{-1}$	1980
3.16 $\mu g/l^{-1}$	3.16 $\mu g/ml^{-1}$	20 µl ethanolic solution of 316 $\mu g/ml^{-1}$	1980

A third treatment was added to the range testing study by adding 50 µg ethanol to certain Pyblast/water dilution treatments. This addition (treated the same as the first treatment with ethanol added once the concentrations) was made so in theory it was a combination of ethanol and Pyblast. Results would show if a higher response (death) in the third treatment is the same as the ethanol treatment. If the higher response in the third treatment is due to the less of the loss of Pyblast form the process of dilution, results should be the same as the water treatment in the first treatment.

Table A.3 List of Pyblast range finding tests carried out on hatchlings stage I and treatments used.

Range tests	Number and type of treatments	Pyblast concentrations used	Controls used
1st	2x both ethanol and diluted water treatments	3.16, 10, 31.6, 100, 316, and 1000 µg/l^{-1}	0 µg/l^{-1} and 0 µg/l^{-1} (solvent)
2nd	1x Pyblast/Water dilution	0, 5, 10, 20, 40 and 80 µg/l^{-1}	0 µg/l^{-1}
	1x Pyblast/Ethanol dilution	0, 2.5, 5, 10, 20 and 40 µg/l^{-1}	0 µg/l^{-1}(solvent)
	1x Ethanol added to two concentrations of Pyblast/water dilutions	10 and 20 µg/l^{-1}	0 µg/l^{-1}
3rd	2x Pyblast/Ethanol and Pyblast/water dilution	0, 1.25, 2.5, 5, 10 and 20 µg/l^{-1}	0 µg/l^{-1} and 0 µg/l^{-1} (solvent)
	1x Ethanol added to two concentrations of Pyblast/water dilutions	2.5 and 5 µg/l^{-1}	0 µg/l^{-1}

A range finding test was carried out using stage I hatchlings (aged 29 days). The generation of exposure solutions and dissolution series of Salmosan powder in water (no solvent used) on is shown in table A.4 below. Five concentrations and one control was used, 0, 0.316, 1.0, 3.16, 10, 31.6 µg/l^{-1} with five hatchlings per concentration and aerated for 96h. The sequence of dilutions carried out to obtain the exposure concentrations are in the table below. From the commercial stock 20 g (50% = 10 g active), a solution of 200 mg/l^{-1} active compound was made. 100 mg powder of commercial stock was weighted and placed into a 250 ml volumetric flask and topped up to almost 250 ml water. A stir bar was added and set on a stirring table for 30 min. It was then removed and topped up to 250 ml.

To obtain a solution of 1 mg/l^{-1} active compound, 1.25 ml of the 200 mg/l^{-1} solution was dispensed into a 250 ml volumetric flask and topped up to 250 ml with water. The exposure concentrations were obtained as dilutions of this solution.

Table A.4 Dilution series protocol of Salmosan powder in water (no solvent used) used in the first range test on stage I hatchlings.

Exposure concentration	1 mg/l^{-1} solution (ml)	Water (ml)
31.6 µg/l^{-1}	3.16 ml	46.84 ml
10 µg/l^{-1}	1 ml	49 ml
3.16 µg/l^{-1}	316 µl	49.684 ml
1 µg/l^{-1}	100 µl	49.9 ml
0.316 µg/l^{-1}	31 µl	49.969 ml
Control	0	50 ml

The same protocol was used in the adult acute toxicity trials however it was scaled up to a 400 mg/l^{-1} solution using 400 mg powder due to the larger 2L exposure vessels. Two crayfish, one male and one female were used per concentration for all range tests and only a water control was used. Concentrations were scaled up based on the results of previous concentrations. The number of range tests and the concentrations (mg/l^{-1}) of Salmosan used in the test is listed in table A.5.

The dissolution series of Salmosan powder in water on adults is shown in the tables A.6, A.7 and A.8 below. From the commercial stock (50% active), make a solution of 200 mg/l^{-1} active compound. Weigh 100mg powder of commercial stock and place into a 250ml volumetric flask and topping up to almost 250ml water. Add a stir bar and set on a stirring table for 30mins. Remove stir bar and top up to 250ml.

To obtain a solution 10 mg/l^{-1} active compound, dispense 12.5ml of the 200 mg/l^{-1} solution into a 250ml volumetric flask and top up to 250ml with water. The exposure concentrations are obtained as dilutions of this solution. Gilson pipettes (1ml & 5ml) and volumetric cylinders were used to measure out the volumes required.

Table A.5 Range test number and concentrations (mg/l^{-1}) of Salmosan used in each test.

Range test	Concentrations used (mg/l^{-1})
1	0, 0.1, 0.316, 1.0, 3.16, 10
2	0, 1.0, 3.16, 10, 31.6, 100
3	0, 2, 6.32, 20, 63.2, 200

Table A.6 Protocol used for the dilution of Salmosan with water on adult crayfish 20-30mm in first range test.

Exposure concentration	1 mg/l⁻¹ solution (ml)	Water (ml)
10 µg/l⁻¹	50	450
3.16 µg/l⁻¹	15.8	484.2
1 µg/l⁻¹	5	495
0.316 µg/l⁻¹	1.58	498.4
0.1µg/l⁻¹	0.5/500µl	499.5
Control		500

Table A.7 Dilution series protocol of Salmosan powder in water (no solvent used) used on adult crayfish 20-30mm in second range test.

Exposure concentration	1 mg/l⁻¹ solution (ml)	Water (ml)
100 µg/l⁻¹	500	0
31.6 µg/l⁻¹	158	342
10 µg/l⁻¹	50	450
3.16 µg/l⁻¹	15.8	484.2
1µg/l⁻¹	5	495
Control		500

Table A.8 Protocol used for the dilution of Salmosan with water on adult crayfish 20-30mm in third range test.

Exposure concentration	1 mg/l⁻¹ solution (ml)	Water (ml)
200 µg/l⁻¹	500	0
63.2µg/l⁻¹	158	342
20 µg/l⁻¹	50	450
6.32 µg/l⁻¹	15.8	484.2
2µg/l⁻¹	5	495
Control		500

The dissolution series of Deltamethrin in ethanol on stage I hatchlings (ethanol present in exposure experiments at 0.1%) is shown in table A.9 below. From the commercial stock solution (50% = 10 g/l⁻¹), an ethanolic solution of 100 µg/ml⁻¹ was made by pipetting 250 µl of commercial stock into a 25 ml volumetric flask and topping up to almost 25 ml with absolute ethanol. The flask was closed with stopper and shaken well to mix.

1000 µl of the 100 µg/mL solution was then pipetted into a 2 ml vial. The vial closed and shaken well to mix. The desired exposure concentrations (0, 0(solvent), 0.1, 0.316, 1.0, 3.16, 10 $\mu g/l^{-1}$) were obtained as serial dilutions of this solution. Controls were untreated controls (50 ml water 2x50 ml) and solvent controls with ethanol 0.1%. 50 µl placed in 50 ml water per beaker.

A series of range finding tests were carried using Deltamethrin on stage I hatchlings (29 days old). For the first trail, five concentrations and two controls were used, 0, 0(solvent), 0.1, 0.316, 1.0, 3.16, 10 $\mu g/l^{-1}$. No aeration supplied to the hatchlings. A sequence of dilutions carried out to obtain 1000x concentrated ethanolic solutions (see table A.9). Note all hatchlings died within one hour in these concentrations so the trial was terminated. For the second trail, five concentrations and two controls were used, 0, 0(solvent), 0.00316, 0.01, 0.0316, 0.1, 0.316 $\mu g/l^{-1}$ with appropriate ethanolic solution made and aerated for 96h.

Table A.9 Protocol used for the dilution of Deltamethrin with ethanol (0.1%) in first range test on stage I hatchlings.

Exposure concentration	**Concentration of 1000x ethanolic solution**	**µl ethanolic solution ($1g/l^{-1}$ = $100\mu g/ml^{-1}$)**	**µl ethanol**
10 $\mu g/l^{-1}$	10 $\mu g/ml^{-1}$	100	900
3.16 $\mu g/l^{-1}$	3.16 $\mu g/ml^{-1}$	31.6	968.4
1 $\mu g/l^{-1}$	1 $\mu g/ml^{-1}$	100 µl ethanolic solution of 10 $\mu g/ml^{-1}$	900
0.316 $\mu g/l^{-1}$	0.316 $\mu g/ml^{-1}$	31.6 µl ethanolic solution of 10 $\mu g/ml^{-1}$	968.4
0.1 $\mu g/l^{-1}$	0.1 $\mu g/ml^{-1}$	10 µl ethanolic solution of 10 $\mu g/ml^{-1}$	990

For adult toxicity trial, the range test was carried out on one male and one female for each concentration. The test used concentrations were 0(solvent), 0.01, 0.0316, 0.1, 0.316, 1.0 $\mu g/l^{-1}$. 1000x concentrated ethanolic solutions were prepared in 10 ml glass vials and 500 µl of the appropriate ethanolic solution was pipetted into 500 ml of water to get the exposure solution. The lower concentrations were removed from this after the trail and additional concentrations were added for the second trial. The second definitive test concentrations were 0 (solvent), 0.1, 0.316, 0.562, 1.0, 1.78 $\mu g/l^{-1}$ were derived from the interval of the dosage range (0-1 µg/l) in the range tests that were carried out giving 0-100% mortality.

Appendix B

Observation pictures during toxicity tests

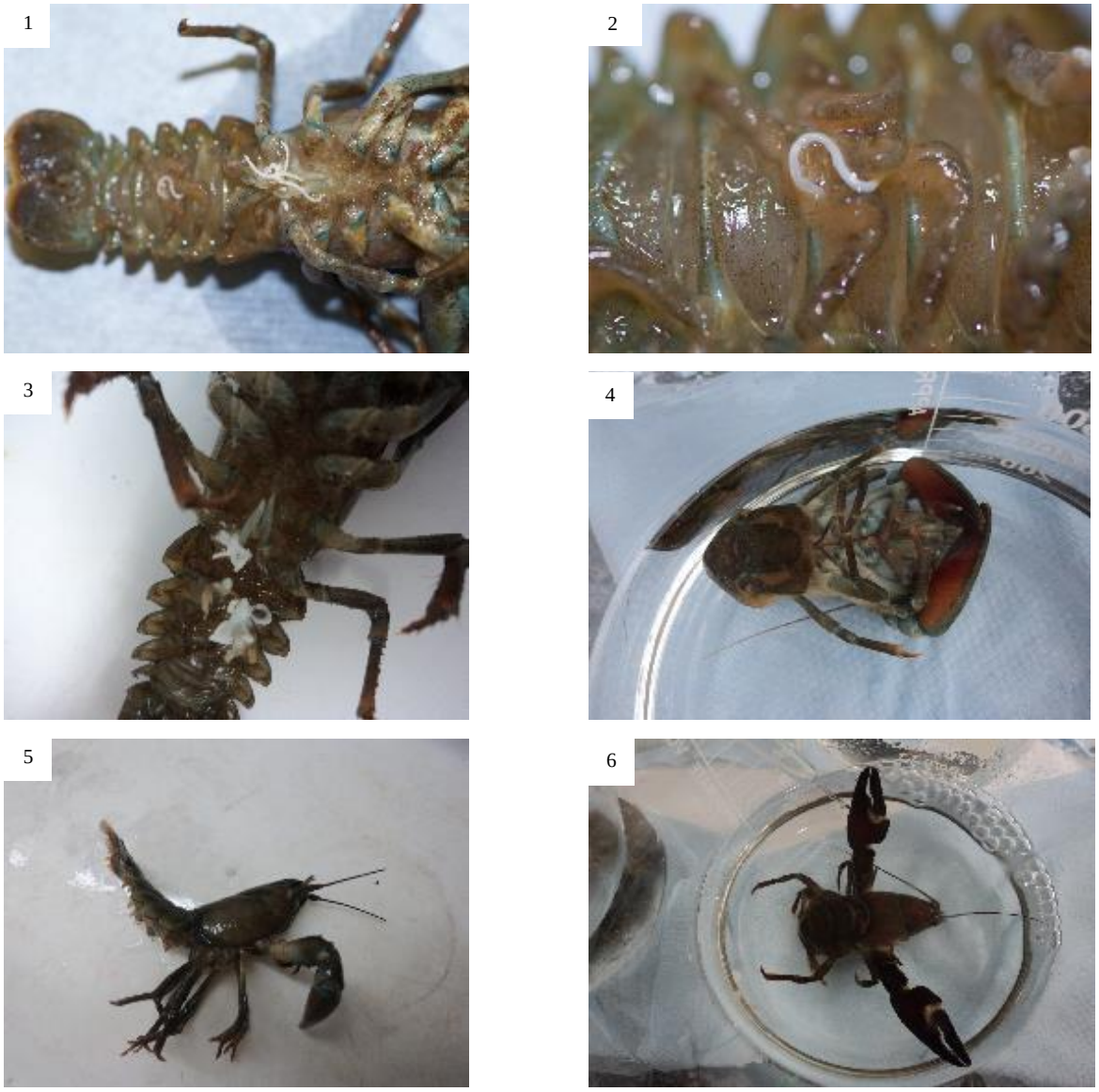


Figure B.1 Observation pictures of unknown white calcified substance secreted by crayfish around the swimmerets (1-3) and some behavioural responses (4-6) observed during Pyblast toxicity trials.

Appendix C

Comparison of LL.2 Model and one way ANOVA in all LC_{50} and EC_{50} toxicity analysis

Table C.1 Model fit values when the model used was compared against one way ANOVA to assess its effectiveness.

Life stage	Dose response	Model used	X^2	*df*	*p*
Stage I families	24h LC_{50}	2	35.101	19	0.01
Stage I families	48h LC_{50}	1	20.005	15	0.1698
Stage I families	24h EC_{50}	2	28.68	19	0.07
Stage I families	48h EC_{50}	1	18.963	15	0.2154
Stage I hatchlings	24h vs 48h LC_{50}	2	174.66	57	0
Stage I hatchlings	24h vs 48h EC_{50}	2	175.76	57	0
Stage II hatchlings	24h vs 48h LC_{50}	2	40.122	33	0.1837
Stage II hatchlings	24h vs 48h EC_{50}	2	38.041	33	0.2506
Stage I vs II	24h LC_{50}	2	112.4	45	0
Stage I vs II	48h LC_{50}	2	102.14	45	0
Stage I vs II	24h EC_{50}	2	115.44	45	0
Stage I vs II	48h EC_{50}	2	98.256	45	0
Deltamethrin Stage I	24h vs 48h LC_{50}	2	20.97	7	0.0038
Deltamethrin Stage I	24h vs 48h EC_{50}	2	29.854	7	1.00e-04
Juveniles	24h vs 48h LC_{50}	2	33.567	51	0.9717
Juveniles	24h vs 48h EC_{50}	2	19.501	51	1
Females	24h vs 48h LC_{50}	2	83.856	65	0.0578
Females	24h vs 48h EC_{50}	1	13.539	25	0.9692
Males	24h vs 48h LC_{50}	2	42.297	73	0.9985
Males	24h vs 48h EC_{50}	2	48.11	73	0.9892
Adult Salmosan	24h vs 48h LC_{50}	2	19.422	27	0.8541
Adult Salmosan	24h vs 48h EC_{50}	1	27.124	23	0.2507
Adult Deltamethrin	24h vs 48h LC_{50}	2	21.015	45	0.9991
Adult Deltamethrin	24h vs 48h EC_{50}	2	30.084	45	0.9571
All pyrethroids on adults	24h LC_{50}	2	87.712	107	0.9132
All pyrethroids on adults	48h LC_{50}	1	82.586	97	0.8514
All pyrethroids on adults	24h EC_{50}	1	158.52	102	3.00e-04
All pyrethroids on adults	48h EC_{50}	1	58.4777	97	0.9993

Appendix D

The following tables show the results from the same data represented in the graphs in chapter four.

Table D.1 Shown are the 24h and 48h LC_{50} estimated values and confidence limits obtained between families of stage I signal crayfish hatchling families when exposed to Pyblast. LC_{50} estimates after Pyblast exposure at different time intervals. Lethal concentration is calculated as total concentration in $\mu g/l^{-1}$ after 24h/48h treatment. Standard errors (SE) are given in parenthesis.

Family	Estimate 24h LC_{50} (± SE)	Lower CL	Upper CL	Estimate 48h LC_{50} (± SE)	Lower CL	Upper CL
1	3.81 (0.89)	2.06	5.57	2.62 (0.54)	1.55	3.69
2	26.24 (6.16)	14.16	38.32	20.99 (4.14)	12.88	29.1
3	17.25 (3.79)	9.82	24.69	7.98 (3.00)	2.08	13.87
4	4.16 (0.96)	2.28	6.04	4.01 (0.58)	2.88	5.15
5	12.60 (2.71)	7.29	17.91	8.62 (1.70)	5.28	11.96

Table D.2 Shown are the 24h and 48h EC_{50} estimated values and confidence limits obtained between families of stage I signal crayfish hatchling families when exposed to Pyblast. EC_{50} estimates after Pyblast exposure at different time intervals. Effective concentration is calculated as total concentration in $\mu g/l^{-1}$ after 24h/48h treatment. Standard errors (SE) are given in parenthesis.

Family	Estimate 24h EC_{50} (± SE)	Lower CL	Upper CL	Estimate 48h EC_{50} (± SE)	Lower CL	Upper CL
1	2.39 (0.63)	1.17	3.62	1.87 (0.66)	0.57	3.17
2	20.08 (4.35)	11.55	28.62	17.38 (6.87)	3.91	30.84
3	15.83 (3.34)	9.27	22.38	5.27 (2.59)	0.2	10.34
4	4.22 (0.94)	2.38	6.06	4.02 (0.58)	2.88	5.15
5	8.59 (1.79)	5.09	12.09	8.04 (1.48)	5.14	10.95

Table D.3 Two tailed *t*-test comparing LC_{50} values between stage I signal crayfish hatchling families at 24h and their significance differences between families. Standard errors (SE) are given in parenthesis.

Hatchling families	Estimate 24h LC_{50}	Standard Error	*t*-value	*p*-value
1-2	-22.43	6.36	-3.58108	**0.0003*** **
1-3	-13.44	3.92	-3.433356	**0.0006*** **
1-4	-0.34	1.29	-0.27099	0.7864
1-5	-8.79	2.86	-3.07367	**0.0021** **
2-3	8.99	7.16	1.25437	0.2097
2-4	22.08	6.27	3.52065	**0.0004*** **
2-5	13.64	6.71	2.03318	**0.0420***
3-4	13.09	3.92	3.33197	**0.0009*** **
3-5	4.65	465	1.00110	0.3168
4-5	-8.44	2.88	-2.93052	**0.0034** **

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.4 Two tailed *t*-test comparing LC_{50} values between stage I signal crayfish hatchling families at 48h and their significance differences between families. Standard errors (SE) are given in parenthesis.

Hatchling families	Estimate 48h LC_{50}	Standard Error	*t*-value	*p*-value
1-2	-18.37	4.17	-4.40255	**0**
1-3	-5.36	3.06	-1.175417	0.0794
1-4	-1.40	0.80	-1.76223	0.078
1-5	-6.00	1.79	-3.35825	**0.0008*** **
2-3	13.01	5.11	2.54373	**0.0110***
2-4	16.97	4.18	4.0623	**0.0000*** **
2-5	12.37	4.47	2.76508	**0.0057** **
3-4	3.96	3.06	1.29287	0.1961
3-5	-0.64	3.46	-0.18545	0.8529
4-5	-4.60	1.80	-2.55931	**0.0105***

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.5 Two tailed *t*-test comparing EC_{50} values between stage I signal crayfish hatchling families at 24h and their significance differences between families. Standard errors (SE) are given in parenthesis.

Hatchling families	Estimate 24h EC_{50}	Standard Error	*t*-value	*p*-value
1-2	-17.69	4.42	-4.00011	**0.0001*** **
1-3	-13.43	4.42	-3.93159	**0.0001*** **
1-4	-1.83	1.10	-1.65769	0.0974
1-5	-6.20	1.89	-3.28419	**0.0010** **
2-3	4.26	5.46	0.78024	0.4353
2-4	15.86	4.47	3.54568	**0.0004*** **
2-5	11.49	4.71	2.43912	**0.0147***
3-4	11.61	3.49	3.32994	**0.0009*** **
3-5	7.23	3.79	1.90668	**0.0566***
4-5	-4.37	2.01	-2.17151	**0.0299***

Significant values are in bold. * <0.05 ** <0.01 ***<0.001

Table D.6 Two tailed *t*-test comparing EC_{50} values between stage I signal crayfish hatchling families at 48h and their significance differences between families. Standard errors (SE) are given in parenthesis.

Hatchling families	Estimate 48h EC_{50}	Standard Error	*t*-value	*p*-value
1-2	-15.51	6.90	-2.24757	**0.0246***
1-3	-3.40	2.67	-1.27410	0.2026
1-4	-2.15	0.88	-2.44598	**0.0144***
1-5	-6.18	1.62	-3.80419	**0.0001*** **
2-3	12.11	7.34	1.64953	0.0990
2-4	13.36	6.89	1.93791	0.0526
2-5	9.33	7.02	1.32818	0.1841
3-4	1.25	2.65	0.47191	0.6370
3-5	-2.78	2.98	-0.93045	0.3521
4-5	-4.03	1.59	-2.52970	**0.0114***

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.7 Shown are both LC_{50} and EC_{50} estimated values and confidence limits calculated after 24h/48h treatment obtained from stage I signal crayfish hatchling families when exposed to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	9.71 (1.06)	7.64	11.78	7.88 (0.89)	6.13	9.62
48h	6.43 (0.72)	5.01	7.84	5.05 (0.61)	3.85	6.24

Table D.8 Shown are both LC_{50} and EC_{50} estimated values and confidence limits calculated after 24h/48h treatment obtained from stage II signal crayfish hatchling families when exposed to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	7.67(1.22)	5.26	10.06	5.44(0.76)	3.96	6.92
48h	5.23(0.78)	3.48	6.97	4.73(0.68)	3.41	6.05

Table D.9 Two tailed *t*-test of LC_{50} and EC_{50} values of Pyblast ($\mu g/l^{-1}$) between 24h and 48h of stage I and II signal crayfish hatchlings and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Life stage	Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
I	24-48	LC_{50}	3.28	1.28	2.56	**0.0102***
		EC_{50}	2.83	1.06	2.66	**0.0078****
II	24-48	LC_{50}	2.44	1.47	1.66	0.0969
		EC_{50}	0.72	0.98	0.72	0.4681

Significant values are in bold. * <0.05 ** <0.01 ***<0.001

Table D.10 Shown are the 24h LC_{50} and EC_{50} estimated values and confidence limits obtained between stage I and II signal crayfish hatchlings when exposed to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Life stage	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
I	9.71(1.06)	7.61	11.81	7.89(0.81)	6.3	9.47
II	7.74(1.20)	5.39	10.08	5.24(0.79)	3.69	6.78

Table D.11 Shown are the 48h LC_{50} and EC_{50} estimated values and confidence limits obtained between stage I signal crayfish hatchling families when exposed to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Life stage	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
I	6.40(0.73)	4.95	7.84	5.13(0.60)	3.96	6.31
II	5.30(0.87)	3.59	7.01	4.36(0.72)	2.94	5.79

Table D.12 Two tailed *t*-tests of LC_{50} and EC_{50} values of Pyblast (µg/l) between stage I and II signal crayfish hatchlings and their significance differences at 24h and 48h observation times. Standard errors (SE) are given in parenthesis.

Exposure time	Stage	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24h	I-II	LC_{50}	1.97	1.6	1.22	0.2191
		EC_{50}	2.64	1.11	2.37	**0.0176***
48h	I-II	LC_{50}	1.1	1.11	0.99	0.3221
		EC_{50}	0.77	0.89	0.86	0.3887

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.13 Shown are both LC_{50} and EC_{50} estimated values and confidence limits calculated after 24h/48h treatment obtained from stage I signal crayfish hatchling families when exposed to Deltamethrin. Lethal and effective concentration is calculated as total concentration in ng/l^{-1}. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	85.76(53.67)	-19.43	190.96	4.82(4.43)	-3.85	13.50
48h	27.47(16.28)	-4.44	59.38	6.82(5.78)	-4.50	18.16

Table D.14 Two tailed *t*-test of LC_{50} and EC_{50} values of Deltamethrin (ng/l) between 24h and 48h in stage I hatchlings and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24-48	LC_{50}	58.30	56.21	1.03	0.2997
	EC_{50}	-2.00	6.44	-0.31	0.755

Table D.15 Shown are both LC_{50} and EC_{50} estimated values and confidence limits calculated after 24h/48h treatment obtained from juvenile signal crayfish when exposed to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	79.34(6.71)	66.19	92.48	6.01(0.88)	4.29	7.73
48h	57.95(4.54)	49.04	66.86	6.35(0.89)	4.6	8.09

Table D.16 Two tailed *t*-tests of LC_{50} and EC_{50} values of Pyblast (µg/l) between 24h and 48h in juvenile signal crayfish and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24-48h	LC_{50}	21.39	8.03	2.6618	**0.0078****
	EC_{50}	-0.33	1.13	-0.2957	0.7675

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.17 Shown are both the 24h and 48h LC_{50} and EC_{50} estimated values and confidence limits obtained from adult female signal crayfish when exposed to Pyblast. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	174.66(16.75)	141.83	207.5	10.48(4.96)	0.77	20.2
48h	118.25(10.54)	97.59	138.91	12.08(2.10)	7.97	16.19

Table D.18 Two tailed *t*-tests of LC_{50} and EC_{50} values of Pyblast (µg/l) between 24h and 48h of adult female signal crayfish and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24-48	LC_{50}	56.41	19.63	2.8739	**0.0041****
	EC_{50}	-1.59	5.38	-0.29614	0.7671

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.19 Shown are both the 24h and 48h LC_{50} and EC_{50} estimated values and confidence limits obtained from adult male signal crayfish when exposed to Pyblast. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	150.90(10.57)	130.19	171.461	9.78(0.79)	8.24	11.33
48h	111.13(7.34)	96.74	125.52	10.40(0.81)	8.81	11.99

Table D.20 Two tailed *t*-tests of LC_{50} and EC_{50} values of Pyblast (µg/l) between 24h and 48h of adult male signal crayfish and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24-48	LC_{50}	39.77	12.8	3.1068	**0.0019****
	EC_{50}	-0.62	1.12	-0.55103	0.5816

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.21 Shown are the LC_{50} estimated values and confidence limits adult males, females and juvenile signal crayfish obtained 24h and 48h exposure to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Life stage	Estimate 24h LC_{50} (± SE)	Lower CL	Upper CL	Estimate 48h LC_{50} (± SE)	Lower CL	Upper CL
Males	154.09(12.10)	130.37	177.8	111.37(7.64)	96.4	126.34
Females	171.05(14.81)	142.02	200.08	94.49(8.46)	77.92	111.07
Juveniles	80.39(7.18)	66.3	94.47	57.92(4.51)	49.09	66.75

Table D.22 Two tailed *t*-tests of LC_{50} values of Pyblast (µg/l) at 24h and 48h of juvenile, adult male and female signal crayfish and their significance differences between life stages and sexes at 24h and 48h. Standard errors (SE) are given in parenthesis.

Time	Life stage	Estimates LC_{50}	Std. Error	*t*-value	*p*-value
24	Females- Juveniles	90.66	16.26	5.57589	**0.000*** **
	Females-males	16.97	18.81	0.90205	0.367
	Juveniles-males	-73.7	13.81	-5.33665	**0.000*** **
48	Females- Juveniles	36.57	9.58	3.8169	**0.000*** **
	Females-Males	-16.88	11.39	-1.4813	0.1385
	Juveniles-Males	-53.45	8.85	-6.0368	**0.000*** **

Significant values are in bold. * <0.05 ** <0.01 ***<0.001

Table D.23 Shown are the EC_{50} estimated values and confidence limits adult males, females and juvenile signal crayfish obtained 24h and 48h exposure to Pyblast. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Life stage	Estimate 24h EC_{50} (± SE)	Lower CL	Upper CL	Estimate 48h EC_{50} (± SE)	Lower CL	Upper CL
Males	9.81(0.79)	4.11	16.39	10.37(0.84)	8.73	12
Females	10.25(3.13)	3.57	7.87	12.13(1.03)	10.11	14.16
Juveniles	5.72(1.10)	8.26	11.35	6.57(0.84)	4.92	8.21

Table D.24 Two tailed *t*-tests of EC_{50} values of Pyblast (µg/l) at 24h and 48h of juvenile, adult male and female signal crayfish and their significance differences between life stages and sexes at 24h and 48h. Standard errors (SE) are given in parenthesis.

Time	Life stage	Estimates LC_{50}	Std. Error	*t*-value	*p*-value
24	Females- Juveniles	4.53	3.32	1.36364	0.1727
	Males-Females	-0.44	3.23	-0.13664	0.8913
	Males-Juveniles	4.09	1.35	3.02082	**0.0025****
48	Females- Juveniles	5.57	1.33	4.1863	**0.000*****
	Males-Females	-1.76	1.33	-1.3280	0.1842
	Males-Juveniles	3.80	1.18	3.2101	**0.0013****

Significant values are in bold. * <0.05 ** <0.01 ***<0.001

Table D.25 Shown are both the 24h and 48h LC_{50} and EC_{50} estimated values and confidence limits obtained from adult signal crayfish when exposed to Salmosan. Lethal and effective concentration is calculated as total concentration in mg/l^{-1}. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC_{50} (± SE)	Lower CL	Upper CL
24h	30.46(5.64)	19.41	41.51	6.24(0.52)	5.22	7.25
48h	15.33(2.82)	9.80	20.84	5.20(0.93)	3.37	7.03

Table D.26 Two tailed *t*-tests of LC_{50} and EC_{50} values of Salmosan (mg/l^{-1}) between 24h and 48h on signal crayfish of mixed sex and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24-48	LC_{50}	15.14	6.3	2.4023	**0.0163**
	EC_{50}	1.04	1.07	0.97172	0.3312

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.27 Shown are both the 24h and 48h LC_{50} and EC_{50} estimated values and confidence limits obtained from adult signal crayfish when exposed to Deltamethrin. Lethal and effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Time	Estimate LC_{50} (± SE)	Lower CL	Upper CL	Estimate EC50 (± SE)	Lower CL	Upper CL
24h	1.76(0.28)	1.2	2.3	0.07(0.01)	0.04	0.1
48h	0.86(0.11)	0.65	1.06	0.09(0.01)	0.06	0.12

Table D.28 Two tailed *t*-tests of LC_{50} and EC_{50} values of Deltamethrin (µg/l) between 24h and 48h on signal crayfish of mixed sex and their significance differences between observation times. Standard errors (SE) are given in parenthesis.

Time	Toxicity value	Estimated difference	Standard Error	*t*-value	*p*-value
24-48	LC_{50}	0.9	0.29	3.08932	**0.002****
	EC_{50}	-0.02	0.02	-0.970369	0.3319

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.29 Shown are the LC_{50} estimated values and confidence limits of adult signal obtained 24h and 48h when exposed the three pyrethroids. Lethal concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Pyrethroid	Estimate 24h LC_{50} (± SE)	Lower CL	Upper CL	Estimate 48h LC_{50} (± SE)	Lower CL	Upper CL
Pyblast	165.75(11.06)	144.08	187.43	104.80(5.66)	93.71	115.9
Deltamethrin	1.69(0.24)	1.23	2.16	0.83(0.09)	0.65	1.02
Salmosan	3030.27(514.17)	2022.51	4038.03	1527.03(305.45)	928.36	2125.72

Table D.30 Two tailed *t*-tests of LC_{50} values of Pyrethroid (µg/l) of adult signal crayfish and their significance differences between pyrethroids at 24h and 48h. Standard errors (SE) are given in parenthesis.

Time	Pyrethroid	Estimates LC_{50}	Std. Error	*t*-value	*p*-value
24	Deltamethrin- Pyblast	-164.06	11.04	-14.8584	**0.000*** **
	Deltamethrin-Salmosan	-3028.58	514.17	-5.8902	**0.000*** **
	Pyblast-Salmosan	-2864.52	514.21	-5.5707	**0.000*** **
48	Deltamethrin- Pyblast	103.97	5.66	18.3661	**0.000*** **
	Deltamethrin-Salmosan	-1526.20	305.45	-4.9965	**0.000*** **
	Pyblast-Salmosan	-1422.23	305.50	-4.6553	**0.000*** **

Significant values are in bold. * <0.05 ** <0.01 ***<0.001

Table D.31 Shown are the EC_{50} estimated values and confidence limits of adult signal obtained 24h and 48h when exposed the three pyrethroids. Effective concentration is calculated as total concentration in $\mu g/l^{-1}$. Standard errors (SE) are given in parenthesis.

Pyrethroid	Estimate 24h EC_{50} (± SE)	Lower CL	Upper CL	Estimate 48h EC_{50} (± SE)	Lower CL	Upper CL
Pyblast	9.43(0.56)	8.32	10.53	10.87(0.65)	0.06	0.13
Deltamethrin	0.07(0.02)	0.04	0.10	0.09(0.02)	9.60	12.15
Salmosan	624.35(68.03)	491.01	757.69	519.81(93.42)	336.72	702.92

Table D.32 Two tailed *t*-tests of EC_{50} values of Pyrethroid (µg/l) of adult signal crayfish and their significance differences between pyrethroids at 24h and 48h. Standard errors (SE) are given in parenthesis.

Time	Pyrethroid	Estimates LC_{50}	Std. Error	*t*-value	*p*-value
24	Pyblast-Deltamethrin	9.36	0.56	16.63332	**0.000*** **
	Pyblast-Salmosan	-614.92	68.03	-9.03866	**0.000*** **
	Deltamethrin-Salmosan	-624.28	68.03	-9.17653	**0.000*** **
48	Pyblast-Deltamethrin	10.78	0.65	16.53533	**0.000*** **
	Pyblast-Salmosan	-508.94	93.42	-5.44779	**0.000*** **
	Deltamethrin-Salmosan	-519.72	93.42	-5.56331	**0.000*** **

Significant values are in bold. * <0.05 ** <0.01 ***<0.001.

Table D.33 Summary table of signal crayfish acute toxicity results (LC_{50} & EC_{50} values and 95% CI) obtained at 24h and 48h from their exposures to pesticides Pyblast, Deltamethrin and Salmosan at 12°C.

Test pyrethroid	Units	Life stage	24h LC_{50}	48h LC_{50}	24h EC_{50}	48h EC_{50}
Pyblast	µg/l	Stage I	9.73	6.43	7.88	5.05
	µg/l	Stage II	7.67	5.23	5.44	4.73
	µg/l	Juveniles	79.34	57.95	6.01	6.35
	µg/l	Adult males	150.90	111.13	9.78	10.40
	µg/l	Adult females	174.66	118.25	10.48	12.08
Deltamethrin	ng/l	Stage I	74.35	26.49	7.70	2.68
	µg/l	Adults	1.76	0.86	0.07	0.09
Salmosan	µg/l	Adults	3046	1533	624	520

Appendix E

Chemical analysis of the active ingredients in Pyblast

Below in table E.1 is the results from each water sample that was analysed for the following active ingredients that are present in the formulation of Pyblast, Cinerin II, Pyrethrin II, Piperonyl Butoxide, Jasmolin II, Cinerin I, Pyrethrin I, Jasmolin I, Cis-permethrin and Trans-permethrin. Water samples were taken from the adult male definitive tests.

Table E.1 Water parameter readings from the active ingredients present in the formulation of Pyblast from each male definitive test.

	Total pyrethrins (nominal) mg/l^{-1}	**Exposure Hr**	**Cinerin II**	**Pyrethrin II**	**Piperonyl butoxide**	**Jasmolin II**	**Cinerin I**	**Pyrethrin I**	**Jasmolin I**	**Cis/Trans-permethrin**	**Pyrethrins total**	**Total pyrethrins (measured)**	**%**
Test 3	Control	0	0.00	0.00	**0.40**	0.00	0.00	0.00	0.00	0.00	**0.0**	**0**	**0.00**
Test 3	50	0	0.24	1.51	**43.17**	0.16	0.38	2.84	0.18	0.00	**5.3**	**35.37**	**70.74**
Test 3	200	0	0.27	1.55	**41.61**	0.16	0.42	3.17	0.22	0.00	**5.8**	**154.68**	**77.34**
Test 3	Control	24	0.00	0.04	**2.20**	0.00	0.01	0.06	0.00	0.00	**0.1**	**0.70**	**0.00**
Test 3	50	24	0.24	1.29	**41.89**	0.14	0.30	1.87	0.12	0.00	**4.0**	**26.33**	**52.66**
Test 3	200	24	0.22	1.14	**39.13**	0.13	0.30	1.93	0.14	0.00	**3.9**	**102.88**	**51.44**
Test 3	Control	48	0.00	0.03	**1.65**	0.00	0.00	0.06	0.00	0.00	**0.1**	**0.56**	**0.00**
Test 3	50	48	0.17	0.85	**36.66**	0.09	0.17	1.02	0.06	0.00	**2.4**	**15.74**	**31.48**
Test 3	200	48	0.19	1.00	**35.37**	0.11	0.27	1.78	0.12	0.00	**3.5**	**92.48**	**46.24**
Test 5	Control	0	0.00	0.00	**0.21**	0.00	0.00	0.00	0.00	0.00	**0.0**	**0**	**0.00**
Test 5	50	0	0.27	1.77	**44.47**	0.17	0.44	3.42	0.23	0.00	**6.3**	**41.97**	**83.94**
Test 5	200	0	0.29	1.75	**45.89**	0.18	0.42	3.47	0.22	0.00	**6.3**	**168.90**	**84.45**
Test 5	Control	24	0.00	0.00	**0.51**	0.00	0.00	0.02	0.00	0.00	**0.0**	**0.12**	**0.00**
Test 5	50	24	0.20	1.08	**42.50**	0.11	0.27	1.82	0.11	0.00	**3.6**	**23.88**	**47.76**
Test 5	200	24	0.45	2.76	**73.15**	0.28	0.88	6.71	0.47	0.00	**11.6**	**308.22**	**154.11**
Test 5	Control	48	0.00	0.00	**0.88**	0.00	0.00	0.00	0.00	0.00	**0.0**	**0**	**0.00**
Test 5	50	48	0.08	0.37	**30.07**	0.04	0.10	0.52	0.03	0.00	**1.1**	**7.59**	**15.18**
Test 5	200	48	0.08	0.36	**36.84**	0.04	0.21	1.29	0.08	0.00	**2.1**	**54.79**	**27.39**