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Detection of pesticide impacts on larval prawns in hatcheries and presence in estuarine intake water

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June 2017

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FRDC Project No 2016-049 IPA APFA

2017

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The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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Executive Summary

The use patterns of pesticides in Australia has changed over the last decade, with the use of pyrethroid and neonicotinoid insecticides increasing. The limited water quality data that is available has measured increased concentrations of the neonicotinoid insecticide imidacloprid in particular. Prawn farms in Australia are predominantly located adjacent to estuaries in Queensland, which are impacted by multiple land uses upstream (e.g. sugarcane farming, banana farming, beef cattle farming and urbanisation). Because of their small size and rapid growth requirements, we would hypothesise that larval prawns would be most susceptible to the impacts of these modern pesticides.

While there is little ecotoxicology data for marine species, early life stages, or Australian species, the information available from studies with crustaceans conducted elsewhere suggests that the concentrations measured in this and other studies may be sufficient to cause impacts on survival, either directly or via inhibition of feeding. However, given the uncertainties in a) the levels of insecticides in farm intake water; b) the impacts of insecticide exposure on prawn larvae; and c) how these compounds interact when they co-occur, we can not adequately predict the impacts of these changes in pesticide use on the survival of post larval phase prawns.

To address these uncertainties, a series of experiments were conducted to determine the sensitivity of post larval prawns to these insecticides. To assess the potential for risk, a series of experiments examined the impact of exposure of post-larval black tiger prawns (*Penaeus monodon*) to modern use insecticides, imidacloprid, bifenthin, and fipronil as well as the organophosphate insecticide chlorpyrifos for comparison. Impacts were measured as decreased survival and feeding inhibition. Post-larval phase prawns were most sensitive to fipronil, bifenthrin, chlorpyrifos, and imidacloprid, in that order. They were comparably sensitive to other crustaceans based on literature studies. Bifenthrin and imidacloprid exposure also reduced the ability of post-larval prawns to capture live prey.

To determine the environmental risk of exposure to these compounds, concentrations of a broad suite of pesticides was measured in prawn farm intake waters. At least some pesticides were measured in every sample. Most of the pesticides detected were measured below concentrations that would be expected to cause harm to prawn larvae, although some herbicides were at concentrations that exceed either ANZECC/ARMCANZ or GBRMPA trigger values, suggesting the possibility of indirect or mixture related impacts. However, in the Mackay Catchment and Logan River, imidacloprid, bifenthrin, fipronil and chlorpyrifos insecticides were measured at concentrations that pose risk to prawn larvae. The potential for interactive effects and the limitations of basing risk on water samples collected at a single time point are discussed.

Introduction

Review of the literature

The purpose of this review is to collate what was known (prior to the onset of our experimental work) about the potential risks from exposure to modern-use pesticides for the survival of farmed post-larval prawns. Although there are hundreds of different pesticides currently in use in Queensland, this study focussed on imidacloprid, bifenthrin, fipronil and chlorpyrifos. Imidacloprid, representative of the neonicotinoid pesticides, was chosen because monitoring data from the Great Barrier Reef (GBR) catchments indicate that its concentration in water samples is increasing rapidly. Bifenthrin and fipronil are representative of classes of compounds that have been associated with aquatic toxicity elsewhere in the world. Chlorpyrifos was chosen for comparative purposes because it is representative of organophosphate insecticides that although being phased out, chlorpyrifos is still one of the most broadly used insecticides (Zhao and Chen, 2016). In the US, the use of chlorpyrifos against termites and for residential applications has been restricted, and it is no longer considered an ecological risk there (Giddings et al., 2014). However, in Australia it is still used for domestic pest control (e.g. it is used in residences) and may pose many of the environmental problems that led to its restriction in the US. This review will cover:

  i.   the patterns of use of these pesticides in Queensland, and where available, the concentrations at which they've been measured; and
  ii.   the current knowledge regarding the physiological impacts on crustaceans from exposure to these insecticides

Risk assessment is dependent on the concentrations of these compounds found in the environment and the sensitivity of the organisms to the contaminant. While we anticipate that their small body size, frequent moulting, low tolerance to starvation, and potential exposure to insecticides in intake water will make the post-larval phase the most sensitive to insecticide exposure of the black tiger prawn (*Penaeus monodon*) life stages, as outlined in the sections below, we do not currently have adequate information about the sensitivity of any Penaeid prawn life stage, or of the concentrations of insecticides to which prawns could be getting exposed to during commercial culture, to enable accurate estimation of the potential risks.

Chemical and Toxicological properties of pesticides

Population growth and increased food demand have led to a concurrent increase in pesticide use (Mugni et al., 2016). Insecticides are commonly used in agriculture to improve crop yield, to eliminate nuisance pests from residential areas, and in public health to control mosquitos and other potential disease vectors (e.g. Stevens et al., 2011). Ideally, an insecticide will be quick acting on the designated "pest species", and have low toxicity to other animals in the environment. As a consequence, many insecticides are nerve agents, and are being designed to be more specific to arthropods, including insects and crustaceans. Even amongst the nerve agents, there are a variety of different compounds with slightly different biochemical properties. These insecticides can be grouped into classes based on their chemical properties and modes of action.

An older class of insecticide is the organophosphate pesticides, such as chlorpyrifos. Organophosphate compounds inhibit acetylcholinesterase activity, an enzyme that resets nerves to "normal" after they have signalled (Halstead et al., 2015). This acetylcholinesterase inhibition results in paralysis and death. Inhibition of acetylcholinesterase is not specific to arthropods, and as a consequence, there have been concerns about the impacts of organophosphate pesticides on non-

target organisms, including humans. Organophosphates are being phased out of use in many countries because of concern regarding their impact on human health and potential carcinogenesis (Weston and Lydy, 2014). Consequently, new classes of insecticides have been used increasingly.

Pyrethroid insecticides, such as bifenthrin, prevent repolarisation of voltage-sensitive ion channels in the membrane of the axon of nerves (Halstead et al., 2015). These have low toxicity to birds and mammals, but higher toxicity to fish and arthropods. The phenylpyrazole insecticide fipronil also interferes with GABA receptors in insect and crustacean nerve cells (Stevens et al., 2011).

Neonicotinid compounds, such as imidacloprid, are specifically designed to alter the normal activity of arthropods nicotinic acetylcholinesterase activity, which are structurally different than those of other animals (Sanchez-Bayo and Hyne, 2014). Imidacloprid binds irreversibly to nicotinic receptors, and exposure may have cumulative effects on organism health (Rondeau et al., 2014). Neonicotinid pesticides have been used in increasing amounts worldwide because they are very effective at eliminating insect pests, yet pose low risks to mammals and fish (Sanchez-Bayo and Hyne, 2014). These pesticides are highly water soluble and can be applied via numerous methods, and are used in both agricultural and urban applications (Sanchez-Bayo and Hyne, 2014). As discussed in more detail in subsequent sections, because these compounds have similar modes of action, they may interact to cause additive or greater than additive toxicity.

Despite the similarity in mode of action, these compounds have quite different chemical properties (Table 1). Pyrethroid pesticides such as bifenthrin are hydrophobic and primarily associated with sediments (Holmes et al., 2008). Once in the water column, these compounds quickly partition into lipid and sediments (Solomon et al., 2001), however increased water-column concentrations can be associated with stormwater run-off events. Although uptake of this compound into tissue is rapid, it is quickly depurated, and as a consequence there is low concern about trophic transfer (Solomon et al., 2001). Fipronil also has relatively poor water solubility and is more likely to be associated with sediment than dissolved in the water column. Chlorpyrifos is also hydrophobic (Kuivila and Foe, 1995), and more often found in sediments than in the dissolved phase (Giddings et al., 2014).

Imidacloprid behaves very differently to the other pesticides discussed because of its low affinity for sediment (Table 1). Imidacloprid is highly water soluble and breaks down in light (Smit et al., 2015), and as a consequence, its environmental persistence is dependent on light (Anderson et al., 2015b), but can persist in turbid or cloudy waters. The compound is persistent in the environment and has a high potential to leach into the aquatic environment (Anderson et al., 2015b) but does not accumulate appreciably in sediments.

Table 1 Chemical properties for the selected pesticides.

Trade name	IUPAC name	CAS number	First use[*]	Water solubility	Soil sorption coefficient	Persistence in water	metabolites
Chlorpyrifos[3]	o,o-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate	2921-88-2	1965	1.4 mg/L	360 to 31,000	Comparatively photostable, lost from water via vaporization.	diethylphosphate and diethylthiophosphate
Bifenthrin[2]	2-methylbiphenyl-3-ylmethyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)- 2,2-dimethylcyclopropanecarboxylate	82657-4-3	1985	<1 μg/L	1.31×10^5 - 3.02×10^5	Photostable and can persist in aerobic waters	2-methyl-3-phenylbenzylalcohol, and 2-methly-3-phenylbenzoic acid
Fipronil[1]	(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)- 4-trifluoromethylsulfinylpyrazole-3-carbonitrile	120068-37-3	1996	2.4 mg/L	825 ± 214	Breaks down with UV – uncertain without the presence of light	fipronil-sulfide and fipronil-desulfiny
Imidacloprid[4]	1-(6-chloro-3- pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	138261-41-3	1994	610 mg/L	249-336	Broken down by photolysis, less stable at high temperature and alkaline pH.	6-chloronicotinic acid and imidazolidine

* Data for first use in the US are publically available. It is assumed that its first use in Australia is comparable

1. Jackson, D.; Cornell, C. B.; Luukinen, B.; Buhl, K.; Stone, D. 2009. *Fipronil Technical Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services.

2. Johnson, M.; Luukinen, B.; Gervais, J.; Buhl, K.; Stone, D. 2010. *Bifenthrin Technical Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services.

3. Christensen, K.; Harper, B.; Luukinen, B.; Buhl, K.; Stone, D. 2009. *Chlorpyrifos Technical Fact Sheet*; National Pesticide Information Center, Oregon State University Extension

4. Gervais, J. A.; Luukinen, B.; Buhl, K.; Stone, D. 2010. *Imidacloprid Technical Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services.

Environmental concentrations of current use pesticides

The concentrations in the aquatic environment of most of these pesticides are unknown for many regions in Queensland, but may be comparatively high because of the agricultural land use adjacent to waterways and the tropical and sub-tropical conditions. Pesticide use can be higher in the tropics due to a variety of environmental factors (Lewis et al., 2016). Lower organic carbon content in soils is associated with increased run-off and risk to aquatic systems, but with increased rates of microbial metabolism as well. As a consequence, field dissipation rates are faster in the tropics than in temperate environments (Lewis et al., 2016).

Very little information is available about the quantities of pesticides used in Queensland. There is publically available information about the products containing each insecticide that are registered for use in a given application (Table 2). Although this does not provide information on whether or not the compounds are used, or in what quantity, it does provide some information on how the products are marketed. All four compounds are registered for use in Queensland and have broad and overlapping applications, including sugar cane, pasture and domestic uses. However, when the number of products containing the selected pesticide are examined, some differences are apparent. For instance, chlorpyrifos is used in pasture, lucerne hay, cotton, and in growing oilseed. Bifenthrin is used more often in products designed to be sprayed on buildings (including industrial, commercial, and residential), on lucerne hay, on turf/ lawns, and on bananas (Table 2). Fipronil is contained in products designed to control fleas on dogs and cats, and in agricultural applications, such as grapes, cotton, bananas and sugar cane. Imidacloprid is used most frequently in horticulture (roses, ornamental plants) followed by agricultural products, including vegetable, stone fruit, cotton, potatoes and sugar cane (Table 2).

Table 2. Most frequently registered uses of the selected pesticide (data from the Australian Pesticides and Veterinary Medicines Authority website).

Imidacloprid	Bifenthrin	Fipronil	Chlorpyrifos
Ornamental plants	Domestic pest control	Flea Control	Pasture
Vegetables	Lucerne hay	Viticulture grapes	Lucerne Hay
Cotton	Turf/ lawn care	Cotton	Oilseed crops
Potatoes and sweet potatoes	Bananas	Potatoes	Domestic pest control
Stone fruit	Pears	Sugar Cane	Cotton
Flea control	Barley	Domestic pest control	Forage crops
Sugar cane	Field peas	Termites	Cereals
Canola	clover	Sorghum	Sunflower
Sunflowers	Wheat	Vegetables	Bananas

Many of these land uses coincide with where prawns are farmed, as outlined in Table 3. For example, cotton is grown in southern Queensland and Northern NSW. Sugarcane and bananas are grown in areas adjacent to prawn farms and are present in many of the catchments. Areas along the Logan River are being rapidly developed and used for residential housing. As a consequence, there is the potential for some of these insecticides to leach into the environment.

Table 3. Land use patterns in catchments containing prawn farms. Only intensive land uses are tabulated, others, including conservation and natural environments, grazing on native vegetation, and production forestry, are not tabulated.

	Clarence River Valley (NSW)	Logan Albert Catchment	Burdekin Catchment (Includes Elliot River, Ayr)	Wet Tropics (includes the Herbert; Johnstone River)	Burnett/Mary Catchment	Mackay Whitsundays Catchment
Residential and farm	✓	13.48%	0.18%	0.18%	2.25%	1.69%
Animal husbandry	✓	0.41%	<0.01%	<0.01%	0.04%	0.09%
Horticulture		0.73%	0.1%	0.1%	0.43%	0.43%
Sugar	†	0.9%	0.75%	7.13%	1.55%	17.89%
Cropping	✓	2.2%	0.96%	0.96%	2.73%	0.03%
Grazing modified pastures[#]	✓	1.35%	<0.01%	<0.01%	0.31%	<0.01%
Total	N.A.	19.07	2.01	8.39	7.31	20.14

† Sugar cane cropping is not mapped separately in the NSW data

does not include grazing on native vegetation

✓ Occurs but not quantified

Although we know that pesticides are used throughout the state, pesticide monitoring information is only available for some of the catchments. Because of the iconic nature of the environment and the $5.6 billion that GBR tourism generates for the Queensland economy, much of the pesticide monitoring undertaken in the region is focussed on the catchments of the Great Barrier Reef (GBR) (Garzon-Garcia et al., 2015). Much of the work that has been done to date in the GBR catchment areas has focussed on the photosystem II-inhibiting herbicides, such as atrazine and diuron, because of their potential impacts on sea grasses and corals (e.g. Devlin et al., 2015). The monitoring sites are focussed on the reef, with the Mary River being the southern-most site where pesticides were analysed. A few generalisations about pesticide behaviour in Queensland Rivers within the monitoring area are: (Devlin et al., 2015):

- Inputs of pesticides into aquatic systems are common after application, following rainfall events and after irrigation, with inputs being highest if application immediately precedes a rainfall event;
- Some pesticides are stable in marine environments, and have low but consistent contamination;
- Wet years typically have greater pesticide loads but lower peak concentrations;
- Water-soluble pesticides are typically transported in the aqueous phase, less water-soluble pesticides are more persistent in sediments
- Pesticide concentrations are inversely correlated to discharge volume

Imidacloprid and chlorpyrifos have been used in sugarcane cultivation and horticulture in the GBR catchment (Devlin et al., 2015). Although few pesticide-use data are available for the region (Devlin et al., 2015), some measured concentrations have been reported. In general, pesticide concentrations were highest in the Tully, Pioneer, N. Johnstone, Herbert and Sandy Creek (Plane) catchments (Garzon-Garcia et al., 2015). Recent monitoring efforts have identified imidacloprid being used at concentrations that are comparable to the photosystem II inhibitors, with an annual load for the GBR catchment estimated at 530

kg/y (Garzon-Garcia et al., 2015). In some catchments, the concentrations of imidacloprid have been measured and have been increasing in recent years to µg/L levels (DSITI unpublished, Figure 1). The other pesticides discussed here were not included in the monitoring program. Fipronil, chlorpyrofos and imidacloprid, but not bifenthrin, have been detected in estuaries (Devlin et al., 2015).
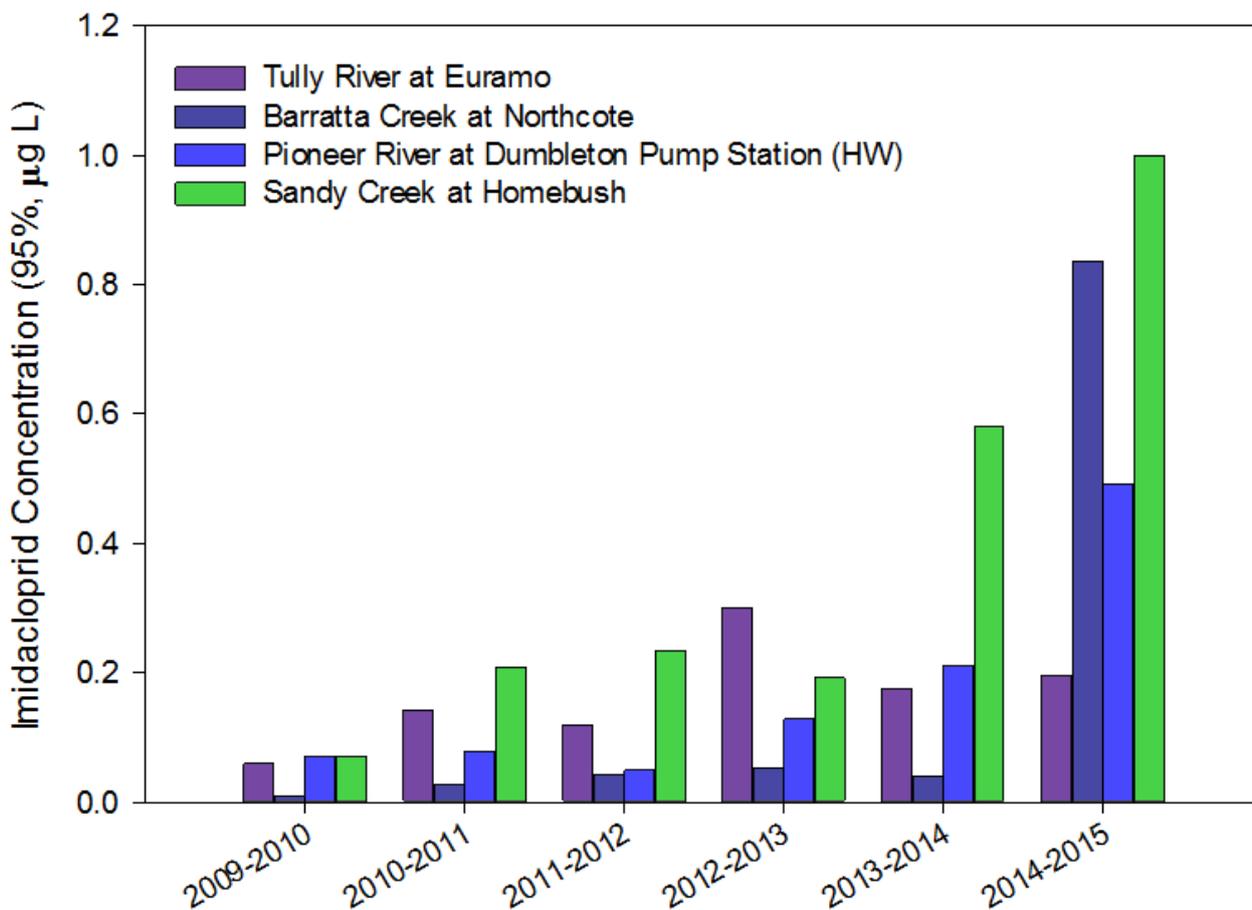
Figure 1. Increases in imidacloprid concentrations measured in Great Barrier Reef catchments (2009-2015)

Neonicotinid pesticides have been detected frequently in other areas around Australia. For example, imidacloprid was measured in 12 of 13 samples collected from rivers in the Sydney region after major rainfall events with concentrations ranging from 40-456 ng/L (Sanchez-Bayo and Hyne, 2014). Other neonicotinid pesticides, including acetamiprid, clothianidin, thiacloprid, and thiamethoxam were also detected, though not as frequently or at as high concentrations (Sanchez-Bayo and Hyne, 2014).

A recent survey reported the concentrations of organic contaminants, including the pesticide chlorpyrifos, in water samples from rivers around Australia (Scott et al., 2014). This study only detected chlorpyifos in two samples, and both were at 5 ng/L (0.005 ppb). However, as the predicted no effect concentrations were 10 ng/L, the levels were still flagged as being "of concern." It did not report which samples had elevated chlorpyrifos, or measure levels of imidacloprid, fipronil, or bifenthrin (Scott et al., 2014).

In other regions in the world, potentially toxic concentrations of these pesticides have been measured in aquatic samples. For example, studies (in Florida, USA) have measured fipronil at 50 ng/L, and fipronil and its metabolites have been measured in California at 30-50 ng/L, which could endanger crustacean

populations (Wu et al., 2015; Weston and Lydy, 2014). These elevated concentrations occurred in residential areas with high amounts of lawn maintenance (Wu et al., 2015). In a US study, Bifenthrin was found in all seven urbanised stream environments surveyed, at concentrations as high as 11 µg/kg in sediments (Kuivila et al., 2012). In inland California, concentrations of chlorpyrifos were high enough to potentially affect the distribution of non-target invertebrates in the early 2000s (Xu et al., 2016). Now that these compounds are being phased out and replaced by pyrethroid pesticides (Ryberg and Gilliom, 2015), these pesticides, including bifenthrin, are causing toxicity in the same region (Xu et al., 2016).

Since chlorpyrifos has been phased out of residential use in the US, there are less frequent reports of it at concentrations greater than 100 ng/L, and it is considered to be low risk there (Giddings et al., 2014). An earlier US study detected chlorpyrifos at between 31 and 42 ng/L in three of twenty samples, but as the other pesticides (diazinon and methidathion) were at much higher concentrations, these concentrations were not thought to be contributing to the toxicity observed in water samples from these sites (Kuivila and Foe, 1995). In Latin America, chlorpyrifos was frequently detected in sediment samples collected in agricultural zones, but was frequently not at concentrations high enough to cause toxicity (e.g. less than 30 ng/g) (Hunt et al., 2016).

Impacts of Insecticides on crustaceans and non-target Aquatic arthropods

The following provides a summary of what is known about the environmental impacts of imidacloprid, fipronil, bifenthrin and chlorpyrifos on crustaceans and non-target aquatic arthropods. Although the concentrations of these pesticides in the aquatic environment are quite low, their specificity for arthropods indicates that non-target crustaceans, such as prawns, may be at risk. Very little work has been done on the toxicity of pesticides to Penaeid prawns or other crustaceans in the region (Devlin et al., 2015) so much of the following review is based on studies conducted elsewhere in the world. Also, much of the recent environmental impact work assessing these compounds has focused on pollinators (reviewed in Sanchez-Bayo and Goka, 2014).

The safety of pesticides prior to their registration for use in the environment has been primarily determined by industry-funded, laboratory-based toxicity tests (Boone et al., 2014). It is thought that some level of contamination is possible without harming aquatic ecosystems. The permissible concentration of each contaminant is based on the risk that exposure to that compound poses to aquatic organisms (Leung et al., 2014). Often a species sensitivity distribution, or SSD, where toxicity test data for a range of receptor species (typically more than 8 species from at least 4 different taxonomic groups) is plotted, and regression analysis is used to identify concentrations that would protect 90, 95 or 99% of species (ANZECC/ARMCANZ, 2000). Under the ANZECC/ARMCANZ (2000) guidelines, a species sensitivity distribution is used to identify concentrations that if found in aquatic environments, should trigger further investigation (Pathiratne and Kroon, 2016). In Australia, water quality guidelines are derived from sublethal toxicity data if available, or where not available, from lethal data with a "safety factor". Recent studies have used this approach to suggest a guideline value of 1.8 µg/L for imidacloprid in tropical fresh waters (Pathiratne and Kroon, 2016), but no comparable values are available for marine species. Similarly, the current ANZECC guidelines for chlorpyrifos is 10 ng/L.

Despite the advantages, this approach does have some shortcomings. The results are expected to be protective of the system as a whole but assumes some level of "functional redundancy" (Leung et al., 2014), and will not necessarily protect all species. These studies are typically done with adult organisms and may not predict impacts on early life stages, and more information is available for freshwater species than marine. Stakeholders (such as aquaculturists or fisheries groups) may prefer assessments carried out on the species they are interested in. Also, the approach is most predictive when the toxicity thresholds are similar across all members of a given taxonomic group. With the large differences in toxicity seen between species in the data sets for these species, the threshold concentrations may not be adequately predictive with only a few toxicity test values.

Laboratory studies

Chlorpyrifos

As shown in the SSD diagram in Figure 2, aquatic organisms in general are sensitive to chlorpyrifos, but crustaceans are seemingly not more sensitive than fish, based on the impacts on survival. The LC_{50}s for crustaceans are typically between 0.1 to 100 µg/L for crustaceans (Giddings et al., 2014); with LC_{50}s of 0.4 µg/L have been reported for some species of amphipod (Mugni et al., 2016), for example. LC_{50}s in fish are typically between 1-1000 µg/L (Giddings et al., 2014). Most studies focussing on sub-lethal endpoints have been conducted in fish and have measured decreased swimming rates, rates of food consumption and of predator avoidance, consequences of acetylcholinesterase inhibition (Giddings et al., 2014). Previous studies with freshwater prawns (*Paratya australiensis)* have shown decreased survival and inhibition of acetylcholine esterase at concentrations as low as 0.03 µg/L (Kumar et al., 2010). In a terrestrial study of the impacts of different modern use pesticides (including imidacloprid, bifentrhin, and chlorpyrifos, but not fipronil) on beneficial insects, chlorpyrifos was found to be one of the most toxic (Fernandes et al., 2016). Because the study was not for aquatic organisms the dose regime was different than the others included in this review, but all of the compounds were toxic at their recommended field application rates. Interestingly, all of the compounds tested reduced the feeding rates of insects at the compounds LC_{50}, suggesting the potential for indirect toxicant induced mortality of non-target organisms (Fernandes et al., 2016). Recovery from acetylcholinesterase inhibition is frequently measured in lab studies (Giddings et al., 2014), however, with food limitation and predation, wild animals may not recover as well as laboratory test species.
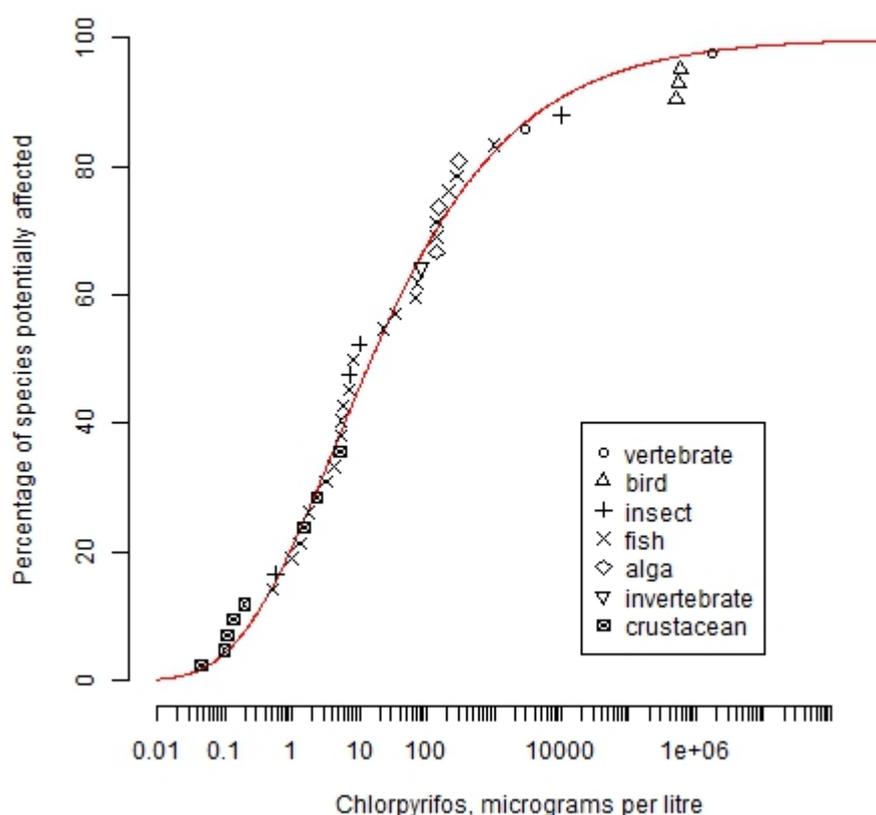
Figure 2. Species sensitivity distribution for chlorpyrifos, based on LC50 concentrations. Data taken from the USEPA (http://www.ipmcenters.org/ecotox/DataAccess.cfm)

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Bifenthrin

Bifenthrin is a fast acting neurotoxin and exerts a toxic impact quickly (Anderson et al., 2015a), in part because it is rapidly bioaccumulated (Solomon et al., 2001). As shown in Figure 3, aquatic animals are more sensitive than birds or plants, but fish are not always less sensitive than crustaceans. Some crustaceans are very sensitive to bifenthrin relative to other taxa, as shown in the SSD curve in Figure 3. For example, the LC_{50} for the amphipod *Hyalella azteca* is approximately 10 ng/L, and growth declines at approximately 6 ng/L (Anderson et al., 2015a). Other studies have reported EC_{50}s between 12 and 250 ng/L in invertebrates (Weston et al., 2015). However, other studies have found that amphipods from central California have orders of magnitude less sensitivity to bifenthrin (Clark et al., 2015), suggesting either rapid adaptation or differences in sensitivity amongst the *Hyallella azteca* species complex (Clark et al., 2015; Weston et al., 2015).
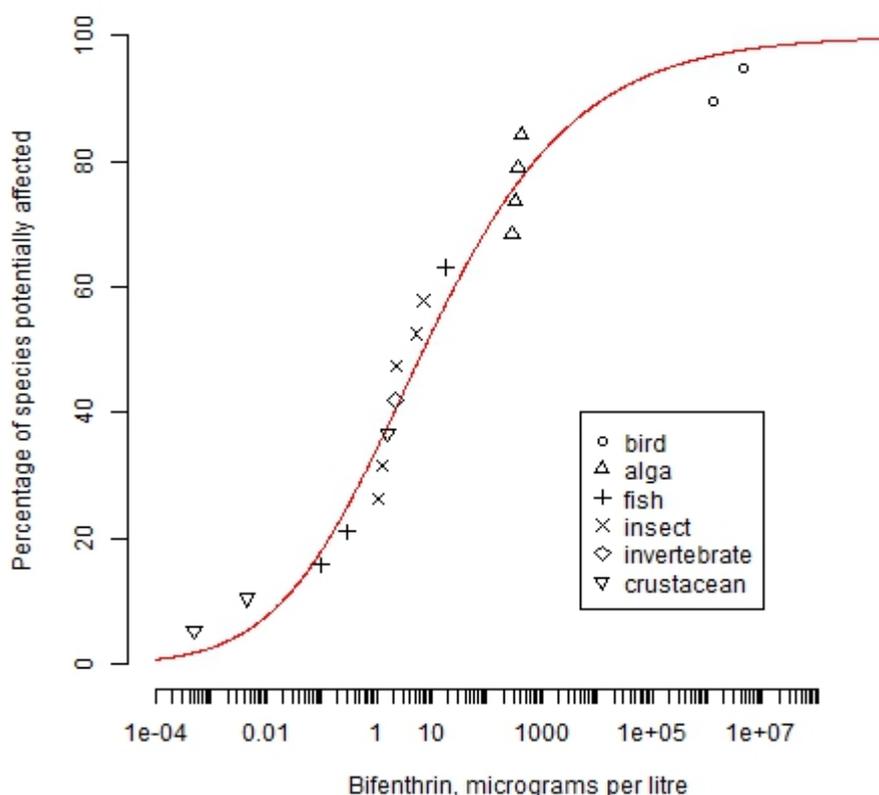
Figure 3. Species sensitivity distribution for bifenthrin, based on LC$_{50}$ concentrations. Data taken from the USEPA (http://www.ipmcenters.org/ecotox/DataAccess.cfm) and from Siegfried et al., 2001.

Some studies have also assessed the sub-lethal effects of exposure to bifentrhin. Decreased motility was found to occur at 1.4 ng/L in the amphipod *Hyalella azteca* or at 53 ng/l in the chironomid *Chironomus dilutus,* contrations that are about ½ the LC_{50} (Hasenbein et al., 2015). In the chironomid *Chironomus tetans,* immobility and decreased growth were measured at 2 ng/L, about 1/3 the LC_{50} (Maul et al., 2008). Isopods exposed to bifenthrin also showed dampened predator avoidance behaviour at sub ng/L concentrations (Huynh et al., 2014). Bifenthrin concentrations in sediment below 100 μg/kg (no correction for organic carbon) have been shown to impair reproduction in the Australian amphipod *Melita plumulosa* (Hook et al., 2014). Exposure to bifenthrin also changed the RNA transcript abundance of some genes associated with moulting and of some associated with the pesticide metabolism (Hook et al., 2014). As a consequence of these low thresholds, there have been concerns about non-target toxicity (Solomon et al., 2001).

Fipronil

Arthropods are amongst the most sensitive taxa to fipronil, as shown in the SSD in Figure 4, and different but related species may have quite different sensitivities to this insecticide (Stevens et al., 2011). For example, the LC_{50}s of different daphnids (waterfleas) can be 50-fold different (Figure 4, Stevens et al., 2011). Fipronil may be more toxic once it is metabolised, as fipronil sulfide and fipronil sulfone are typically two- to three-fold more toxic than the parent compound (Weston and Lydy, 2014). While the vertebrates and other non-arthropod taxa are less sensitive to fipronil than the insects and crustaceans, the difference is not as stark as for imdiacloprid (discussed in subsequent sections).
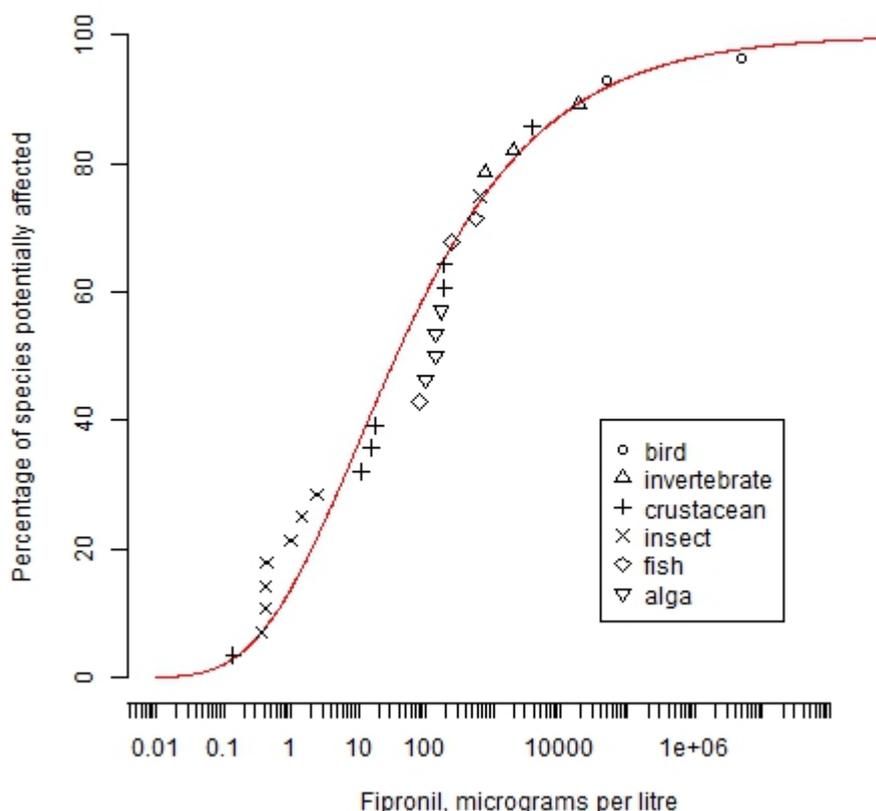
Figure 4. Species sensitivity distribution for fipronil, based on LC_{50} concentrations. Data taken from the USEPA (http://www.ipmcenters.org/ecotox/DataAccess.cfm) and from Stevens et al. (2011).

Few sub-lethal endpoints exist for crustaceans exposed to fipronil. The concentrations that inhibited growth and mobility in the chironomid *Chironomus tetans* were not appreciably different than those which cause increased mortality (Maul et al., 2008). Fipronil concentrations in sediment below 50 μg/kg (no correction for organic carbon) have been shown to impair reproduction in the Australian amphipod *Melita plumulosa* (Hook et al., 2014). *Daphnia pulex* is only expected to have declines its reproductive rate at concentrations that approach the 48-hour LC_{50} (Stark and Vargas, 2005). Exposure to fipronil also changed the RNA transcript abundance of some genes associated with moulting and pesticide metabolism (Hook et al., 2014). Typically risk assessment has prioritised tests focussing on survival, rather than sub-lethal endpoints such as behaviour, but recent studies have emphasised that crustaceans that are immobile are unlikely survive in the field (Weston and Lydy, 2014).

Imidacloprid

Fish, algae, molluscs, and other non-arthropod life forms life forms are comparatively insensitive to imidacloprid (Anderson et al., 2015b), to the extent where their toxicity values do not fall in the same distribution as the arthropods (Figure 5). As shown in the species sensitivity distribution in Figure 2, while crustaceans in general are sensitive, there is a very wide range in the sensitivity of crustaceans to imidacloprid, with LC_{50} values ranging from 1 to 52,500 μg/L (reviewed in Smit et al., 2015). Cladocerans, the most commonly used ecotoxicology test species, are comparatively insensitive to imidacloprid, and as a consequence, the risks of environmental release may have been underestimated (Hayasaka et al., 2013). Non-target insects and worms have also been shown to be very sensitive to imidacloprid, with toxic effects occurring between 0.7 and 300 μg/L, depending on the species (reviewed by Smit et al., 2015). A recent SSD-based study of freshwater tropical test data suggested that sensitive taxa some catchments in the GBR area (where monitoring data for Queensland exists) may be at risk from toxic impacts from imidacloprid (Pathiratne and Kroon, 2016). Previous studies have identified a 24-hour LC_{50} for blue crab (*Callinectes sapidus*) larvae of 10 μg/L, with larvae being more sensitive than juveniles, and the commercial formulation more potent than the pure compound. Imidacloprid also decreased the moulting frequency, and increased the number of dead after moulting (Osterberg et al., 2012). Mesocosm based studies have found NOECs (no observed effect concentrations) between 0.6 and 4 μg/L (reviewed in Smit et al., 2015).
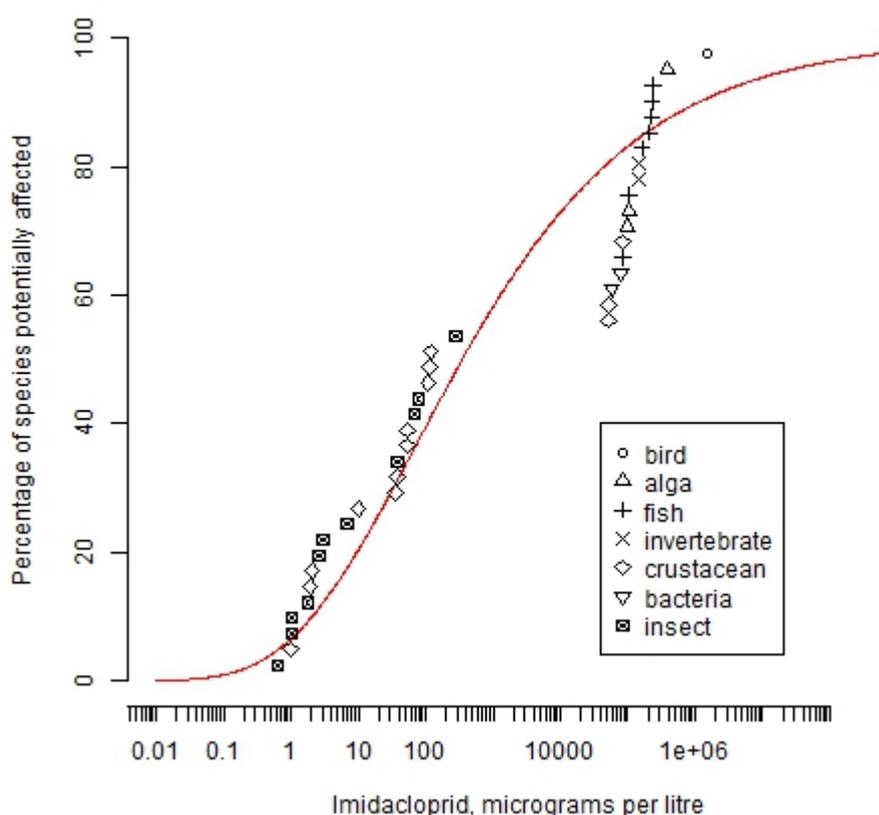
Figure 5. Species Sensitivity distribution for imidiacloprid, based on LC$_{50}$ concentrations. Data from USEPA (http://www.ipmcenters.org/ecotox/DataAccess.cfm) and Anderson et al. (2015). Note that arthropods seemingly have their own curve to the left of the other taxa, indicating that they are much more sensitive.

In addition to impacts on survival, behavioural changes have been observed at imidacloprid concentrations ranging between 0.1-12.9 μg/L (Anderson et al., 2015b). In laboratory tests, exposure to imidacloprid inhibited feeding in amphipods at all concentrations tested (>810 ng/L) (Agatz et al., 2014). However, at all but the highest concentration tested (10 μg/L), feeding increased once amphipods were transferred to clean

water. In another study, amphipods exposed pulsed and constant imidacloprid concentrations had reduced movement, including feeding, and ultimately reduced lipid content, leading to mortality (Nyman et al., 2013).

Imidacloprid is not thought to bioaccumulate (Smit et al., 2015). In a terrestrial food chain, imidacloprid taken up via trophic transfer has been shown to exert toxicity (Douglas et al., 2015). Slugs were shown to accumulate imidacloprid from their food, and to pass it on to beetle predator. The predatory beetles accumulated a lethal dose of imidacloprid. Field-collected slugs contained enough imidacloprid to be toxic to their predators (Douglas et al., 2015).

Australia currently has no water quality guidelines for imidacloprid (Pathiratne and Kroon, 2016). Different water quality criteria have been set in different places around the world. In the US, the water quality criteria was 1.05 μg/L, which exceeds the LC_{50} for the most sensitive species (Smit et al., 2015). In Canada, the guideline value is 0.23 μg/L (Anderson et al., 2015). In Europe, the guideline values are approximately 10 ng/L, which are frequently exceeded. A continuous exposure standard of 8 ng/L, and a "pulse" exposure standard of 200 ng/L was recommended (Smit et al., 2015).

Mixtures

Since these compounds have similar modes of toxic action, these compounds may interact and cause greater than predicted effects. In addition, the insecticides discussed above are not used in isolation, rather they are used in combination with other pesticides (including herbicides, fungicides, and insecticides such as methoprene that have different modes of toxic action). Furthermore, *in vitro* studies have identified interactions between the nicotinic acetylcholine receptor (the target for neonicotinid insecticides) and acetyl cholinesterase (the target for organophosphate receptors), suggesting that these compounds may exert a greater than additive toxicity (Shao et al., 2013). As highlighted in the literature studies below, when crustaceans are exposed to these compounds in concert, impacts can be surprisingly greater than expected.

Other types of pesticides may also change the sensitivity of crustaceans to insecticides. Ergosterol biosynthesis-inhibiting fungicides increased the toxicity of α-cypermethrin (a different neonicotinid pesticide) to the water flea *Daphnia magna* as much as twelve fold, even though *Daphnia* were much more sensitive to α-cypermethrin than to the fungicides (Norgaard and Cedergreen, 2010). The imidazole fungicide prochloraz in particular increased the potency of α-cypermethrin. It is not certain whether the toxicity increased because of increased bioavailability or a result of the inhibition of the enzymes that normally metabolise pesticides (Norgaard and Cedergreen, 2010). In bees, potential for synergistic toxicity from mixtures of neonicotinid and pyrethroid pesticides with ergosterol inhibiting fungicides has been noted (Sanchez-Bayo and Goka, 2014). Herbicides may also alter the sensitivity of crustaceans to insecticides. For example, even though grass shrimp embryos are comparatively insensitive to atrazine, exposure to simultaneous exposure to atrazine, imidacloprid and fipronil was more toxic than exposure to imidacloprid and fipronil alone (Key et al., 1997).

Other compounds are deliberately put into pesticide formulation to increase their efficacy, and these may increase their environmental risk as well. Surfactants such as alkylphenol ethoxylates are commonly co-applied with herbicides and other pesticides to increase their uptake by the weeds being targeted (Xie et al., 2005; Kroon et al., 2015). Their presence could also increase the bioavailability of the insecticides being studied. Piperonyl butoxide (PBO) inhibits the mixed function oxidases that animals normally use to metabolise pesticides, and as a consequence, are used to increase the effectiveness of mosquito control (Hunt et al., 2016). Although PBO alone has low toxicity to aquatic organisms, it can increase the toxicity of pyrethroid compounds (Hunt et al., 2016). However, PBO decreases the toxicity of organophosphate compounds.

Field-based studies

Microcosm and mesocosm experiments with chlorpyrifos concentrations as high as 0.1 μg/L did not show impacts on communities, although concentrations higher than this did cause declines in the abundance of fish (Giddings et al., 2014). Crustaceans were often the most sensitive organism in these studies, and it was occasionally noted that the loss of crustacean grazers led to algal blooms (Giddings et al., 2014).

Toxicity studies in pesticide-contaminated areas of the US have noted declines in crustaceans associated with bifentrhin and fipronil. A study in a tidal wetland in San Francisco, CA, USA, compared the toxicity (as paralysis) of winter storm water runoff to the amphipod *Hyallela azteca* and the freshwater midge *Chironnmus dilutus* (Weston et al., 2015). They found toxicity via immobility, though not lethality, in stormwater from urbanised that could be attributed to fipronil and bifenthrin. Agricultural runoff was not toxic, though this study was conducted outside of the growing season (Weston et al., 2015). A separate study of agricultural areas in central California (USA) found toxicity associated with field collected water and sediment samples, associated with chlorpyrifos, pyrethroid pesticides (including bifenthrin), or a mixture of the two in sediments and with chlorpyrifos and diazanon in water sediments (Anderson et al., 2014). They also found decreases in field populations of macroinvertebrates (Anderson et al., 2014).

Other California, USA, studies have found toxicity related to pyrethroid pesticides, primarily bifenthrin, in urban creeks (Holmes et al., 2008; Phillips et al., 2010). The initial study compared the pesticide concentrations in the sediment to mortality of the amphipod *Hyalella atzeca*. They found widespread toxicity, most acutely in heavily urbanised areas, and only bifentrin was present in sufficient quantities to explain the toxicity (Holmes et al., 2008). Chlorpyrifos was also measured in this study, and was thought to potentially contribute to the toxicity observed (Holmes et al., 2008). They also referred to other work documenting the loss of macroinvertebrates in the areas from which their sediments were collected (Holmes et al., 2008). Another study using field-collected sediments in California, USA, used a Toxicity Indentification Evaluation (TIE) approach to identify the causative agents of toxicity to the amphipod *Hyalella atzeca*. It identified bifenthrin as the most likely cause of the toxic responses, as the concentrations from sediments in the sediments studied ranges from 0.79-7.65 μg/g organic carbon, whereas the LC_{50} was 0.52 μg/g organic carbon (Phillips et al., 2010). Other pyrethroid pesticides, such as cyfluthrin and cypermethrin, may have also contributed to the toxicity observed (Phillips et al., 2010).

Another study examining the impact of bifenthrin concentrations following rainfall events in the Sacramento River, USA on endangered steelhead salmon found no impact, as measure either through traditional or molecular methods, on the fish themselves (Weston et al., 2015). However, the concentrations measured (as high as 15 ng/L) were sufficient to cause mortality in the caddisfly larvae that the salmon prey upon, suggesting the possibility of indirect effects (Weston et al., 2015). Similar studies in the Pacific Northwest of the US did not find as great an impact (Weston et al., 2011). One third of all sediment samples collected in this study had detectable pyrethroids, and toxicity was measured with only two of them. However, the authors argued that the low temperatures that occur in this region could increase the potency of bifenthrin (Weston et al., 2011).

Some studies have also measured problems in field sites with imidacloprid. In streams in the Netherlands contaminated with imidacloprid, there was a significant decrease in the densities of macrofaunal invertebrates. These declines start at concentrations as low as 37-64 ng/L (Van Dijk et al., 2013). In rice paddies, changes in community structure are observed at imidacloprid concentrations greater than 1 μg/L (Anderson et al., 2015).

We have implemented the current study to address the uncertainty around both the concentrations of insecticides measured in Queensland and the sensitivity of prawn larvae (*Penaeus monodon*) to these insecticides. Lethal and sublethal responses of prawns to chlorpyrifos, bifenthrin, fipronil and imidacloprid will be determined, and the concentrations of a suite of compounds, including these insecticides, in the intake water collected at prawn farms will be measured. If the concentrations measured approach 1/10 th the EC_{50}, the larvae would be considered "at risk" and subsequent studies of mixtures and cumulative stressors may be warranted.

Objectives

1. Perform a desktop study of the potential impacts of modern use insecticides on the rearing of larval prawns.

2. Determine whether environmentally relevant concentrations of pesticide (imidacloprid, bifenthrin, fipronil, and chlorpyrifos) affect the survival and feeding behaviour of prawn larvae.

3. Analyse the intake waters from selected farms for the concentrations of pesticides, including the selected insecticides, to determine whether the concentrations approach those that could be harmful.

Method

Toxicity Assessments

Prawns

Post larval stage black tiger prawns *Penaeus monodon* (PL-10's, approximately 20 days post hatch and 10 days post metamorphosis from the mysis stage) were obtained from cultures from ongoing breeding trials as part of ongoing work conducted as part of the ARC Research Hub for Advanced Prawn Breeding (Bribie Island, Queensland). Prawns were maintained on commercial feed and live artemia, and fed every three hours between 7 am and 8 pm. The water quality recorded during experiments is provided in Table 4.

Table 4. Range of water quality parameters measured in our experimental set up

Parameter	Range
Temperature	28.4-32.3°C
Dissolved oxygen (% saturation)	84.5-95%
Dissolved oxygen (mg/L)	5.27 - 5.89 mg/L
Salinity	38.2 - 39.7 ppt
pH	8.03-8.19

Exposure Solutions

Pure compounds of each pesticide were ordered from Sigma Aldrich. Stock pesticide concentrations were made by dissolving the entire amount provided by the manufacturer into methanol. To create exposure solutions with the appropriate insecticide concentration, the stock solution was added to the glass jar and the methanol was evaporated off under a fume hood. Filtered sea water was added to each jar The range finder experiments were conducted using nominal concentrations only. Jars were lightly aerated using Teflon tubing, and exposures were carried out under red lights to minimise stress to the prawn post larvae, which are normally benthic and not accustomed to ambient light.

Range finder experiments

To determine the pesticide concentration range over which post-larval prawns are sensitive, a quick survival experiment that bracketed the range of sensitivities reported for other crustaceans was performed. Three replicates of ten prawns each were distributed into log step 10 concentrations for each of the selected insecticides. After 24 hours, the number of PL's surviving was counted.

Daily renewal tests

The first series of experiments used daily renewal of solutions in an attempt to keep exposure concentrations constant. Ten post larval prawns were added to one of five replicate flasks for each insecticide

concentration. After 24 hours, surviving prawns were counted transferred to a new test solution. "Used" solutions were filtered for chemical analysis.

Static exposure tests

The second series of experiments was similar to the first except that it employed a 48 hour static design. Each concentration had 5 replicates containing 10 post larval phase prawns. After 24 hours, the number of surviving prawns was tallied. Prawns that had jumped and adhered to the mouth of the jar were removed from the experiment. At 48 hours, surviving prawns were counted.

Feeding Inhibition

Feeding inhibition was measured as a diminished ability of the prawn post larvae to capture live prey (artemia nauplii). Experiments were conducted in the morning when the animals had been fasting. Approximately 50 newly hatched Artemia nauplii were counted and put into a tube. Each prawn post larval stage replicate was fed one tube. Two hours later, the number of Artemia nauplii surviving was counted using a dive light at the side of the flask and the Artemia's phototactic behaviour. Results were normalised to the number of surviving post larval prawns. For the highest pesticide concentrations, artemia were exposed for two hours without prawns to determine if the pesticides altered survival or phototaxis, but no changes were observed.

Statistical analysis

Differences in survival and number of artemia nauplii consumed per surviving post larval prawn were tested to determine whether they were greater than would be expected due to chance. T tests assuming equal variance were carried out in Sigmaplot (version 12.5). LC_{50} concentrations were calculated using ToxCalc (ToxCalc Version 5.0.23, Tidepool Scientific Software).

Analytical Chemistry

Sample Collection

Farms were selected for study such that a large geographic distribution was captured (Figure 6). Where there were multiple farms in the same catchment, the farm with the most upstream location in the estuary was chosen. Three replicate water samples (one litre) were collected by the farmers at the intake points before water treatment and after a rain event. Samples were stored at 4°C until shipment at 4°C to the CSIRO Land and Water analytical laboratories in South Australia.

Chemical Analysis

All analyses were carried out with appropriate QA/QC consideration, including matrix matched calibration, accurate mass for identification, use of laboratory blanks, standards bracketing the samples and recovery and reproducibility assessments during analysis.

Analytical grade pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg Germany). Stock solutions were prepared in methanol at the concentration of 1000 mg/L. Working solutions in the concentration range of 1–100 μg/L were prepared by appropriate dilution in blank matrix solution in order to match the matrix associated with the study solutions. Calibrations were also carried out using matrix matched solutions. All mass spectrometry (MS) grade solvents were purchased from Merck (Darmstadt,

Germany). The water used to prepare the solutions or mobile phase was purified in a Milli-Q-plus system (Millipore, Bedford, MA, USA).

Owing to low concentrations in the experimental and environmental samples, the water samples needed to be pre-concentrated using a standard solid phase extraction (SPE) procedure. For this purpose Meg Bond Elute Plexa (500mg 6ml, Agilent technologies, CA USA). SPE cartridges were used for sample clean-up and pre-concentration. Prior to loading the cartridges with seawater samples, SPE cartridges were conditioned with 3×2 mL of methanol, according to standard protocol. One litre of a seawater sample was passed through the SPE cartridge at a flow rate of 5 mL/minute. After loading the samples, the salts on the SPE cartridge were washed off with 50 mL of mill-Q water and then the cartridges were dried under vacuum. Following this the cartridges were eluted with three lots of 3 mL of methanol. The combined extracts were evaporated to dryness under a gentle stream of nitrogen and were reconstituted in 1 mL of methanol prior to use for residue analysis.

The residue analysis was carried out using a Liquid Chromatograph (LC) coupled with Triple Time of Flight mass spectrometry (LC - Triple TOF 5600, SCIEX, USA). Using gradient elution method, the two mobile phases used for separation of various pesticides were A) methanol and B) 10mM ammonium formate in 0.01% formic acid. The gradient elution scheme was chosen based on the chemistry of the molecule. An aliquot of 10 μL of MeOH extract was injected onto the LC column (Kinetex 2.6u C18 100*2.10mm, Phenomenex, USA). The total run time was 18 minutes. Accurate mass for each compound was used for identification and quantification. The concentrations were calculated using matrix-matched calibration curves using Analyst TF 1.7.1 software. Analytes included in the survey are shown in Table 5.

Figure 6. Location of farms where samples were taken. Approximate sampling locations are shown with letters, and catchment locations are also marked. Image, Google Maps.

Table 5 Level of detection (μg/L) of pesticides measured in this study

Insecticides		Herbicides		Fungicides		Others	
Analytes	*Level of detection (μg/L)*	*Analytes*	*Level of detection (μg/L)*	*Analytes*	*Level of detection (μg/L)*	*Analytes*	*Level of detection (μg/L)*
Bifenthrin	0.005	2,4-D[1]	0.001	Difenconazole	0.005	*Surfactant "boosters"*	
Carbaryl	0.005	Ametryn	0.005	Metalaxyl	0.005	4-nonylphenol	0.025
Chlorfenvinphos	0.005	Atrazine	0.0025	Myclobutanil	0.005	4-nonylphenol-mono-ethoxylate	0.005
Chlorpyrifos	0.005	Diuron	0.001	Prochloraz	0.005	4-tert-octylphenol	0.005
Clothianidin	0.001	Hexazinone	0.005	Pyraclostrobin	0.005	4-nonylphenol-di-ethoxylate	0.01
Cypermethrin	0.01	MCPA[3]	0.005	Pyrimethanil	0.005	*Corrosion Inhibitor*	
DEET[2]	0.005	Metolachlor	0.005	Trifloxystrobin	0.005	Benzotriazole	0.005
Diazinon	0.01	Simazine	0.005				
Fipronil	0.005	Tebuthiuron	0.001				
Imidacloprid	0.001						
Indoxacarb	0.01						
Permethrin	0.01						
Pirimicarb	0.01						

1 2,4-D is 2,4-Dichlorophenoxyacetic acid; 2 DEET is N,N-Diethyl-meta-toluamide;   3 MCPA is 2-methyl-4-chlorophenoxyacetic acid

Results and Discussion

Toxicity Assessments

Range Finder Results

The results from the range finder experiment are presented in Table 6. For the subsequent work with fipronil, concentrations less than 0.5 µg/L were used. For Bifenthrin, we used concentrations less than 5 µg/L, for chlorpyrifos, concentrations less than 1 µg/L were used. For imidacloprid, a larger range of concentrations were used to bracket both those that were expected to cause lethal toxicity (100-500 µg/L) and those that have been reported in Queensland Rivers (around 1 µg/L).

Table 6. Results obtained during the range finder study

Insecticide	Dose, µg/L	Survival
	Control	100 ± 0
	Solvent Control	96.7 ± 5.8
Fipronil	0.1	90 ± 17.3
	1	0 ± 0
	10	0 ± 0
	100	0 ± 0
Bifenthrin	0.001	100 ± 0
	0.01	90 ± 10
	0.1	86.7 ± 11.5
	1	90 ± 17.3
Imidacloprid	1	93.3 ± 5.8
	10	93.3 ± 5.8
	100	83.3 ± 28.9
	1000	60 ± 17.3
Chlorpyrifos	0.01	96.7 ± 5.8
	0.1	96.7 ± 5.8
	1	0 ± 0
	10	0 ± 0

Fipronil

The range finder experiments found 100% mortality at 100, 10 and 1 µg/L (Table 6), so remaining experiments were conducted at nominal concentrations of 0.0625-0.5 µg/L. A statistically significant decrease in survival relative to control was measured at all concentrations tested (Figure 7). No statistically significant impairment of prey capture ability was noted (Figure 8), perhaps because the data were confounded by differences in individual survival.
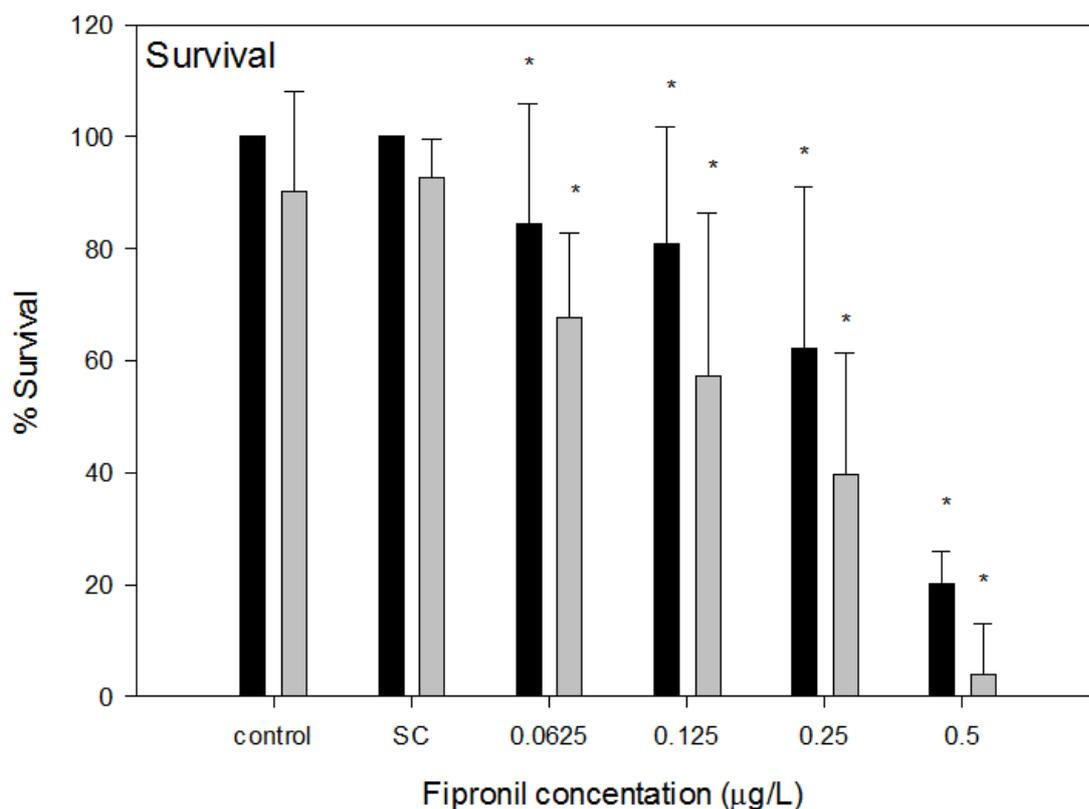
Figure 7. Survival (average $\pm$ standard deviation) of post larval phase prawns exposed to different concentrations of fipronil. Concentrations provided are nominal. The black bar shows survival at 24h, the grey bar shows survival at 48 hours. SC denotes solvent control. All exposure concentrations have significantly lower survival than control, as denoted by the asterix (*).
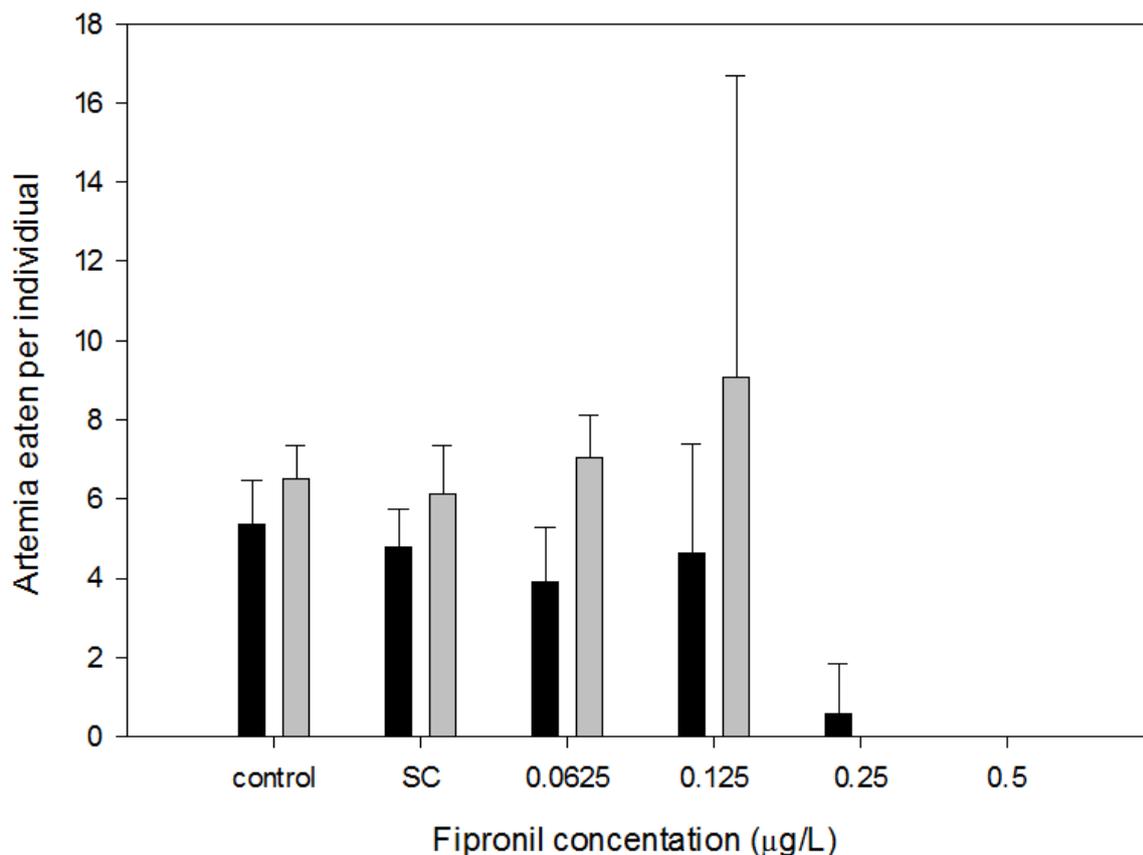
Figure 8. Average artemia consumption (mean ± standard deviation) of post larval phase prawns exposed to different concentrations of fipronil, normalized to surviving individual. Concentrations provided are nominal. The black bar shows prey capture ability at 24h, the grey bar shows prey capture ability at 48 hours. SC denotes solvent control.

Imidacloprid

The range finder experiments found low survival of post larval prawns at 1000 µg/L (Table 6), so subsequent experiments used concentrations less than 250 µg/L. The first survival experiment measured significantly decreased survival in all concentrations tested relative to control and solvent control at the 48 hour time point (Figure 9). It also had poor control survival at 72 hours, presumably because the prawn larvae were being handled daily, so it was repeated with a static design, which required less handling of the organisms. When feeding inhibition was examined, a trend towards decreased prey capture ability was measured in all doses, but none of the changes were significant, in part due to high variability in survival and consequently, prey capture rates (Figure 10).
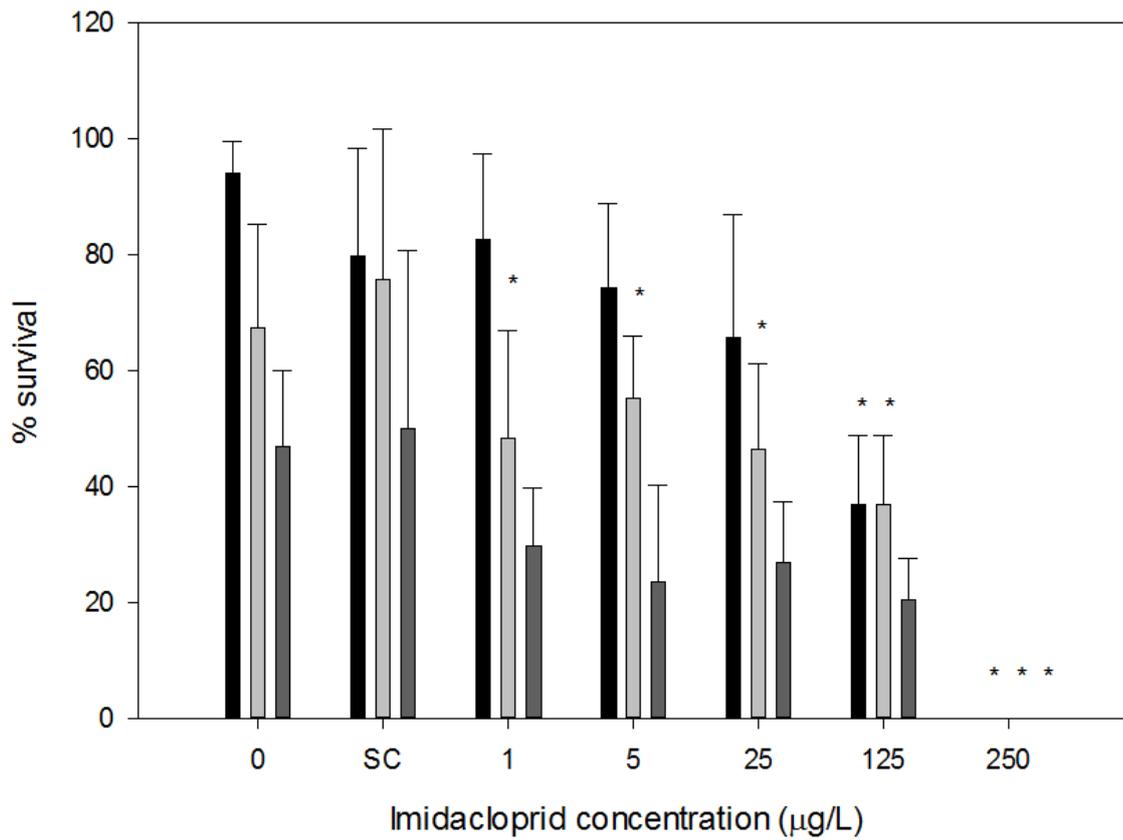
Figure 9. Average survival (mean $\pm$ standard deviation) of post larval phase prawns exposed to imidacloprid. Concentrations provided are nominal. The black bar shows survival at 24h, the pale grey bar shows survival at 48 hours, the dark grey bar shows survival at 72 hr. SC denotes solvent control. All exposure concentrations recorded significantly lower survival than control at 48 hours, as denoted by the asterix (*).
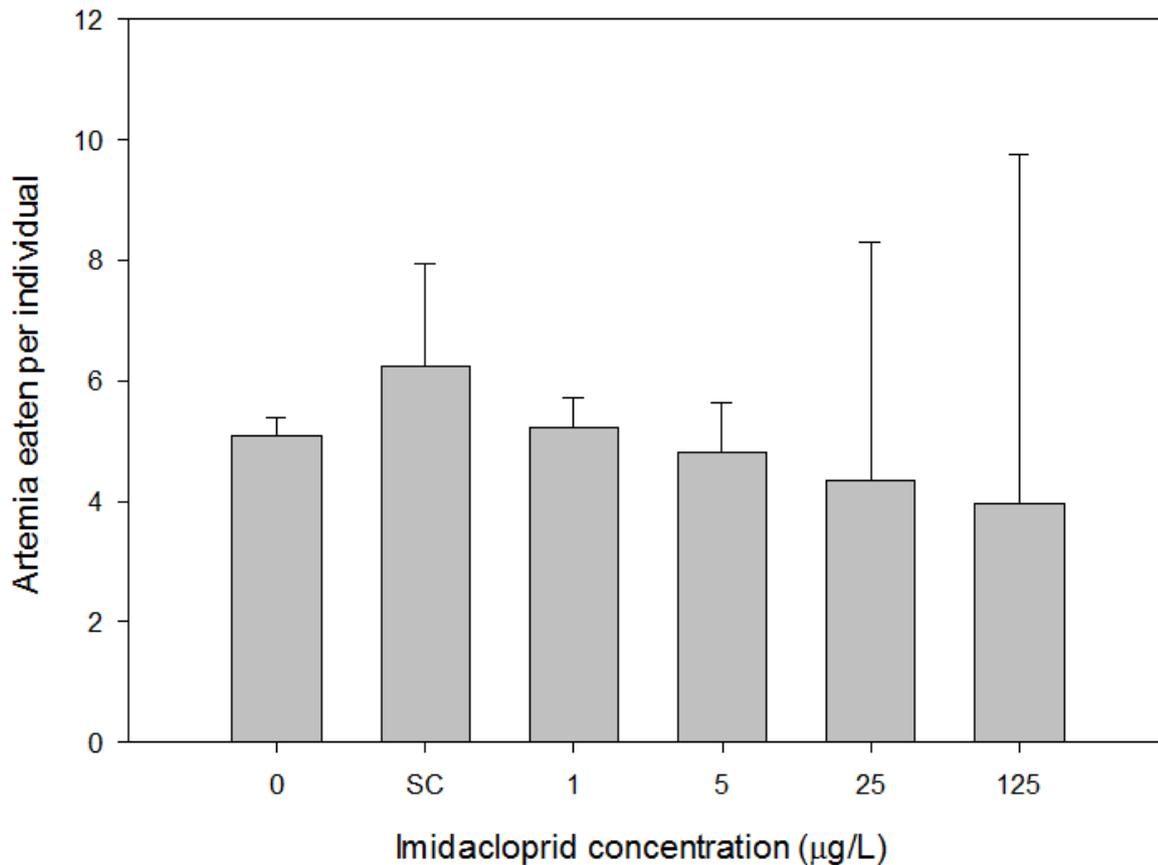
Figure 10. Average artemia consumption (mean $\pm$ standard deviation) of post larval phase prawns exposed to imidacloprid for 24 hours, normalized to surviving individual. Concentrations provided are nominal. SC denotes solvent control.

In the second experiment, specifically examining feeding inhibition, we focussed on concentrations that bracket those which have been reported in Queensland Rivers. For this experiment, we changed the design so that the exposures were static and the animals were not handled daily. Control survival was higher, and only survival in the 2 μg/L concentration at 48 hours was different than control (Figure 11). When feeding inhibition was examined, statistically decreased prey capture ability was measured at every dose, but not at every time point. At 24 hours, all doses except the 1 μg/L dose had a diminished capacity to catch live prey, and at 48 hours, all doses except the 2 μg/L had a diminished capacity to catch live prey (Figure 12).
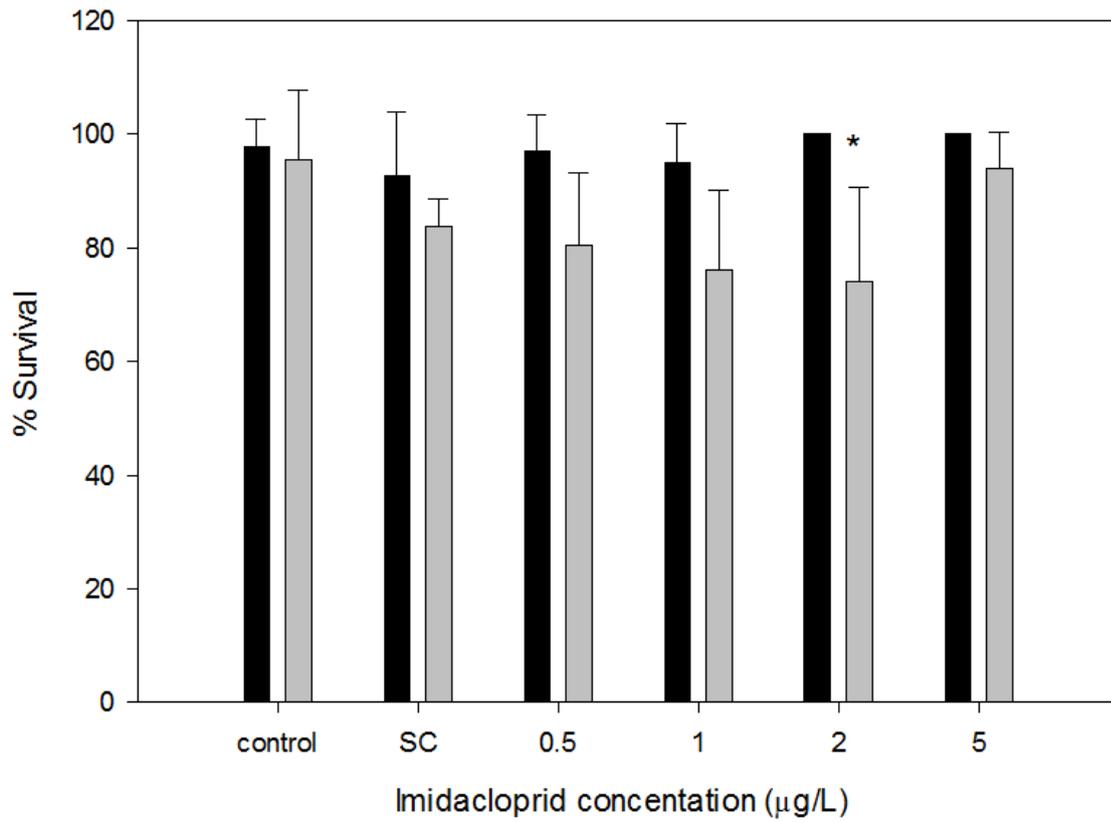
Figure 11. Average survival (mean ± standard deviation) of post larval phase prawns exposed to different concentrations of imidacloprid. Concentrations provided are nominal. The black bar shows survival at 24h, the grey bar shows survival at 48 hours. SC denotes solvent control. Only 2 µg/L at 48 hours had significantly lower survival than control, as denoted by the asterix (*).
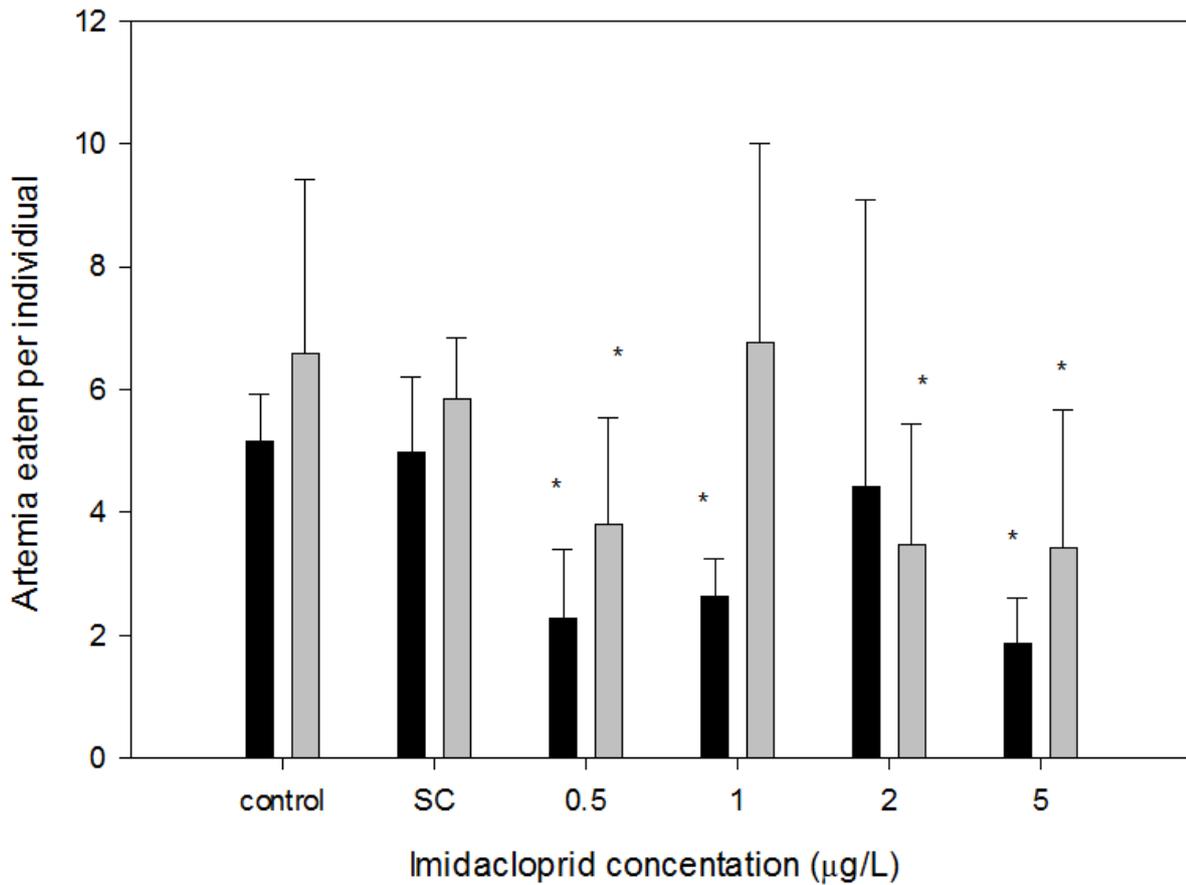
Figure 12. Average Artemia consumption (mean ± standard deviation) of post larval phase prawns exposed to different concentrations of imidacloprid, normalized to surviving individual. Concentrations provided are nominal. The black bar shows prey capture ability at 24h, the grey bar shows prey capture ability at 48 hours. SC denotes solvent control. At 24 h, all doses except 2 µg/L are statistically different than control. At 48 h, all doses except 1 µg/L are statistically different than control, as denoted by the asterix (*).

The third survival experiment used higher concentrations to assess survival, and the static exposure design. Again, control survival was much higher, but we did not get a significant concentration survival relationship (Figure 13). At 48 hours, only two of the concentrations (5 and 50 µg/L) were significantly different than control. The other two doses had a trend towards decreased survival, but the differences were not significant (25 µg/L, P =0.076; 125 µg/L P=0.071). The feeding inhibition experiment was not repeated a third time.
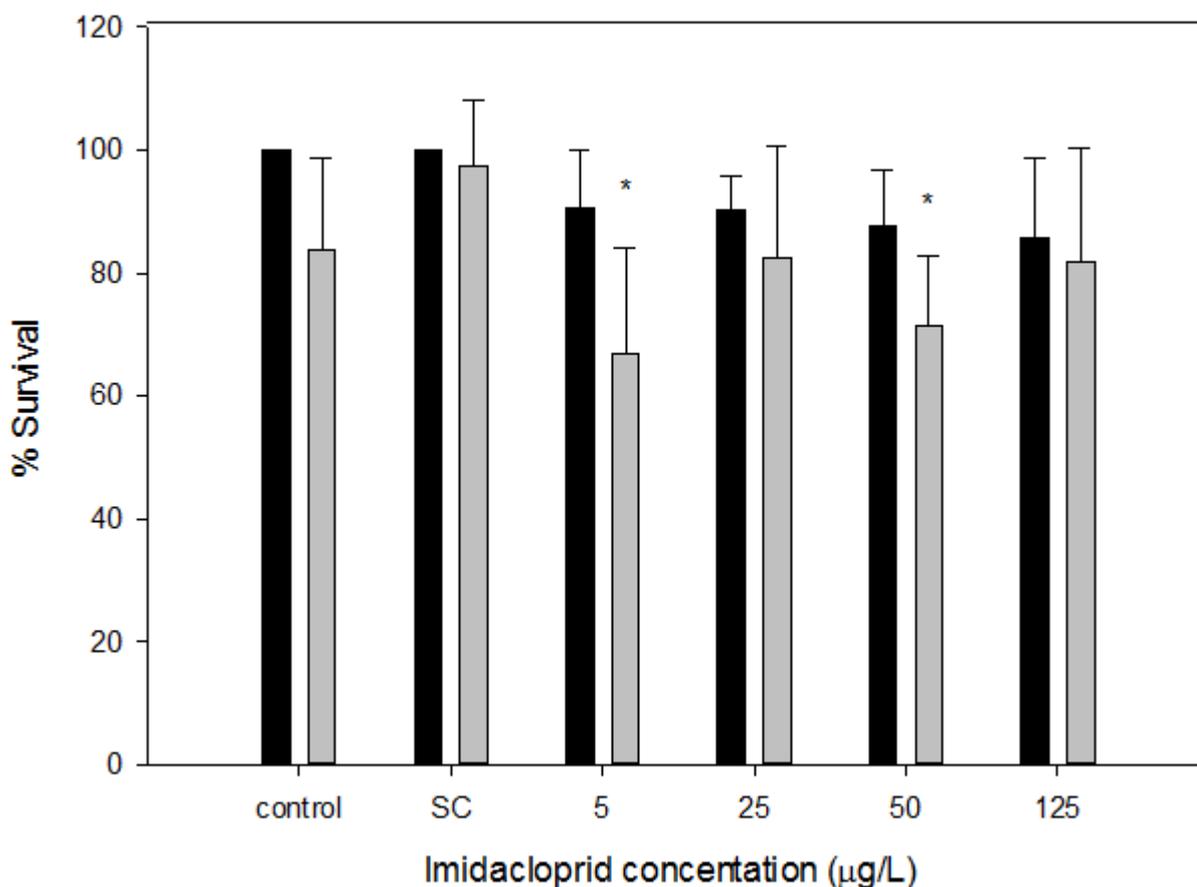
Figure 13. Average survival (mean ± standard deviation) of post larval phase prawns exposed to different concentrations of imidacloprid. Concentrations provided are nominal. The black bar shows survival at 24h, the grey bar shows survival at 48 hours. SC denotes solvent control. Only 5 and 50 µg/L at 48 hours had significantly lower survival than control, as denoted by the asterix (*).

Bifenthrin

The range finder tests indicated that bifenthrin exposures above 1 µg/L caused mortality (Table 6), so in subsequent tests bifenthrin toxicity was measured using concentrations of 1 µg/L and below. Bifenthrin was only tested using the static renewal design. As shown in Figure 14, survival is significantly lower than control at the two highest doses only, though there is an insignificant trend (p=0.062) towards decreased survival at 0.25 µg/L. No post larval prawns survived the 1 µg/L dose. All doses tested did significantly inhibit the ability of the post larval prawns to capture live prey at 24 hours, as shown in Figure 15. At 48 hours, the lowest dose is no longer significant. Whether this is recovery or the data being confounded by differences in survival can not be determined at this point.
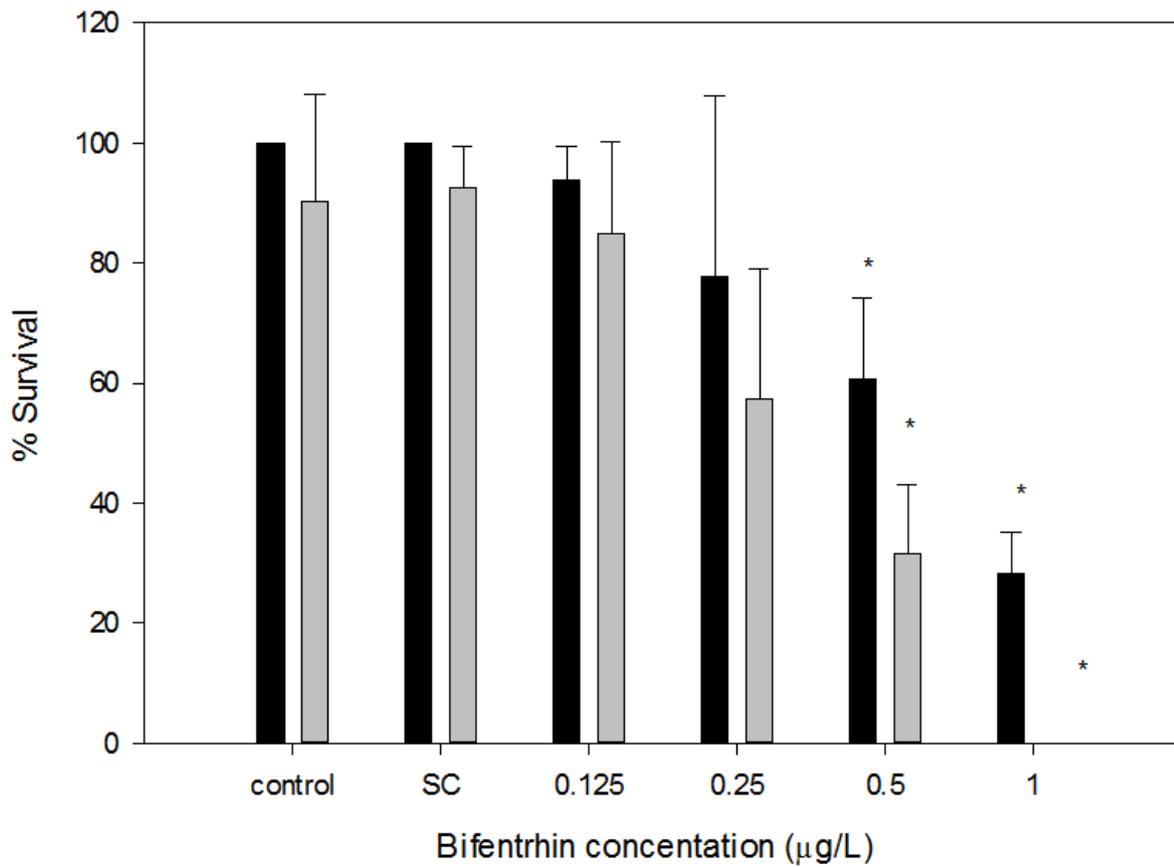
Figure 14. Average survival (mean ± standard deviation) of post larval phase prawns exposed to different concentrations of bifenthrin. Concentrations provided are nominal. The black bar shows survival at 24h, the grey bar shows survival at 48 hours. SC denotes solvent control. Only 0.5 and 1 µg/L at 48 hours had significantly lower survival than control, as denoted by the asterix (*).
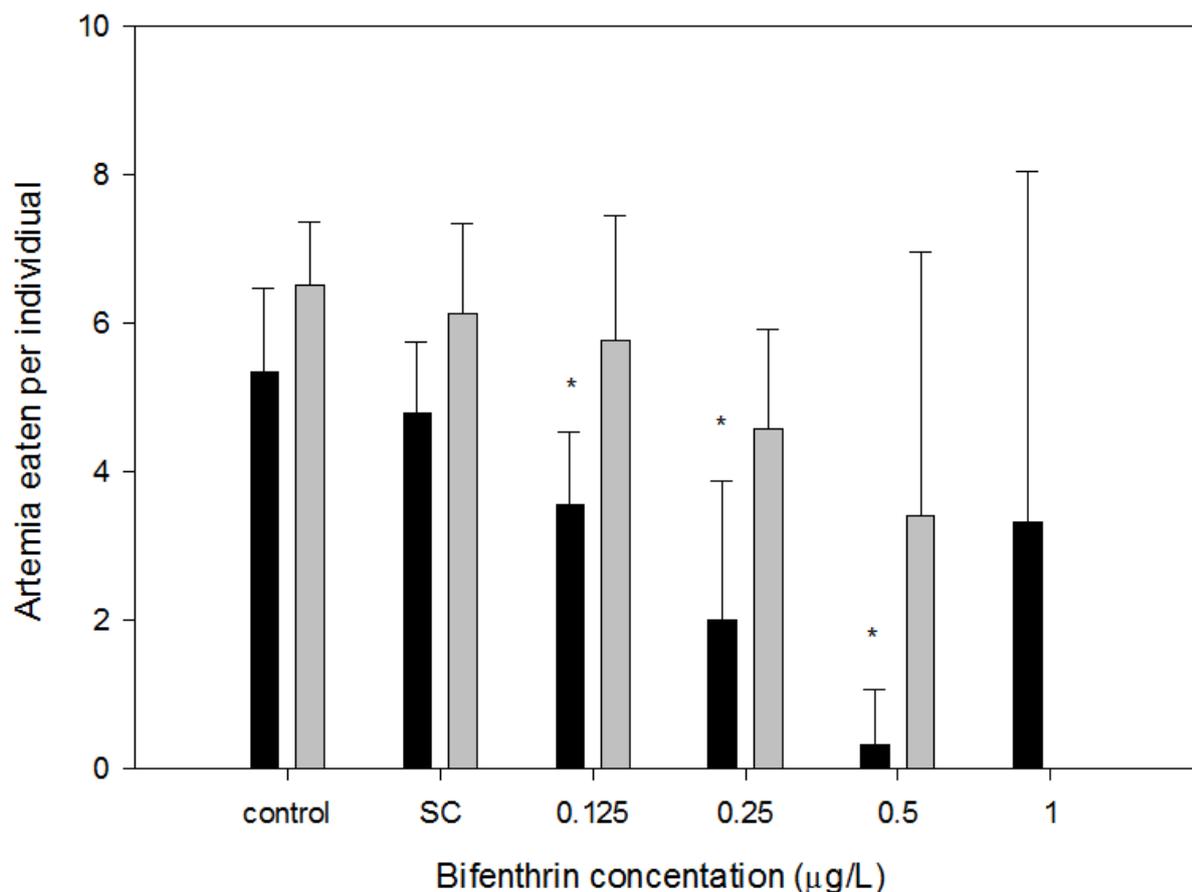
Figure 15. Average artemia consumption (mean ± standard deviation) of post larval phase prawns exposed to differing concentrations of bifenthrin, normalized to surviving individual. Concentrations provided are nominal. The black bar shows prey capture ability at 24h, the grey bar shows prey capture ability at 48 hours. SC denotes solvent control. At 24 h, all doses are statistically different than control, as denoted by the asterix (*).

Chlorpyrifos

In the range finder experiments, no post larval prawns survived in concentrations greater than 1 µg/L (Table 6). Therefore, in subsequent testing, exposures focussed on concentrations of 0.5 µg/L and less. Chlorpyrifos toxicity was measured twice, first in daily renewal tests, then in static tests. As shown in Figure 16, survival was diminished in the 0.5 µg/L treatment after 48 and 72 hours. However, when the test was repeated, no change in survival was observed (data not shown), perhaps because the prawns used were day 15 instead of day 10. In addition, no feeding inhibition was measured in prawn post larvae exposed to Chlorpyrifos (data not shown).
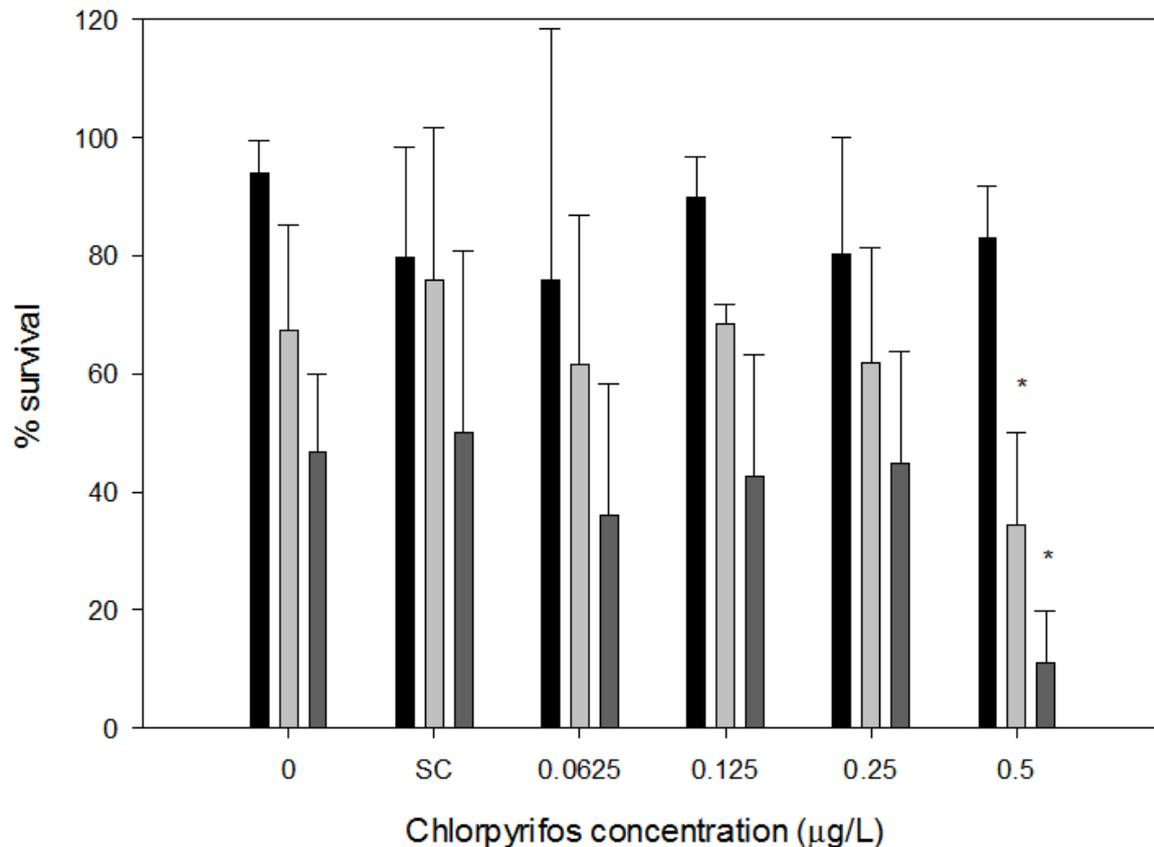
Figure 16. Average survival (mean ± standard deviation) of post larval phase prawns exposed to differeing concentrations of Chlorpyrifos. Concentrations provided are nominal. The black bar shows survival at 24h, the pale grey bar shows survival at 48 hours, the dark grey bar. 0.5 ug/L doses have diminished survival, relative to control, at 48 and 72 hours, as denoted by the asterix (*).

In this series of experiments, lethal toxicity was measured in post-larval prawns exposed to the four different insecticides. Feeding inhibition was measured in two of the four tested compounds. However, the experiments were constrained by the narrow window of opportunity to work with this life stage. Since post-larval prawns were available for a few weeks only, short exposures were necessary. As chlorpyrifos, in particular, is more toxic as it is metabolised, greater toxicity may have been found with longer exposures. Also, the animals were sensitive to handling, so the solutions could not be renewed. As a consequence, the toxicity of imidacloprid may have been underestimated. In addition, the animals sensitivity to being handled meant that it was not practical to isolate them to measure feeding inhibition, meaning that our measures may not have been as precise as would be desired. Nevertheless, these few experiments provide all the data that, to our knowledge, exist in the scientific literature on the sensitivity of post-larval prawns to modern insecticides and thus will be very helpful in their risk assessment.

Of the insecticides studied, fipronil had the greatest effects on post-larval prawns, as measured via survival (Table 7). Imidacloprid had the least impact on survival. However, both imidacloprid and bifenthrin inhibited feeding, which would impair survival if the exposure was prolonged.

Table 7. LC_{50} and LC_{10} values for the insecticides included in this study. Values are based on nominal concentrations.

Pesticide	LC_{50}, µg/L	LC_{10}, µg/L
Imidacloprid	175.00	3.00
Bifenthrin	0.36	0.13
Fipronil	0.20	0.02
Chlorpyrifos*	0.48	NC

* Chlorpyrifos LC_{50} values are based on the first experiment and the 48-h data. AnLC_{10}value could not be derived.

Our study showed that black tiger prawns post-larvae (20 days post hatch) have sensitivities to neonicotinoid and pyrtheroid insecticides that are comparable to other crustaceans. For instance, our data show an LC_{50} for imidacloprid of 175 µg/L, which is similar to 110 and 119 µg/L reported for amphipods and isopods, respectively (Anderson et al., 2015b). *Ceriodaphnia* and another amphipods were reported to have much lower LC_{50} values – approximately 2 µg/L (Anderson et al., 2015b). Black tiger prawns post larvae also have an intermediate sensitivity to bifenthrin with an LC_{50} of 0.36 µg/L, between the 1.6 µg/L reported for *Daphnia* and the 0.0045 µg/L reported for mysids (USEPA OPP ecotoxicology pesticide database, http://www.ipmcenters.org/ecotox/DataAccess.cfm). Their sensitivity to fipronil is comparable to mysids ($LC_{50 of}$ 0.2 µg/L reported here, compared to 0.14 µg/L), one of the most sensitive species to this compound. The sensitivity of black tiger prawns post larvae to chlorpyrifos, which has an LC_{50} of 0.48, is comparable to other, previously reported data for prawns. *Penaeus aztecus* has an LC_{50} of 0.2 µg/L, and *Palaemonetes pugio* has an LC_{50} of 1.5 µg/L (USEPA OPP ecotoxicology pesticide database, http://www.ipmcenters.org/ecotox/DataAccess.cfm).

The studies described are based on nominal, not measured, concentrations, so comparisons of the results to field data should be made cautiously. Never the less, when toxicity data are compared to the concentrations of pesticides reported in the field, we can begin to characterise the risk these compounds may pose. Imidacloprid causes feeding inhibition at nominal water concentrations similar to those reported in Queensland rivers that can be as high as 1 µg/L (Turner et al., unpublished) and as high as 0.45 µg/L in waterways of the Sydney metropolitan area (Sanchez-Bayo and Hyne, 2014). The concentrations of fipronil (0.03-0.05 µg/L) measured elsewhere in the world (Wu et al., 2015; Weston and Lydy, 2015) are greater than the LC_{10} concentrations measured in this study, indicating the potential for impacts for prawn PL's in the Queensland waterways under investigation. The concentrations of bifenthrin measured in the US are less than a factor of 10 below those at which feeding inhibition was measured (0.125 mg/L), again suggesting a moderate potential for impacts. The Chlorpyrifos levels that have been reported in Australia (0.005 µg/L; Scott et al., 2014) are one hundred fold less than the levels at which we measure toxicity (LC_{50} is approximately 0.5 µg/L) suggesting that the risk is lowest for this insecticide, especially as the use of this pesticide is declining. However, these risk estimates are based on chemical concentrations measured elsewhere. A more accurate characterisation of risk will be done when the project is complete and pesticide concentrations in the farm intake waters are available.

Although the toxicity studies described in this report help to characterise risk by determining the sensitivity of post larval prawns to modern use insecticides, they are not sufficient on their own. Currently, there is not sufficient insecticide water quality monitoring data to determine the levels of insecticides in the catchments where prawn farms are located, especially for rivers outside the Great Barrier Reef catchment area. The second part of this project addresses that data short fall.

This portion of the study suggests that there may be some risk as feeding inhibition was measured at concentrations of imidacloprid that have been measured in some catchments in the GBR region (DSITI, unpublished), and in the Sydney metropolitan area (Sancez-Bayo and Hyne, 2014). The concentrations of bifenthrin and fipronil reported elsewhere in the world may also cause toxicity in post larval prawns if similar insecticide levels are measured in intake waters. However, this project alone will not be sufficient to

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fully characterise the risks posed by insecticides on prawn farming operations. The catchments used for prawn farming may contain a suite of different and potentially interacting compounds, studies examining the potential interactions of different pesticides should be conducted as well. Many of the compounds studied, including fipronil, partition to sediments, studies should be conducted examining the role of a sedimentary route of exposure on post larval prawns. Finally, these compounds could be impacting the health of wild crustaceans, including brood stock. These impacts may be the result of direct toxicity or may instead result from impacts on smaller invertebrates that make up the food supply for prawn post larvae and early life stages of many aquatic species.

Chemical Analyses

Insecticides

Imidacloprid was detected in every catchment except the Clarence River and the Burdekin River catchment (Table 8). In some cases, the concentrations were only slightly above the detection limits, but in the Mackay catchment, concentrations ranged from 0.2 -0.42 μg/L imidacloprid. This is consistent with values in the literature, where concentrations between 0.1- 5 μg/L imidacloprid can be measured in other agriculturally impacted water systems worldwide (Sanchez Bayo et al., 2016). Feeding inhibition was measured in this project at imidacloprid concentrations greater than 0.5 μg/L, and previous studies have measured sub-lethal effects at concentrations as low as 0.065 μg/L imidacloprid (van Dijk et al., 2013; Figure 17). Measured concentrations are about half the 1 μg/L imidacloprid at which mortality is reported for ostracods, the most sensitive of the crustaceans (reviewed by Anderson et al., 2015b) (although it is much lower than the LC50 reported in this work). In the Burnett-Mary and Logan catchments, the concentrations measured were lower, but still exceeded European water quality standards of 0.01 μg/L imidacloprid (Table 9; Smit et al., 2015).

Fipronil was detected at only one site, in the Logan catchment (Table 8). Of the catchments surveyed, this catchment has the highest proportion of residential land use. This finding is consistent with the overseas literature, which primarily finds fipronil in residential and urban environments (e.g. Wu et al., 2015, Weston and Lydy, 2014). As shown in Figure 17, the highest concentration measured (0.07 μg/L) would be expected to cause mortality of post larval stage prawns, as determined by both this project and studies reported in the US EPA database. However, fipronil would be expected to partition into sediments (e.g. Wu et al., 2015, Weston and Lydy, 2014), so accumulation in the pond sediments may pose a greater risk to post-larval prawns than concentrations measured in the intake water.

Bifenthrin was also detected only in samples from the Logan River catchment (Table 8). Again, the higher proportion of residential land use in this catchment relative to the others may explain this finding. The highest concentration measured (20 ng/L) is in the range where mortality has been reported in the literature (Ding et al., 2012; Harwood et al., 2013), although it is below the concentrations of 0.1 and 0.5 μg/L bifenthrin, respectively, at which mortality and feeding inhibition were measured in this study (Figure 17) . Like fipronil, bifenthrin would be expected to bind to sediments (Holmes et al., 2008), so water column measurements may underestimate the risk of bifenthrin exposures to prawn larvae.

Chlorpyrifos was detected in samples from the Logan River, and occasionally at concentrations just above the levels of detection in other samples (Table 8). The highest concentration measured (40 ng/L), is above the trigger values (e.g. the concentrations at which toxic effects to some component of the ecosystem have a greater probability of occurring) (ANZECC/ARMCANZ 2000; GBRMPA, 2014). The highest concentration is about half of the 80 ng/L where sub-lethal impacts have been reported in literature studies (Figure 17), and approximately one tenth of the LC50 measured of 480 ng/L in this project (summarised in Section 4). These concentrations also exceed GBRMPA guideline trigger values (Table 3). As with fipronil and bifenthrin, chlorpyrifos partitions to sediment, so water column measures may underestimate the risk (Giddings et al., 2014).

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As shown in Figure 17 and Table 8, the insect repellent DEET (N,N-diethyl-meta-toluamide) was found at comparatively high concentrations (greater than 1 μg/L) at one site in the Wet Tropics. Other surveys in Queensland have also reported elevated concentrations of DEET (DSITI, pers. comm.). There is very little information about the environmental toxicity of DEET in either the scientific literature or the US EPA ecotoxicology database, but the data that do exist suggests that DEET is overtly toxic only at very high concentrations (g/L). This is consistent with DEET's known mode of action as a deterrent, meaning that insects avoid contact with the substance but it does not kill them. However, some studies have suggested that exposure to DEET may make food less palatable to decapod crustaceans (Aggio et al., 2012). Because of this, it is possible that simultaneous exposure to DEET and some of the other insecticides may exacerbate feeding inhibition.

Clothiandin, a neonicotinoid insecticide with similar toxic impacts to imidacloprid, was measured at 0.005 μg/L in samples from the Logan River only. These concentrations were much lower than the 1 μg/L levels expected to cause a toxic impact (Figure 17). All other insecticides included in the study (listed in Table 5) were below the levels of detection at all sites.

Table 8. Pesticide concentrations measured in three replicate samples collected from intake waters at participating prawn farms. Only those compounds detected in at least one sample are reported. Mean concentrations are presented, with the range of concentrations measured provided in brackets. Values that are italicised are less than the levels of reporting, as given in Table 1. Bold concentrations exceed guideline values lowest water quality guideline values as provided in Table 9. The highest measured concentrations are denoted with an asterix (*).

			Insecticides (µg/L)					
Catchment	**Farm**	**Date**	**Bifenthrin**	**Chlorpyrifos**	**Clothianidin**	**DEET[a]**	**Fipronil**	**Imidacloprid**
Clarence, NSW	A	20-Mar-17	*<0.0025*	0.0076 (*<0.005*-**0.0179**)	*<0.001*	*<0.001*	*<0.005*	*<0.0025*
Logan, QLD	B	22-Sep-16	0.0091 (0.0027-0.0206*)	0.0247 (0.0063-**0.0430***)	0.0049 (0.0048-0.0049*)	*<0.001*	0.028 (*<0.005*-0.0738*)	**0.0136** (0.0047-**0.0297**)
Burnett-Mary, QLD	C	4-April-17	*<0.0025*	*<0.005*	*<0.001*	*<0.001*	*<0.005*	**0.0146** (**0.0115-0.0173**)
Mackay, QLD	D	13-Dec -17	*<0.0025*	0.0036 (*<0.005*-0.0057)	*<0.001*	0.0056 (0.0012-0.0116)	*<0.005*	**0.3450 (0.271-0.415*)**
Burdekin, QLD	E	19-April-2017	*<0.0025*	*<0.005*	*<0.001*	0.046 (0.040-0.049)	*<0.005*	*<0.0025*
Wet Tropics	F	27-Sept-2016	*<0.0025*	*<0.005*	*<0.001*	0.0208 (0.0206-0.0210)	*<0.005*	0.0028 (0.0027-0.003)
Wet Tropics	G	27-Sept-2016	*<0.0025*	*<0.005*	*<0.001*	1.3912 (1.1615-1.6315*)	*<0.005*	0.0029 (0.0026-0.0032)

			Herbicides (μg/L)						
						Herbicides (μg/L)			
Catchment	Farm	Date	2,4-D[b]	Atrazine	Diuron	Hexazinone	MCPA[c]	Metalochlor	Tebuthiuron
Clarence, NSW	A	20-Mar-17	0.0136 (0.0126-0.01450	0.0092 (0.0077-0.0110)	0.0176 (0.0163-0.0187)	0.0036 (0.0034-0.0038)	*<0.005*	0.0097 (0.0096-0.0098)	0.0021 (0.0017-0.0023*)
Logan, QLD	B	22-Sep-16	0.058 (0.0543-0.0644*)	0.0038 (0.0029-0.0042)	*<0.001*	*<0.0025*	0.014 (0.0134-0.0145)	0.188 (0.174-**0.211**)	*<0.001*
Burnett-Mary, QLD	C	4-April-17	0.0297 (0.0276-0.0311)	0.127 (0.120-0.136)	0.0144 (0.0137-0.0153)	0.0034 (0.0032-0.0036)	*<0.005*	**0.150 (0.147-0.155)**	*<0.001*
Mackay, QLD	D	13-Dec -17	0.0444 (0.044-0.045)	0.184 (0.148-0.206)	0.2570 (0.2350 – 0.2740)	0.050 (0.0482-0.0516*)	0.0222 (0.0213-0.0226)	0.0064 (0.0060-0.0067)	*<0.001*
Burdekin, QLD	E	19-April-2017	*<0.001*	0.0027 (0.0025-0.0030)	0.0039 (0.0034-0.0043)	*<0.0025*	*<0.005*	*<0.005*	*<0.001*
Wet Tropics	F	27-Sept-2016	0.004 (0.0015-0.0065)	*<0.0025*	*<0.001*	*<0.0025*	*<0.005*	*<0.005*	*<0.001*
Wet Tropics	G	27-Sept-2016	0.0502 (0.0489-0.0519)	0.433 (0.403-0.471*)	**1.069 (0.933-1.178*)**	0.0027 (0.0025-0.0030)	0.0583 (0.0546-0.0618*)	**0.529 (0.497 – 0.553*)**	*<0.001*

| Catchment | Farm | Date | Other compounds (μg/L) | | | | Fungicide | Corrosion inhibitor |
| | | | Adjuvants | | | | | |
			4-n -Nonylphenol	4-Nonylphenol-mono-ethoxylate	4-tert-Octylphenol	4-Nonylphenol-di-ethoxylate	Metalaxyl	Benzotriazole
Clarence, NSW	A	20-Mar-17	*<0.025*	*<0.005*	*<0.005*	*<0.005*	*<0.005*	*<0.005*
Logan, QLD	B	22-Sep-16	*<0.025*	0.0151 (0.010-0.0211)	*<0.005*	0.0090 (0.006-0.0142)	*<0.005*	0.0729 (0.0678-0.0776)
Burnett-Mary, QLD	C	4-April-17	*<0.025*	0.0062 (<0.005-0.0101)	*<0.005*	0.0037 (<0.005-0.0060)	0.0059 (0.0053-0.0064)	*<0.005*
Mackay, QLD	D	13-Dec -17	*<0.025*	0.0151 (<0.005-0.0154)	0.0055 (0.0041-0.0067)	0.0068 (0.0052-0.0097)	*<0.005*	*<0.005*
Burdekin, QLD	E	19-April-2017	*<0.025*	*<0.005*	*<0.005*	*<0.005*	*<0.005*	*<0.005*
Wet Tropics	F	27-Sept-2016	*<0.025*	*<0.005*	*<0.005*	*<0.005*	*<0.005*	*<0.005*
Wet Tropics	G	27-Sept-2016	*<0.025*	*<0.005*	*<0.005*	*<0.005*	*<0.005*	*<0.005*

[a] DEET is N,N-diethyl-meta-toluamide; [b] 2,4-D is 2,4-dichlorophenoxyacetic acid; [c] MCPA is 2-methyl-4-chlorophenoxyacetic acid
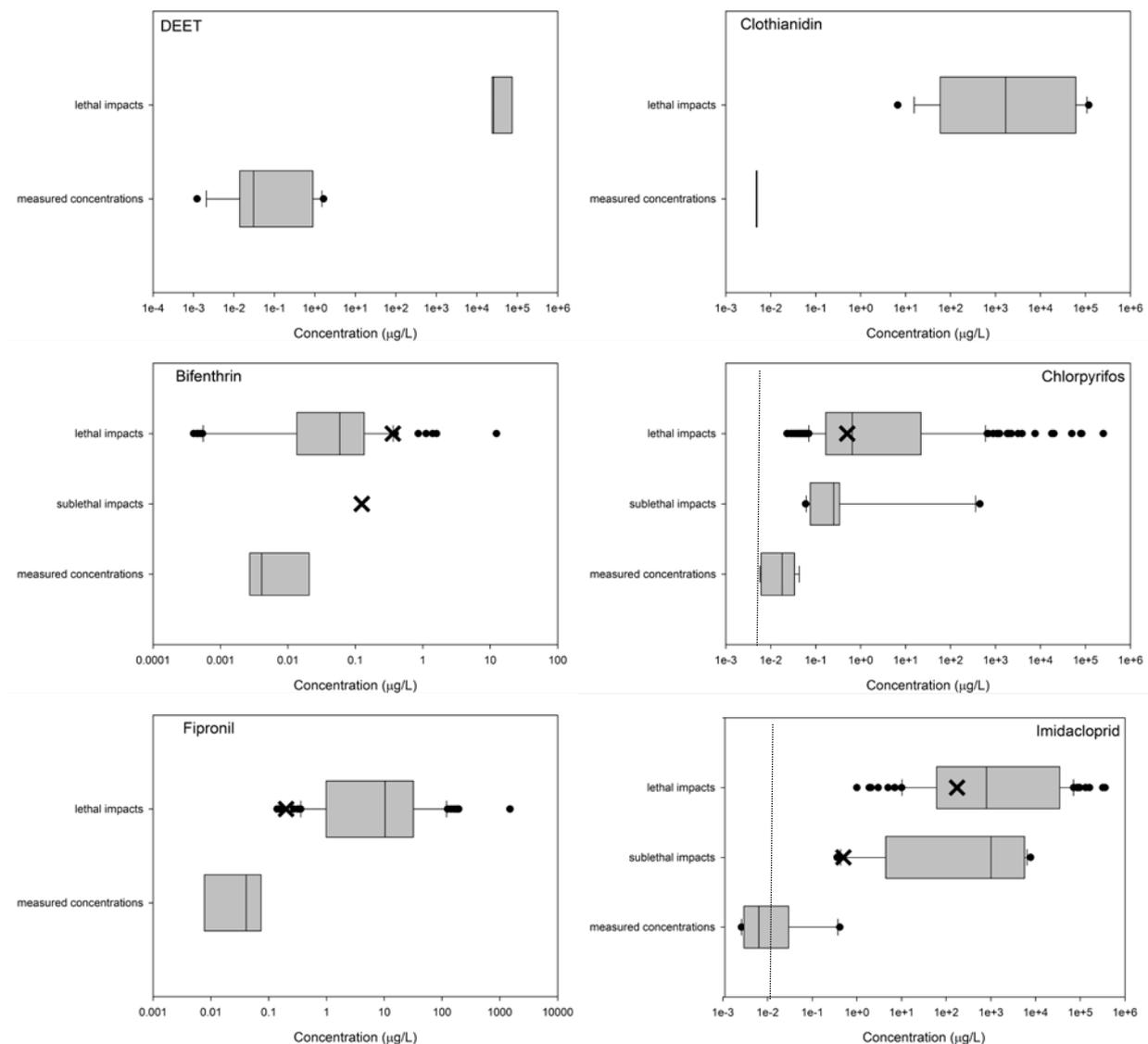
Figure 17. Measured concentrations of each insecticide compared to concentrations at which lethal and sublethal impacts have been reported in crustaceans. The range of concentrations measured and toxicity thresholds from literature studies, obtained from the US EPA's ecotoxicity database, (**https://cfpub.epa.gov/ecotox/**) are shown using the box plots, toxicity studies from our previous work with prawn larvae are shown with X. For the box plot, the median concentration is shown with the center bar, the out edges of the box denote the 25th and 75th percentile, whiskers show the 10th and 90th percentiles, and dots denote outliers. Dashed lines indicate the lowest water quality guidelines as provided in Table 9, where available

Table 9. ANZECC/ARMCANZ and GBRMPA water quality guideline values for pesticides measured in prawn farm intake waters. No guideline values are given for bifenthrin, clothianidin, DEET, fipronil, nonylphenol or the other alkyl phenol ethoxylates, metalaxyl or benzotriazole. European guideline values are used for imidacloprid as they are the only ones available. Dashes denote that values are not provided in these databases.

Pesticide	ANZECC/ARMCANZ Freshwater Trigger Value[a] (µg/L)	ANZECC/ARMCANZ Marine Trigger Value[a] (µg/L)	GBRMPA Trigger Value[a] (µg/L)	European Guideline Values[b] (µg/L)
Chlorpyrifos	0.01	0.009	0.0005	
Imidacloprid	-	-	-	0.01
2,4-D	36	36	0.8	
Atrazine	18	18	0.6	
Diruon	0.2	1.8	0.9	
Hexazinone	75	75	1.2	
MCPA	1.4	1.4	-	
metolachlor	0.02	0.02	-	
Teburthiuron	-	-	0.02	

[a] The ANZECC/ARMCANZ water quality guidelines are for the protection of 95% of species, the GBRMPA trigger values are for the protection of 99% of species. Data obtained from: http://www.agriculture.gov.au/water/quality/guidelines or from http://www.gbrmpa.gov.au/managing-the-reef/how-the-reefs-managed/water-quality-in-the-great-barrier-reef/water-quality-guidelines-for-the-great-barrier-reef
[b] obtained from Smit et al., 2015

Herbicides

Herbicides were the compounds most frequently encountered and were measured in concentrations as high as 1 µg/L (Table 8). Despite this, these concentrations are often below the ANZECC and GBRMPA trigger values shown in Table 9 (ANZECC/ARCANZ, 2000, GBRMPA, 2010) and below the concentrations that have been shown to be toxic to crustaceans (Figure 18). 2,4-D (2,4-dichlorophenoxyacetic acid) was detected in every sample, but at concentrations less than 0.1 µg/L, whereas toxic impacts in crustaceans have only been reported at concentrations greater than 1000 µg/L. Concentrations of 2,4D are also below GBRMPA guideline trigger values of 0.2 µg/L (Table 9). Atrazine was detected in all but one intake point, and at concentrations as high as 0.5 µg/L, which is approaching the GBRMPA trigger values. The highest measured concentrations are still lower than 100 µg/L at which sub-lethal impacts have been reported or 1000 µg/L where mortality occurs in crustaceans (Figure 18). Diuron and hexazinone were detected in samples from the Mackay, Wet Tropics, Burnett-Mary and Clarence catchments, with diuron concentrations greater than 1 µg/L in the Wet Tropics, exceeding the GBRMPA trigger values of 0.9 µg/L (Table 9). These concentrations are still lower than the 1000 µg/L diuron concentrations that cause mortality in crustaceans, or the 100 µg/L hexazinone concentrations that cause sublethal impacts in crustaceans (Figure 18). MCPA and metolachlor were also frequently detected at concentrations as high as 0.5 µg/L, which exceeds

ANZECC/ARMCANZ guidelines (Table 9). These compounds have not been well studied from an ecotoxicological perspective, but the data that do exist suggest that toxic impacts in crustaceans occur at concentrations greater than 1000 μg/L. Tebuthiuron was measured in concentrations only slightly above the levels of detection in samples from the Logan River, and are below GBRMPA trigger values. These levels are not expected to have a toxic impact. No other herbicides (Table 5) were measured in the samples collected for this study.

Although the concentrations of herbicides measured in this survey would not be expected to directly impact the prawn larvae, exposure to herbicides at these concentrations may increase the toxic impacts of exposure to the insecticides. For example, mixtures of atrazine, imidacloprid and atrazine were more toxic to grass shrimp than exposure to either imidacloprid or fipronil alone, even though the grass shrimp were comparatively insensitive to atrazine (Key et al., 1997). The potential for toxic impacts of exposures to mixtures should be considered.
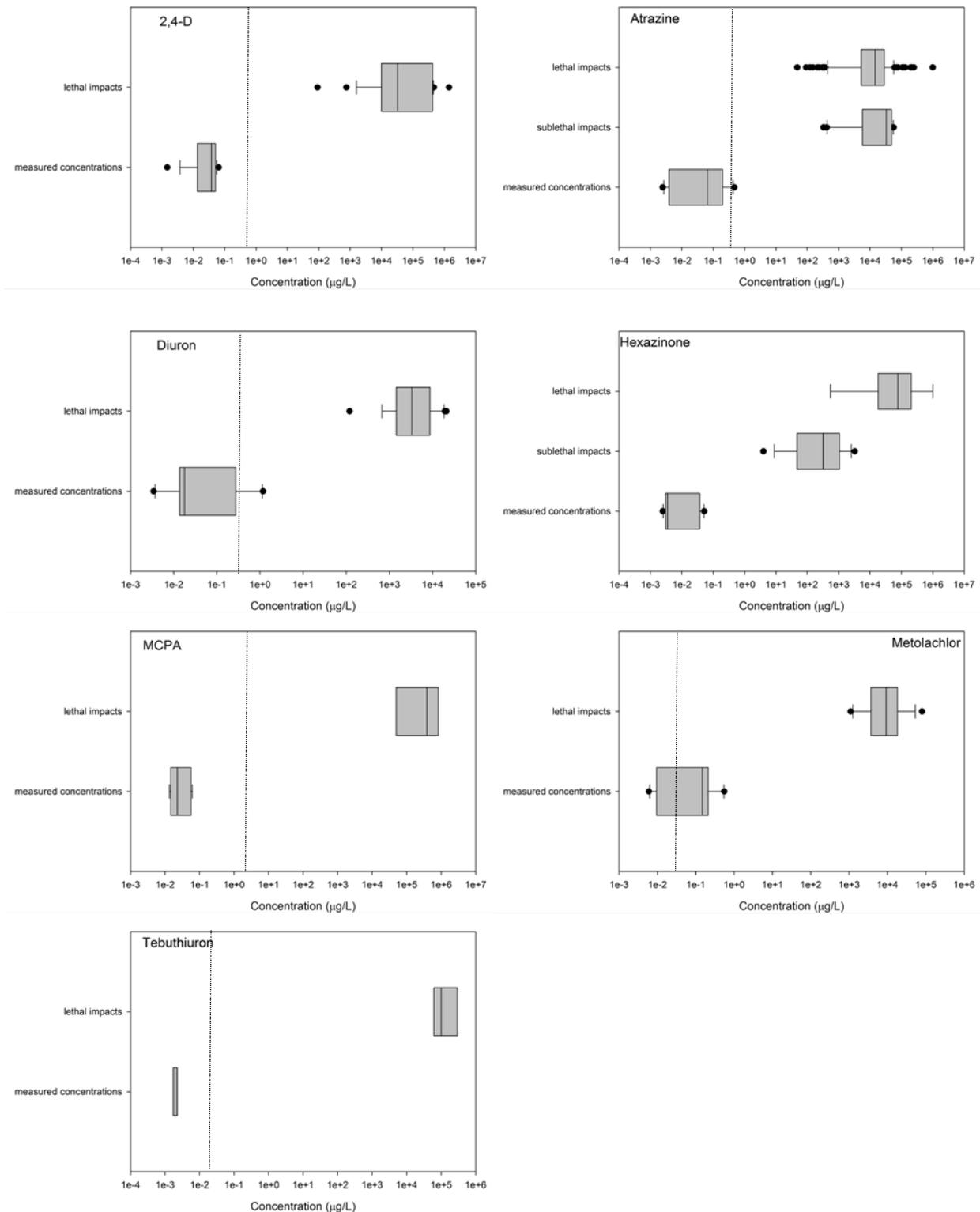
Figure 18. **Measured concentrations of each insecticide compared to concentrations at which lethal and sublethal impacts have been reported in crustaceans. The range of concentrations measured and toxicity thresholds from literature studies, obtained from the US EPA's ecotoxicity database, (https://cfpub.epa.gov/ecotox/) are shown using the box plots. For the box plot, the median concentration is shown with the center bar, the out edges of the box denote the 25th and 75th percentile, whiskers show the 10th and 90th percentiles, and dots denote outliers. Dashed lines indicate water quality guidelines, where available.**

Other Compounds

The surfactant nonylphenol is commonly used to increase the absorption and as a consequence efficacy of many pesticides (e.g. Kroon et al., 2015). Although nonylphenol was not detected in these samples, its break down products, including 4-Nonylphenol-mono-ethoxylate, 4-tert-Octylphenol, and 4-Nonylphenol-di-ethoxylate (Careghini et al., 2015) were detected in all catchments except the wet tropics (Table 8). The concentrations of these compounds measured in the water is quite low, beneath those that would be expected to cause sublethal toxic effects or mortality. However, these compounds may increase the uptake of the other pesticides by prawns. Also, these compounds also bind to sediments, so the concentrations measured in the water column would be expected to be much lower. Risk may be higher than would be predicted from the water column concentrations.

Other compounds were occasionally detected in these water samples, typically at concentrations that were far lower than those known to cause toxic effects in crustaceans (Figure 19). These compounds included the fungicide metalxyl in the Burnett/Mary Catchment and the corrosion inhibitor benzoatriazole in the Logan catchment. While these compounds have not been well studied from an ecotoxicological perspective, they would still be considered to pose little environmental risk. Other compounds were included in the survey (Table 5), but were not detected.
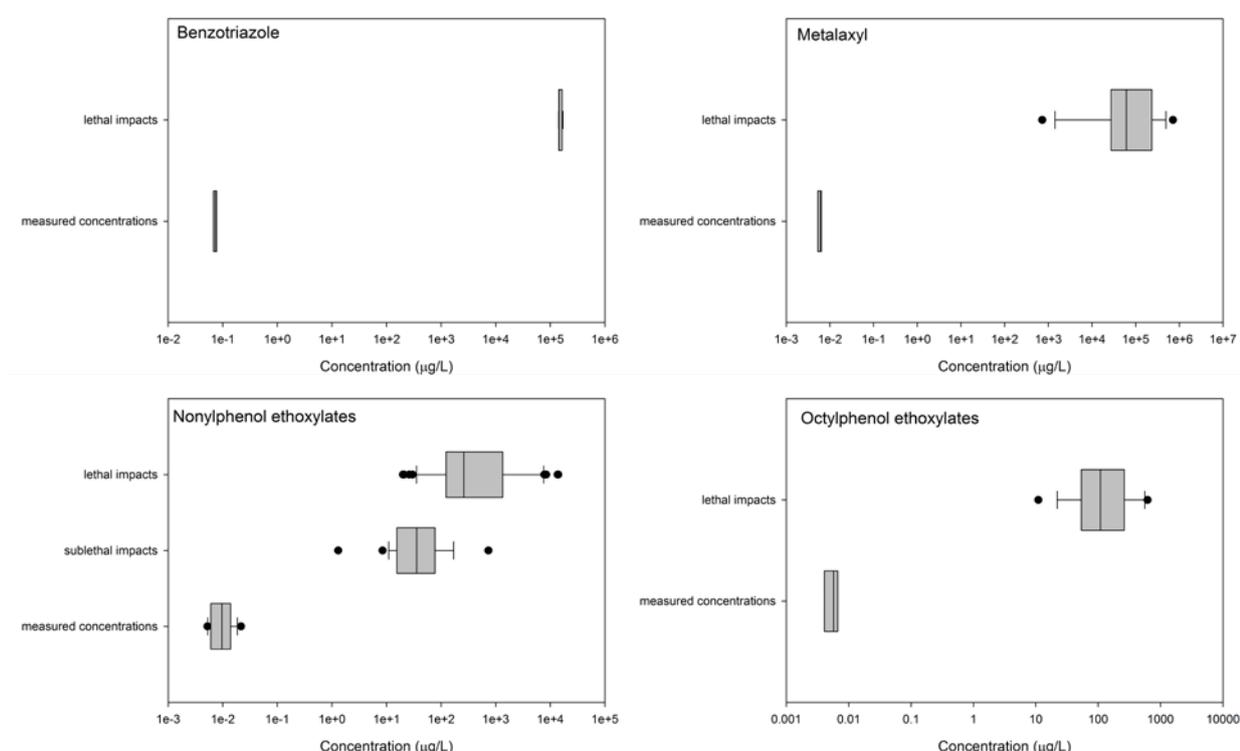
Figure 19. Measured concentrations of other analytes compared to concentrations at which lethal impacts have been reported in crustaceans. The range of concentrations measured and toxicity thresholds from literature studies, obtained from the US EPA's ecotoxicity database, (https://cfpub.epa.gov/ecotox/) are shown using the box plots. For the box plot, the median concentration is shown with the center bar, the out edges of the box denote the 25th and 75th percentile, whiskers show the 10th and 90th percentiles, and dots denote outliers.

Limitations

This study provides important information about the potential impacts of exposure to pesticides for prawn farmers, however, there are some important limitations. One is that each catchment is represented by a single sampling period. Samples were typically collected after a rain event during the growing season when pesticide concentrations were expected to be highest (Devlin et al., 2015; Garcia-Garzon et al., 2015), but

none the less, we can not be certain they represent the highest concentrations as we do not know when pesticides are applied. The samples from the Burdekin, however, were collected outside of the peak period of pesticide application, and a few weeks after Cyclone Debbie, which made landfall 31 March 2017, so the system may have been flushed. Concentrations measured in this sample may not be typical of the watershed as a consequence and may have lower than normal pesticide concentrations. Continuous monitoring with passive samplers may be a better way to calculate exposure.

This study also assumes that the risk is driven by the water borne pesticides. For imidacloprid, which is highly water soluble (Smit et al., 2015), this assumption is probably valid, but the other insecticides, such as bifenthin, fipronil and chlorpyrifos, are more likely to bind to sediment (Holmes et al., 2008, Giddings et al., 2014). These pesticides may build up in ponds over time and may pose a risk to prawn aquaculture that is greater than would be predicted by a single water column measure. Additional sediment based studies may be required to properly assess the risk.

Another important qualification of this study is that the risk is based on single exposure only, and does not evaluate chemical mixtures. The vast majority of laboratory studies, including those conducted as part of this project, test the impacts of exposure to single compounds, even though it's known that most environments are contaminated with a mixture of different classes of compounds. Compounds with similar modes of action may have an additive effect, and compound that inhibit pesticide metabolising enzymes may have a synergistic effect.

Table 10. The values used for risk assessment. The maximum concentration is the highest in any of the replicate samples in Table 8. The sublethal and lethal literature values were taken from the USA EPA pesticide database. Where available, the lowest water quality guideline value is provided (Table 9).

Insecticides (µg/L)								
	Values Derived From This Study				Literature Values			
Compound	LC$_{50}$	Feeding inhibition	Max. Concentration	Catchment	Sublethal	LC$_{50}$	References	Water quality guideline
Bifenthrin	0.36	<0.125	0.02	Logan	0.004	0.005	Ding et al., 2012; Harwood et al., 2013	-
Chlorpyrifos	0.48	-	0.043	Logan	0.0076	0.0023	Deanovic et al., 2013; Asselman et al. 2014	0.0005[b]
Clothianidin	n.d.	n.d.	0.005	Logan	6.67	12.52	De Perre et al., 2015	-
DEET[a]	n.d.	n.d.	1.63	Wet Tropics	24000	300	Ralston-Hooper et al., 2009	-
Fipronil	0.2	-	0.074	Logan	0.155	0.14	US EPA, 1992, Weston and Lydy, 2014	-
Imidacloprid	175	<0.5	0.415	Mackay	0.36	2.07	Chen et al., 2010; Sanchez-Bayo and Goka, 2012	0.01[c]

Table 11 (Cont.)

Herbicides (μg/L)							
	Values Derived From This Study				Literature Values		
Compound	LC$_{50}$	Feeding inhibition	Max. Concentration	Catchment	Sublethal	LC$_{50}$	References	Water quality guideline
2,4-D[b]	-	-	0.0644	Logan	2800	91.2	Soto et al., 2000, US EPA, 1992	0.8[b]
Atrazine	-	-	0.471	Wet Tropics	330	48	Noppe et al., 2007; Kallqvist and Romstad, 1994	0.6[b]
Diuron	-	-	1.178	Wet Tropics	120	1100	US EPA, 1992	0.9[b]
Hexazinone	-	-	0.0516	Mackay	4	550	Tatum et al., 2012; Thompson et al., 1990	1.2[b]
MCPA[c]	-	-	0.0618	Wet Tropics	830000	50000	Davies et al., 1994, US EPA, 1992	1.4[a]
Metolachlor	-	-	0.553	Wet Tropics	1100	1410	US EPA, 1992, Foster et al., 1998	0.02[a]
Tebuthiuron	-	-	0.0023	Clarence	62000	-	US EPA, 1992	0.02[b]
Other Compounds (μg/L)							
Nonylphenol	-	-	0.0211	Logan	0.5	20	Hong and Li, 2007; Jordao et al., 2016	-
Octylphenol	-	-	0.0067	Mackay	13	11	Ra et al., 2008, Anderson et al., 2001	-
Benzotriazole[*]	-	-	0.0776	Logan	-	147300	Li, 2013	-
Metalaxyl	-	-	0.0064	Burnett-Mary	12500	730	US EPA, 1992,	-

* data is only available for flatworms
a ANZECC water quality trigger values; b GBRMPA water quality trigger values; c European water quality guideline valu

Conclusions and Implications

Review of the international scientific literature suggested the possibility of risk of toxic impacts in prawn post larvae exposed to pesticides present in Queensland estuaries, however, given the range in sensitivities of different crustaceans to imidacloprid in particular and the potential for dilution of contaminants in estuarine environments, this risk needed to be confirmed. Our toxicological assessments in this study suggested that prawns have sensitivities to imidacloprid, chlorpyrifos, bifenthrin and fipronil at concentrations that are comparable to other crustaceans, and are susceptible to imidacloprid and bifenthrin induced feeding inhibition. Furthermore, our analysis of the pesticide concentrations in prawn farm intake waters suggests that in at least some samples, concentrations of all four insecticides are high enough to cause negative impacts on post larval growth and survival (Table 10). Some of these measured pesticide concentrations are also above the lowest concentrations that have been reported to be harmful to crustaceans in literature studies (Table 10). In addition, herbicides were found at elevated concentrations, in some cases above water quality criteria (Table 10). Although these measured herbicide concentrations are much below those that cause direct harm to crustaceans, they could cause indirect impacts by disrupting either algal cultures or the food chain, or could cause interactive impacts.

Recommendations

For this project, the results will be further disseminated via a presentation at the upcoming Australian Prawn Farmer's Association conference (Gold Coast, QLD, 1-2 August). The results are also being prepared for publication, ideally in Ecotoxicology and Environmental Safety. We are in ongoing discussions with Queensland Department of Science, Information Technology and Innovation to partner with them to do additional estuarine monitoring for pesticide contamination.

Further development

While this initial study confirms the potential for impacts, further studies are needed to properly assess any negative consequences. Some of the areas for future study include:

- Assessment of pesticide concentrations in pond sediments as well as the toxicological impacts associated with these exposures;

- Additional assessment of the behavioural changes associated with insecticide exposure, including prolonged or repeated exposures;

- Assessment of the impacts of exposures to chemical mixtures;

- Assessment of the efficacy of water treatment systems of potentially impacted farms.

- Additional monitoring at regular intervals to determine seasonal variation of pesticide concentrations.

Extension and Adoption

The project has been extended and communicated to the community as a whole through conversations with members of the Australian Prawn Farmer's Association and conversations with state agencies (including Queensland Department of Science, Information Technology and Innovation, Queensland Department of Environment and Heritage Protection, New South Wales Department of Primary Industries). The research will be communicated to the broader scientific community via publication and presentations at the APFA conference.

Glossary

Acetylcholinesterase: an enzyme that breaks down neurotansmitters , stopping nerves from signalling and allowing them to return to a resting state.

Depuration: Excreting a compound from the body.

EC$_{50}$: The concentration required to impact 50% of a population. Used for endpoints other than survival.

Enzyme: A protein that carries out a specific function (usually a biochemical reaction) in a cell.

Enzyme inhibition: when a chemical binds to an enzyme and prevents it from carrying out that chemical reaction.

Ergosterol: a compound found in the cell membrane of fungi

In vitro: studies conducted in cell lines are other simulations of living systems.

LC$_{50}$: The concentration required to kill 50% of a population. Inversely related to toxicity (compounds with a low LC$_{50}$ are very toxic, compounds with a high LC$_{50}$ are less toxic).

Metabolite: A break down product or the end product of metabolism.

Mixed Function Oxidases: enzymes that break down organic compounds in cells.

Mode of action: The physiological pathway by which a contaminant causes toxicity.

Neonicotinoid Insecticide: a class of insecticide that are similar to nicotine and inhibit insect nervous systems by altering nicotinic acetylcholinesterase receptors, causing effects similar to acetylcholinestase inhibition.

Organophosphate Insecticide: an organic phosphorus containing pesticide that causes toxicity via nerve damage by inhbiting acetylcholine esterase.

Pesticide load: The amount of pesticide entering a system in year. Pesticide loads are typically modelled.

Pesticide Concentration: The measured amount of pesticide in a volume of sample. Pesticide concentrations are typically measured.

Phenylpyrazole insecticide: a class of compounds that inhibits the insect nervous system by blocking GABA gated nerve channels.

Photosystem II inhibiting herbicides: a class of compounds that disrupt the proteins that plants use to capture energy from light during photosynthesis

Pyrethriod Insecticide: any insecticide created from the pyrethrin compounds normally produced by Chrystanthemums. They prevent voltage sensitive gates in the nerve from closing.

Range finder: A series of experiments used to identify the concentrations at which an organism is sensitive; e.g. the concentrations at which subsequent studies will be conducted.

Regression Analysis: using the mathematical relationships between two variables to make predictions.

Species Sensitivity Distribution: also SSD. A plot that enables both the comparison of the sensitivity of different taxa to a compound and enables the prediction of save concentrations of that compound.

Toxicity Identification Evaluation: also TIE. A series of toxicity tests designed to identify the toxic agent in a complex mixture.

Trigger value: a pesticide concentration that indicates increased possibility of negative consequences.

Water Solubility: The amount of a compound that will dissolve in water.

Project materials developed

Hook, S.E., Doan, H., Gonzago, D., Musson, D., Du, J., Kookana, R., Sellars, M., Kumar, A. in preparation. Modern use pesticides impacts on prawn aquaculture: a risk assessment for North Eastern Australia. *To be submitted to Ecotoxicology and Environmental Safety*.

Appendices

Project staff

Name	Department	Role
Sharon Hook	CSIRO Oceans and Atmosphere	Experimental Design; Toxicological Assessments; Data Analysis; Report Preparation; Project Management
Melony Sellars	CSIRO Agriculture and Food	Experimental Design; Report Preparation; Communication
Anu Kumar	CSIRO Land and Water	Experimental Design; Data Analysis; Report Preparation
Hai Doan	CSIRO Land and Water	Toxicological Assessments; Chemical Analysis
Debra Gonzago	CSIRO Land and Water	Toxicological Assessments
Dean Musson		Toxicological Assessments; Animal Husbandry
Jun Du	CSIRO Land and Water	Chemical Analysis
Rai Kookana	CSIRO Land and Water	Chemical Analysis; Data Analysis

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