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The impacts of modern-use pesticides on shrimp aquaculture: An assessment for north eastern Australia

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ABSTRACT

The use of pyrethroid and neonicotinoid insecticides has increased in Australia over the last decade, and as a consequence, increased concentrations of the neonicotinoid insecticide imidacloprid have been measured in Australian rivers. Previous studies have shown that non-target crustaceans, including commercially important species, can be extremely sensitive to these pesticides. Most shrimp farms in Australia are predominantly located adjacent to estuaries so they can obtain their required saline water, which support multiple land uses upstream (e.g. sugar-cane farming, banana farming, beef cattle and urbanisation). Larval and post-larval shrimp may be most susceptible to the impacts of these pesticides because of their high surface area to volume ratio and rapid growth requirements. However, given the uncertainties in the levels of insecticides in farm intake water and regarding the impacts of insecticide exposure on shrimp larvae, the risks that the increased use of new classes of pesticide pose towards survival of post-larval phase shrimp cannot be adequately predicted. To assess the potential for risk, toxicity in 20 day past hatch post-larval Black Tiger shrimp (*Penaeus monodon*) to modern use insecticides, imidacloprid, bifenthin, and fipronil was measured as decreased survival and feeding inhibition. Post-larval phase shrimp were sensitive to fipronil, bifenthrin, and imidacloprid, in that order, at concentrations that were comparable to those that cause mortality other crustaceans. Bifenthrin and imidacloprid exposure reduced the ability of post-larval shrimp to capture live prey at environmentally realistic concentrations. Concentrations of a broad suite of pesticides were also measured in shrimp farm intake waters. Some pesticides were detected in every sample. Most of the pesticides detected were measured below concentrations that are toxic to post-larval shrimp as used in this study, although pesticides exceed guideline values, suggesting the possibility of indirect or mixture-related impacts. However, at two study sites, the concentrations of insecticides were sufficient to cause toxicity in shrimp post larvae, based on the risk assessment undertaken in this study.

1. Introduction

Population growth and the emerging middle class are expected to lead to an increasing demand for high quality protein for consumption, and specifically for seafood. As traditional fishing stocks are frequently either fished at their maximum sustainable yield or overexploited (e.g. Pauly and Zellar, 2016), it is anticipated that much of the increased demand for seafood will come from aquaculture (Tacon and Metian, 2013). Aquaculture yields one billion dollars a year in Australia, and shrimp aquaculture has a value of $60 million, primarily from north east Queensland (Stephan and Hosbawn, 2014). Aquaculture needs to be conducted in areas with good water quality, both to ensure adequate growth and survival of the organisms and to maintain market desirability. In north east Australia, traditional aquaculture is conducted in estuarine areas with additional land uses, such as residential development and agriculture (e.g. DSITIA, 2012). These land uses have been associated with declines in water quality and pesticide run off. Agriculture, specifically sugar-cane farming, has been identified as one of the greatest sources of sediments, nutrients and pesticides to catchments in the Great Barrier Reef region (Brodie et al., 2012; Smith et al., 2012), many of which are adjacent to shrimp farms. Elevated insecticide concentrations have also been measured in residential areas in the US, particularly in areas with highly manicured lawns and gardens (e.g. Weston and Lydy, 2014; Wu et al., 2015).

Changes in the useage patterns of insecticides may lead to increased risks associated with pesticide exposure for shrimp farms. Because of

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environmental concerns about the effects of organophosphate pesticides, their use is being phased out because of concerns about their neurological impacts on humans and fish (e.g Laetz et al., 2009; Eskenazi et al., 2007). They are being replaced by other types of insecticides: chiefly pyrethroids and neonicotinoids (Giddings et al., 2014). 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 gamma-aminobutyric acid (GABA) receptors in insect and crustacean nerve cells (Stevens et al., 2011). Neonicotinoid compounds, such as imidacloprid, are specifically designed to alter the normal activity of arthropods (including insects and crustaceans) nicotinic acetylcholinesterase activity, which are structurally different to 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). Previous studies have shown a wide range in sensitivities in crustaceans (reviewed in Anderson et al., 2015b), with daphnids and other cladocerans having low sensitivities (LC_{50} values greater than 10 000 μg/L), whereas ostracods are highly sensitive (LC_{50} values approximately 1 μg/L). Neonicotinoid 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 insecticides can also persist in the environment. Bifenthrin and fipronil are hydrophobic and primarily associated with sediments (Holmes et al., 2008). Once in the water column, these compounds quickly partition into lipids and sediments (Solomon et al., 2001), however, increased water-column concentrations can be associated with stormwater run-off events. Imidacloprid is highly water-soluble and has a high potential to leach into the aquatic environment (Anderson et al., 2015b). Imidacloprid breaks down in light (Smit et al., 2015), but can persist in turbid or cloudy waters.

The concentrations in the aquatic environment of most of these pesticides are unknown for many regions in north east Australia (northern New South Wales and Queensland), but are expected to be comparatively high because of the agricultural land use adjacent to waterways and the tropical and sub-tropical conditions. Very little information is available about the quantities of pesticides used in northern New South Wales and Queensland. All three compounds are registered for use in Queensland and have broad and overlapping applications, including sugarcane, pasture and domestic uses. 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 Great Barrier Reef (GBR) environment and the $5.6 billion per year that tourism to the reef generates for the Queensland economy, much of the pesticide monitoring undertaken in the region is focussed on the catchments of the 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). 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 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 (Turner et al., 2017). The other pesticides discussed here were not included in the monitoring program. Fipronil, chlorpyrofos and imidacloprid, but not bifenthrin, have been detected in estuaries in the Great Barrier Reef Catchment area (Devlin et al., 2015).

The risk that modern-use insecticides poses to shrimp aquaculture cannot be determined without information about the concentrations of pesticides in the intake waters and information about the susceptibility of sensitive life stages of commercially important species. We have implemented the current study to address the uncertainty around both the concentrations of insecticides measured in coastal areas in north eastern Australia and the sensitivity of shrimp post larvae, approximately 20 days post hatch, (*Penaeus monodon)* to these insecticides. Lethal and sublethal responses of shrimp to bifenthrin, fipronil and imidacloprid were determined, and the concentrations of a suite of compounds, including these insecticides, in the intake water collected at shrimp farms were measured. Risk was determined by comparing the highest measured concentrations of these compounds to the concentrations at which toxic impacts have been reported, both in this study and in the literature.

2. Materials and methods

2.1. Experimental shrimp

The Black Tiger shrimp, *Penaeus monodon,* is the most commercially important cultivated penaeid species in Australia and is a common aquaculture species in southeast Asia (Motoh, 1985). Juvenile shrimp typically develop in estuarine environments, and the species is valued for it large size and rapid growth (Motoh, 1985). Despite its commercial importance, it is not commonly used as a test species in environmental toxicology.

First generation post-larval (PL) Black Tiger shrimp *Penaeus monodon* (PL10, approximately 20 days post-hatch and 10 days post-metamorphosis from the mysis stage) were produced and reared on-site at the CSIRO Bribie Island Research Centre (BIRC), Queensland, Australia. Shrimp were maintained on commercial feed and live *Artemia*, and fed every three hours between 7 a.m. and 8 p.m. and were kept at 30 °C. The water quality recorded during was temperature between 28.4–32.3 °C, dissolved oxygen between 5.27–5.89 mg/L (84.5–95% saturation), salinity between 38.2 − 39.7 ppt, and pH between 8.03 and 8.19. Care was taken to reduce stress on the animals during the experiments.

2.2. Pesticide solutions

Pure compounds of each pesticide were ordered from Sigma Aldrich (Sydney, Australia). Stock pesticide solutions were made by dissolving the entire amount (100 mg) provided by the manufacturer into 10 millilitres of methanol, which was then diluted 1 in 10 to make a 1 g per litre stock solution, which was diluted in methanol if necessary to create low concentrations. To create exposure solutions with the appropriate insecticide concentration for the range-finder, static and feeding inhibition experiments, the appropriate amount of diluted stock solution was added to the glass jar and the methanol was quickly evaporated off in a fume hood. Filtered sea water (500 mL – the test volume) was then added to each jar. During exposure of shrimp to pesticide, water in the jars was lightly aerated using Teflon tubing, and exposures were carried out under red lights to minimise stress to the post-larvae, which are normally benthic and not accustomed to ambient light.

2.3. Range-finder experiments

To determine the range over which PL shrimp are sensitive, a survival experiment that bracketed the range of sensitivities reported for other crustaceans (derived from USEPA, 1992) was performed. Three replicates of ten shrimp each were distributed into log step 10 concentrations of each of the selected insecticides. After 24 h of exposure, the number of PLs surviving was counted. The range finder experiment used concentrations of 0.1, 1, 10 and 100 μg/L fipronil; 0.001, 0.01, 0.1 and 1 μg/L bifenthrin, and 1, 10, 100, and 1000 μg/L imidacloprid.