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Feeding response in marine copepods as a measure of acute toxicity of four anti-sea lice pesticides

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ABSTRACT

Anti-sea lice pesticides used in salmon aquaculture are released directly into the environment where non-target organisms, including zooplankton, may be exposed. The toxicity of four pesticides to field-collected copepods was examined in 1-h exposures with lethality and feeding endpoints determined 5-h post-exposure using staining techniques. Copepods were immobilized within 1 h, at aquaculture treatment concentrations of deltamethrin (AlphaMax®), cypermethrin (Excis®), and hydrogen peroxide (Interox®Paramove™50). All organisms showed vital staining, indicating immobilized organisms were still alive, thus LC50s were not determined. Feeding on carmine particles was inhibited and EC50s ranged from 0.017 to 0.067 μg deltamethrin/L, 0.098–0.36 μg cypermethrin/L, and 2.6–10 mg hydrogen peroxide/L, representing 30- to 117-fold, 13- to 51-fold, and 120- to 460-fold dilutions of the respective aquaculture treatments. No effects were observed in copepods exposed to azamethiphos (Salmosan®) at 5-times the aquaculture treatment. Acute exposure to three of the four pesticides affected feeding and mobility of copepods at environmentally-realistic concentrations.

1. Introduction

Sea lice are ectoparasites of many species of fish and are a serious problem in salmon aquaculture (Roth et al., 1993; MacKinnon, 1997). The species that infect cultured Atlantic salmon in Canada are parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus*. Treatment of fish often involves immersion in a pesticide bath for up to 1 h by placing impervious skirts or tarps around aquaculture cages or pumping fish into specialized well boats. The pesticide is then released from the aquaculture cage or well boat and disperses as a plume, with the fate of the chemicals depending on water movement and chemical characteristics. Studies of pesticide dispersion from aquaculture sites have found that effluent plumes are detectable 2–5.5 h post-release at distances 0.9–3 km from the cage site, with pesticide concentrations that represent 1/1000–1/2000 of the pre-release concentrations (Ernst et al., 2001). Thus indigenous (or non-target) organisms may potentially be exposed to anti-sea lice pesticides released from aquaculture sites. In particular, pelagic organisms such as zooplankton may be entrained in effluent plumes and exposed for hours (Willis et al., 2005). Copepods are typically the most abundant organisms in the zooplankton of coastal ecosystems and an important component of marine and estuarine food webs (Verity and Smetacek, 1996; Gerber, 2000; Turner, 2004). Planktonic copepods have a similar life cycle as ectoparasitic copepods (sea lice) and also may be adversely affected by anti-sea lice pesticides (Willis et al., 2005).

Studies have examined the toxicity of the pyrethroids cypermethrin and deltamethrin to marine copepods, as these chemicals have been used in anti-sea lice pesticides. For example, eggs, nauplii, and adults of the species *Acartia tonsa* were exposed to cypermethrin for 96 h (Medina et al., 2002) or for 2–5 d (Barata et al., 2002a) and examined for lethality, clutch size, and feeding rates. Similarly, the toxicity of both cypermethrin and deltamethrin to another species *Tisbe battagliai* was examined in 4- to 6-d lethality tests with eggs, nauplii, and adult females, as well as 6-d tests examining feeding rates and clutch size in adults (Barata et al., 2002b). However, these studies used standard exposure durations of marine toxicity tests which do not reflect the very acute exposures (i.e., up to a few hours) expected to occur with

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aquaculture effluents. Using a more realistic exposure scenario, delayed mortality (up to 144 h post exposure) of adult *A. tonsa* was observed following 1- and 24-h pulse exposures to concentrations of cypermethrin close to the aquaculture treatment concentration (Medina et al., 2004). This suggests that effects can occur following short, pulsed exposures and that tests reflecting these conditions may be more appropriate than previous standard exposure durations.

Survival is a common endpoint in zooplankton studies such as those by Barata et al. (2002a) and Medina et al. (2002, 2004). Typically survival is evaluated by examining individuals under a dissecting scope for indications of movement, heartbeat, etc., which can be time consuming and limit the numbers of organisms that can be examined. Sublethal effects such as immobility and decreased feeding are also ecologically-relevant endpoints as they may impair survival, growth, and reproduction. These sublethal effects may occur well below lethal thresholds, particularly for acute exposures, and have sometimes been shown to be more sensitive than lethal endpoints for copepods (e.g., Barata et al., 2002a). Investigation of the potential effects of aquaculture effluents on copepods thus requires consideration of alternative experimental designs and use of techniques that will facilitate assessment of both lethal and sublethal endpoints. For example, staining techniques have been used to differentiate between alive and dead organisms in marine/estuarine copepod populations. A protocol for staining copepods with neutral red (a vital stain taken up into live tissues) was first described by Dressel et al. (1972) and has since been evaluated and developed into a protocol for field samples by Tang et al. (2006) and Elliot and Tang (2009). Filterable particles such as carmine in the digestive tract demonstrate feeding and have been used with copepods and other zooplankton as another indicator of relative organism health (e.g., Dressel et al., 1972; Jawecki et al., 2011). The use of such staining methods in laboratory-based toxicity tests would enable rapid and objective determination of lethality and feeding endpoints in a large number of zooplankton. This improves statistical power, which is particularly important for (sublethal) endpoints that may be highly variable or for populations with unknown "health" or exposure history (i.e., field samples).

The purpose of the present study was to determine the effects of four anti-sea lice pesticides on copepods from local zooplankton communities in southwest New Brunswick (NB), Canada; this is an area where salmon aquaculture and treatment with anti-sea lice pesticides are prevalent. The goal was to develop a laboratory test method representative of potential environmental exposures, and with easy-to-assess, robust, and relevant endpoints that relied on staining techniques. The present study addresses some of the data gaps in the literature by examining.

- Acute exposures that are environmentally-relevant;
- Potential for delayed toxicity;
- Lethal and sublethal effects;
- Relative toxicity of several chemicals used to control sea lice; and
- Actual anti-sea lice pesticide formulations instead of technical grade chemicals.

2. Materials and methods

2.1. Selection of anti-sea lice pesticides

Four anti-sea lice pesticide formulations were selected for this study because they are either currently or have been used to combat infestations of sea lice in eastern Canada. AlphaMax® was used under an emergency registration from Health Canada's Pest Management Regulatory Agency (HC-PMRA) in 2009 and 2010 (HC-PMRA, 2010). Excis® was applied experimentally in southwest New Brunswick in the mid 1990's, but is used extensively in other jurisdictions (Chang and McLelland, 1996, 1997). Interox®Paramove™50 and Salmosan® have been given emergency registration status and are being used to combat sea lice infestations in southwest New Brunswick (HC-PMRA, 2013a,b).

The anti-sea lice formulations AlphaMax® and Excis® are emulsifiable concentrates containing 1% of the synthetic pyrethroids deltamethrin or cypermethrin as the active ingredients, respectively. Pyrethroids affect nerve transmission by interfering with sodium (Na^+) channels resulting in depolarization and repetitive firing of the nerve ending, leading to eventual paralysis and death (Miller and Adams, 1982; Crane et al., 2011; Haya et al., 2005). Both pesticides are effective against all attached stages of sea lice including adults (Haya et al., 2005; Burridge et al., 2010). Treatment of salmon is a 40 min bath with AlphaMax® at a target concentration of 2.0 μg deltamethrin/L (SEPA, 2008) or a 1-h bath with Excis® at a target concentration of 5.0 μg cypermethrin/L in tarped cages (SEPA, 1998), herein referred to as "aquaculture treatment concentrations".

Interox®Paramove™50 is a liquid formulation made up of 50% hydrogen peroxide. The suggested mechanisms of action of hydrogen peroxide are mechanical paralysis, peroxidation of lipid and cellular organelle membranes by hydroxyl radicals, and inactivation of enzymes and DNA replication (Cotran et al., 1989). Most evidence supports toxicity via mechanical paralysis, as bubbles form in the gut and haemolymph and cause sea lice to release and float to the surface (Bruno and Raynard, 1994). The formulation is not effective against larval sea lice and its effectiveness against pre-adult and adult stages has been inconsistent (Mitchell and Collins, 1997). It is applied in a bath treatment at 1200–1800 mg hydrogen peroxide/L for 40 min, but the effectiveness is temperature dependent, and it has been suggested that treatment with these concentrations may not be effective below 10 °C (Treasurer et al., 2000).

Salmosan® is a wettable powder formulation consisting of 47.5% azamethiphos, an organophosphate pesticide that inhibits acetylcholinesterase (AChE) activity and causes repetitive firing of nerves (Baillie, 1985). The formulation is effective against pre-adult and adult stages of the sea louse, but it does not affect larval stages (SEPA, 2005). It is applied as a bath treatment at 100 μg azamethiphos/L for 30–60 min in well boats and tarps and at 150 μg/L in skirt treatments.

2.2. Organism collection and toxicity tests

Zooplankton samples composed almost completely of copepods (see SI Table 1) were collected monthly between January and May, 2013, from Passamaquoddy Bay, NB. Zooplankton samples were collected using vertical tows (max. 50 m depth) with a 75-cm diameter, 150-μm mesh plankton net. Contents of tows were transferred to glass jars with seawater, and stored in a cooler in the dark during transit to the laboratory (DFO – St. Andrews Biological Station, St. Andrews, NB). Zooplankton were distributed among large beakers which were topped up with sand-filtered (0.2 μm) seawater (source Pasmaquoddy Bay; ~30 parts per thousand) to reduce crowding and held overnight (max. 2 nights) in darkness and in a temperature-controlled room (at 9 °C) prior to use in toxicity studies. Toxicity tests were conducted at the same temperature under fluorescent lighting of ~250 lux.

Following initial method development and range finding studies, a testing protocol was established with a defined concentration series for each pesticide. Toxicity tests with each of the four