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The effects of emamectin benzoate or ivermectin spiked sediment on juvenile American lobsters (*Homarus americanus*)

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ABSTRACT

This study examined the effects of a range of ½-log concentrations of emamectin benzoate (commercially applied as SLICE®) and ivermectin (commercially applied as IVOMEC®) on juvenile American lobster, *Homarus americanus*. Phase I of the research assessed acute (up to 4 days) and chronic (30-day) toxicity of sediment dosed with the active ingredients emamectin benzoate (EMB) formulated as SLICE® or ivermectin (IVM) formulated as IVOMEC® at various nominal concentrations (EMB: 15, 48, 150, 475 and 1500 ng g^{-1} wet sediment; IVM: 3, 9.5, 30, 95 and 300 ng g^{-1} wet sediment) on juvenile Atlantic lobster (stages IV). Phase II evaluated sublethal effects (e.g., growth, moulting success) of all lobster surviving past the 30 day exposure period, over an additional 41 days. Chemical analysis of EMB and IVM in sediment samples from the exposure tanks revealed a strong linear association (R^2 values 0.99 and 0.98 for EMB and IVM, respectively) between nominal dose and measured concentration of compound. EMB exposure concentrations at very high levels (≥ 343.3 ng g^{-1}) were acutely toxic to juvenile lobster such that 100% of lobsters had died after 13 days of exposure. The maximum cumulative mortality of lobsters exposed to the highest concentrations of EMB and IVM was 100% after 10 days and 25 days, respectively. The 10-day LC_{50} estimates (± 95% CI) for EMB and IVM were 250.23 ± 90.4 and 212.14 ± 202.64 ng g^{-1}, respectively. Using abnormal behaviour as an indicator, the 15-day EC_{50} estimates (± 95% CI) for EMB and IVM were 96.19 ± 51.42 and 15.82 ± 6.93 ng g^{-1}, respectively. The NOEC (no observed effect concentration) for abnormal behaviour was 0.0 ng g^{-1} for each product and the LOEC (lowest observed effect concentration) was 8.8 and > 3.0 ng g^{-1} for EMB and IVM, respectively. Observations on sublethal effects included delayed moulting to stage VI and reduced growth at higher exposure concentrations for both therapeutants. Using failure to moult to stage V or VI as an indicator, the 15-day EC_{50} estimates (± 95% CI) for EMB and IVM were 32.72 ± 18.26 and 14.00 ± 12.43 ng g^{-1}, respectively. The NOEC for failure to moult to stage V only was 343.3 and 14.7 ng g^{-1} for EMB and IVM, respectively. Whereas, the LOEC was 1066.7 and > 61.0 ng g^{-1} for EMB and IVM, respectively. The concentrations of EMB and IVM tested in the present study were acutely toxic to juvenile lobster exposed to the highest dosages (343.3 and 1066.7 ng EMB g^{-1} and 61.0 and 300.0 ng IVM g^{-1}). There was significant evidence of chronic toxicity, longer exposure increased mortality with LT_{50} values decreasing with increasing test material concentration.

1. Introduction

Sea lice (predominantly *Lepeophtheirus salmonis*) are large, marine ectoparasitic copepods of salmonids having a circumpolar distribution in the Northern hemisphere. These copepod parasites can cause significant health problems for farmed Atlantic salmon, especially when the stocking densities can be unnaturally high. If untreated, large numbers of sea lice can cause skin and tissue damage resulting in fish stress and mortality. The global economic impact associated with loss of stock, product downgrades at harvest and the cost involved in monitoring and managing sea lice infection has been estimated to exceed US $480 M annually (Costello, 2009; Johnson et al., 2004). In 2016, Marine Harvest ASA, the world's largest salmon farming company, reported that sea lice treatments cost the company CDN$115.8 million to grow 380,621 t of salmon or approximately CDN$300 per tonne (http://marineharvest.com/investor/annual-reports/). In 2015, the

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Atlantic coast of Canada produced 29,000 t of salmon. If the same sea lice cost rates were applied to Canadian farmers, it is estimated that sea lice cost the eastern Canadian salmon farming industry CDN$8.7 million.

Emamectin benzoate (EMB) and ivermectin (IVM) belong to a class of chemicals called avermectins that are formulated as an in-feed medication (e.g., EMB, trade name SLICE®) to reduce infection of sea lice on farmed Atlantic salmon. These compounds affect membrane permeability by inhibiting nerve impulse transmission via opening glutamate-gated chloride channels of neurons, resulting in hyperpolarization of nerve cells and muscle tissue in arthropods, leading to paralysis of the neuromuscular system (Turner and Schaeffer, 1989); (Davies and Rodger, 2000; Horsberg, 2012). IVM is the main active pharmacological ingredient (API) for the commercial product IVOMEC®; has a high octanol-water partition coefficient (log K_{ow} = 3.2) (Halley et al., 1989) and a low water solubility (4 mg L^{-1}) (Fisher and Mrozik, 1989) indicating that it has the potential to sorb to organic particulate material and surfaces, thus should readily bind to marine sediments. Typical in-feed prescription would depend on the age of the fish and environment temperatures but, on average, it ranges from 50 µg kg^{-1} of fish biomass twice weekly with 1-week interval to 200 µg kg^{-1} of fish biomass every two weeks from June to November (Davies and Rodger, 2000; DFO, 1996). Internationally, SLICE® has been developed as an alternative to other sea lice therapeutants, and was approved for use in Canada to control sea lice in 2014 by Health Canada's Veterinary Drugs Directorate. EMB is the main API for the commercial product SLICE®. The recommended dosage of EMB, administered as SLICE® is 50 µg kg^{-1} of fish biomass per day for a duration of 7 consecutive days (Bright and Dionne, 2004). EMB has a high octanol-water partition coefficient (log K_{ow} = 5) and has a low water solubility (5.5 mg L^{-1}). Due to the lipophilic character, the molecule has a high affinity to organic matter and will persist for a long time in the marine environment (SEPA, 2004).

In the vicinity of fish farms, if these drugs are being poorly absorbed by fish (Davies and Rodger, 2000; Horsberg, 2012; Roth et al., 1993), a high percentage of active ingredient would be released into the environment through faeces and urine (and uneaten pellets) where it ends up in sediments and it may exceed concentrations above the threshold that causes ecological effects (DFO, 2013; Page and Burridge, 2014). Consequently, the concern with these therapeutants is the potential exposure to sediment-associated benthic arthropods and, especially, their toxicity to non-target crustaceans (Ernst et al., 2001; Waddy et al., 2007). At an Atlantic salmon farm in Scotland, EMB in fish food occurred during treatment and up to four months post-treatment largely in particulate form via uneaten feed, faecal material and in soluble form in urine, but was confined to sediment within 25–100 m from treated pens (Telfer et al., 2006). A maximum sediment level of 2.73 µg EMB kg^{-1} wet weight was found at 10 m from the treated cages. In the same study, quantifiable concentrations of EMB were also found in blue mussels deployed up to 100 m from the treatment cages after one week post-treatment (< 1.00 µg EMB kg^{-1} wet weight). In a study conducted on the Pacific coast of Canada (DFO, 2012), the highest levels of EMB (Site A = 35 ng g^{-1} and site B = 0.33 ng g^{-1}) were found in surface sediment collected at the edge of the treated net pens between 2 and 3 weeks post-treatment. However, trace levels of EMB (0.12 ng g^{-1}) could still be detected in sediment collected up to 150 m from the treated net pens (DFO, 2012). Similar (but fewer) studies have been reported for the environmental fate of IVM. Using aquatic mesocosms as models, chronic responses (up to 97 days) were observed in the ecosystem structure and function when sediment was spiked with a nominal concentration of IVM at 30 ng g^{-1} and long-term effects (> 229 days) were identified among some organisms with sediments spiked with IVM at 1000 ng g^{-1} (Sanderson et al., 2007). The rapid sorption of EMB and IVM in sediments greatly reduces bioavailability to pelagic organisms but, once associated with sediments, the potential exists for exposure of benthic organisms via sediment particles (by ingestion or contact) or from interstitial water (Hamer et al., 1992; Power and Chapman, 1992; Widenfalk, 2002). Consequently, there is concern that IVM or EMB present in sediment could have chronic effects (e.g., growth or moulting impairment) on benthic species including high-value commercial species as the American lobster, *Homarus americanus*.

The American lobster is one of the most economically important shellfish worldwide and comprises the highest-value fishery in Canada. The salmon culture industry in the Bay of Fundy, Eastern Canada, is located in a region of high lobster productivity, where over 12,280 metric tonnes of lobster were landed in 2014 (DFO, 2015, 2016). Most therapeutants to treat sea lice on farmed salmon in the Bay of Fundy are used in the spring, summer and fall, when lobster larval stages are released in the water column and juveniles are settling and foraging (Lawton and Lavalli, 1995). Consequently, the lobster fishing industry has concerns about the use of therapeutants in the coastal environment. Initial studies reported the acute toxicity (LC_{50}) of EMB to be > 589 and > 644 ng g^{-1} (in the diet) for juvenile and adult lobsters, respectively, following voluntary consumption of dosed feed pellets (Burridge et al., 2004). Given the difficulty to evaluate what quantity of pellet food was consumed by the lobsters and lack of mortality above 55%, this study couldn't establish a confident estimate of EMB toxicity. However, sublethal doses and chronic exposures associated with EMB feeding have been shown to disrupt the moult cycle of ovigerous female lobsters (Waddy et al., 2007, 2010). Similar literature exists for IVM LC_{50} estimates for fish and marine invertebrates (Davies and Rodger, 2000). However, most studies employed a spiked water exposure regime rather than IVM spiked sediment which is likely the more relevant of the two scenarios given what is known about the fate of these products after a treatment.

To date, no studies to evaluate the effects of either EMB or IVM present in sediment on American lobster have been published. Regarding the long half lives of EMB and IVM (> 100 days as determined in Davies et al., 1998), these therapeutants will most probably reside in sediments close to fish farms, and regarding the benthic behaviour of stage IV to VI lobsters, this study is highly relevant from an exposure pathway perspective for lobster. Consequently, the present study was designed to provide the first key ecotoxicology data and sublethal effects associated with the response of juvenile (stages IV-VI) American lobster when exposed to varying concentrations of EMB and IVM following chronic exposure (30 days) to dosed sediment. The primary objectives were to produce ecotoxicology information (LC_{50}, LT_{50}, EC_{50}, LOEC, NOEC) associated with exposure and identify any sublethal effects (e.g., growth and moulting impairment) beyond the 30-day exposure.

2. Materials and methods

2.1. Study design overview

This study, separately but concurrently, examined the effects of a range of concentrations of emamectin benzoate (EMB) and ivermectin (IVM) within sediment on survival and moulting success of juvenile American lobsters, *Homarus americanus*. Sediment samples were mixed with different amounts of commercially available premix to meet nominal API for each therapeutant and placed in replicate exposure jars within a gently aerated static bath of filtered seawater. All sediment and test compound weights presented herein are wet weights. Moisture content of the sediment was between 23% and 25%. An initial EMB nominal concentration (1500 ng g^{-1}) was used as a 'high dose' exposure treatment, combined with four additional ½-log dilutions (target doses: 475, 150, 48 and 15 ng g^{-1}). For IVM, an initial nominal concentration (300 ng g^{-1}) was used as a 'high dose' exposure treatment, combined with four additional ½-log dilutions (target doses: 95, 30, 9.5 and 3 ng g^{-1}). These concentrations were chosen to represent "excessively high" and "low" ranges with intermediate values that mimic concentrations measured in sediment near aquaculture sites. A seawater