The lethality of the anti-sea lice formulation AlphaMax® (deltamethrin) to adult American lobster (*Homarus americanus*) during chronic or pulse dose exposures

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ABSTRACT

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The pyrethroid pesticide formulation AlphaMax® (1% w/v deltamethrin) has been used in Canada under an Emergency Drug Release (EDR) at a recommended treatment concentration of 2.0 µg deltamethrin L^{-1} to treat farmed salmon for infestations of the copepod parasites, *Lepeophtheirus salmonis* and *Caligus elongatus* (sea lice). The adult American lobster (*Homarus americanus*) was exposed chronically (continuously) for 10 days and the resulting lethal threshold was 14.7 $ng \cdot L^{-1}$ based on measured water concentrations of deltamethrin. Adult American lobster exposed to pulse doses (15 or 30 minute) to either low (8 to 13 $ng \cdot L^{-1}$) or high (32 to 66 $ng \cdot L^{-1}$) measured concentrations of deltamethrin, 4 times a day for 6 days at 3 temperatures (5, 10 and 14 °C) were affected as early as the first day for most treatments. Despite consistent mixing methods and uniform concentrations within experiments measured concentrations of deltamethrin differed significantly between experiments particularly at the higher target concentrations. Regardless, all lobsters exposed to the higher concentrations for 30 minutes died over the course of the study at all 3 temperatures. Greater than 50% of lobsters exposed to the higher concentrations for 15 minutes died with a greater number dying at the lower temperatures. There was mortality at 5 °C for both the 15 and 30 minute pulse dose treatment groups at the lower concentration (11 and 13 $ng \cdot L^{-1}$) of deltamethrin and at 10°C for the 30 minute pulse dose group (10 $ng \cdot L^{-1}$) but not for the lower exposure concentrations at 14 °C indicating a temperature effect on response. Lab based experiments at higher temperatures may underestimate risk to non-target crustaceans during the cooler months. Sublethal effects related to lobster condition were observed at concentrations as low as 8 $ng \cdot L^{-1}$. These results indicate that chronic or pulse dose of AlphaMax® at low concentrations may cause lethal and sublethal effects (immobility) in the commercially important non-target species, the American lobster.

RÉSUMÉ

Lyons, M.C., Burridge, L.E., Wong, D.K.H. and MacKeigan, K.G. 2017. The lethality of the anti-sea lice formulation AlphaMax® (deltamethrin) to adult American lobster (*Homarus americanus*) during chronic or pulse dose exposures. Can. Tech. Rep. Fish. Aquat. Sci. 3217: v + 18p.

La formulation de pesticides pyréthroïdes AlphaMax® (1 % p/v de deltaméthrine) a été utilisée au Canada dans le cadre d'un programme de distribution de médicaments d'urgence (DMU) à une concentration de traitement recommandée de 2,0 µg de deltaméthrine L^{-1} pour traiter le saumon d'élevage contre les infestations de parasites

copépodes, *Lepeophtheirus salmonis* et *Caligus elongatus* (poux du poisson). Le homard adulte (*Homarus americanus*) a été exposé de façon chronique (continue) pendant dix jours et le seuil létal qui en a résulté était de 14,7 ng·$L^{-1,}$ selon les concentrations de deltaméthrine mesurées dans l'eau. Le homard adulte exposé à des doses intermittentes (15 ou 30 minutes), à des concentrations de deltaméthrine mesurées faibles (de 8 à 13 ng L^{-1}) ou élevées (de 32 à 66 ng L^{-1}), quatre fois par jour pendant six jours, à trois températures (5, 10 et 14 °C), a été touché dès le premier jour pour la majorité des traitements. Malgré une combinaison constante des méthodes et des concentrations uniformes dans le cadre des expériences, les concentrations de deltaméthrine mesurées ont différé considérablement d'une expérience à l'autre, en particulier pour les concentrations cibles les plus élevées. Quoi qu'il en soit, tous les homards exposés aux concentrations les plus élevées pendant 30 minutes sont morts au cours de l'étude, aux trois températures. Plus de 50 % des homards exposés aux concentrations les plus élevées pendant 15 minutes sont morts; un plus grand nombre étant mort aux températures les plus basses. Une mortalité a été constatée à 5 °C dans les groupes de traitement à doses intermittentes de 15 et de 30 minutes, à la concentration la plus faible (11 et 13 ng L^{-1}) de deltaméthrine, et à 10 °C dans le groupe à doses intermittentes de 30 minutes (10 ng L^{-1}), mais pas pour les concentrations d'exposition plus faibles à 14 °C, indiquant un effet de la température sur la réponse. Des expériences en laboratoire à des températures plus élevées sont susceptibles de sous-estimer les risques pour les crustacés non visés au cours des mois les plus frais. Les effets sublétaux liés à la condition du homard ont été observés à des concentrations de seulement 8 ng L^{-1}. Ces résultats indiquent qu'une dose chronique ou intermittente d'AlphaMax®, à de faibles concentrations, peut entraîner des effets létaux ou sublétaux (immobilité) chez le homard, une espèce non-ciblée importante sur le plan commercial.

INTRODUCTION

Sea lice, the common name for copepodid ecto-parasites of Atlantic salmon are a problem for fish farms around the world (Burridge et al., 2010). Severe infestations of sea lice often result in costs to fish farmers either in loss of product or in the cost of combating the infestations (Haya et al., 2005). A number of pesticides have been used to combat sea lice infestations in Canada since sea lice first became a problem in 1994 when two species, *Lepeophtheirus salmonis* and *Caligus elongatus* infested salmon in southwest New Brunswick (SWNB) (*cf* Burka et al., 1997, Burridge, 2003, Burridge et al., 2010).

Pyrethroid insecticides are among the most toxic insecticides known (Fairchild et al., 2010). They are synthetic analogues of natural pyrethrins that have low mammalian toxicity and greater environmental stability than the natural pyrethrins (Fairchild et al., 2010). The mechanism of action of the pyrethroids involves interference with nerve membrane function, primarily by their interaction with sodium (Na^+) channels (Miller and Adams, 1982) which results in depolarization of the nerve ending. In the case of the pyrethroid deltamethrin this interaction results in repetitive firing of the nerve ending resulting in eventual paralysis and death (Crane et al., 2011). Marine crustaceans are generally more sensitive to pyrethroids than marine fish (Clark et al., 1989) and among the pyrethroids, deltamethrin is often the most toxic to crustaceans in comparative tests (Haya, 1989). Pyrethroids are unlikely to be accumulated to a significant degree in fish and aquatic food chains since they are rapidly metabolized (Kahn, 1983) although Alonso et al. (2012) demonstrated pyrethroid bioaccumulation in marine mammals. Deltamethrin has a very low water solubility (< 0.2 $\mu g \cdot L^{-1}$) and a log K_{ow} of 4.6 (Tomlin, 1997) indicating that deltamethrin can adsorb onto suspended particles (Fairchild et al., 2010) and persist in sediments for weeks and may be desorbed and affect benthic invertebrates (Haya et al., 2005). Several recent publications have addressed its use in or near marine waters (Fairchild et al., 2010; Crane et al., 2011; Alonso et al., 2012; Burridge et al., 2014; Ernst et al., 2014; Van Geest et al., 2014a, 2014b, 2014c).

Pesticide use in Canada is regulated by Health Canada's Pest Management Regulatory Agency (PMRA). The therapeutants available for use to combat infestations of sea lice and the corresponding treatment protocols are tightly regulated and therapeutants can only be used under prescription from a licensed veterinarian (Burridge et al., 2014). There are provisions for Emergency Drug Release (EDR) and 'off-label' use of drugs and pesticides. AlphaMax®, the aquaculture formulation of deltamethrin, Pest Control Products Act (PCPA) registration number 29330 (Health Canada, 2013) was used under an EDR from Health Canada in the fall of 2009 and the summer of 2010 in SWNB (Burridge and Van Geest, 2014).

AlphaMax® is an emulsifiable concentrate (EC) formulation of deltamethrin which makes up 1% of the formulation. The recommended treatment of salmon against sea lice is a 30 minute bath with AlphaMax® with a target concentration of 2.0 µg deltamethrin L^{-1} (Health Canada, 2013). Deltamethrin is effective against all attached stages including adults, and therefore less frequent treatments should be required than with pesticides such as organophosphates; 5-6 week intervals rather than 2-3 week intervals, respectively (Haya et al. 2005). However, Whyte et al. (2014) and Arriagada et al. (2014) reported variable efficacy of deltamethrin for different life stages of sea

lice. After treatment, the chemical is allowed to disperse into the surrounding water. Exposure of non-target organisms to deltamethrin from a single treatment may be via water, sediment or through ingestion of particles (Burridge et al., 2014). The potential of these releases to affect wild non-target organisms is determined by a combination of exposure and the toxicity of the product to the organisms. The exposure is determined by discharge and natural transport and dispersal processes. The effect is determined by the concentration and duration of exposure and the sensitivity of the organisms.

The nature of the salmon aquaculture industry in SWNB is such that many farms are in close proximity to each other and to areas of traditional lobster fisheries (Figure 1) (Burridge et al., 2000b). The successful co-existence of aquaculture industry activities with fish, fish habitat and commercial fishing is dependent on understanding the effects of pesticides on non-target marine organisms. It has been well documented that some pesticides can be quite toxic to American lobster (*Homarus americanus*). There have been fewer studies on the effects of repeat or chronic exposure of pesticides to non-target organisms. Ernst et al., (2001) simulated pesticide treatment releases in SWNB and reported that toxic concentrations of cypermethrin, another pyrethroid, have been shown to persist for up to 5 hours after a single treatment. The effects of short-term pulse exposures using sensitive organisms with observations post-exposure for delayed effects or recovery are essential to properly evaluate risk (Ernst et al., 2001). Burridge et al. (2000a) exposed lobsters to cypermethrin and used scaling analysis to predict that the pesticide could be found at concentrations above the lethal threshold for over 3 h after a single treatment. Given the number of cage sites in close proximity to each other, the number of treatments likely to occur over relatively short time frames and many factors influencing the transport and discharge, the potential range of exposure concentrations and times likely to be experienced by *in situ* non-target organisms is large (Page and Burridge, 2014).

Of all the environmental parameters, temperature has the most pervasive influence on survival, growth, and reproduction of juvenile and adult lobsters. Juvenile and adult lobster exist at water temperatures that vary seasonally from less than 0°C to approximately 25°C and metabolic processes are accelerated by elevated temperature (Aiken and Waddy, 1986). Anthropogenic changes to the environment are usually somewhat less important because their influence is usually limited to small areas or brief periods of time but all unfavourable environmental conditions cause stress, and stress exaggerates the impact of an adverse environment (Aiken and Waddy, 1986). Contaminants at sublethal concentrations can stress the lobster and make it less able to withstand situations that would not normally be a problem (Aiken and Waddy, 1986). Pyrethroids are atypical in that they become more toxic at colder temperatures (Weston et al., 2009; Hardwood et al., 2009).

In this study we exposed adult American lobster continuously for 10 days to the formulation AlphaMax®. A range of exposure concentrations equivalent to dilutions of the anti-louse treatment concentration of 2.0 µg deltamethrin L^{-1} were used. It is difficult to assess the risk of pesticide use to lobster, particularly when exposure is intermittent, as is the case during sea lice treatments. If non-target organisms are exposed, it is likely to be repeated for short periods of time (minutes) (Burridge et al., 2000b). We investigated the effect of water temperature in combination with pulse doses of AlphaMax® over 6 days on adult American lobster. These exposures were designed to simulate conditions at an aquaculture site where over the course of a

day, 4 cages of salmon in close proximity to each other would be treated with pesticide, consecutively, and that this would be repeated daily for 6 days. Prior to any type of regulation by Health Canada it was considered that, over a 6 day period, with 4 cages treated each day, non-target crustaceans could be exposed chronically to effluent from the treatments or to pulses (minutes) of the pesticide up to 24 times (Dr. Michael Beattie, Province of New Brunswick, Department of Agriculture, Aquaculture and Fisheries, St. George, New Brunswick, Canada, pers. comm.).

MATERIALS AND METHODS

SOURCE OF PESTICIDE

AlphaMax® (manufactured by Pharmaq AS, Norway) was obtained through Dr. Michael Beattie, Province of New Brunswick, Department of Agriculture, Aquaculture and Fisheries, St. George, New Brunswick, Canada. The AlphaMax® formulation indicated 10 g deltamethrin per liter which is 1% active ingredient. It was stored in the dark at room temperature.

EXPERIMENTAL ANIMALS

Adult American lobster (*Homarus americanus*) were purchased from the Bay of Fundy commercial fisheries through Misty Harbour Seafood, Saint John, New Brunswick, Canada and transported to the St. Andrews Biological Station, St. Andrews, New Brunswick, Canada where they were held for several weeks in fibreglass tanks with flow-through filtered seawater (~30 psu salinity) at ambient water temperature. They were fed shrimp or herring daily and prior to exposure animals were tagged so that individual lobster could be identified. Five female and five male lobster were used for each exposure concentration. Average weight of lobsters used for the 10 day chronic exposures was 504 ± 35 g (n=150). Average weight of lobsters used for the 6 day pulse exposures was 707 ± 181 g (n=180).

ADULT LOBSTER – 10 DAY CHRONIC (CONTINUOUS) EXPOSURES

Ten lobster (5 female and 5 male) were placed in each of 5- 400 L fibreglass tanks so that 50 adult lobster were used in each of three replicate experiments. Chronic exposures were conducted in 300 L of flow through seawater (3 $L \cdot min^{-1}$). Stock solutions of deltamethrin (15, 45, 150 and 450 $ng \cdot mL^{-1}$), were prepared daily in dechlorinated water using the formulation AlphaMax®. Target exposure concentrations in the exposure tanks were 5, 15, 50 and 150 $ng \cdot L^{-1}$ for exposure 1. These exposure concentrations represent 0.25%, 0.75%, 2.5% and 7.5% of the recommended treatment concentration for sea lice (2 $\mu g \cdot L^{-1}$ of deltamethrin). As a result of significant and rapid loss of experimental lobsters at the highest concentration during the first replicate, target exposure concentrations were reduced to 1.67, 5, 15 and 45 $ng \cdot L^{-1}$ for exposures 2 and 3. These exposure concentrations represent 0.083%, 0.25%, 0.75% and 2.25% of the recommended treatment concentration for sea lice (2 $\mu g \cdot L^{-1}$ of deltamethrin). Deltamethrin stock solutions prepared for replicates 2 and 3 were 5, 15, 45 and 135 $ng \cdot mL^{-1}$. One tank of 10 tagged lobsters was left as a seawater control. The treatments were started by adding the appropriate amount of test solution required to bring each tank up to the desired concentrations. Mariotte

bottles of the stock solutions were calibrated to gravity feed the treatment solutions at a flow rate of 1 $mL \cdot min^{-1}$ directly into the stream of the incoming water supply of each treatment tank to ensure complete mixing. Mariotte bottles were replenished daily with freshly prepared stock solutions. The volume of stock solution used in the Mariotte bottles over each 24 h period was measured and an estimate of the concentration in each exposure tank was calculated. Water samples were collected (900 mL or 3600 mL) from each exposure tank daily to confirm the presence of deltamethrin. Mortalities were removed and noted and the activity and behavior of the live animals were monitored. Effects were noted including animals shedding claws, animals lying on their sides or backs, slowed or twitching pleopod movement, immobility (not moving, but response to gentle prodding) and a moribund state (immobile, limp body with no response to stimulus). Lobsters were considered dead when there was no movement of pleopods even with gentle prodding. The lobsters were fed daily and food consumption was monitored. Measured (ambient) water temperatures were between 11.9°C and 13.2°C (12.7 ± 0.5, mean ± SD) for the first 10 day exposure, 12.8°C and 13.4°C (13.2 ± 0.2, mean ± SD) for the second exposure and between 13.7°C and 14.5°C (14.0 ± 0.2, mean ± SD) for the third exposure. The surviving lobsters were transferred to clean seawater at the end of the 10 day exposure. The exposures took place in July and August 2010 and the surviving lobster were held and monitored until January 2011.

ADULT LOBSTER – 6 DAY PULSE DOSE EXPOSURES AT 3 TEMPERATURES

The pulse dose experiments were performed at 3 ambient temperatures: 5°C, 10°C and 14°C. Exposures were conducted as follows: six 200 L glass aquaria were filled to 150 L with seawater. Two aquaria were spiked with 30 mL of a stock solution of deltamethrin (0.1 $\mu g \cdot mL^{-1}$) made daily from the formulation AlphaMax® for a 20 $ng \cdot L^{-1}$ (deltamethrin) predicted exposure concentration and two aquaria were spiked with 150 mL of the deltamethrin stock solution for a 100 $ng \cdot L^{-1}$ (deltamethrin) predicted exposure concentration. These exposure concentrations represent 1% and 5% of the recommended treatment concentration for sea lice (2 $\mu g \cdot L^{-1}$ of deltamethrin). Two aquaria were left as seawater controls. Sixty tagged adult lobster were used in each experiment. Ten lobsters (5 female and 5 male) were placed in each aquaria for either 15 or 30 minutes and then transferred to clean flowing seawater. Aquaria were emptied and replenished with freshly prepared exposure water after each exposure. After two hours from the beginning of the exposures the lobster were transferred back into the aquaria for another 15 or 30 minutes. This allowed for four exposures each day and the lobster were left in flowing seawater overnight. This sequence of exposures was repeated for 6 days. Over the 6 day period, lobster were exposed 24 times to the same concentration and for the same length of time. This exposure regime was recommended by a veterinarian from the Province of New Brunswick, Department of Agriculture, Aquaculture and Fisheries, St. George, New Brunswick. Mortalities were removed when observed and the time was noted. Lobsters were considered dead when there was no movement of pleopods even after gentle prodding. Effects were monitored and noted as in the 10 day chronic exposure. Water samples were collected (900 mL) from exposure tank daily to confirm the presence of deltamethrin. Water temperature was measured daily. The 5°C exposures took place in January 2011, the 10°C exposures in June 2010 and the 14°C exposures in August 2010.

WATER ANALYSIS

Water samples were collected during all experiments. Samples for deltamethrin analysis were collected (900 mL or 3600 mL) into IChem 200 amber bottles, preserved with dichloromethane (50 mL·L^{-1} water) and held at 4 °C until they were transferred to the Research and Productivity Council in Fredericton, New Brunswick, Canada where they were extracted and then analysed by gas chromatography with electron capture detection (GC/ECD) according to the United States Environmental Protection Agency (US EPA) method 3510C and 8081B (US EPA, 2007). The reporting limit was 5 ng·L^{-1}. For the purposes of calculation, any sample reported as being < the level of detection was assigned a value of 4.9 ng·L^{-1}. This is an arbitrary assignment but allows calculations to be performed and adds another level of conservatism to our LC_{50} result.

LC_{50} DETERMINATION

An LC_{50} was determined for each 10 day exposure according to Stephan (1977) using the computer program Toxstat and the average LC_{50} was determined. The CI was then calculated as the interval around the mean LC_{50}. Measured water concentrations were used for the calculations.

RESULTS

All exposure concentrations reported herein are as the measured active ingredient deltamethrin in seawater.

ADULT LOBSTER – 10 DAY CHRONIC (CONTINUOUS) EXPOSURES

Mean measured concentrations of deltamethrin in water for the 10 day chronic (continuous) exposures are presented in Table 1. Measured water concentrations of deltamethrin were generally lower than the corresponding nominal concentrations regardless of what day they were collected. There were exceptions to this in test 1 where the measured concentrations were generally higher than the nominal concentrations.

The time of first observed behavioral effects are presented in Table 1. There were no behavioral effects seen at lower concentrations between 1.7 and 4.2 ng·L^{-1}. Behavioral responses were noted at 7.7 ng·L^{-1} in the first exposure. One lobster was immobile as early as 71 h from the beginning of the exposure and subsequently died on day 10. Behavioral responses were noted as early as 47 h at a concentration of 10.8 ng·L^{-1} in the second test and as early as 52 h at a concentration of 6.6 ng·L^{-1} in the third test (Table 1). At that time, all lobster appeared to be affected in the second test and one lobster had shed both claws and other lobster showed twitching movements in the third test. Most lobster in the 27.3 ng·L^{-1} exposure tank of the first test were affected as early as 48 hours from the beginning of the test. Several animals were immobilized, on their backs or looked weak and one animal later shed both claws. In the second and third tests, effects were seen much earlier- T=3 h and T=4 h at similar concentrations of deltamethrin- 32.0 ng·L^{-1} and 24.9 ng·L^{-1}. Some were lying on their backs, some with twitching movements of legs and some lethargic. The exposure of lobster to 24.9 ng·L^{-1} deltamethrin was stopped on the third day in the

last test. The eight animals that had been presumed dead and two lobster that were in very poor shape were removed from the 24.9 $ng \cdot L^{-1}$ exposure tank and placed in clean flowing seawater. Some lobster that had been presumed dead appeared to be recovering but the final number dead in the third 24.9 $ng \cdot L^{-1}$ exposure group was eight. The two remaining lobster that were in very poor shape on the third day were not exposed again. By the fourth day they appeared to have recovered in clean seawater. They displayed no behavioral problems, except one was on its back briefly. Several lobster exposed to 60 $ng \cdot L^{-1}$ and 81 $ng \cdot L^{-1}$ in the first exposure were affected within the first 3 to 6 hours of the first day. Some lobster were standing high on their claws, some were lying on their backs with slight movements in their legs or pleopods and had no response to stimuli. All 10 lobster exposed to 81 $ng \cdot L^{-1}$ deltamethrin were dead by 10.5 hours on the first day of exposure in the first experiment. Consequently this exposure concentration was dropped in the second and third experiments.

Mortality was recorded and daily results are shown for each test in Table 1. The LC_{50}s for Alphamax (reported as the concentration of deltamethrin) were determined as 19.0, 12.2 and 12.8 $ng \cdot L^{-1}$ based on measured concentrations for each of the three replicate tests. The average LC_{50} was 14.7 $ng \cdot L^{-1}$ with a 95% confidence interval of 8.3-21.1 (Table 3).

ADULT LOBSTER – 6 DAY PULSE DOSE EXPOSURES AT 3 TEMPERATURES

Mean measured concentrations of deltamethrin in the exposure water are presented in Table 2. Measured concentrations of deltamethrin in water were consistently lower than the corresponding target concentrations regardless of whether they were collected at T=0 h (before lobster were placed in the aquaria) or at the end of the 15 or 30 minute exposures. Mean measured deltamethrin concentrations ranged from 32% to 66% of target concentrations. The concentration of deltamethrin in water was consistent within each experiment. There was no significant difference in concentration at the lower exposure regime with concentrations ranging from 8 to 13 $ng \cdot L^{-1}$ during the three experiments (Table 2). At the higher concentrations, however, the concentration of deltamethrin varied significantly between experiments and ranged from 32 to 66 $ng \cdot L^{-1}$ (Table 2). The reason for this difference is unclear.

The time of first observed effects are presented in Table 2. Mortality was recorded and daily results are shown for each temperature and exposure concentration in Table 2. There were no mortalities in the seawater controls. Lobster that were severely affected in the treatment tanks were left in the recovery tanks and not exposed again. Severely affected lobster appeared moribund, laying on their backs or sides and had very little movement of pleopods. Some severely affected lobster died during and after the 6 day exposure and some lobster recovered.

Despite the differences in deltamethrin concentrations at the higher concentrations the biological effects are consistent. All lobsters exposed to the higher concentration for 30 minutes died during the study regardless of temperature. Greater than 50% of those exposed to the higher concentration for 15 minutes died in all cases. Behavioural responses were noted in the first day at all the higher concentration exposures regardless of concentration, exposure time or exposure temperature (Table 2). From a risk perspective, exposure to these concentrations for even a short period of time appears to be hazardous.

Differences in responses at different temperatures were noted at the lower concentrations:

5^0C exposures

Some lobster were affected on the second day of the 15 minute- 13 ng·L^{-1} pulse dose group and the 30 minute- 11 ng·L^{-1} pulse dose at 5^0C (Table 2). At the beginning of the sixth and final day, seven lobster from the 30 minute pulse dose group and one lobster from the 15 minute pulse dose group were severely affected so were not exposed again. At the end of the sixth day of exposures, six more lobster in the 15 minute exposure group and the final three lobster in the 30 minute exposure group were severely affected. Two days post exposure, seven lobster from the 15 minute- 13 ng·L^{-1} pulse dose group had recovered and four lobster from the 30 minute- 11 ng·L^{-1} exposure group had recovered.

10^0C exposures

One lobster was affected on the second day in the 30 minute- 10 ng·L^{-1} pulse dose group at 10^0C. On the fifth day, five lobster in the 30 minute- 10 ng·L^{-1} exposure group were dead and four were on their backs but still moving. On the sixth exposure day two lobster were affected in the 15 minute- 9 ng·L^{-1} group. One lobster had shed its crusher claw and one was on its back.

14^0C exposures

The lobster in the 15 minute- 9 ng·L^{-1} and 30 minute- 8 ng·L^{-1} groups appeared unaffected throughout the 6 day exposures. One lobster in the 30 minute- 8 ng·L^{-1} group died 2 weeks post end of exposures.

DISCUSSION

Measured water concentrations of deltamethrin were generally lower than the corresponding target concentrations in this study and the reason for this is unknown. There were exceptions to this and the mortality that occurred in the 15 ng·L^{-1} exposure tank in test 1 may be attributed to the higher measured exposure concentration of 27.3 ng·L^{-1} (Table 1). Measured deltamethrin concentrations ranged from 32 to 66% of nominal concentrations (100 ng·L^{-1}) for the 6 day pulse dose exposures with higher measured concentrations at the lowest test temperature of 5^0C (Table 2).

The generally lower measured concentrations in this study are consistent with other investigators (Burridge et al., 2014; Ernst et al., 2014; Van Geest et al., 2014c). Deltamethrin concentration data from the field during a net pen tarp treatment showed the measured value to be 37% of the intended treatment concentration (Ernst et al., 2014). They reported that after filtering the treatment water collected from the net pen, the proportion of deltamethrin was always greater in the particle phase, by approximately 3-4 times, than in the aqueous phase. The particle-bound deltamethrin would also have a greater tendency to sequester to sediments and concurrent and sequential applications of many pens within bay areas may represent a cumulative loading to sediments, potentially exposing benthic communities (Ernst et al., 2014). Ernst et al. (2014)

spiked water samples and found recoveries of deltamethrin were low (32-46%) compared to results reported in freshwater. They suggest that the concentrations of deltamethrin reported for environmental samples in their study likely substantially underestimate real concentrations and therefore established toxicity thresholds would probably underestimate the toxic potential of deltamethrin in those samples. The daily estimates of deltamethrin concentrations in the exposure tanks in the present study determined from volume of stock solutions dripped into the tanks were similar to the nominal concentrations but water analysis showed generally lower measured concentrations. Further research should be conducted to determine why there is a loss of deltamethrin in treatment water when analyzed as this is important information in the commercial application of AlphaMax® (DFO, 2013; Burridge et al., 2014).

The LC_{50} of 14.7 $ng \cdot L^{-1}$ (deltamethrin) for adult lobster exposed to AlphaMax® continuously for 10 days is <1% of the recommended anti-louse treatment concentration of 2 $\mu g \cdot L^{-1}$. Behavioral effects were seen as early as 48 h from the beginning of the 27.8 $ng \cdot L^{-1}$, 10.8 $ng \cdot L^{-1}$ and 6.6 $ng \cdot L^{-1}$ exposures and as early as 3 h from the beginning of the 24.9 $ng \cdot L^{-1}$ and 32 $ng \cdot L^{-1}$ exposures. There are LC_{50} data in the literature based on 24 h or 1 h deltamethrin exposures to adult lobster but not on repeated exposure or chronic exposures. The effect of temperature on responses and the potential effect of long term exposure are also important in assessing risk. Adult American lobster exposed to pulse doses for 6 days at 3 temperatures (5, 10 and 14 °C) were affected as early as the first day for most treatments. The cumulative effect of the 30 minute pulse doses was greater than the cumulative effect of the 15 minute doses at all 3 temperatures. Mortality was dose dependant with greater mortality in the higher concentration treatment groups as compared to the lower concentration treatment groups for all 3 temperatures. Lobsters exposed to 8 $ng \cdot L^{-1}$ and 9 $ng \cdot L^{-1}$ deltamethrin at 14 °C were not affected during the 6 day test. Pyrethroids have been shown to be more toxic at lower temperatures (Weston et al., 2011, Sparks et al., 1983). Hardwood et al. (2009) found that pyrethroid toxicity change resulted from a combination of increased accumulation of parent compound and increased nerve sensitivity, exacerbating the toxicity of pyrethroids at a lower temperature. The strong dependence of pyrethroid toxicity on temperature has important ramifications for predicting their environmental effects (Weston et al., 2009). Lab based experiments at higher temperatures may dramatically underestimate risk to non-target crustaceans during the cooler months. The dependence of toxicity on temperature also has ramifications for possible treatment protocols and risks to non-target organisms in the environment may be less if treatments are carried out during warmer months of the year. The Health Canada label for Alphamax® states that extra precautionary measures should be exercised if treatments are performed at low water temperatures. At water temperature $<6^{o}C$, the product's safety margin is reduced (Health Canada, 2013). Traditionally winter, when water temperatures drop, is a time when sea lice will die naturally and there is seldom a new set of lice on a farm from December to March (ACCFA, 2011). With the extended warm water temperatures experienced in recent years sea lice bath treatments have continued until the end of January 2015 and January 2016. Low water temperatures experienced after this time kept sea lice numbers relatively low on most farms until mid-May leading into the traditional strategic spring treatment (ACCFA, 2016). Bath treatments are used when water temperatures reach 10°C from as early as June to as late as January (ACCFA, 2016).

Burridge et al. (2014) report the 1 h and 24 h lethal thresholds for deltamethrin in adult lobster to be in the same range, 18.8 and 15 $ng \cdot L^{-1}$, respectively (Table 3). This is an indication that the effect of deltamethrin in the AlphaMax® formulation occurs very quickly, after an exposure as

short as 1 h and at low concentrations and this is consistent with the results of this study. It is interesting to compare the time of first behavioral effects observed between the chronic and pulse exposures in this study. Regardless of whether doses were pulsed for 15 and 30 minutes or chronic, effects were seen quickly. Behavioral effects were seen within 1 to 7 hours on the first day of pulse exposures of 32 to 66 $ng \cdot L^{-1}$ of deltamethrin and generally within 3 to 6 hours on the first day of the chronic exposure of 24.9 to 81.5 $ng \cdot L^{-1}$. At the lower exposure concentrations at 5 and 10°C effects were seen as early as 24 hours from the beginning of the pulse exposures of 10 to 13 $ng \cdot L^{-1}$ of deltamethrin and as early as 47 hours from the beginning of the chronic exposure of 6.6 to 10.8 $ng \cdot L^{-1}$. Some of the lobsters classified as severely affected recovered after a period of time in clean water. This was somewhat surprising as this had not been noted in studies with lobsters and another pyrethroid, cypermethrin. (Burridge unpublished results). Fairchild et al. (2010) reported a LC_{50} of 28.2 $ng \cdot L^{-1}$ for stage IV lobster larvae exposed continuously for 4 days which is in the same range of the LC_{50} values reported for this study (Table 3). They saw animals immobile in the first 24 hours of the test at 3 nominal exposure concentrations (3.2 $ng \cdot L^{-1}$, 10 $ng \cdot L^{-1}$ and 32 $ng \cdot L^{-1}$). Larval stage I and stage III lobsters are even more sensitive to AlphaMax® (Table 3). Burridge et al. (2000b) found that adult American lobster exposed repeatedly to the pyrethroid, cypermethrin stopped directed movements and their claws were either crossed or extended laterally. Lobsters eventually rolled onto their backs and all movement stopped except for sporadic twitching of the legs. Some lobsters were on their backs after three exposures and were still alive after nine exposures. From a risk perspective recovery of lobster in the wild may not be successful as moribund lobster or lobster that shed claws could be subject to predation. The results of the present study confirm the observation that AlphaMax® acts quickly. These data are interesting in terms of concentrations in commercial application of the AlphaMax® formulation that may cause effects on non-target organisms after the release of treatment waters from multiple cages over several days.

Page and Burridge (2014) used a hazard quotients approach (i.e. the ratio of estimated *in situ* exposure concentration (C_{th}) to the level of effect concentration (C_{loe})) in an effort to give a more field oriented perspective on the spatial and temporal scales upon which therapeutant toxicity potentials may occur. Estimates of the potential toxicity of treatment target concentrations of deltamethrin were calculated as the ratio C_{th}/C_{loe}. When this ratio is greater than 1, the treatment or exposure concentration is greater than the effect concentration and the bath treatment is indicated as having potential to cause the specified effect. Conversely, when the ratio is less than 1, the treatment concentration is less than the effect concentration and the therapeutant bath is interpreted as being unlikely to cause the specified effect. Page and Burridge (2014) suggested that when the ratio is greater than 10, there is a strong potential for effects. The ratio was calculated in the present study as 136 when using the 10 day LC_{50} of deltamethrin as the C_{loe}. The deltamethrin treatment concentration of 2 $\mu g \cdot L^{-1}$ was used for C_{th} in the ratios as this is the recommended bath treatment concentration used to treat Atlantic salmon for sealice in SWNB. This ratio indicates that there is a strong potential for effects on adult lobster exposed to AlphaMax® and that under current aquaculture scenarios there would be potential for risk to lobster from chronic exposure to AlphaMax®.

Page and Burridge (2014) calculated the *in situ* dilution times of 3.73 h and 5.77 h using the adult lobster 10 day LC_{50} of 14.7 $ng \cdot L^{-1}$ (Page and Burridge, 2014). During the dilution time the length of the dispersing plume may range from 575 m to 986 m and the distance the patch may

travel away from the treated cage ranges from 1.3 km to 2.1 km based on the LC_{50} (Page and Burridge, 2014). These estimates are based on a single discharge from a tarped cage. Page and Burridge (2014) used the location of salmon aquaculture sites in SWNB (Figure 1) to estimate the potential overlapping zones of influence following bath treatments. They showed the potential for therapeutants in the discharge plume, assuming a circular zone of influence of 2 kilometers, to overlap should multiple treatments occur in a given area, as well as where there may be potential for discharge plumes to interact with the shallow near-shore or intertidal areas. While Page and Burridge based their calculations on a single release, our thresholds are based on a 10 day chronic exposure and therefore should be interpreted carefully since it is unlikely that in normal circumstances *in situ* organisms will experience the reported LC_{50} concentrations continuously for 10 days (Page and Burridge, 2014).

Ernst et al. (2014) exposed the amphipod, *Eohaustorius estuarius* to samples taken outside the edge of a net pen after the release of an actual treatment with AlphaMax®. The plume from the treatment produced an EC_{50} (mortality plus paralysis) to *E. estuarius* in short term (1 h) exposures up to 350 m from the edge of the net pen. Since bath treatments can be conducted within an hour of each other, these numbers suggest that it is quite possible for the plumes to overlap (DFO, 2013). Burridge et al. (2000b) reported that pesticide dispersion after sea lice treatments have been studied in SWNB and the concentration and time of exposure likely to be experienced by benthic invertebrates (such as lobster) near treated cages was predicted. The worst case scenario would be for benthic organisms bathed in a fairly uniform "patch" of treatment effluent that is close to the shoreline where horizontal velocities of water are often reduced relative to those offshore. In these situations, the organisms may be exposed to concentrations greater than about one-tenth the LC_{50} for as long as several hours (Burridge et al., 2000b).

Van Geest et al. (2014a) found inhibition of feeding in marine copepods to be a sensitive endpoint. They ranked AlphaMax® as having the greatest effect on feeding inhibition when compared to three other anti-sea lice treatments. Van Geest et al. (2014c) investigated the toxicity of AlphaMax® by exposing the amphipod *Echinogammarus finmarchicus* that inhabits the near-shore environment of SWNB where cage aquaculture sites are located. They reported 1- and 24-h LC_{50}s of deltamethrin in water to be 70 $ng \cdot L^{-1}$ and 9.4 $ng \cdot L^{-1}$, respectively. They suggest that organisms in the vicinity of effluent plumes could potentially be exposed to toxic concentrations of deltamethrin for sufficient duration to cause lethality or immobility, even delayed effects after the short 1-h exposure. They also reported a 10-d LC_{50} of deltamethrin to be 16 $ng \cdot g^{-1}$ for amphipods exposed to sediment spiked with deltamethrin at a range of 6-200 $ng \cdot g^{-1}$. Van Geest et al. (2014b) studied the toxicity of AlphaMax® to the polychaete worm *Nereis virens* and observed no considerable mortality in sediment spiked at relatively high concentrations but observed sublethal effects related to burrowing behavior and worm condition/ mobility that could affect long term survival and growth. They suggested the potential for exposure of worms would be dependent on the extent of pesticide accumulation in sediment (influenced by treatment frequency, and physical, chemical and oceanographic conditions). Ernst et al. (2001) reported that for all releases of pesticide and dye in their SWNB study, the dye plume entered the intertidal zone, and contacted the bottom where it could have an effect on benthic species, particularly arthropods. They found that the use of the pyrethroid, cypermethrin creates the potential for lethal plumes, from treatment of a single cage, which might cover up to a

km^2. Since treatment of multiple cages is the operational norm, area-wide effects of cypermethrin on sensitive species cannot be discounted (Ernst et al. 2001). Cypermethrin is very toxic to crustaceans, but to a lesser extent than deltamethrin (Burridge and Van Geest, 2014, Van Geest et al., 2014a, Van Geest et al., 2014c). Burridge et al. (2000a) reported a 24-h LC_{50} of cypermethrin in Excis® for adult lobster of 140 $ng \cdot L^{-1}$ compared to a reported 24-h LC_{50} of deltamethrin in AlphaMax® for adult lobster of 15 $ng \cdot L^{-1}$ (Burridge et al., 2014) (Table 3). Area-wide effects of deltamethrin on sensitive non target species cannot be discounted after multiple treatment water releases in the same area.

CONCLUSION

We have attempted to mimic in these lab based studies the repeat or chronic exposure of a commercially important species, the American lobster to deltamethrin that may occur in SWNB where the successful co-existence of aquaculture industry activities with other commercially fished species is dependent on understanding the unwanted effects of the pesticide on non-target species. The studies are ecologically relevant in that chronic and pulse doses of AlphaMax® at low levels caused effects quickly, including immobility and mortality in the commercially important American lobster. Although a 10 day chronic exposure may seem unrealistic, deltamethrin has an affinity for organic material and can persist in the sediments for weeks (Haya et al., 2005). It may desorb and affect benthic invertebrates like the American lobster through ingestion of contaminated organic particles as well as from water (Burridge et al, 2014). The data presented here are consistent with previously published data with respect to lethal and sublethal effects happening quickly regardless of the exposure length. Unexpectedly, some of the lobster classified as severely affected recovered after a period of time in clean water. From a risk perspective moribund animals in the wild may be subject to predation before recovery can occur. Directed studies could help describe the potential for affected lobsters to survive exposure to AlphaMax®. The cumulative effects of multiple short exposures to deltamethrin were dependent on water temperature with deltamethrin being more toxic to the American lobster at lower temperatures. Water temperature should be considered when using pyrethroids as anti-louse treatments as risk to non-target crustaceans is greater at lower temperatures. Data from this study should strengthen the risk assessment of AlphaMax® use as an anti-louse formulation. Although AlphaMax® is not currently registered for use in Canada against sea lice, it is by far the most toxic compound ever registered, past or present. It's future use should be regulated accordingly and monitored closely in water and sediment around aquaculture sites.

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Table 1. Record of mortality and time of first behavioral effects observed for 10 day chronic exposure of adult lobster to AlphaMax®.

Test #	Temperature (°C) mean ± SD	Measured deltamethrin concentration ($ng \cdot L^{-1}$) mean ± SD	Time of first behavioral effects observed	Day									
				1	2	3	4	5	6	7	8	9	10
				# Dead/ 10 Exposed									
1	12.7 ± 0.5	<5 (control)	no effects	0	0	0	0	0	0	0	0	0	0
		7.7±3.7	T=71 h	0	0	0	0	0	0	0	0	0	1
		27.3±3.8	T=48 h	0	0	0	0	3*	3	3	3	6	6
		60.0	T=6 h	3	6	8	8	8	8	9	9	9	10
		81.5±12.0	T=3 h	10	10	10	10	10	10	10	10	10	10
2	13.2 ± 0.2	<5 (control)	no effects	0	0	0	0	0	0	0	0	0	0
		1.7±0.3	no effects	0	0	0	0	0	0	0	0	0	0
		4.2±1.2	no effects	0	0	0	0	0	0	0	0	0	0
		10.8±2.3	T=47 h	0	0	0	0	0	1	1	3	4	5
		32.0±17.0	T= 3 h	0	10	10	10	10	10	10	10	10	10
3	14.0 ± 0.2	<5 (control)	no effects	0	0	0	0	0	0	0	0	0	0
		1.9±0.6	no effects	0	0	0	0	0	0	0	0	0	0
		2.4±1.1	no effects	0	0	0	0	0	0	0	0	0	0
		6.6±1.4	T=52 h	0	0	0	0	0	0	0	0	0	0
		24.9±5.7	T=4 h	4	6	8**	8	8	8	8	8	8	8

*Several lobster were on their backs as early as the third day of the 27.3 $ng \cdot L^{-1}$ exposure.
**The 24.9 $ng \cdot L^{-1}$ exposure of test 3 was stopped on the third day with the remaining two lobsters in very poor shape; they subsequently recovered.

Table 2. Record of mortality and time of first behavioral effects observed for 6 day pulse dose exposure of adult lobster to AlphaMax®.

Temperature (°C) mean ± SD	Exposure duration (min)	Measured deltamethrin concentration ($ng \cdot L^{-1}$) mean ± SD	Time of first behavioral effects observed	# Dead/ 10 Exposed					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
4.9 ± 0.2	15	<5 (control)	no effects	0	0	0	0	0	0
	30	<5 (control)	no effects	0	0	0	0	0	0
	15	13±6	T=24 h	0	0	1	1	1	3
	30	11±2	T=30 h	0	3	3	3	5	6
	15	66±6	T=3 h	0	8	8	8	8	8
	30	62±16	T= 3 h	0	10	10	10	10	10
10.5 ± 0.4	15	<5 (control)	no effects	0	0	0	0	0	0
	30	<5 (control)	no effects	0	0	0	0	0	0
	15	9±2	T=120 h	0	0	0	0	0	0
	30	10±5	T=24 h	0	1	2	3	5	5
	15	33±7	T=3 h	0	8	10	10	10	10
	30	32±4	T=7 h	0	7	10	10	10	10
13.8 ± 0.1	15	<5 (control)	no effects	0	0	0	0	0	0
	30	<5 (control)	no effects	0	0	0	0	0	0
	15	9±3	no effects	0	0	0	0	0	0
	30	8±2	no effects	0	0	0	0	0	1*
	15	41±14	T=4 h	0	0	0	3	3	6
	30	45±14	T=1 h	7	7	7	7	7	10

* lobster died 2 weeks post exposure.

Table 3. LC_{50} values of AlphaMax® (as deltamethrin) for American lobster taken from the literature. Estimates are calculated as the mean of replicate bioassays (N) and are based on measured concentrations of deltamethrin (except when noted). Dilution factors are based on a prescribed treatment concentration of 2 $\mu g \cdot L^{-1}$.

Test animal/ Exposure time	LC_{50} ($ng \cdot L^{-1}$)	95% CI	N	Dilution factor	Reference
Adult lobster - 10 day chronic (continuous)	14.7	8.3-21.1	3	140	This study
Adult lobster - 1 h pulse + 95 h clean water	18.8	3.9-33.6	3	110	Burridge et al. (2014)
Adult lobster – 24 h	15.0	11-19	3	130	Burridge et al. (2014)
Stage IV lobster – 4 day chronic (continuous)	28.2	12.6->32	1	70	Fairchild et al. (2010)[a]
Stage III lobster – 4 day chronic (continuous)	4.74	2.96-7.60	1	400	Fairchild et al. (2010)[a]
Stage III lobster – 4 day chronic (continuous)	3.74	2.27-6.22	1	535	Fairchild et al. (2010)[a]
Stage III lobster – 1 h pulse + 16 days clean water	36.5	25.0-53.3	1	55	Fairchild et al. (2010)[a]
Stage III lobster – 16 day chronic (continuous)	4.45	3.69-5.36	1	450	Fairchild et al. (2010)[a]
Stage I lobster – 1 h pulse + 95 h clean water	3.4	1.5-6.0	2	590	Burridge et al. (2014)

[a]Fairchild et al. (2010) values are based on nominal concentration.

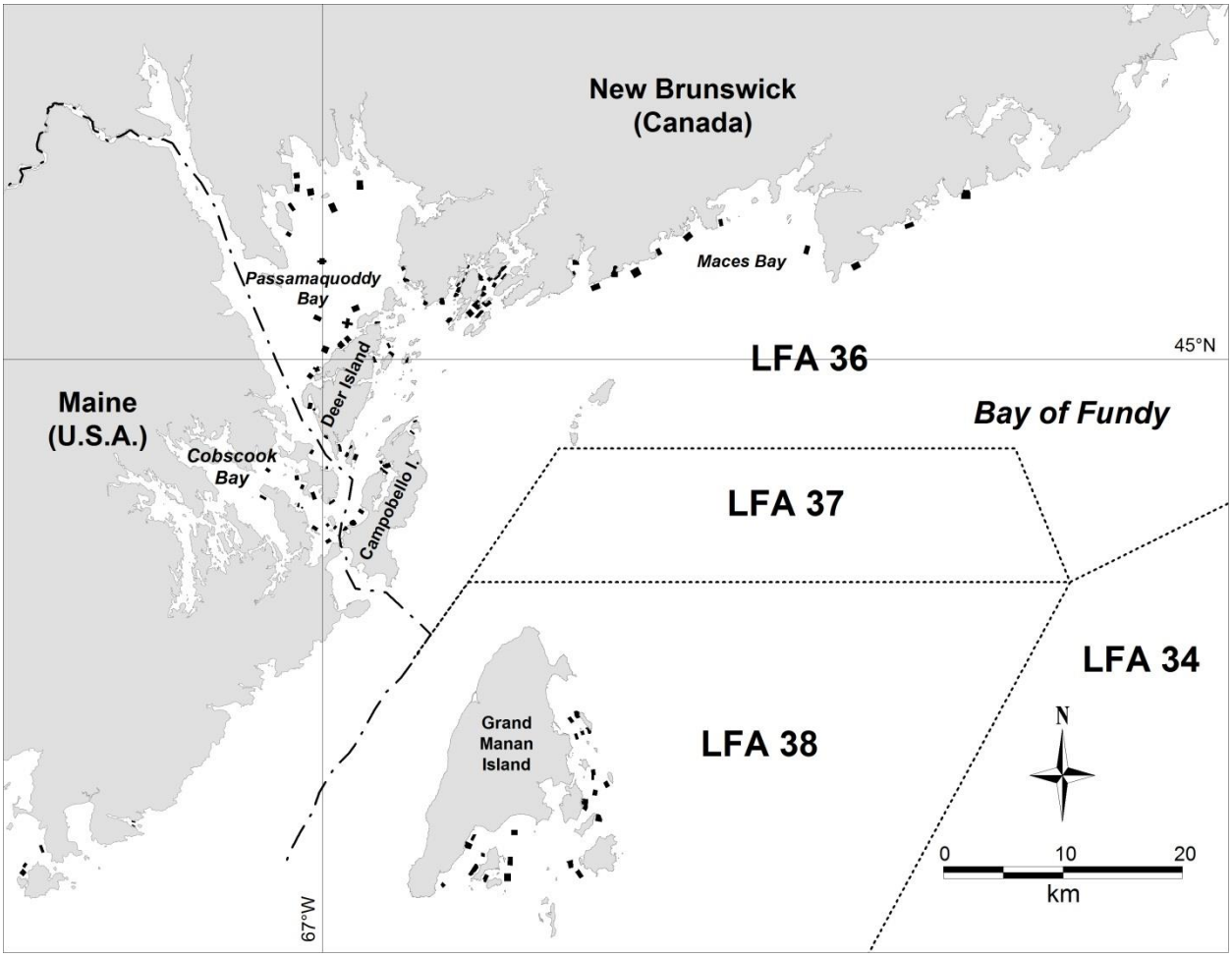

Fig. 1. Aquaculture site locations (black squares and rectangles) and Lobster Fishing Areas (LFA) in southwest New Brunswick, Canada.