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The toxicity of the anti-sea lice pesticide AlphaMax® to the polychaete worm *Nereis virens*

J.L. Van Geest [a,b,*], L.E. Burridge [a], K.A. Kidd [b]

[a] Fisheries and Oceans Canada, St. Andrews Biological Station, 531 Brandy Cove Road, St. Andrews, NB E5B 2L9, Canada
[b] University of New Brunswick, Biology Department & Canadian Rivers Institute, 100 Tucker Park Road, Saint John, NB E2L 4L5, Canada

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ABSTRACT

Polychaete worms have been suggested as a commercially valuable, extractive species to use in Integrated Multi-Trophic Aquaculture (IMTA) to remove organic materials (fish feces) released from salmon aquaculture. However, pesticides used to control parasitic sea lice infestations on salmon are also released from fish farms and non-target organisms may be exposed to these chemicals. In laboratory studies, the polychaete *Nereis virens* was exposed via water and sediment to the anti-sea lice pesticide AlphaMax® (active ingredient, deltamethrin). Worms exposed in water for 48 h exhibited mortality and impaired mobility in up to 100% of organisms, only at greater than 2-times the prescribed aquaculture treatment concentration. This would suggest negligible risk to worms from acute environmental exposure to AlphaMax® in water. Low mortality (≤20%) occurred in 7- or 30-d tests with sand or sediment spiked at relatively high concentrations (up to 0.72 μg deltamethrin/g), but sublethal effects related to burrowing behavior and worm condition were observed at concentrations as low as 11 μg/g. Therefore, the long-term survival, growth, and ability of worms to perform their ecosystem function of processing organic waste could be affected, depending on the extent of deltamethrin accumulation in sediment. Environmental concentrations of deltamethrin in sediment near aquaculture sites are not presently known and are needed to assess risk to non-target organisms.

1. Introduction

Salmon aquaculture has grown in the last 30 years to meet the increasing demands of a worldwide market. Excess feed and fish feces may accumulate under and around salmon cages. Biodegradation of this waste can lead to oxygen deficiency, changes in sediment biochemistry, and reduced water quality resulting in adverse effects on both the fish farm and the surrounding environment (Gray et al., 2002; Holmer et al., 2005; Sutherland et al., 2007). Selection of higher-flow locations for aquaculture sites and highly controlled feeding rates has been used to reduce the organic accumulation under cages. Another strategy termed Integrated Multi-Trophic Aquaculture (IMTA) attempts to mitigate some of the potential effects by co-cultivating "extractive" species from different trophic levels in close proximity to fish cages. For example, organic wastes are used by suspension- or deposit-feeders which are then harvested for commercial purposes.

Polychaete worms have been identified as potential extractive species for IMTA because they are highly efficient detritivores that process sediment, often dominate the benthic community in areas of high organic loading (Kutti et al., 2007; Mirto et al., 2012; Sutherland et al., 2007; Weston, 1990), and are of commercial value in the live-bait industry and production of aquaculture feed (Olive, 1999). Polychaetes have also been used to bioremediate organically enriched sediment under fish farms (Kinoshita et al., 2008). *Nereis virens* is the main polychaete species of interest since it is a common, cosmopolitan intertidal predator and deposit feeder and is available from some commercial hatcheries (e.g., in England, Wales, and Maine, USA), which culture this species because collection from natural populations cannot meet market demands (Olive, 1999).

As is the case with all intensive culture of animals, diseases and infestations of parasites are often present at aquaculture sites. When treatment is required, antibiotics as well as drugs and pesticides are applied to control parasitic sea lice and other diseases (Roth et al., 1993). These chemotherapeutants are tightly regulated and can only be used under the prescription from a licensed veterinarian. One such chemotherapeutant, the anti-sea lice pesticide formulation AlphaMax®, is an emulsifiable concentrate containing 1% of the synthetic pyrethroid deltamethrin as the active ingredient. Pyrethroids affect nerve transmission by interfering with sodium (Na^+) channels (Miller and Adams, 1982) which results in the depolarization and repetitive firing of the nerve endings and leads to eventual paralysis and death (Crane et al., 2011; Haya et al., 2005). The recommended treatment of salmon against sea lice is a 40 minute bath in AlphaMax® with a target concentration of 2 μg deltamethrin/L (SEPA, 2008). Following treatment, the

* Corresponding author at: Fisheries and Oceans Canada, St. Andrews Biological Station, 531 Brandy Cove Road, St. Andrews, NB E5B 2L9, Canada.

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pesticide is allowed to disperse into the environment. Although AlphaMax® is registered or approved for use in a number of salmon-producing nations, it was applied in Canada under emergency registration only in 2009 and 2010. Deltamethrin has a very low water solubility (<2 μg/L) and a log K_{ow} of 4.6 (Tomlin, 1994) and is not expected to persist in water but, like other pyrethroids, tends to sorb to suspended particulate matter or sediment (Solomon et al., 2001). The half-life for deltamethrin in marine sediments has been estimated at approximately 140 days (Gross et al., 2008), thus it is considered a persistent compound based on criteria of the European Commission's REACH program (>180 days in marine sediment or >120 days in freshwater or estuarine sediment; ECHA, 2012). Therefore, multiple sea lice treatments may result in accumulation of this compound in sediments near cage sites (Gross et al., 2008), where it could persist and affect sediment-dwelling invertebrates (Haya et al., 2005). The combination of the emulsifiers and hydrophobic deltamethrin in AlphaMax® suggests that non-target organisms may be exposed via water, sediment, or ingestion of contaminated organic particles. Deltamethrin has the potential to be bioaccumulative based on a log K_{ow} greater than 4.5 (ECHA, 2012). Therefore, benthic organisms potentially exposed to deltamethrin in sediment could accumulate this compound and be a source of dietary exposure for other organisms. Typically, pyrethroids are considered unlikely to accumulate to a significant degree in aquatic food chains since they are rapidly metabolized by fish and mammals (Alonso et al., 2012; Kahn, 1983); however, low to moderate bioaccumulation has been documented in both fish and marine mammals (Alonso et al., 2012; Tjeerdema, 2012).

In general, pyrethroids are more toxic to non-target insects and crustaceans than to other phylogenetically distant invertebrates, with deltamethrin being one of the most toxic pyrethroids (Mian and Mulla, 1992). Deltamethrin has been shown to be extremely toxic to marine crustaceans (e.g., sea lice, lobster, shrimp) with 1-hour (h) LC50s (i.e., lethal thresholds) for lobster and shrimp ranging from 3.4 to 142 ng/L, well below the prescribed aquaculture treatment concentration (Burridge et al., 2014; Fairchild et al., 2010). However, there is no data on its toxicity to polychaetes and the release of AlphaMax® could result in exposure of worms to deltamethrin. The purpose of the present study was to determine the toxicity of deltamethrin, in the AlphaMax® formulation, to the polychaete *N. virens* under laboratory conditions, including the potential for bioaccumulation of this compound.

2. Materials and methods

2.1. Organisms and general conditions

Laboratory experiments were conducted at Fisheries and Oceans Canada, St. Andrews Biological Station, in St. Andrews, New Brunswick (NB), Canada. All water used for holding and testing organisms was the main laboratory supply of sand-filtered (0.2 μm) seawater (~30 parts per thousand salinity, source Passamaquoddy Bay, NB). Organisms were provided a 12:12 hour light:dark photoperiod with a maximum light intensity of 2 lx.

Juvenile polychaetes, *N. virens*, were obtained from the University of Maine's Centre for Cooperative Aquaculture Research (Freeport, ME, USA). Worms were held (10–90 d) in aquaria with flow-through seawater at ambient temperature with silica sand (#00 from Shaw Brick, Saint John, NB) as a substrate. On a weekly basis, salmon fish food pellets (2 mm, Skretting North America, Bayside, NB, Canada) were provided ad libitum to worm cultures.

2.2. Toxicity of AlphaMax® in water

A 48-h test was conducted to assess the toxicity of AlphaMax® in water. Exposure concentrations were chosen after conducting preliminary range-finding tests. A stock solution of AlphaMax® was prepared by mixing the formulation in seawater and then spiked into 600 mL of seawater in 1-L glass jars to give target nominal concentrations of 2.5, 5, 10, 20, and 40 μg deltamethrin/L. The 600-mL volume of test solution in water-only exposures matched the volume of overlying water used in subsequent tests with sand or sediment. Jars were sealed and placed on a reciprocating shaker at high speed for 5 min, and then placed in a water bath at an ambient seawater temperature of 13–14 °C. At test initiation, water from one replicate jar/test concentration was collected for measurement of water quality (temperature, pH, dissolved oxygen, salinity) and deltamethrin. The test was static with no aeration of water and no substrate was provided. One worm was added per replicate (n = 10/treatment) shortly after preparation of test treatments. Average wet weight of worms was 4.71 ± 1.46 g (n = 60). At 3, 6, 12, and 24 h, 40-mL aliquots of exposure water were removed from each replicate in the 10 μg/L treatment and pooled for chemical analysis to measure pesticide concentrations over time. At test termination, water was pooled from 2 to 3 replicates for water quality and deltamethrin analyses. Water samples for analysis of deltamethrin were preserved with dichloromethane (DCM; ~5% v/v in sample) and shaken for 15 min. All samples were refrigerated at ~4 °C until chemical analysis. Worms were considered dead when there was no movement, response to stimulus, or visible movement of blood in the dorsal vessel. Other varying degrees of effects were noted and the severity of effect was categorized, including a slowed or reduced mobility, immobility (not moving, but response to stimulus), and a moribund state (immobile, no response to stimulus, but movement of blood visible in the dorsal vessel).

2.3. Toxicity of AlphaMax® in sand or sediment

Due to the concentrated nature of AlphaMax® (10 g deltamethrin/L), stock solutions were prepared by dilution of the formulation in acetone for spiking sand or sediment. The chemical was spiked into jars containing sand/sediment and water, rather than into dry sand/sediment, as this more reflects field conditions in which the chemical is added to the water. All concentrations reported herein are as the active ingredient deltamethrin and expressed on a dry weight (dw) basis in sand/sediment. Target nominal concentrations are based on the assumption of 100% adsorption of deltamethrin to sand/sediment.

To determine the toxicity of deltamethrin from an additional route of exposure, a 7-day test was conducted with AlphaMax® spiked into commercial silica sand (99.5% sand, total organic carbon <0.1%). The concentration series for this test was selected based on a preliminary range finding test. On the day before the start of the test (Day −1), 200 g dry silica sand and 600 mL seawater were added to 1-L glass jars and 1-mL volumes of acetone-based stock solutions were added to give target nominal concentrations of 0.125, 0.25, 0.5, 1, and 2 μg/g. Jars were sealed and placed on a reciprocating shaker at high speed for 5 min, and then placed in a water bath at an ambient seawater temperature of 13–14 °C overnight with overlying water in each test jar aerated using a Pasteur pipette placed through a hole in a loosely fitted lid. At test initiation (Day 0), overlying water and sand were collected from one replicate/test concentration for measurement of water quality and deltamethrin analyses. One worm was added per replicate (n = 10/treatment) with an average wet weight of 3.03 ± 0.96 g (n = 60). Worms were monitored for burrowing behavior (presence on sand surface) and general condition (if visible) daily throughout the test.

At test termination, overlying water was pooled from three replicates for water quality and deltamethrin analyses. Worms were recovered from jars, rinsed, and their condition noted. They were blotted dry, weighed individually, and then frozen (−80 °C). Categorization of survival/condition endpoints included damaged sections, hindered mobility, both of these sublethal effects, and mortality. Sand was collected from one replicate/test concentration for chemical analysis and frozen (−20 °C) until chemical analysis.

To determine the impact of substrate type on the toxicity of deltamethrin, the 7-d test was repeated using sediment collected at low tide from Passamaquoddy Bay (deltamethrin concentration below method detection limit of <0.005 μg/g) and the same target nominal concentrations. Sediment was pressed through a 2 mm sieve to remove gravel and shells (without addition of water), and stored at ~4 °C until use. The sediment was composed mostly of sand (99%) with a total organic carbon content of 0.2–0.3%. In the test set-up, 250 g of wet sediment was added to jars, which was equivalent to 200 g dry weight based on its moisture content. The test was conducted at a controlled temperature of 12 ± 1 °C in a water bath. Average wet weight of worms on Day 0 was 1.98 ± 0.56 g (n = 60). Worms were monitored for burrowing behavior and endpoints were the same as those described for the 7-d sand test.

To examine effects on *N. virens* from a more chronic exposure to deltamethrin, a 30-d test was conducted with sediment using the same target nominal concentrations as the 7-d tests (excluding the 2 μg/g nominal treatment). Additional replicates with worms were included in the 0.5 μg/g treatment to accommodate time-point sampling for deltamethrin analysis in sediment and water on Days 3, 6, 13, and 20. The test was conducted at a controlled temperature of 12 ± 1 °C in a water bath. Average wet weight of worms on Day 0 was 1.93 ± 0.70 g (n = 54). Due to the longer test duration, worms were fed two salmon fish food pellets (2 mm) twice per week, and uneaten food was removed immediately prior to subsequent feeding. No feces were visible for removal at feeding intervals. The endpoints that were assessed were the same as those described for the 7-day tests.

2.4. Chemical analyses

Samples were analyzed for deltamethrin at the University of New Brunswick (Saint John, NB, Canada) following standard methods, including associated quality assurance/quality control procedures. For water samples, a liquid–liquid extraction was completed using a separatory funnel and DCM (US EPA, 1996a). Each sample was extracted two times with DCM, which was then collected for analysis. The extract was concentrated using a Büchi Rotavapor R200 and further concentrated with an N-Evap™ 112 nitrogen evaporator to a final volume in isooctane.

Sediment and biota samples were stored frozen until analysis. Samples were freeze dried, and the percent moistures were determined gravimetrically. The freeze-dried samples were extracted using an Accelerated Solvent Extractor (ASE 300) from Dionex, with 50:50 DCM: hexane (US EPA, 1996b). Extracts were concentrated using a Büchi Rotavapor R200 and further concentrated with a N-Evap™ 112 nitrogen evaporator to a final volume in 50:50 DCM:hexane. Percent lipid was determined gravimetrically in an aliquot of the extract from biotic samples. Extracts for sediment and biota were run through a J2 Scientific Automated Gel Permeation Column to remove the heavier contaminants that may interfere with the quantification of deltamethrin (US EPA, 1996c). Samples were re-concentrated and the "clean" extract was added to a Florisil column and eluted with a series of non-polar to polar solutions. Separate fractions containing polychlorinated biphenyl (PCB) surrogates/standards and deltamethrin were solvent transferred to isooctane, and then further concentrated to final extracts.

PCB 30 and PCB 204 were added to samples as surrogates prior to extraction. PCB 103 and PCB 198 were added as internal standards to final extracts just prior to analysis using gas chromatography with electron capture detection (GC-ECD) and quantification using an internal standard calibration, based on standard methods (Hladik et al., 2009; US EPA, 1995). Quality assurance/quality control procedures performed on each set of 10 samples included: surrogates, calibration checks, method blank, and a method spike. The method detection limits were 0.005 μg/L and 0.005 μg/g for water and sediments/sand, respectively.

Grain size and total organic carbon (TOC) content of sand and sediment and TOC in overlying water were measured by the Research and Productivity Council (Fredericton, NB, Canada). TOC in overlying water from both sand and sediment tests was below the reporting limit of 0.5 mg/L.

2.5. Test endpoints

An LC50 or EC50 (i.e., concentration causing lethality or other effect in 50% of organisms) with 95% Confidence Intervals (C.I.) was determined for each test. These endpoints were determined according to Stephan (1977), using the computer program Toxstats and Probit, (Trimmed) Spearman–Karber, or binomial methods where appropriate, and were based on measured concentrations of deltamethrin. For sand or sediment, the concentrations measured at the beginning and end of each test were averaged for each treatment and used to calculate endpoints. For water, average measured concentrations over the test duration were determined based on the methods of Zitko et al. (1977), except where noted. For treatments for which time-point sampling occurred, an average concentration for that treatment was calculated based on the area under the exponential degradation curve fit to the measured data. The ratio between this time-weighted average and the time (T) = 0 h measured value was used to calculate adjusted average concentrations for the other treatments based on the corresponding T = 0 h measured data.

Growth was measured as the average percent change in individual wet weight at the end of the test (7 or 30 d) relative to Day 0 values. This endpoint did not consider feeding rates in the 30-d test because relative growth was compared within test types. Worms from extra replicates for time-point sampling during the 30-d sediment test were not included in calculation of growth endpoints.

Concentrations of deltamethrin in whole-body tissue samples were normalized for lipid content. Biota-sediment accumulation factors (BSAFs) were calculated as the mean lipid-normalized tissue concentration (n = 2 from pooled organisms) divided by the TOC-normalized average sand/sediment concentrations. TOC values of 0.1 and 0.25% were used for sand and sediment, respectively.

3. Results

3.1. Toxicity of AlphaMax® in water

3.1.1. Chemical concentrations

Measured concentrations of deltamethrin at T = 0 h in the 48-h water-only test ranged from 71 to 117% of nominal concentrations (Table 1). Concentrations were measured above the solubility limit of deltamethrin because AlphaMax® forms an emulsion due to the presence of emulsifiers. Over the 48 h, there was an average loss of 70% of deltamethrin in all treatments. In the 10 μg/L nominal treatment sampled over time, the concentration of deltamethrin decreased exponentially and loss of deltamethrin ranged from 29% of the initial concentration at 3 h to 77% at 48 h (Fig. 1).

3.1.2. Biological effects

After 1 h of exposure, worms in the 7.6 and 22 μg/L (average measured concentrations, hereafter) treatments appeared kinked or coiled, but still exhibited a lot of movement. After 24 h, all worms in the 4.4 μg/L treatment were curled or upside down, showing some movement, while those in the 7.6 and 22 μg/L treatments exhibited little movement or were immobile (data not shown).

After 48 h, 50% of worms in the two highest treatments were dead and varying degrees of effects were noted in all other individuals (Fig. 2). Surviving organisms in these treatments were either immobile or moribund (see Section 2.2 for definitions). All surviving worms in the 4.4 μg/L treatment were mobile, but slow when compared to control worms. Worms were not affected in the two lowest treatments or

Table 1
Measured concentrations of deltamethrin from AlphaMax® in water 48-h test with *Nereis virens* (n = 1/concentration).

Nominal concentration μg/L	T = 0 h μg/L	% of nominal	T = 48 h μg/L	Average[a] μg/L
0	0.013	–	nm	–
2.5	1.8	73	0.43	0.84
5	3.5	71	1.0	1.6
10	9.4	94	2.1	4.4
20	16	82	8.1	7.6
40	47	117	13	22

nm — not measured.
[a] Adjusted average based on Zitko et al. (1977) using average area under the exponential degradation curve of 10 μg/L measured data.

control. The estimated 48-h LC50 (death as endpoint) was 16 μg/L (95% C.I. 8.7–95) based on adjusted average concentrations. However, since other effects were observed indicating somewhat or severely debilitated organisms, EC50s for these combined effects were determined. The 48-h EC50s (95% C.I.) for all effects and only the most severe effects (i.e., immobile to dead) were 2.7 (1.6–4.4) and 5.4 (4.6–6.2) μg/L, respectively.

3.2. Toxicity of AlphaMax® in sand or sediment

3.2.1. Chemical concentrations

In AlphaMax®-spiked sand, measured concentrations of deltamethrin on Day 0 ranged from 26 to 53% of target nominal concentrations in sand (Table 2). In spiked sediment, measured concentrations on Day 0 were 17–24% and 15–38% of nominal values in the 7- and 30-d tests, respectively (Table 2). On average, measured concentrations were 50% lower in sediment than in sand. In many treatments, sand/sediment concentrations changed minimally between Days 0 and 7, although concentrations were variable and inconsistent between treatments. In the 30-d test, sediment concentrations decreased between Days 0 and 30 in all treatments by an average of 47%. In the 0.5 μg/g nominal treatment of the 30-d test, there was a ~20% decrease between Days 0 and 3 or 6, which is within the range of variation measured in the 7-d tests. This was followed by a 55% decrease between Days 6 and 13, with the concentration of deltamethrin remaining relatively constant thereafter to Day 30 (Fig. 3).

Deltamethrin measured in overlying water on Day 0 of sand/sediment tests ranged from approximately 0.73 to 12 μg/L, and concentrations were higher in higher AlphaMax® treatments (Table 2). On Day 7, concentrations in water overlying sand remained elevated or were increased from Day 0 values, whereas for sediment tests the overlying water concentrations decreased to ≤1.7 or ≤0.11 μg/L on Days 7 and 30, respectively. The concentration of deltamethrin in overlying water of the 0.5 μg/g nominal treatment decreased exponentially over the 30 d (Fig. 3).

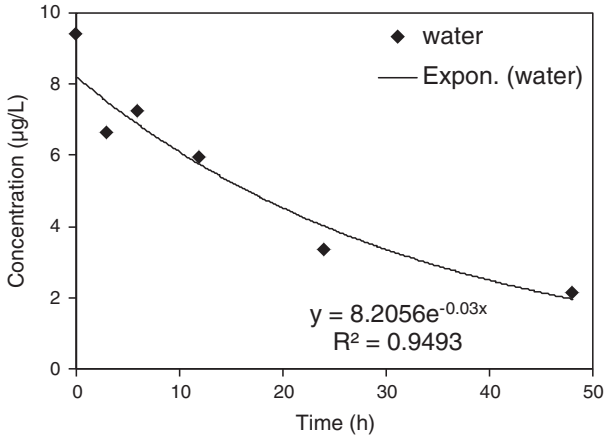

Fig. 1. Loss of deltamethrin from AlphaMax® formulation in water during 48-h test with *Nereis virens*. T = 0 nominal water concentration was 10 μg/L (n = 1/time).

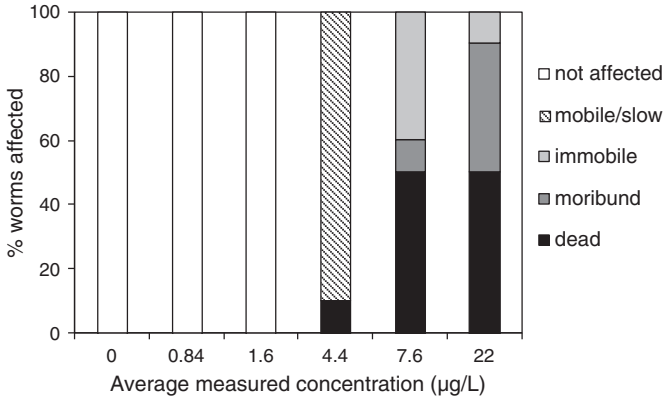

Fig. 2. Percentage of *Nereis virens* exhibiting varying degrees of effects with exposure concentration (μg/L deltamethrin) in 48-h test of AlphaMax® in water (n = 10 worms/treatment).

3.2.2. Burrowing behavior

In the 7-d sand test, after 3–7 h of exposure, 80–100% of the worms in the 0.32 and 0.72 μg/g treatments were observed on the sand surface and many remained there for the duration of the test (Fig. 4). Worms in the 0.11 and 0.22 μg/g treatments burrowed initially on Day 0, but up to 80–90% emerged or partially emerged onto the sand surface on Days 1 to 3 (Fig. 4). Worms in the control and 0.055 μg/g treatments remained burrowed. In the 7-d test with sediment, worms burrowed immediately and remained burrowed throughout the test, except those in the 0.4 μg/g treatment of which approximately half emerged from the sediment on Day 1 and remained on the surface for the duration of the test (Fig. 4). In the 30-d sediment test, worms burrowed immediately and remained burrowed throughout the test.

3.2.3. Survival and condition

By Day 7 in AlphaMax®-spiked sand, 10–20% of worms had died in the 0.11 to 0.72 μg/g treatments, and the condition of the surviving worms was affected to varying degrees (Fig. 5). Some worms showed signs of damage to their tail sections or tail sections were broken off. Other worms had sections of their bodies in which the segments appeared rigid and contracted, and this caused pooling of blood in the dorsal blood vessel behind those sections and hindered their mobility considerably (Fig. 6). The greatest percentage of worms exhibiting damaged sections and/or rigidity and hindered mobility was observed in the highest test concentration. The estimated 7-d EC50s (95% C.I.) determined for all levels of effect and only the most severe effects (i.e., rigid-hindered mobility to dead) were 0.13 (0.10–0.17) and 0.23 (0.15–0.38) μg/g, respectively.

In spiked sediment, 20% of worms died in the 0.4 μg/g treatment by Day 7 and the condition of surviving worms was affected only in the two highest treatments (0.16–0.4 μg/g). Similar effects on worm condition were observed compared to the 7-d sand test, but to a lesser extent in all but the highest treatment (Fig. 5). The estimated 7-d EC50s (95% C.I.) determined for all effects and for only the most severe effects (i.e., rigid-hindered mobility to dead) were 0.20 (0.16–0.25) and 0.23 (0.20–0.27) μg/g, respectively. No mortality was observed in the 30-d sediment test. Worms did not appear to be affected other than one organism in the highest treatment (0.18 μg/g), which was observed on the sediment surface on Day 30, exhibiting slow movement, and its body was soft.

3.2.4. Growth

During the 7-d test in sand, all worms including the controls lost weight. Average loss of wet weight from Day 0 values ranged from 7 to 17%. No trend was associated with treatment concentration (Fig. 7A), and there were no statistically significant differences between treatments (ANOVA, $p \geq 0.050$). In the 7-d sediment test, worms in all but the

Table 2
Measured concentrations of deltamethrin in sand, sediment, and overlying water in 7- and 30-d tests of AlphaMax®-spiked sand/sediment with *Nereis virens*.

Nominal concentration μg/g	Day 0			Day 7 or 30		Average	
	Sand μg/g	Water μg/L	% of nominal sand	Sand μg/g	Water μg/L	Sand μg/g[a]	Water μg/L[a,b]
7-day sand							
0	<0.005	0.051	–	nm	nm	–	–
0.125	0.057	1.5	46	0.053	0.13	0.055	0.80
0.25	0.13	1.5	53	0.077	1.6	0.11	1.5
0.5	0.13	1.8	26	0.30	3.0	0.22	2.4
1	0.32	3.2	32	0.32	8.4	0.32	5.8
2	0.72	8.9	36	nm[c]	12	0.72	10
7-day sediment							
0	<0.005	0.031	–	nm	nm	–	–
0.125	0.030	1.5	24	0.036	0.18	0.033	0.24
0.25	0.048	3.4	19	0.048	0.30	0.048	0.54
0.5	0.11	4.9	21	0.052	0.58	0.079	0.78
1	0.17	7.9	17	0.15	1.1	0.16	1.3
2	0.42	9.5	21	0.38	1.7	0.40	1.5
30-day sediment							
0	<0.005	0.037	–	nm	nm	–	–
0.125	0.019	0.73	15	0.011	0.02	0.015	0.11
0.25	0.06	3.2	24	0.03	0.08	0.045	0.50
0.5	0.19	6.3	38	0.076	0.11	0.133	0.99
1	0.22	12	22	0.140	0.10	0.180	1.9

nm — not measured.
[a] Average of Days 0 and 7 or 30 measured data.
[b] For 7- and 30-d sediment tests, adjusted average based on Zitko et al. (1977) using average area under the exponential degradation curve of measured overlying water data from 0.5 μg/g treatment in 30-d AlphaMax®-spiked sediment test.
[c] Sample accidentally not collected.

control and lowest treatment typically lost an average wet weight of 5–21%, with weight loss increasing with treatment concentration (Fig. 7A). Weight loss in the 0.4 μg/g treatment was significantly greater than in all other treatments (ANOVA–Tukey, $p < 0.001$). Weight loss was typically greater in the 7-d test with sand than with sediment, which would suggest a substrate effect; however, this difference was minimized when weight loss was corrected for control values (i.e., control subtracted from value; Fig. 7B). Food was provided in the 30-d test because of the longer duration and the weight loss observed in the 7-d tests. As a result, worms gained an average wet weight of 11–30% in 30 d and there were no significant differences in average weight gain between treatment concentrations (ANOVA, $p \geq 0.050$; data not shown).

3.2.5. Bioaccumulation

Deltamethrin was measured in whole-body tissue, on a subset of treatments from the 7-d AlphaMax®-spiked sand and sediment tests, to examine the potential for bioaccumulation and to examine the relationship between tissue concentrations and the level of effects observed. Worms from spiked treatments bioaccumulated deltamethrin compared to those in control treatments (Table 3). Tissue concentrations did not increase with exposure concentration in sand/sediment, apart from the lowest treatment in the 7-d sand test. Biota-sediment accumulation factors (BSAFs) decreased with increasing sand/sediment concentration. Bioaccumulation of deltamethrin was higher in worms exposed to AlphaMax® in sediment than sand, when tissue concentrations (both dry weight and lipid-normalized; see SI Table 1) and corresponding BSAFs were compared. This possibly could be due to a higher proportion of sediment in the gut (i.e., unpurged organisms) and/or greater interaction with sediment since worms typically remained burrowed compared to the large proportion of worms emerged in the sand test. Comparison of tissue data between treatments and tests did not indicate that there was a threshold tissue concentration associated with the biological effects observed.

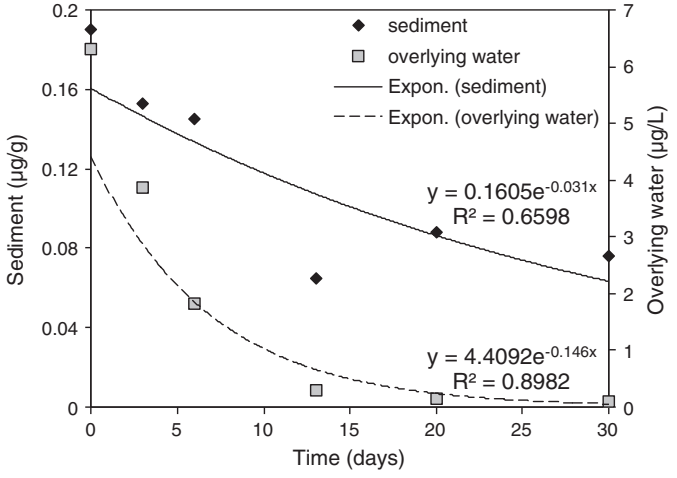

Fig. 3. Change in concentration of deltamethrin in sediment and overlying water during 30-d test of AlphaMax®-spiked sediment with *Nereis virens*. T = 0 nominal sediment concentration was 0.5 μg/g (n = 1/time).

3.2.6. Summary of biological effect concentrations

A summary of the deltamethrin concentrations causing various effects in worms across all tests is shown in Table 4. The lowest concentrations in which emergence of worms was observed varied somewhat between tests, from 0.11 to 0.4 μg/g. In terms of worm survival and condition, the lowest concentrations causing any effect and the EC50 values derived for all effects and the most severe effects were similar between sand and sediment tests. This was also mirrored when effect concentrations were determined on an overlying water basis for these tests. Additionally, effect concentrations did not vary in magnitude between the extent of effects (i.e., lowest concentration causing an effect and EC50s). Therefore, the concentrations at which effects occur were fairly consistent between tests.

4. Discussion

One of the objectives of the present study was to contrast the toxicity of deltamethrin (in the AlphaMax® formulation) in commercial sand that is homogenous and relatively inert with natural sediment that was similar in composition in terms of particle size, but contains higher

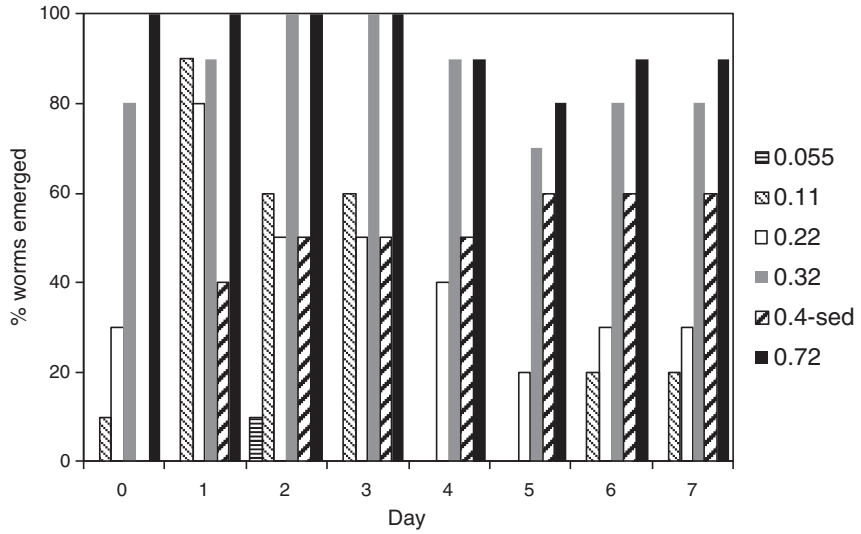

Fig. 4. Percentage of *Nereis virens* emerged on the sand surface for each exposure concentration (μg/g measured deltamethrin) throughout 7-d test of AlphaMax®-spiked sand (0.4-sed from 7-d sediment test, other concentrations not shown). Worms found dead on the sand surface were removed but still included in subsequent counts of emergence (n = 10 worms/treatment).

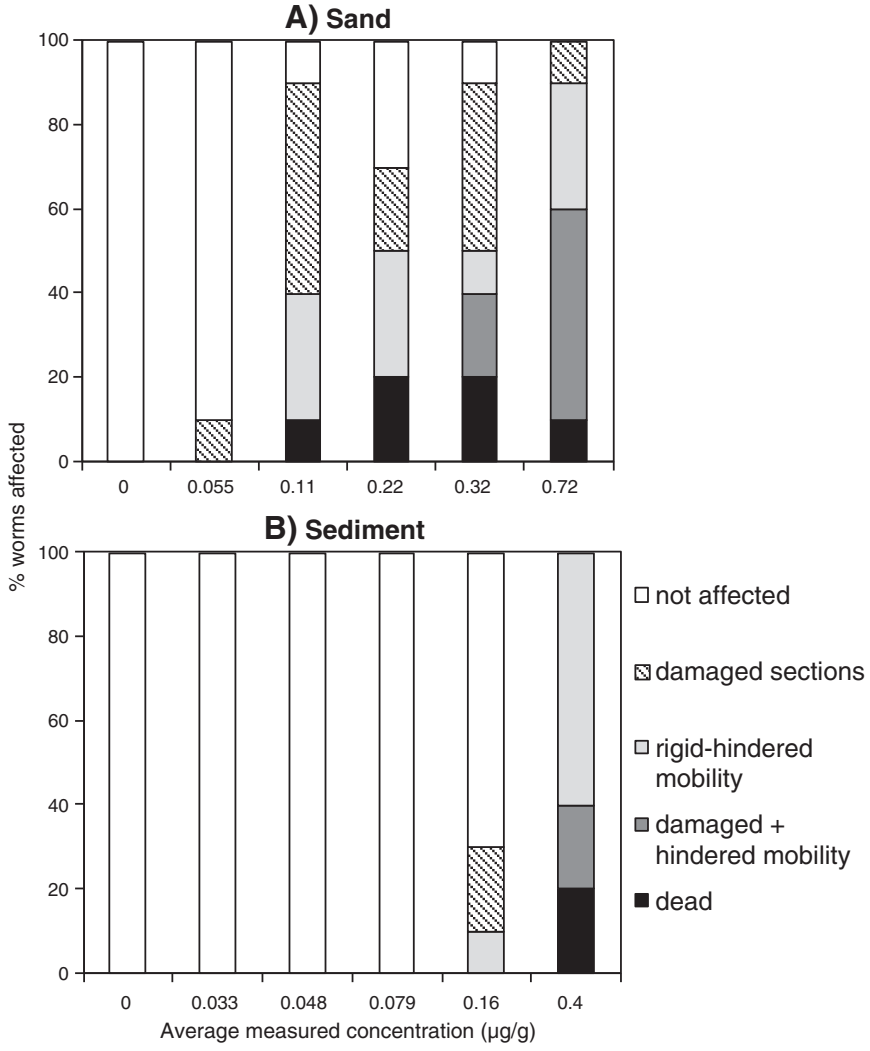

Fig. 5. Percentage of *Nereis virens* exhibiting varying degrees of effects for each exposure concentration (μg/g deltamethrin in sand/sediment) in 7-d test of AlphaMax®-spiked (A) sand or (B) sediment (n = 10 worms/treatment).

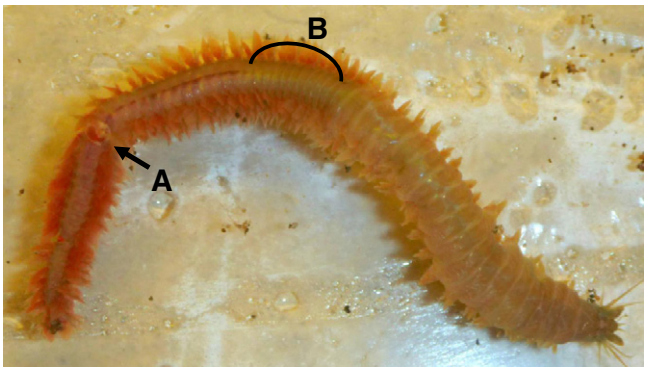

Fig. 6. Image of affected condition of *Nereis virens* showing (A) tail damage and (B) sections of rigid, contracted segments that caused pooling of blood in dorsal blood vessel behind those sections and hindered mobility.

organic matter and is more representative of environmental conditions. Although the same methods were used for spiking sand and sediment, average measured concentrations were 50% lower in sediment than sand. These differences could partly be due to the slightly higher TOC content of the sediment (0.2–0.3% versus <0.1% in sand), but with a low TOC in both matrices, the reasons for this disparity are not known. Initial concentrations of deltamethrin in overlying water of the 7-d sediment test were up to 2.7-times higher than those from the sand test. This could be due to additional dissolved organic carbon in the overlying water from the sediment, which can enhance solubility of pesticides (Zhu and Selim, 2002). This may also have contributed to the lower initial sediment concentrations of deltamethrin. However, water concentrations in the sediment exposures decreased by an average of 87% over time, while those in the sand test remained elevated or increased by up to 2.7-times. Therefore, slight differences in the physicochemical properties between the sand and sediment may have influenced the extent and rate of sorption or desorption of deltamethrin,

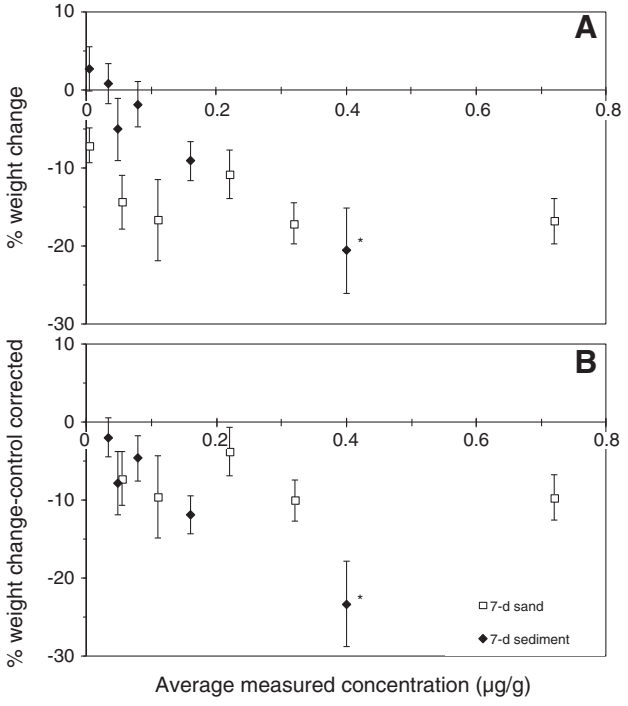

Fig. 7. Wet weight change (%) of *Nereis virens* with exposure concentration (µg/g deltamethrin in sand/sediment) in 7-d tests of AlphaMax®-spiked sand or sediment. (A) Absolute values and (B) corrected for control. Data are mean and standard error. *Significant difference from other treatments in test (ANOVA–Tukey, $p < 0.001$).

Table 3
Whole-body tissue concentrations of deltamethrin in *Nereis virens* and biota-sediment accumulation factors (BSAFs) from 7-d tests of AlphaMax®-spiked sand or sediment. Tissue data are means (n = 2 replicates/treatment) of pooled organisms.

Sand or sediment concentration µg/g[a]	Tissue concentration µg/g lipid	BSAF[b]
7-day sand		
0	<0.017	nc
0.055	0.61	0.01
0.11	1.1	0.01
0.22	1.2	0.006
0.32	1.0	0.003
0.72	1.2	0.002
7-day sediment		
0	<0.053	nc
0.033	nm	–
0.048	nm	–
0.079	3.0	0.09
0.16	2.4	0.04
0.40	2.0	0.01

nm — not measured because biological response not different from the next highest treatment.
nc — BSAFs not calculated for controls.
[a] Average of Days 0 and 7 measured data.
[b] BSAF = mean lipid-normalized tissue concentration/TOC-normalized sediment concentration.

causing these different trends in overlying water and sediment concentrations between tests.

Lower toxicity of deltamethrin in terms of impaired survival, behavior, and condition of worms was observed in tests conducted with sediment compared with those for sand at similar measured exposure concentrations. These differences are not likely due to higher exposure to deltamethrin from overlying water because average measured concentrations were similar between these treatments (1.3 and 1.9 µg/L in sediment tests versus 1.5 and 2.4 µg/L in corresponding treatments of sand tests). Additionally, the difference in biological effects observed was not related to whole-body tissue concentrations of deltamethrin, which were higher in the sediment test. Emergence of worms was not observed at concentrations below 0.4 µg/g of deltamethrin in sediment, but occurred in sand treatments as low as 0.11 µg/g. Fewer worms emerged from AlphaMax®-treated sediments and those emerging did so later than those in sand exposures at similar concentrations of deltamethrin. In combination, these results suggest that toxicity of deltamethrin was reduced in sediment when compared to sand.

Loss of weight occurred in most worms, but was greater in the 7-d test with sand than with sediment, including the controls, and may be due to a nutritional source of carbon in the sediment. However, when weight loss was standardized to that of the control animals, these differences between sand and sediment were minimized. Significant growth effects (weight loss) related to exposure to deltamethrin were only found in the highest sediment treatment (0.4 µg/g), and may have been influenced by burrowing behavior (by lack of sediment ingestion) as this was the only sediment treatment in which emergence was observed. In the sand test, emergence was observed in all but the control and lowest deltamethrin treatment. Therefore, any growth effect related to deltamethrin exposure in sand may have been reduced because of the lack of interaction or exposure to sand, although emerged organisms were exposed to elevated deltamethrin concentrations in the overlying water.

The initial concentrations of deltamethrin measured on Day 0 in overlying water of sand/sediment tests ranged from 0.73 to12 µg/L and were within the exposure concentrations (0.84–22 µg/L) of the 48-h water-only test. This enables some comparison of toxicity between the water-only and sand/sediment tests. The magnitude of effects observed in the water-only tests (i.e., proportion of dead or moribund organisms) was greater than tests with sand or sediment. This is likely due to the higher initial exposure concentrations in water-only

Table 4
Summary of deltamethrin concentrations causing effects[a] in *Nereis virens* from toxicity tests of AlphaMax®-spiked sediment and (overlying) water.

Test	Sediment μg/g				Water[b] μg/L			
	Lowest concentration		EC50[c] (95% C.I.)		Lowest concentration	EC50[c] (95% C.I.)		LC50[d] (95% C.I.)
	Emergence (Day)	Any effect	All effects	Severe effects[e]	Any effect	All effects	Severe effects[e]	Mortality
48-h water-only	–	–	–	–	4.4	2.7 (1.6–4.4)	5.4 (4.6–6.2)	16 (8.7–95)
7-d sand	0.11 (D1)	0.11	0.13 (0.10–0.17)	0.23 (0.15–0.38)	1.5	1.3 (0.68–1.9)	3.2 (2.0–5.8)	nc
7-d sediment	0.4 (D1)	0.16	0.20 (0.16–0.25)	0.23 (0.20–0.27)	1.3	1.3 (1.2–1.4)	1.4 (1.3–1.4)	nc
30-d sediment	0.18 (D30)	0.18	nc	nc	1.9	nc	nc	nc

nc — not calculated; effect not observed in 50% of organisms.
[a] Effects include emergence, mortality, and affected condition of worms.
[b] Water concentrations greater than the solubility limit of deltamethrin (2 μg/L) exist because the pesticide is present as an emulsion.
[c] EC50; concentration causing an effect in 50% of organisms.
[d] LC50; concentration causing lethality in 50% of organisms.
[e] Severe effects defined as rigid-hindered mobility, damaged + hindered mobility, moribund, and mortality.

experiments as these worms were affected rapidly, within 1 to 24 h. Effects in the water-only test were only observed when worms were exposed to an average concentration ≥4.4 μg/L over 48 h. Average concentrations of deltamethrin in overlying water only exceeded this value in the two highest treatments of the 7-d sand test. Worms did not burrow in these treatments, so the elevated concentrations in the overlying water presumably had a larger contribution to the observed toxicity. When the effects observed in the sand/sediment tests were related to deltamethrin concentrations in overlying water, the effect concentrations (for all effects) were lower than those from the water-only tests. This can likely be attributed to the longer exposure duration in the tests with substrate and to the contribution of multiple routes of exposure to deltamethrin toxicity. However, these values derived on the basis of overlying water concentrations differ by less than an order of magnitude from those for water-only exposures.

Most work on the toxicity of deltamethrin to marine organisms has been focused on crustaceans because of their extreme sensitivity. Exposure of lobsters and shrimp to AlphaMax® from as short as 1 h to as long as 16 d resulted in LC50 values that are ng/L concentrations (Burridge et al., 2014; Fairchild et al., 2010), orders of magnitude below the 48-h LC50 derived for worms in the present study. In toxicity tests, aquatic worms typically have been found to be less sensitive compared to other aquatic invertebrates. For example, 96-h LC50s for cypermethrin (a pyrethroid similar to deltamethrin) for the polychaete *Polydora cornuta* were one to three orders of magnitude higher than for arthropods such as amphipods, shrimp, and lobsters exposed to the same pesticide formulation or the technical grade chemical (Ernst et al., 2001). No published data exist on the toxicity of deltamethrin in sediment to marine species, and information in the freshwater literature is almost as limited. Amweg et al. (2005) reported a 10-d LC50 of 0.010 μg/g dw for the amphipod *Hyalella azteca*. Åkerblom et al. (2008) determined a 28-d LC50 of 0.011 μg/g dw for the midge *Chironomus riparius* exposed to spiked artificial sediment, and noted that mortality did not occur when natural sediment was spiked with deltamethrin up to 0.17 μg/g. In the present study, the lowest sediment concentration at which effects to worms occurred was 0.11 μg/g, confirming their tolerance relative to other invertebrates.

Reported environmental concentrations of deltamethrin in sediment near aquaculture operations are scant. Chemical monitoring for therapeutants in sediment around aquaculture sites is not a requirement in Canada. One jurisdiction where monitoring does occur is Scotland, where the Scottish Environmental Protection Agency (SEPA) conducts annual screening surveys of sediments around marine fish farms. However, deltamethrin has been not analyzed in those surveys, despite its use there. SEPA data does exist for cypermethrin, a similar pyrethroid used in the sea-lice pesticide Excis®, from four surveys conducted between 2003 and 2006. These surveys reported concentrations of cypermethrin in sediment ranging from 0.03 to 7.19 ng/g dw (SEPA, 2004–2007). Deltamethrin has a similar log K_{ow} as cypermethrin and aquaculture treatments with AlphaMax® and Excis® are similar (2 and 5 μg/L as active ingredient, respectively), so presumably deltamethrin accumulation in sediment around fish farms would not differ considerably from that observed for cypermethrin. However, the lack of environmental monitoring data for deltamethrin in sediment around cage sites remains a large source of uncertainty regarding the potential for effects to benthic invertebrates.

5. Conclusions

The toxicity of sea-lice pesticide AlphaMax®, with the active ingredient deltamethrin, has been studied for sensitive non-target organisms such as crustaceans, but not for polychaete worms, a potential extractive species in IMTA. The toxicity data in the present study suggest that under current aquaculture scenarios there would be negligible risk to worms from acute exposure to AlphaMax® in water because effects were only observed at concentrations higher than the prescribed treatment for sea-lice. However, the chemical properties of deltamethrin dictate that it will likely sorb to organic particles, and as a result may accumulate and persist in sediment. While no considerable mortality was observed in sediment spiked at relatively high concentrations, the observed sublethal effects related to burrowing behavior and worm condition/mobility could affect long term survival, growth, and the ability of worms to perform their ecosystem function of processing organic waste under cages. Additionally, the bioaccumulation of deltamethrin by worms represents a potential source of dietary exposure for other organisms. 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) and the methods/duration in which worms are held under aquaculture cages. Environmental concentrations of deltamethrin in sediment related to use in aquaculture are not presently known, which remains the largest source of uncertainty in predicting potential exposure and risks to non-target organisms, including IMTA extractive species.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2014.03.044.

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