Pergamon

Marine Pollution Bulletin Vol. 42, No. 6, pp. 433–444, 2001

Printed in Great Britain

PII: S0025-326X(00)00177-6

0025-326X/01 $ - see front matter

REPORTS

Dispersion and Toxicity to Non-target Aquatic Organisms of Pesticides Used to Treat Sea Lice on Salmon in Net Pen Enclosures

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Pesticides are used extensively in the finfish aquaculture industry to control sea lice infestations on farmed salmon. The most prevalent method of use is to enclose a net pen with an impervious tarpaulin and mix a pesticide solution within that enclosure. After treatment for short periods (1 h) the pesticide solution is released to the environment. Concerns have been raised that there is a potential risk to non-target aquatic organisms from those releases. The fate of dispersing pesticide solutions was measured after six simulated treatments in the Lower Bay of Fundy, New Brunswick. Three simulated treatments were done with azamethiphos and three with cypermethrin. Rhodamine dye was added to all pesticide solutions in order to facilitate tracking of the dispersing plume through real-time measurements of dye concentrations by a flow-through fluorometer coupled with a differential global positioning system (DGPS). Water samples were obtained from within the plumes at various times after release and analysed for pesticide content and toxicity to a benthic amphipod *Eohaustorius estuaris*. Dye concentrations were detectable for time periods after release which varied from 2 to 5.5 h. Distances travelled by the dye patches ranged from 900 to 3000 m and the dye concentrations at the final sampling period were generally 1/200–1/3000 the pre-release concentrations and cypermethrin concentrations were generally 1/1000–1/2000 the pre-release concentrations. Cypermethrin concentrations in water samples were closely correlated with dye concentrations, indicating that dye analyses were an accurate surrogate for cypermethrin concentrations. Most samples taken after the releases of azamethiphos were not toxic to test organisms in 48 h exposures and none were beyond 20 min post-release. By contrast, almost all samples taken after the release of cypermethrin, even up to 5-h post-release, were toxic. Data indicate the potential to cause toxic effects over areas of hectares from a single release of cypermethrin.

Keywords: aquaculture; pesticides; azamethiphos; cypermethrin; sea lice; toxicity; dispersion.

Introduction

The magnitude of the finfish aquaculture industry, particularly with salmon (*Salmo salar*) in the Lower Bay of Fundy, New Brunswick, increased dramatically during the 1980s and 1990s. As the intensity and extent of culture practices increased, so too did the incidence of sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) infestation on farmed fish (MacKinnon, 1997).

Since high infestation levels can cause reduced market value due to cosmetic effects as well as decreased fish health, costing the industry up to 20% of the total yearly market value (MacKinnon, 1997), aquaculturists have sought control methods. A number of chemicals have been used to manage sea lice, including dichlorvos, pyrethrum, hydrogen peroxide, azamethiphos, and cypermethrin (IMO, 1989). At present, there is only one product registered for use in Canada under the authority of the Pest Control Products Act: Salmosan, which

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contains the active ingredient azamethiphos, an organophosphorous insecticide. As it is highly effective, there is presently a desire on the part of the aquaculture industry to use the product Excis, which contains the active ingredient cypermethrin, a synthetic pyrethroid pesticide (Pahl *et al.*, 1996). Although cypermethrin is authorized for use in a number of countries, including the USA, it is not presently registered for operational use in Canada for sea lice control.

The methods presently used to treat fish infected with sea lice involve the release to the environment of pesticide solutions after relatively short (1 h) exposures. Concerns have been raised regarding the potential effect of discharged sea lice treatment chemicals on non-target organisms due to their known toxicity to arthropod species (McLeese *et al.*, 1980; Mian and Mulla, 1992; Siegfried, 1993).

Effects of chemicals in the environment are a function of exposure as well as toxicity, and limited studies conducted have not documented significant effects on non-target species under field conditions with the use of the most highly toxic chemicals such as cypermethrin (Hogans, 1997). That may be due to the methods used in previous studies, which have involved the placement of test organisms in cages around the treatment site and the difficulty in predicting the transport path of the released pesticide solution. Previous studies have also not been able to document pesticide dispersion characteristics for long periods after release, or for great distances from the release site (Dobson and Tack, 1991; Pahl *et al.*, 1996).

This study was conducted to improve the understanding of the disappearance rate of pesticide solutions from net pen aquaculture sites under various conditions, and to determine the toxicity of treatment solutions to selected aquatic organisms after they have been released to the environment. This was done by mixing a visible and photoactive dye with the pesticide solution to track the movement of the patch of pesticide/dye over time, and taking water samples from within the dye patch at periodic intervals to measure the pesticide concentration and to assess its toxicity to non-target organisms, as well as assessing the toxicity of a dilution series of the treatment mixture.

Materials and Methods

Laboratory screening for suitable test organisms

To select organisms, which would be suitable for use in field studies, a range of taxa were tested for their sensitivity to Salmosan and Excis formulation by Environment Canada, Atlantic Region, Toxicology Laboratory in Moncton, NB, Canada. Test organisms included a bacterium (*Vibrio fischeri*), three amphipods (*Amphiporeia virginiana, Gammarus* spp and *Eohaustorius estuarius*), a polychaete (*Polydora cornuta*), the adult green sea urchin (*Strongylocentrotus droebachiensis*), two other invertebrates (*Brachionus plicatilis* and *Artemia salina*), a fish (*Gasterosteus aculeatus*), and a fertilization inhibition test using sea urchins (*Lytechinus pictus*). The test toxicant was the pesticide formulation as it came from the commercial container which was diluted with seawater.

For toxicity tests in which mortalities were observed, an LC50 (the concentration at which half the test organisms die) and its 95% confidence limits were calculated according to the method of Stephan (1977). When a response other than mortality was measured, an EC50 which measured immobilization, based on loss of mobility, although movement in appendages might still have been present, and its 95% confidence limits were calculated, except for the fertilization test in which concentrations inhibiting various levels of fertilization are estimated (IC25 and IC50). The results of those tests are summarized in Table 1. All concentrations throughout the report are expressed as a weight to volume ratio (either mg/l or μg/l). Most organisms tested were not sensitive to the insecticides at their treatment dosages, exceptions being the three amphipod species.

To select the organisms to be used in the dispersion study, these tests were rated for sensitivity, test volume requirements, ease of testing, availability of test organisms, and ecological relevance. The amphipod *E. estuarius* was chosen as most appropriate. As a tracer dye (Rhodamine WT) would be used in field studies, *E. estuarius* was also tested for toxicity to the dye–methanol formulation, as well as for the combined toxicity of the dye and the insecticides. Those results are summarized in Table 1 and indicate the dye produced no toxic effects.

Field study of treatment solution dispersion

The study was conducted over a period of two years. Three dispersion events were observed in October 1996, and three in September 1997. Treatment site locations were all in the Lower Bay of Fundy near St. George, New Brunswick (Figs. 1 and 2), whose water exchanges are high with tidal amplitudes of about 8 m. At two of the sites (Deadmans Harbour, Letang Harbour), treatments occurred just prior to an ebbing tide, while the other four treatments occurred prior to a flooding tide. Sites chosen were representative of a range of dispersive energy conditions which would be suitable for local net pen operations. The Back Bay site had an extremely high dispersion potential, while Deadmans Harbour had comparatively low water movement. The remainder of the sites were what would be locally categorized as moderate current regimes. In all cases, the treatments were simulated operational treatments using a single 50 m circumference circular cage float fitted with a 40 m treatment tarpaulin. No net pen was used and no fish were present in the tarpaulin. The tarpaulin was weighted on the inside to ensure its bag-like shape approximately 3 m in depth, and contained a water volume similar to an operational treatment. Filling the tarpaulin with water was accomplished by lowering the lip of the bag against the current. As a requirement of the research permit issued by the Pest Management Regulatory Agency, all sites were located at

TABLE 1

Toxicity results for azamethiphos and cypermethrin to marine organisms.[a]

Test organism	Azamethiphos concentration (μg/l)	Cypermethrin concentration (μg/l)	Rhodamine WT dye concentration (μg/l)	Methanol concentration (μg/l)
V. fischeri (Bacterium, Microtox test) 15 min[b]	EC50 11000 (1880–64500)[a]	EC50 > 4950		
L. pictus (sea urchin) fertilization test 20 min[b]	IC50 6840 (4880–8095) IC25 3340 (1150–4810)	IC50 2560 (2540–2590) IC25 1330 (1310–1340)		
Sticklebacks (Fish, G. *aculeatus* 96) h[b]	LC50 190 (140–250)	LC50 8.1 (5.4–12.2)		
Gammarus spp (amphipod) 96 h[b]	LC50 < 5	LC50 0.36 (0.22–0.57)		
Rototox M (Rotifer, *Brachionus plicatilis* 24 h[b]	LC50 > 10,000	LC50 > 500		
Artotox M (Brine shrimp, *Artemia salina* 24 h[b]	LC50 > 10,000	LC50 > 500		
E. estuarius (amphipod) 48 h[b]	LC50 > 20[i] EC50 3.0 (2.1–4.4)[i]	LC50 > 1[i] EC50 < 0.05[i]	LC50 > 1000	LC50 > 5000
P. comuta (juvenile polychaete) 96 h exposure, then 96 h clean seawater[b]	LC50 2310 (650–590,000)	LC50 27.8 (9.9–87.0)		
S. droebachiensis (adult sea urchins) 96 h exposure, then 96 h clean seawater[b]	LC50 > 1000	LC50 > 50		
A. virginiana (amphipod) 48 h exposure[b]	Not tested	LC50 7.42 (4.24–10.6) EC50 0.030 (0–.06)		
A. virginiana (amphipod) 48 h exposure, then 48 h clean seawater[b]	Not tested	48 h LC50 6.86 (4.41–9.30) 48 h EC50 0.0034 (0.002–0.005) 48 + 48 h LC50 0.012 (0–0.02)		
M. bahia 96 h[c]	Not tested	LC50 0.056		
M. bahia 96 h[d]	Not tested	LC50 0.005		
Palaemonetes pugio 96 h[e]	Not tested	LC50 0.016		
Penaeus duorarum 96 h[d]	Not tested	LC50 0.036		
Homarus americanus 96 h[f]	Not tested	LC50 0.040[g]		
H. americanus 48 h (stages I to IV plus adults)[h]	LC50 1.0 to 3.6			

TABLE 1 (CONTINUED)

H. americanus 48 h (stages I to IV)[g]		LC50 0.06 to 0.18
C. septemspinosa 96 h[f]	Not tested	LC50 0.010
Uca pugilator 96 h 96 h[d]	Not tested	LC50 0.20
Crassostrea virginica 96 h[d]	Not tested	LC50 370
C. gigas 96 h[d]	Not tested	LC50 > 2300
Cyprinodon variegatus 96 h[d]	Not tested	LC50 1.0
Salmo salar 96 h[f]	Not tested	LC50 2.0

[a] Numbers in brackets are 95% confidence limits.
[b] Performed in the Environment Canada Toxicology Laboratory, Atlantic Region.
[c] Clark *et al.*, 1989.
[d] Hill, 1985.
[e] Clark *et al.*, 1987.
[f] McLeese *et al.*, 1980.
[g] Burridge *et al.*, 1999.
[h] Burridge *et al.*, 2000.
[i] In 100 mg/l Rhodamine WT.

least 1 km from shellfish holding facilities, and 500 m from any finfish aquaculture site.

Approximately, 1 h prior to slack tide, the tarpaulin was filled with water and 50 kg of Rhodamine (WT) dye solution (20% Rhodamine) was added (total of 10 kg Rhodamine per treatment). An exception to this was the Letang site, where 45 kg of Rhodamine solution was used. The Rhodamine WT was mixed with an appropriate amount of methanol (determined by hydrometer readings) to make it of the same density as seawater. At the time the dye was added, sufficient quantity of either Salmosan (azamethiphos, used in the 1996 study) or Exis (GPRD 01-cypermethrin, used in the 1997 study) was mixed in seawater and added to the tarpaulin enclosure at four or five separate locations. The theoretical final concentration within the enclosure was 100 μg/l in the case of azamethiphos and 5 μg/l in the case of cypermethrin.

Pesticide additions were done according to operational procedures by a professional applicator who had been certified by the New Brunswick Department of the Environment. Quantities of pesticide required to provide theoretical treatment concentrations were determined using volume estimates calculated from tarpaulin depth measurements obtained using a weighted line at three separate points. Water samples were taken within the treatment tarpaulin for subsequent dye and pesticide measurements. In 1996, pesticide mixing was accomplished by recirulating water with a 100 l/min water pump for approximately 20 min. In 1997, the solution within the treatment tarpaulin was mixed for approximately 1 h by means of a high volume of air (ca. 150 l/min) passed through two large airstones. The pesticide/dye solution was allowed to flow from the treatment tarpaulin by first releasing the downcurrent edge and retrieving the tarpaulin from the upcurrent side. Site conditions during dye release are presented in Table 2. Immediately after release, the dye plume behaviour was monitored by continuously measuring the fluorescence of the water using a flow-through fluorometer (Turner, Model 10 AU 005) fitted with an optical lamp and filters to detect the fluorescence excitation and emission maxima (554, 570 nm) for Rhodamine. To provide near real time measurement of Rhodamine concentration, the flow-through fluorometer received water from a submersible pump fitted to a 30 m length of 15 mm ID hose, which was weighted and could be lowered to provide concentration with depth. Depth was recorded from a metered block in 1996, by an electronic transducer in 1997. The fluorometer was calibrated using dilutions of a standard dye solution and a regression was performed for the resulting multi-point curve to obtain the relationship between fluorometer output and dye concentration.

Dye concentrations were continually recorded on a Campbell Scientific Instruments CR10 datalogger programmed to record pressure, fluorescence, and time every two seconds. The CR10 was connected to a laptop PC through a serial port to allow display of data during

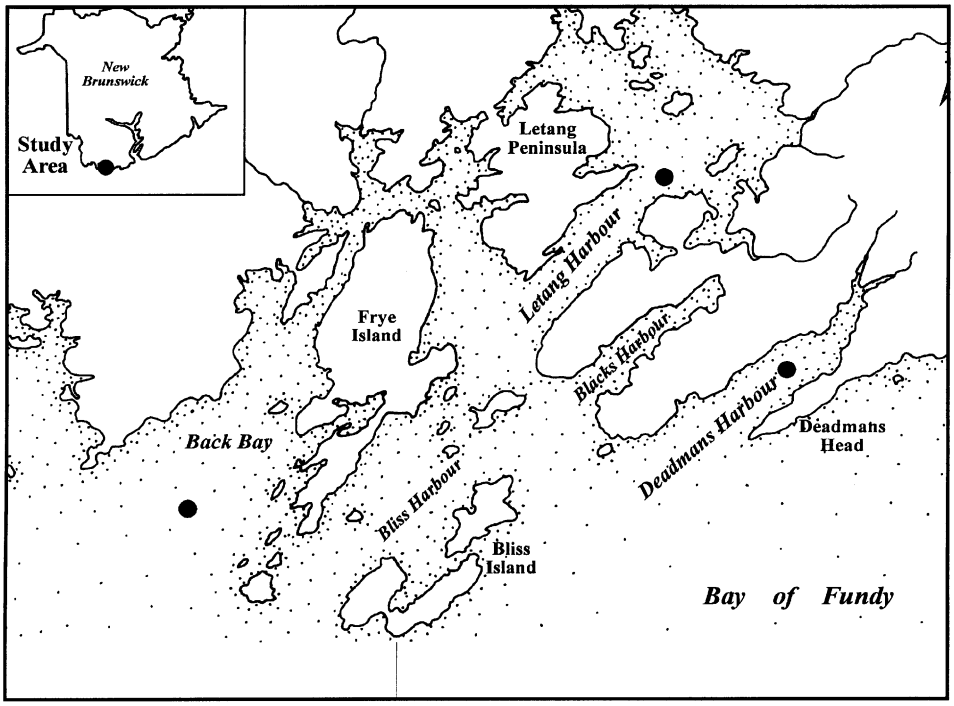

Fig. 1 Location of sites for release of simulated sea lice treatments in 1996.

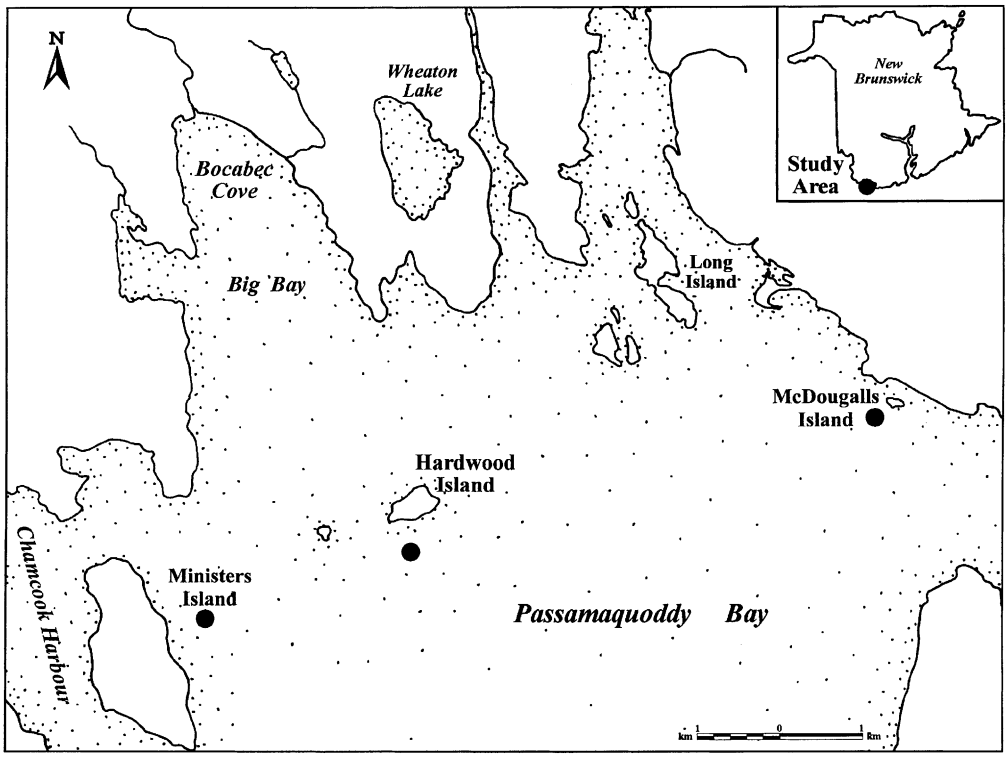

Fig. 2 Location of sites used for release of simulated sea lice treatments in 1997.

recording. The vessel position was measured using an onboard differential global positioning system (DGPS), which was continually logging data from an acoustic doppler current profiler (ADCP) fixed to the side of the vessel.

The DGPS, fluorescence, and pressure data were combined and processed using Microsoft Excel. The position, fluorescence, and depth data were than imported into TECPLOT for interpolation and plotting.

TABLE 2
Site conditions during dye release.

Site	Depth (m)	Tide (ADST)
Back Bay	29	Flooding
Deadmans Harbour	8.2	Ebbing
Letang Harbour	9.1	Ebbing
Ministers Island	16.5	Flooding
Hardwood Island	18.2	Flooding
McDougalls Island	7.3	Flooding

On a continuous basis after release, longitudinal and lateral transects as well as depth profiles of the dispersing dye patches were conducted for as long as the dye could be detected, or to change of tidal flow. Vertical profiles were performed by lowering the pump at specific locations and transects were performed by towing the pumping system through the dye patch at specific depths.

Grab water samples for pesticide (in duplicate) and bioassay analysis were obtained from the net pans before release and at various times directly from the fluorometer discharge and exact times (nearest second) noted in order to facilitate correlation of pesticide with Rhodamine concentrations. The water samples for pesticides analyses were collected in 1-l amber glass bottles, which had been pre-cleaned by detergent washing followed by sequential solvent rinses of hexane and acetone. To each 800 ml sample, approximately 50 ml of dichloromethane was added within 30 min of collection and were shaken vigorously by hand for 1 min. They were maintained at 4°C until subsequent additional extraction and analysis. The water samples for bioassay analyses were collected in 4 l clean food-grade plastic pails and transported back to the toxicity laboratory for testing within 6 h of collection.

Toxicity evaluation of field collected samples

Eohaustorius estuarius were purchased from a supplier (Northwestern Aquatic Sciences) and acclimated to 15°C and to a salinity of 30 ppt. Tests were performed on 1 l samples in mason jars with 10 animals per treatment. A photoperiod of 16 h of light, 8 h of darkness, and a temperature of 15 ± 1°C were maintained. All tests were conducted within 6 h of sample collection. The duration of the tests were 48 h. At test termination animals in some tests were transferred to clean seawater for an additional 48 h to check for recovery. Samples were not aerated throughout the test, but dissolved oxygen content was measured at the initiation and termination of the tests.

Samples from the treatment net pen prior to release were analysed as a dilution series. The concentrations tested ranged from 100% to 0.001% depending on the toxicity of the sample, and included a control. All other samples were analysed as a single concentration (100%) with a control sample for approximately every 10 samples. The control and dilution water was the natural seawater supply at St. Andrews Biological Station, St. Andrews, New Brunswick, Canada. As an additional control, water samples were taken from the sites of the 1997 study prior to mixing and release of pesticides, for use as site controls.

At the termination of the test, animals were removed from the test solutions and checked under a microscope. Animals were considered dead if there was no movement of any appendages. Animals were considered immobile if swimming had ceased but movement of any body appendage continued.

For the dilution series, an EC50 (immobilization) was calculated. Both mortality and immobility were included in the effects. For single concentration tests, % survival and % immobilized were determined.

A reference toxicant test using cadmium chloride in water-only exposures was run with each group of test organisms and the 96-h LC50 value was compared with historic warning limits for this species. Concentrations of cadmium chloride tested were 32, 18, 10, 5.6, 3.2, 1.8, 1.0 mg/l, as well as a clean seawater control.

Results

The mixing of solutions within the tarpaulin (Table 3) was much more consistent in 1997, when mixing within the net pen was accomplished by airstream-induced convection, than in 1996 when mixing was done by recirculating pumping.

1996 dye dispersion

Back Bay. This was a very high energy site, which would be at the upper limit of suitability for an operational finfish aquaculture site because of the high water movement. The dye was released at 15:35 ADST at the onset of a flooding tide and remained in the upper 5 m

TABLE 3
Fluorescence values of samples from tarpaulin enclosure prior to release.

Location	Year	*N*	Mean fluorescence (μg/l)	Standard deviation	Coefficient of variation[a]
Deadmans Harbour	1996	3	4055	4002	98.6
Letang Harbour	1996	5	159207	94769	59.5
Ministers Island	1997	3	50833	12850	25.3
Hardwood Island	1997	3	41033	899	2.2
McDougalls Island	1997	3	56433	1103	2.0

[a] Coefficient of variation – standard deviation as a percentage of the mean value.

of the water column for the first hour following the release of the tarp. Peak concentrations of the dye immediately after release were between 150 and 250 μg/l. As the strong flood tide proceeded, the dye was drawn into Letite Passage and rapidly dispersed by the high velocity currents.

The contour plots of the vertical profiles revealed that the dye became rapidly mixed throughout the water column with higher concentrations found at a depth. The maximum cohesive dye plume dimension occurred within 37 min of release at which time, it was approximately 200×470 m^2 and the concentrations within the plume ranged from 4 to 200 μg/l. The measurable dye was dispersed within two hours, after having travelled approximately 3 km from the release site, at which time peak concentrations were 10–20 μg/l resulting in an order of magnitude dilution of the initial measured concentrations after release (no samples for Rhodamine concentration determination were taken from inside the tarpaulin prior to release).

Deadmans Harbour. The dye was released at the onset of the ebb tide. This site had very low water flows and represented the lower limit for dispersive force from operational sites in this area. The plots of dye concentrations reveal that the plume separated into three patches, which remained in the top 10 m for approximately 20 min. The immediate surface concentrations were approximately 1000 μg/l, while subsurface peaks were approximately 100 μg/l.

The next sampling period, which took place close to an hour later indicated that the dye had been mixed down to a depth of 15 m. The surface portion of the plume spread out and moved seaward of the harbour, but there were still trace amounts of dye at the release site two hours after the release. A transect of vertical profiles performed along the length of the harbour revealed an elongated submerged patch that remained at mid-water depth (the depth of the harbour increases seaward through the harbour). The remaining transects of vertical profiles performed across the harbour revealed several submerged patches throughout the harbour of low concentrations of 10 μg/l. Those low concentrations at the bottom could still be detected approximately 5 h after release. At 4 h after the dye release, the plume was approximately 1270×480 m^2 in dimension and had concentrations, which ranged from 4 to 15 μg/l, which were 1/1000–1/270 the concentrations within the tarpaulin prior to release (Table 4).

Letang Harbour. The dye was released at the onset of an ebbing tide. After release, the dye patch moved rapidly seaward and mixed quickly (within 1 h) to a depth of 15 m. The maximum dimension of the plume occurred 1.4 h after release when it was approximately 450×650 m^2 (Table 4). The concentrations at that time ranged from 4 to 40 μg/l. The dye patch was followed for approximately 3 h, at which time concentrations of 20 μg/l were still being measured (1/8000 the measured dye concentration in the tarpaulin prior to release). Dye by that time had mixed to a depth of 25 m. That patch moved approximately 1200 m from the release site before it could no longer be measured.

1996 toxicity tests

When azamethiphos was used as the treatment solution, all of the net pen samples immobilized the test organisms at concentrations, which ranged from 1.6% to

TABLE 4

Maximum dye plume dimensions after release.

Location	Time after release (h)	Maximum width (km)	Maximum length (km)	Concentration range at maximum area (μg/l)	Maximum distance of leading edge from point of release (km)
1996 study					
Deadmans Harbour	4:13	0.478	1.265	4–15	1.173
Letang Harbour	1:38	0.446	0.663	4–40	1.234
Back Bay	0:37	0.216	0.468	4–200	3.012
1997 study					
Ministers Island	5:29	0.895	0.479	<3–15	1.166
Hardwood Island	5:13	0.942	1.423	<3–15	2.063
McDougalls Island	3:37	0.185	0.593	<3–15	0.907

TABLE 5

Toxicity to *E. estuarius* water samples taken from net pens treated with azamethiphos in 1996.

Site	Mean EC50 as % of treatment[a]	Mean EC50 as nominal pesticides solution concentration (μg/l)	Number of tests
Back Bay	7.7	7.9	3
Deadmans Harbour	12.1	12.1	4
Letang Harbour	1.6	1.6	5

[a] EC is for immobilization expressed in percent of net pen solution.

TABLE 6

Toxicity of post-release samples of azamethiphos to the amphipod *E. estuarius* (1996 Azamethiphos Results).[a]

Sample time (hours and minutes)	Number of samples causing no observable effects on the amphipod *E. estuarius*	Number of samples causing 10–90% immobilization of the amphipod *E. estuarius*	Number of samples causing 100% immobilization of the amphipod *E. estuarius*
0–0.5 h	4	0	4
0.5–1 h	10	0	0
1–2 h	16	0	0
2 h–5 h 28 min	10	0	0

[a] Samples from all study locations combined.

12.1% dilutions of the treatment solution (1.6–12.1 μg/l azamethiphos nominal), (Table 5). This represents an 8–62-fold dilution of the actual treatment concentration in the net pens.

Very few of the samples taken after the release of azamethiphos treatment solutions were toxic to the test organism (Table 6). None of the four samples taken after the release at Back Bay displayed any toxicity. Only one of the 27 samples (30 min after release from the net pens) caused immobilization after the Deadmans Harbour release. For the Letang Harbour release, three of samples caused immobilization up to 5 min post-release from the net pens. Later samples for Deadmans Harbour and Letang Harbour release were not toxic.

1997 dye dispersion

The concentration of cypermethrin in the samples obtained from within the tarpaulin prior to release ranged from 34 to 43 μg/l (Table 7), which was approximately 7–9 times the intended concentration. That was the result of a consistent over calculation of the tarpaulin volume by the pesticide applicator. They probably represent a worst case situation for initial concentrations during operational use of the product.

Ministers Island. The dye was released at the onset of flood tide. The dye was detected at depths of 7 m within the first half hour. The plume remained coherent and moved in a northerly direction along the east side of Ministers Island. Approximately, 4 h after release, the plume entered the intertidal zone of Ministers Island and was in contact with the bottom to a depth of 5 m. The plume was observed until 5.20 h after release, at which time it was approximately 480 × 900 m^2 and had dye concentrations, which ranged from 3 to 15 μg/l (maximally 1/3000 the tarpaulin concentration). The leading edge of the plume was measurable at 1166 m from the release site (Table 4). In order to quantify the decrease in cypermethrin concentration with time, a regression of average cypermethrin concentration against time was calculated. The average concentration was calculated on the arithmetic average of the two sequential samples taken at the same time. The regression (Fig. 3) indicates the maximum concentration of cypermethrin decayed exponentially with time. The 1/2

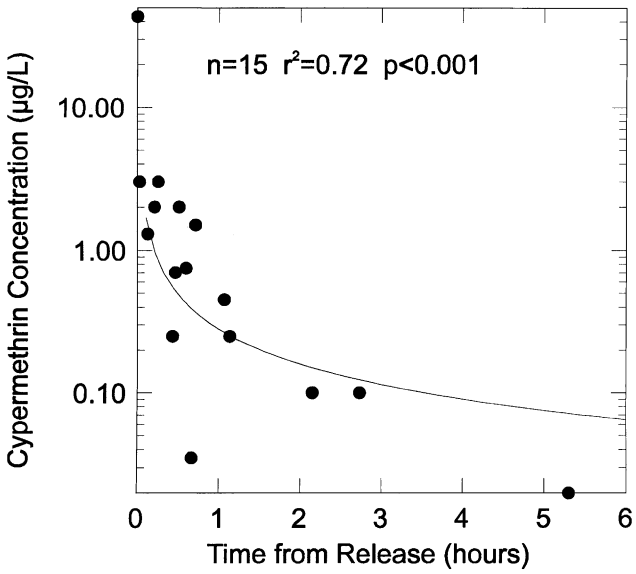

Fig. 3 Decrease in cypermethrin concentration over time for Ministers Island release.

TABLE 7

Toxicity of *E. estuarius* of water samples taken from net pens treated with cypermethrin in 1997.

Site	Mean cypermethrin concentration of treatment solution (μg/l)	Mean 48 h LC50 in % of treatment solution (and peticide concentration)	Mean 48 h EC50 in % of treatment solution (and pesticide concentration)	Number of tests
Ministers Island	43.3 (40–50)[a]	2.4 (1.0)	<0.1 (0.04)	6
Hardwood Island	34.0(±5.5)[b]	8.5–>10 (3.1–>3.6)	0.02 (0.007)	4
McDougalls Island	40	>1.0 (>0.004)	0.02 (0.008)	3

[a] Range of measurement.
[b] Standard deviation of measurement.

disappearance time (time to reach 1/2 net pen concentration) was within 10 min of release. Trace pesticide concentrations (0.02 μg/l) could be measured within the plume at the last sample time (5.5 h after release). Those concentrations were approximately 1/2000 the mean concentration within the tarpaulin prior to release.

Hardwood Island. At the onset of flood tide, the dye moved slowly in a northerly direction, with minimal dispersion horizontally and little vertical mixing in the first half hour. By the end of the first hour, the plume had not dispersed substantially and was within 5 m of the surface. At that time, the plume had contacted the intertidal zone. Shortly after two hours, the plume moved around the eastern tip of Hardwood Island and dispersed in a northerly direction, at which time it had mixed to a depth of 7 m. At approximately 4 h after release, the maximum measured dimension of the plume was 900×1400 m^2, with concentrations, which ranged from 3 to 15 μg/l, or maximally 1/270 the pre-release concentrations. At the termination of observations, the leading edge of the dye plume was 2 km from the release point (Table 4). The regression of cypermethrin concentration (arithmetic average of two sequential samples) with time (Fig. 4) indicates an exponential decay with a 1/2 disappearance time (time to 1/2 net pen concentration) of several minutes post-release. Again, pesticide (0.04 μg/l) was measured within the dye plume when the final sample was taken at approximately 4 h post-release. Those concentrations were 1/900 the net pen concentrations.

McDougalls Island. The dye was released at the beginning of flood tide. Dye was detected at depths of 7 m within 15 min of the release, and began to break into several small plumes within 45 min. Within 1.5 h, the plume had extended to the mouth of a small cove behind McDougalls Island. At that time relatively high concentrations of dye (>250 μg/l) were still being detected near the release site at a depth of 4 m, and it was evident that the surface portion of the plume was moving faster than deeper portions. The plume began to move into the cove behind McDougalls Island (the direction of the wind at that time) approximately 3 h after release, and by 4 h the entire intertidal and subtidal benthic zone of the cove was contacted. At that time, the plume was approximately 200×600 m^2 and dye concentrations were 3–15 μg/l or 1/3700 the original dye concentration. The reduction of cypermethrin concentration within the

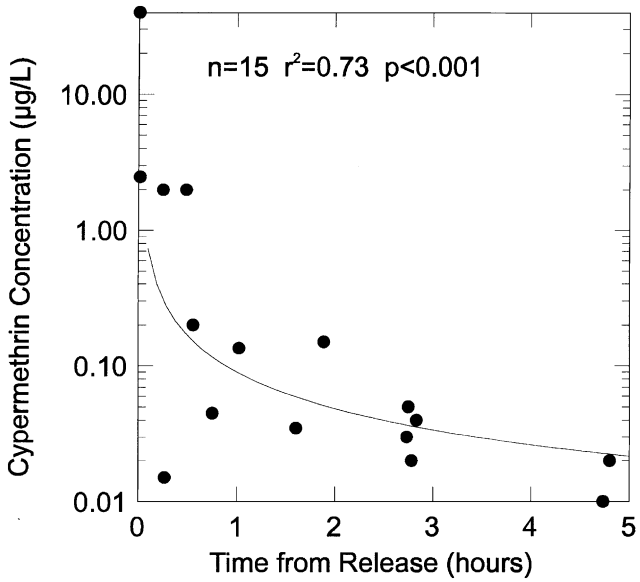

Fig. 5 Decrease in cypermethrin concentration over time for McDougalls Island release.

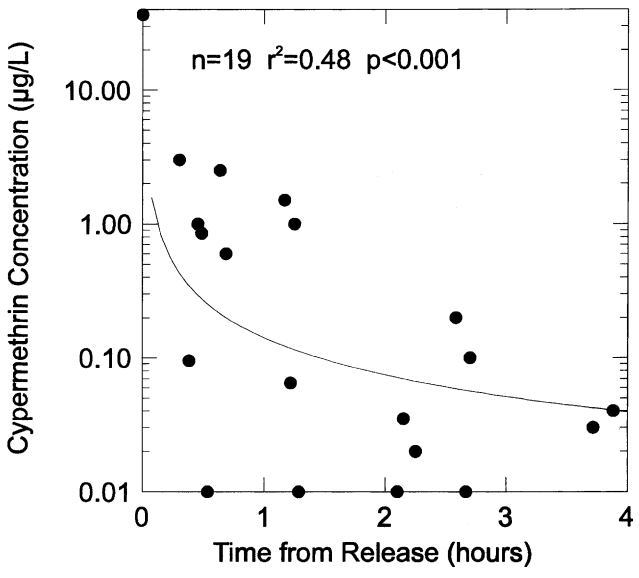

Fig. 4 Decrease in cypermethrin concentration over time for Hardwood Island release.

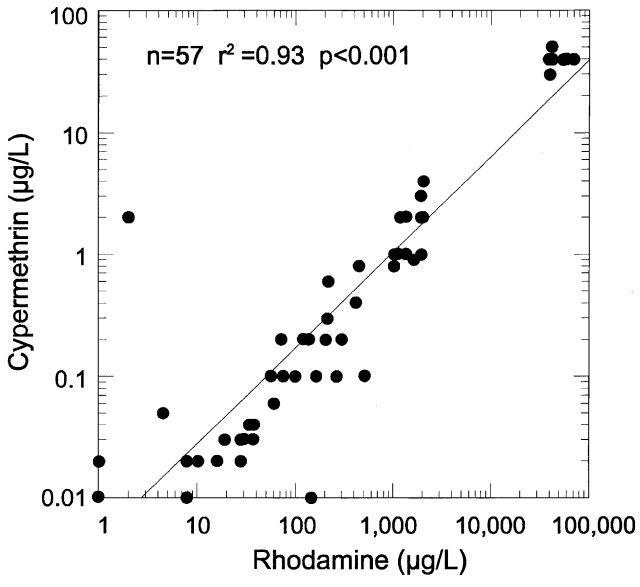

Fig. 6 Correlation of cypermethrin and Rhodamine concentrations from all releases in 1997.

plume was again exponential with the disappearance 1/2 time (time to 1/2 net pen concentration) being within several minutes of release (Fig. 5). At the time the final sample was taken (4.75 h post-release there was still approximately 0.02 μg/l cypermethrin within the plume which represented about 1/2000 the original net pen concentration.

Fig. 6 presents the regression of Rhodamine concentration with measured pesticide concentration for samples taken at the same time. Only the first of the sequential cypermethrin residue samples taken were used in the regression, since those were closest to the time mark noted from which Rhodamine concentrations were recorded. Pesticide concentrations measured were likely the maximum for that time period since samples were usually obtained when maximum Rhodamine concentrations were noted. The regression indicates a correlation, which was significant (ANOVA $p < 0.001$).

1997 toxicity tests

All net pen samples of the cypermethrin solution were highly toxic to the test organism producing 48 h LC50s, which ranged from 2.4% to >10% of the treatment solution (1.0–3.6 μg/l cypermethrin), (Table 7). The 48 h EC50 for immobilization was as low as 0.02% of the treatment solution (0.008 μg/l cypermethrin), and animals did not recover when placed in clean seawater for an additional 48 h. Most remained immobilized and further mortality occurred. The EC50 values (immobilization) represent up to a 5000-fold dilution of the actual concentrations in the net pens.

In contrast with the 1996 release using azamethiphos, most of the 1997 samples taken after release of cypermethrin from the net pens were toxic to *E. estuarius* (Table 8). After the release at Ministers Island, 16 of 17 samples taken up to three hours after release, which was the last sampling period, were toxic causing immobilization. After the Hardwood Island release, 25 of 29 samples taken up to 5 h post-release, which was the last sampling period, were toxic. For the McDougalls Island release, 19 of 20 samples taken up to five hours post-release, which was the last sampling period, were toxic. In all cases, the pre-release control samples were found to be not toxic, and the 10 laboratory control tests resulted in no mortality or immobilization after 48 h.

Immobilization was the most commonly observed toxic response, with 60 of 66 post-release samples causing 50% or greater immobilization of test animals. Immobilization was produced by some samples which had no detectable residues of cypermethrin (<0.01μg/l). When animals were transferred to clean water after the 48 h exposure period, recovery was not observed. Immobilization caused by cypermethrin under these test conditions appears to be irreversible.

Discussion

Dispersion

Poor mixing within tarpaulins during the 1996 releases preclude reliable estimates of dilution based on original concentrations during that year. The lack of adequate mixing within the tarpaulin may also have affected the subsequent behaviour of the plume after release; however, the plume was mixed to a depth below that which would have occurred in the tarpaulin (ca. 3 m) within one hour of release, and after that time the plume would probably behave as if thorough mixing had occurred within the tarpaulin. In addition, the lack of pesticide residue measurements in 1996 limits our ability to discuss behaviour of pesticides; however, it is assumed that the good correlation between cypermethrin concentration and Rhodamine concentration obtained in 1997 (Fig. 6) would also apply to azamethiphos since it has water solubility characteristics similar to cypermethrin.

Dye concentrations were detectable for time periods after release, which varied from 2 to 5.5 h, and distances which ranged from 900 to 3000 m. A previous study, which used visual detection of Rhodamine dispersion from operational net pen treatments to sample water for pesticide concentration was not able to detect those pesticides beyond 25 m from the net pen, nor more than 1.5 h after treatment (Dobson and Tack, 1991). Our ability to follow the dye plume for longer periods was probably due to the fact that we used greater volumes of Rhodamine (approximately 500 times that of Dobson and Tack, 1991), and fluorometric sensitivity was greater than visual detection. Those authors were also of the opinion that the presence of a net pen had a substantial effect in slowing the release of the treatment solution. It is noted that the rate of dis-

TABLE 8

Toxicity of post-release samples of cypermethrin to the amphipod *E. estuarius* (1997 Cypermethrin Results).[a]

Sample time (hours and minutes)	Number of samples causing no observable effects on the amphipod *E. estuarius*	Number of samples causing 10–90% immobilization of the amphipod *E. estuarius*	Number of samples causing 100% immobilization of the amphipod *E. estuarius*
0–0.5 h	0	1	13
0.5–1 h	1	0	7
1–2 h	1	1	14
2 h–5 h 17 min	2	3	23

[a] Samples from all study locations combined.

persion once the dye left the net was much slower (between 1/10 and 1/100 those from this study), and the effect that the presence of a net pen would have had in the higher energy conditions of the Bay of Fundy remains uncertain.

The 1997 releases provided data by which dispersion rates could be compared reliably with original concentrations within the tarpaulin. For those three events, which represent the moderate dispersion conditions for that locality, final concentrations of dye reached a three order of magnitude reduction between 3 and 5 h post-release. Cypermethrin concentration reductions appear to behave in a similar manner, given the trace quantities and the highly variable sampling milieu.

Toxicity

The 1996 study showed a low toxic risk for azamethiphos to non-target organisms when released from net pens under the conditions used in this study.

In this study, cypermethrin demonstrated lethal effects at concentrations of <0.4 μg/l to *Gammarus* spp, <1.0 μg/l to *E. estuarius*, and immobilization at <0.0125 μg/l to *E. estuarius* and 0.003 μg/l to *A. virginiana*. Other authors report mortality at concentrations of 0.04 μg/l to lobsters (*Homarus americanus*) 0.01 μg/l to shrimp (*Crangon septemspinosa*), (McLeese *et al.*, 1980), and 0.005 μg/l to *Mysidopsis bahai* (Hill, 1985). Such concentrations are extremely low, being 1–3 orders of magnitude lower than the intended treatment concentrations. Such dilutions were not achieved until 3–4 h post-release in this study, indicating that the plume retained its toxicity for substantial time periods after release.

It is also known that the octonol/water partition coefficient of cypermethrin is very high ($k_{ow} = 2.1 \times 10^6$), which indicates that it would be strongly bound to organic matter (Crossland, 1982). Nets that have been in the water for any period of time become coated with organic debris and sessile organisms, which would serve as a substrate for cypermethrin binding. Fish within the net pen during treatment would also have reduced initial pesticide concentrations through uptake mechanisms. The magnitude of pesticide reduction by such mechanisms is difficult to quantify. Undoubtedly the above factors, combined with over application of pesticide (up to approximately nine times the intended concentration), indicate that the initial concentrations of pesticide at the time of release produced exposure potentials, which exceed normal single pen application.

It must be noted that the above toxicity information was developed using exposure times of 48 h, whereas toxic concentrations of cypermethrin have only been shown to persist for up to 5 h (this study). The effects of short-term pulse exposures using sensitive organisms with observations post-exposure for delayed effects or recovery have not been reported in the literature, and are essential to properly evaluate risk. Hogans (1997) did not document lethal effects on sensitive organisms such as lobster, which were arrayed in cages moored around a net pen, which was treated with cypermethrin, indicating that pulsed exposures may not be as toxic; however, the study was also not able to measure cypermethrin residues after release of the treatment solution, and so it cannot be determined whether pesticide plumes contacted the cages.

It should also be noted that after all 1997 releases reported here, the dye plume entered the intertidal zone, and contacted the bottom where it could have an effect on benthic species, particularly arthropods.

In summary, the combined results of the study indicate that the use of azamethiphos for sea lice control presents a low to moderate environmental risk. However, the use of 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.

The authors wish to acknowledge Blythe Chang for logistics support, Randy Losier for able equipment management and advice in the field, Christine Garron for field support, Bill Hogens for advice and pesticide mixing and the Captain and crew of the CCG RV Pandalus III and M.V. Scott. The donation of EXIS from Grampian Pharmaceuticals for the trials is appreciated. The donation of a polar circle and treatment tarpaulin by the N.B. Salmon Growers Association for the trials was most welcome.

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