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Screening of the sea lice medications azamethiphos, deltamethrin and cypermethrin

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Screening of the sea lice medications azamethiphos, deltamethrin and cypermethrin
Screening av lusemidlene azametifos, deltametrin og cypermetrin

Summary- sammendrag

The occurrence and environmental risk of selected antiparastic pesticides used in the treatment of sealice are reported.

Forekomsten og miljørisiko av utvalgte antiparasittiske stoffer brukt til å behandle laks i oppdrettsanlegg som er smittet med lakselus.

4 emneord

Lakselus, azametifos, cypermetrin, akvakultur

4 subject words

Sea lice, azamethiphos, cypermethrin, aquaculture

Front page photo

Sigurd Øxnevad

Abstract

The screening of new and emerging contaminants entering the environment is of increasing concern to environmental authorities. On behalf of the Norwegian Environment Agency (Miljødirektoratet), the Norwegian Institute for Water Research (NIVA) investigated the occurrence of three selected veterinary medicines used in the aquaculture industry during a screening program in 2014.

The screening survey investigated the occurrence of three compounds; azamethiphos, an organophosphate, and the two pyrethroids, cypermethrin and deltamethrin, used for the treatment of sea lice infestation in salmonid aquaculture. Three fish farms were selected that had reported recent treatment with azamethiphos and possible historical treatment with cyperemthrin, but no recent recored treatment with deltamethrin. All three pesticides are used as a topical bath treatment resulting in a pulsed release of high concentrations of pesticides into the environment immediately post treatment.

Azamethiphos was detected in the water in the vicinity of a fish farm 1 week post treatment but the measured concentrations were all below the Environmental Quality Standard reported in the UK (< 40 ng/L). Neither cypermethrin nor deltamethrin were detected in water samples.

Pyrethroid treatment had not occurred in recent weeks and no cypermethrin or deltamethrin was detected in sediment samples indicating that there was no accumulation from any historical use, although cypermethrin was detected in some blue mussel samples at trace concentrations, which were all below reported effects concentrations. Cypermethrin was detected in blue mussels collected from two fish farms and also from a location almost 5 km from a fish farm. It is likely that other fish farms added to this accumulation.

The authors conclude that there is minimal environmental risk with current practices 1-2 weeks post treatment with azamethiphos but cannot rule out potential acute risk to non-target species immediately following sealice treatment. There was no evidence of pyrethroid accumulation in sediment from historical usage but again, potential acute risk to non-target species immediately after sealice treatment cannot be discounted.

Sammendrag

Screening av nye miljøgifter og deres tilsig i miljøet er av økende bekymring for miljømyndighetene. På vegne av Miljødirektoratet har Norsk institutt for vannforskning (NIVA) i screeningprogrammet 2014 undersøkt forekomsten av utvalgte veterinærlegemidler som brukes i oppdrettsnæringen.

Screeningundersøkelsen kartla forekomsten av tre veterinærlegemidler; azamethiphos, en organophosphateforbindelse og to pyretroider, cypermetrin og deltametrin, som alle brukes til behandling av lakselus innen akvakultur med laksefisk. Det ble valgt ut 3 oppdrettsanlegg til undersøkelsen som alle nylig hadde rapportert behandling med azamethiphos og mulig tidligere behandling med cypermetrin men ikke deltametrin.

Alle tre legemidlene brukes som en topikal behandling, som medfører en frigjøring av legemiddelet i høye konsentrasjoner umiddelbart etter behandling.

Azamethiphos ble påvist i vannet i nærheten av ett av oppdrettsanleggene 1 uke etter behandling, men de målte konsentrasjonene var alle under miljøstandarden (EQS) som benyttes i Storbritania (<40 ng/L). Verken cypermetrin heller deltametrin ble påvist i vannprøver.

Pyretroid behandling hadde ikke pågått de siste ukene før prøvetaking og hverken cypermetrin eller deltametrin ble funnet i sedimentprøvene. Dette indikerer at tidligere bruk av disse stoffene ikke resulterer i akumulering i sedimentene. Lave konsentrasjoner av cypermetrin ble likevel detektert i noen blåskjellprøver. Nivåene i blåskjellprøvene var lavere enn de konsentrasjonene som er påvist å gi en effekt. Cypermetrin ble detektert i blåskjell fra to oppdrettsanlegg, samt fra en prøvetakingsstasjon nesten 5 km fra et oppdrettsanlegg. Det er derfor sannsynlig at også andre oppdrettsanlegg i området har bidratt til akkumuleringen av cypermetrin.

Forfatterne konkluderer med at det er minimal miljørisiko knyttet til dagens praksis 1-2 uker etter behandling med azamethiophos. Vi kan derimot ikke utelukke potensiell akutt fare for andre arter umiddelbart etter lakselusbehandlingen. Det var ingen bevis for akkumulering av pyrethroid i sediment som skyldes historisk bruk. Det kan imidlertid ikke utelukkes en potensiell akutt fare for andre arter umiddelbart etter lakselusbehandlingen.

Contents

1. Introduction

Aquaculture is one of Norway's most important industries with the export of salmon and trout worth over 46 billion Norwegian kroner (>5 billion Euro) in 2014 (Norwegian Seafood Council, 2015). Owing to it being such a significant industry, the health of the farmed fish is an important consideration. The aquaculture industry uses a broad range of chemicals to optimise fish health and productivity. Therapeutics such as antibiotics and antiparasitics, antifoulants, disinfectants and antiseptics (Costello et al., 2001), are all used and by their nature, the majority are designed to be toxic to target species under certain conditions. Of particular concern are chemicals released to the environment which have the potential to cause adverse undesired effects on non-target species, and antiparasitic treatments are one such group of chemicals (Langford et al., 2014).

Sea lice (*Lepeophtheirus salmonis)* are a parasitic copepod causing a detrimental effect on fish health and therefore on economic status (Johnson and Kabata, 2004; Langford et al., 2014). A sea lice infestation results in sub-epidermal hemorrhage which can lead to secondary infections and stress. Sea lice can survive for long periods in their larval stage before needing to attach to a host and therefore have the potential to spread over a wide area. As a result monitoring and reporting of sea lice are mandatory. When the first incidence of sea lice are reported in spring (usually early March), systematic treatment from south to north occurs and then sea lice are treated when required throughout the season with various licensed medications with the aim of preventing the appearance of gravid females. Over the last 25 years, the quantity of sea lice medication being used has been increasing corresponding with an increase in farmed fish numbers and an increase in sea lice resistance to treatments, and usage patterns have changed (Figure 1).

Organophosphates, dichlorvos and metrifonate, were the first treatment options available in Norway, metrifonate use dominated until the mid-1980s followed by dichlovos until the late 1990s (Denholm et al., 2002). The development of resistance coupled to the low therapeutic index (similarity in the concentration toxic to fish, versus the concentration toxic to sea lice) and hazards to personnel, resulted in organophosphates being phased out by the late 1990s. These were replaced by emamectin, a number of chitin synthesis inhibiting compounds and pyrethroids. Extensive resistance to emamectin, followed by reports of adverse environmental impact of the chitin synthesis inhibitors has resulted in an orders of magnitude increase in the use of azamethiphos, deltamethrin and cypermethrin over the last 15 years and a doubling in just the last 5 years (Figure 2).

Treatment to sea lice is possible in 2 forms, in feed or topical treatment. Azamethiphos, deltamethrin and cypermethrin are 3 of the topical treatments available and were the focus of this study. Topical treatment involves surrounding individual fish cages with a tarpaulin or transferring the fish to a well-boat so they are enclosed, and dosing the water with the medication for a specific time period dependant on the active ingredient and water temperature. These bath treatments are considered topical treatments because the sea lice absorb the active ingredient from the water column rather than via their fish host. After the treatment, the tarpaulins are removed which results in a potentially toxic plume of water entering the marine environment, the extent of which is dependent on the water currents, water quality and weather conditions.

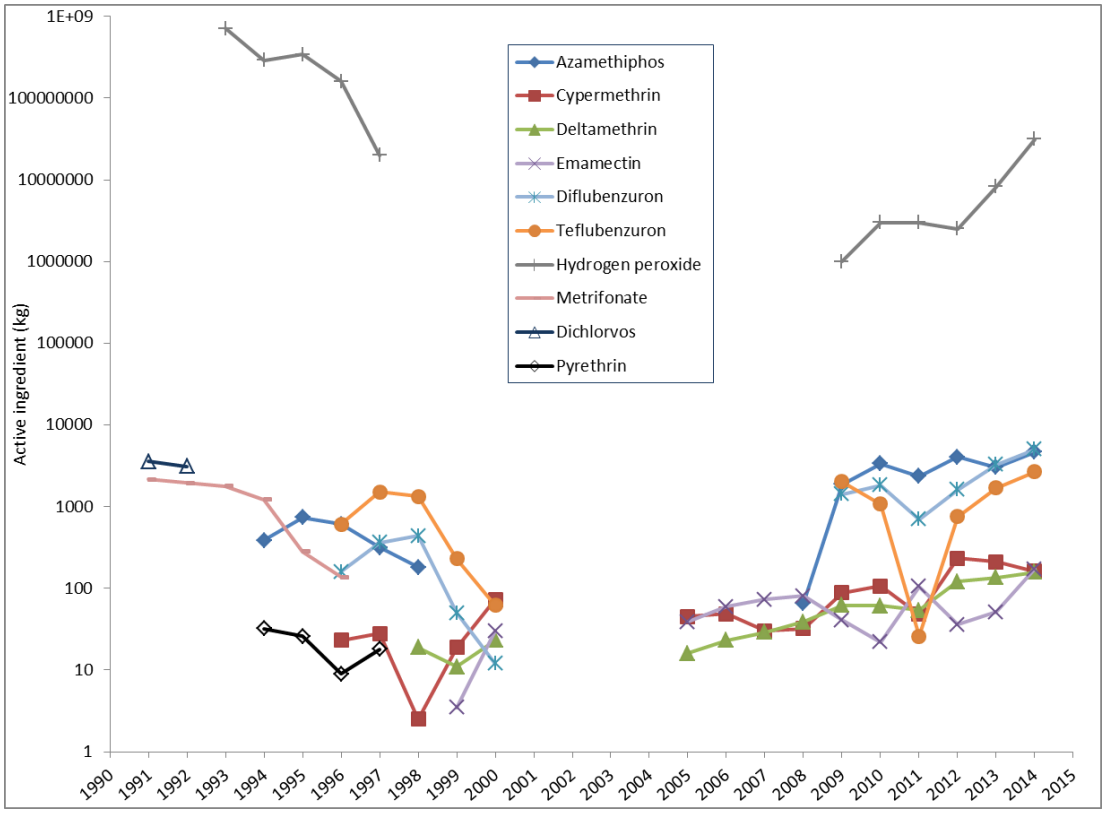


Figure 1. Antiparasitic medication usage in Norway (Denholm et al., 2002; Norwegian Institute of Public Health, 2015)

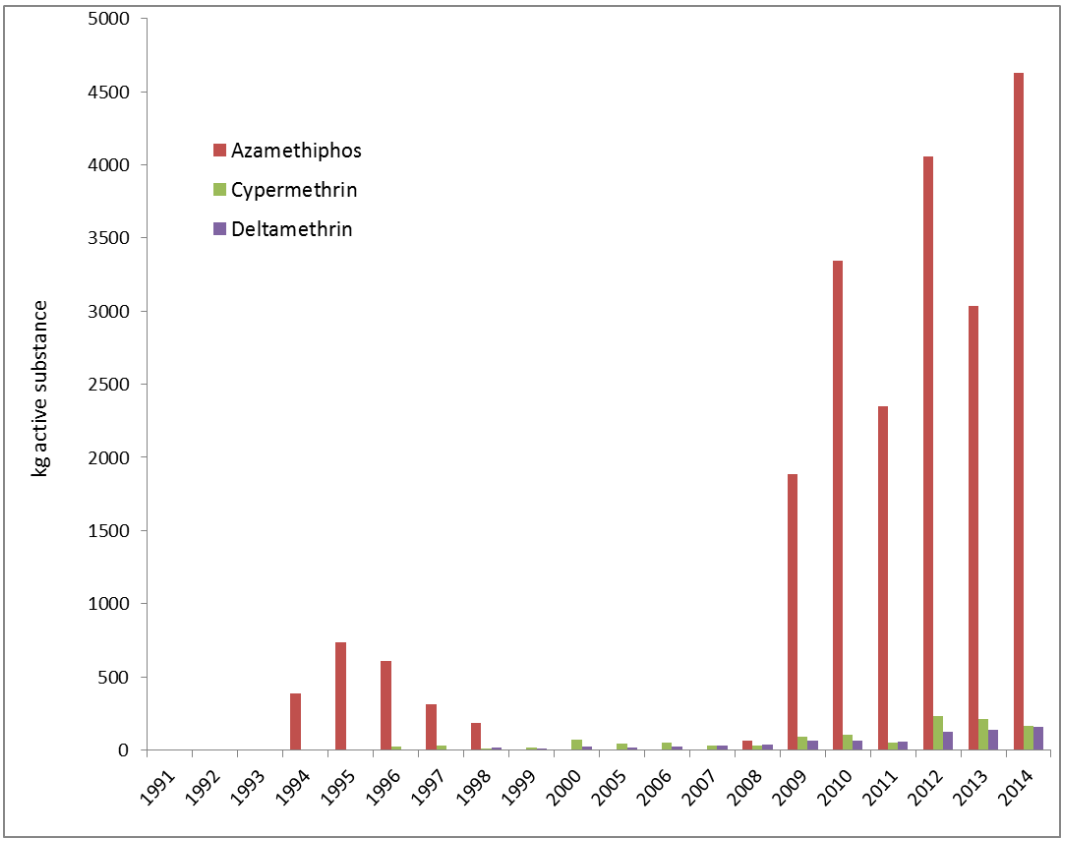


Figure 2. Usage of azamethiphos, cypermethrin and deltamethrin in Norway (Denholm et al., 2002; Norwegian Institute of Public Health 2015)

1.1 Azamethiphos

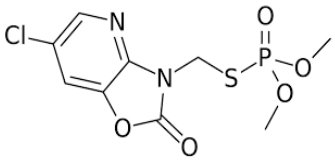
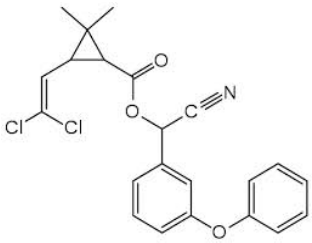
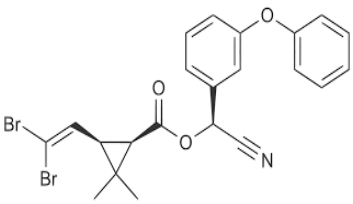
Azamethiphos is currently the only organophosphate licenced for use in Norwegian aquaculture and has been in use since 2008, and with the exception of hydrogen peroxide, it is used in the largest volumes. The dose concentration required is 100 µg/L with an exposure time of 20-40 minutes depending on water temperature. It is a neurotoxic acetylcholinesterase inhibitor that acts by causing the neurotransmitter, acetylcholine, to accumulate, resulting in sea lice nervous system disruption leading to paralysis (Mora et al. 1999; Galloway and Handy, 2003; Domingues et al., 2010) . This mode of action can have detrimental effects on non-target species as well. Azamethiphos was developed for the control of terrestrial pests and considered safe for mammals but the change in use may have detrimental effects on aquatic non-target species.

1.2 Cypermethrin and deltamethrin

The group of chemcials classed as pyrethroid insecticides contain some of the most effective insecticides in use today and show significantly greater toxicity to sealice and non-target crustaceans than to fish. Pyrethroids have a different mode of action to azamethiphos, and disrupt the sodium ion channel (Belden and Lydy, 2006), the result is also a neurotoxic effect leading to paralysis, as with azamethiphos. Pyrethroids are synthetic derivatives of *Chrysanthemum cinerariaefolium* extracted from the flowers. Cypermethrin and deltamethrin are both licensed for use in Norway.

Deltamethrin is more toxic to crustaceans than cypermethrin which is reflected in the treatment dose required for sealice treatment. The required dose concentration for cypermethrin is 5 µg/L and for deltamethrin is 3 µg/L, both with an exposure time of 30 minutes.

Table 1. Chemical characteristics of sea lice medications
Azamethiphos, cypermethrin and deltamethrin

	Azamethiphos	Cypermethrin	Deltamethrin
Commercial formulation	Salmosan®	Excis®/Betamax®	AlphaMax®
Active ingredient dose concentration	100 µg/L	5 µg/L	3 µg/L
Chemical group	Organophosphate	Pyrethroid	Pyrethroid
Formula	$C_9H_{10}ClN_2O_5PS$	$C_{22}H_{19}Cl_2NO_3$	$C_{22}H_{19}Br_2NO_3$
Structure			
CAS number	35575-96-3	52315-07-8	52918-63-5
RMM	324.7	416.3	505.2
Chemical name	dimethyl [(6-chloro-2-oxo-2H,3H-[1,3]oxazolo[4,5-b]pyridin-3-ylmethyl)sulfanyl]phosphonate	cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	(S)-cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate
Water solubility	1100 µg/L	4 µg/L	<2 µg/L

2. Materials and Methods

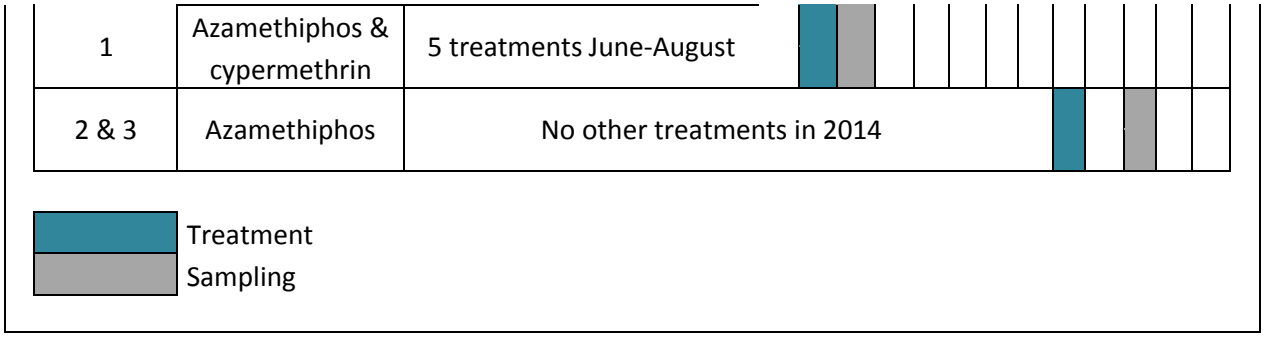
2.1 Sampling

2.1.1 Description of sampling sites

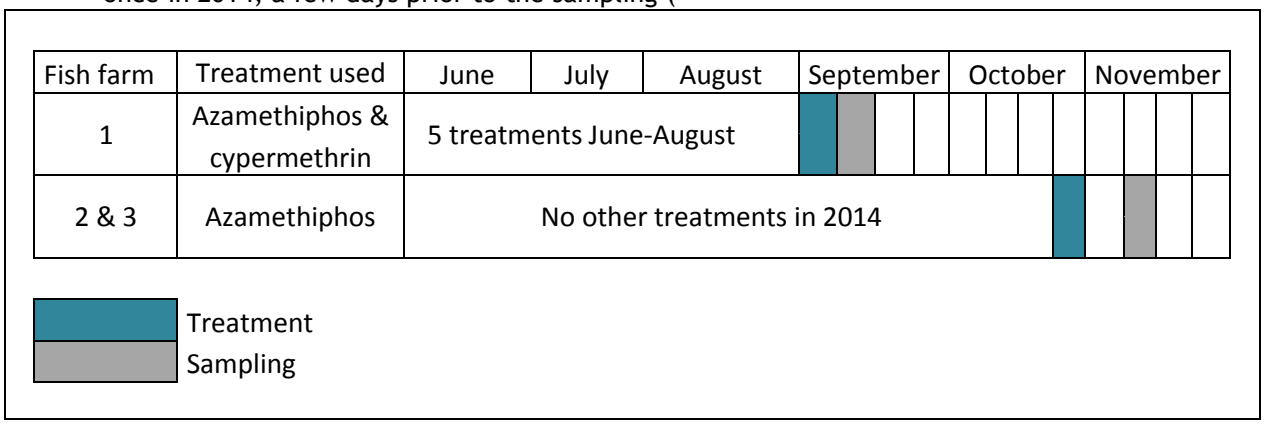
Samples were collected from three fish farm locations on the west coast of Norway, from two fish farms in Hordaland and one fish farm in Sogn og Fjordane (Figure 3).

- Fish farm 1 in Sogn og Fjordane County. This farm had treated the fish with azamethifos and cypermethrin in the week prior to the sampling (

Fish farm	Treatment used	June	July	August	September	October	November



- Figure 4). The fish farm in addition had treated the fish five times with azamethifos and cypermethrin since June 2014. The fish farm has 10 cages (130 m diameter). Depth under the fish farm is 120 to 160 meters. There was no reported treatment with deltamethrin.
- Fish farms 2 and 3 in Hordaland County. These farms had treated the fish with azamethifos only once in 2014, a few days prior to the sampling (



- Figure 4). There are four more fish farms in the fjord area owned by the same company, and those fish farms had also been treated with azamethifos during the last two weeks. . There was no reported treatment with deltamethrin. The depth under fish farm 2 is 100 to 170 meters, and 80 to 150 meters under fish farm 3, with increasing depth close outside the fish farms.

Fish farm 1
Samples of water, sediment, polychaetes and blue mussels were collected on 8th and 9th of September 2014. Water and sediment were collected on five stations. Blue mussels were collected on the fish farm and on four stations on the nearby shoreline.

Fish farm 2 and 3
Samples of water, sediment, polychaetes and blue mussels were collected on 14th and 15th of November 2014. Water and sediment were collected on five stations. Blue mussels were collected on the fish farm and on two stations on the nearby shoreline.

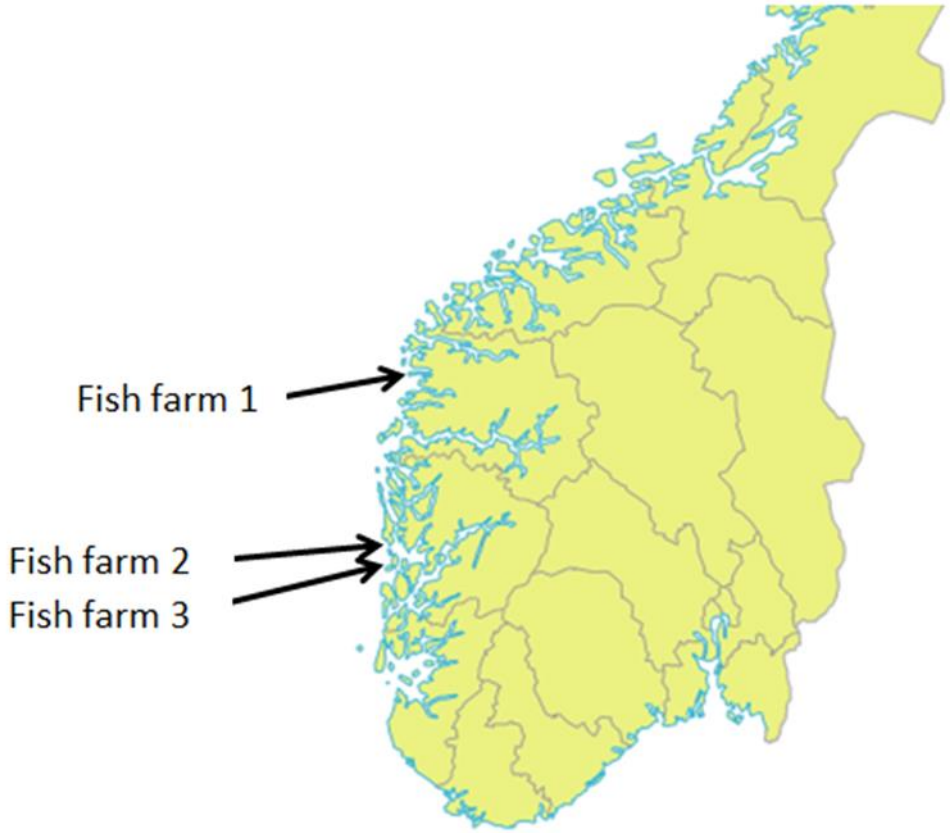


Figure 3. Sampling locations

Fish farm	Treatment used	June	July	August	September	October	November
1	Azamethiphos & cypermethrin	5 treatments June-August					
2 & 3	Azamethiphos	No other treatments in 2014					

Treatment
Sampling

Figure 4. Treatment and sampling schedule

At each location the following samples were collected:

- Water
- Sediment
- Blue mussel (*Mytilus edulis*)

- Bristle worm (Polychaete)

The distance between the fish farms and sampling stations is summarized in table 1.

2.1.2 Sample collection

Samples of biota and sediment were put in clean, baked (400°C) jars. Samples of water and sediment were collected in a transect from the fish farms, the direction of which was determined by the practicalities of sample collection in very deep waters beyond the depth of the sampling equipment, or in shallow waters close to the shore where water was too shallow to collect a representative sample. Blue mussels were collected on the fish farms rigs and from locations on the nearby shoreline. The water samples were put in clean baked (400°C) bottles (2.5 L). The water samples were taken from the surface. Sediment samples were collected from five stations using a Van Veen grab (Figure 5). Sediment samples were taken from the upper 2 cm of the sediment. At each station sediment samples from two grabs were collected and then stored in buckets. Afterwards the sediment was sieved using 0.5 mm and 0.1 mm sieves (Figure 6), and bristle worms (Figure 7) were then picked out by hand using a pair of tweezers.

30-50 blue mussels were collected on each station. The mussels were frozen (-20 °C) upon arrival at NIVA from the field. The soft tissue of at least 30 blue mussels were mixed into one bulk sample from each station.

Table 2. Approximate distance between the fish farms and sampling stations

	Distance from fish farm 1 (m)	Distance from fish farm 2 (m)	Distance from fish farm 3 (m)
Water 1	0	0	0
Water 2	100	150	200
Water 3	400	300	320
Water 4	700	350	430
Water 5	1000	500	670
Sediment 1	0	0	0
Sediment 2	100	150	210
Sediment 3	400	300	320
Sediment 4	700	350	430

Sediment 5	1000	500	670
Bristle worm 1	0	0	0
Bristle worm 2	100	150	210
Bristle worm 3	400	300	320
Bristle worm 4	700	350	430
Bristle worm 5	1000	500	670
Blue mussel 1	0	0	0
Blue mussel 2	1800	1000	1200
Blue mussel 3	1500	4800	1300
Blue mussel 4	1200	-	-
Blue mussel 5	1400	-	-

Figure 5. The Van Veen grab used for sediment sampling (photo: Sigurd Øxnevad, NIVA).

Figure 6. Sieving of sediment samples. Sediment collected from directly under the fish farm was dark grey, and smelled of H_2S (photos: Sigurd Øxnevad, NIVA).

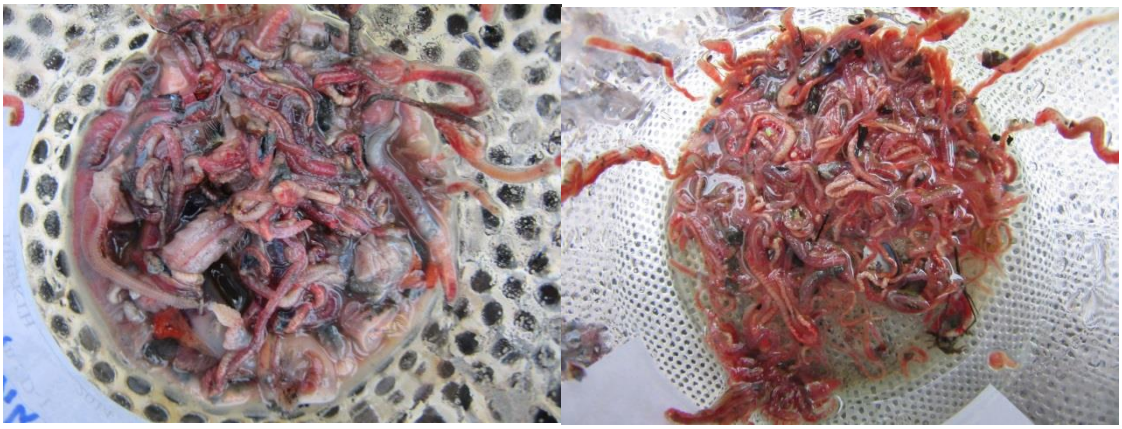


Figure 7. Samples of bristle worms (photos: Sigurd Øxnevad, NIVA).

2.2 Analytical Methodology

All glassware was heated treated at 400°C before use.

Atrazine-d_5 and trans-cyfluthrin-d_6 were used as internal standards for azamethiphos and pyrethoid analysis respectively and added to all samples before extraction.

Methods were developed based on modifications of those reported earlier (García-Rodríguez et al., 2008; 2012; Ikonomou and Surridge, 2012).

With each batch of samples extracted, 2 matrix matched spiked control samples and a blank lab control were extracted for quality control purposes. The spiked control samples were used to calculate limits of detection, recovery efficiency and analytical uncertainty (Table 3).

Table 3. Method recoveries, uncertainty and detection limits

	Azamethiphos		Cypermethrin		Deltamethrin	
	LOD	Recovery (%) ± RSD	LOD	Recovery (%) ± RSD	LOD	Recovery (%) ± RSD
Water/particulate (ng/L)	0.1	60 ± 4	5	143 ± 7	1	68 ± 21
Sediment (ng/g dry wt)	0.05	68 ± 1	6	101 ± 6	3	123 ± 16
Biota (ng/g wet wt)	0.1	40 ± 1	2	70 ± 3	2	182 ± 29

2.2.1 Water Extraction

Water (2.5 L) was filtered using pre-cleaned GFC (0.4 µm) filters (Whatman). 100 ng of internal standard was added to each sample prior to solid phase extraction (SPE). Oasis® HLB extraction cartridges (200 mg) were pre-conditioned with 6 ml methanol (MeOH) followed by 12 ml ultrapure water. Samples were then applied under vacuum at a flow rate of approximately 4 ml/min. After sample extraction, SPE cartridges were rinsed with approximately 10 ml ultrapure water to remove residual salts. After drying under vacuum, azamethiphos and pyrethroids were eluted with ethyl acetate (EtAc). Eluants were evapourated under nitrogen (36 ºC) to approximately 1 ml.

An aliquot of extract was taken for azamethiphos analysis, and the remaining extract was taken for further cleanup before pyrethroid analysis. Extracts were applied to LC-florosil® cartridges (1 g, Supelco) and eluted with 10 % diethyl ether in hexane (approximately 15 ml) and then evapourated under nitrogen (36 ºC) to approximately 150 µl.

2.2.2 Particulate Extraction

Particulates collected on pre-cleaned GFC (0.4 µm) filters (Whatman) were extracted by sonication with 20 ml dichloromethane (DCM) for 1 hour and extracts were concentrated under a gentle stream of nitrogen (36 ºC) to approximately 1 ml and then filtered through 0.45 µm PTFE filter. Extracts were divided into 2 aliquots, 1 for direct analysis of azamethiphos, and the other was solvent exchanged to cyclohexane before analysis of pyrethroids.

2.2.3 Sediment Extraction

Freeze dried sediment (2 g) was extracted by sonication with 20 ml DCM for 1 hour, and the DCM was decanted before repeating the extraction process for a second time. The combined extracts were concentrated under a gentle stream of nitrogen (36 ºC) to approximately 1 ml and then filtered through 0.45 µm PTFE filter. Extracts were cleanup up by gel permeation chromatography (GPC). GPC was carried out on an Alliance 2695 system (Waters, Milford MA, USA) with two sequential Envirogel GPC clean-up columns (19 x 300 mm and 19 x 150 mm) and DCM as a mobile phase at a flow rate of 5 ml/min. The 12.0 – 20.3 min fraction was collected and further processed for analysis. Extracts were evapourated under nitrogen (36 ºC) to approximately 1 ml and half was taken directly for azamethiphos analysis. The remaining half was solvent exchanged to cyclohexane and applied to LC-florosil® cartridges (1 g, Supelco) and eluted with 10 % diethyl ether in hexane (approximately 15 ml) and then evapourated under nitrogen (36 ºC) to approximately 150 µl.

2.2.4 Biota Extraction

Blue mussels and bristle worms (5-6 g) were extracted by sonication with approximately 20 ml EtAc for 1 hour, and the EtAc was decanted before repeating the extraction process for a second time. The combined extracts were concentrated under a gentle stream of nitrogen (36 °C) to approximately 1 ml and then filtered through 0.45 µm PTFE filter. Extracts were cleaned up by GPC as with sediment extracts with a slight adjustment to the fraction collection time, and for biota, the 12.35-20.30 min fraction was collected. Extracts were evapourated under nitrogen (36 °C) to approximately 1 ml and half was taken directly for azamethiphos analysis. The remaining half was solvent exchanged to cyclohexane and applied to LC-florosil® cartridges (1 g, Supelco) and eluted with 10 % diethyl ether in hexane (approximately 15 ml) and then evapourated under nitrogen (36 °C) to approximately 150 µl.

2.2.5 Azamethiphos Analysis

UPC2-MS/MS analysis of azamethiphos was performed on a Waters ACQUITY UPC^2 system coupled to a Quattro Premier XE (Waters, Sweden). The flow rate of the CO_2-based mobile phase containing 15 mM ammonium acetate MeOH/acetonitrile (50/50) as mixed co-solvents was 1.2 ml/min, with a gradient elution from 97% CO_2 to 60% CO_2 over 5 mins, with separation using a UPC^2 BEH 2-EP column (1.7 µm x 3 x 100 mm) (Waters, Sweden). The retention time for azamethiphos was 2.2 mins and 2 mass transitions were used for quantification and qualification; 325.1 → 139.0 and 325.1 → 183.1 respectively.

2.2.6 Pyrethroid Analysis

Pyrethroids were analysed by gas chromatography coupled to an electron capture detector (GC-ECD) (Hewlett-Packard 6890 GC / ^{63}N µECD detector). The injector was in splitless mode (1.25 mins) at 300 °C. A DB-5 (30 m x 0.25 mm x 0.25 µm) column was used (J&W Scientific). The GC oven was held at 90 °C for 2 mins, then ramped at 20 °C/min to 200 °C for 2 mins, 3 °C/min to 280 °C for 10 mins, and 20 °C/min to 325 °C and held for 2 mins. Hydrogen was the carrier gas at a flow of 1 ml/min and nitrogen was the makeup gas at flow of 30 ml/min with the detector set to 320 °C.

Deltamethrin exists as 1 isomer where as cypermethrin exists as 8 isomers which are separated into 4 pairs of cis- and trans- isomers by GC-ECD analysis.

2.3 Supporting parameters

2.3.1 Particle Size Analysis

Wet sediment was shaken by mechanical fractionater with < 63 µm sieves. Dry weight measurements were used for the particle size calculations.

2.3.2 Sediment TOC

Freeze dried sediment sample aliquots (0.5-10 mg) were heated in a furnace at 1,800 °C in the presence of oxygen free helium. The carbon dioxide gas produced was passed through a chromatography column and the total organic carbon was measured.

2.3.3 Water DOC

Samples (4 ml) were injected into an inorganic carbon chamber and 0.5 ml 21% phosphoric acid was added. The inorganic bound carbon from carbonates, bicarbonates and dissolved CO_2 is released to an NDIR detector for CO_2 quantification.

3. Results

The results of all parameters determined are presented in Tables 4-6 for each individual fish farm.

Azamethiphos was detected in water samples up to 1 km from the treated fish farms at concentrations of between < 0.4 ng/L and 26 ng/L (Figure 8). At fish farms 2 and 3, where treatment with azamethiphos occurred 1 to 2 weeks prior to sampling, the water concentrations were all less than 1 ng/L. However, at fish farm 1, where treatment with azamethiphos occurred in the week prior to sampling, the concentration was 26 ng/L at the fish farm with a decreasing concentration measured with an increasing distance from the farm, and the lowest concentration was 0.5 ng/L which was measured 1 km from fish farm 1. Azamethiphos was measured in 1 particulate sample from fish farm 1 (0.2 ng/L water) and was below the limits of detection in all other matrices.

Cypermethrin was detected in blue mussel samples at fish farms 2 and 3 (Figure 9). Blue mussels collected from fish farm 2 and 3 had concentrations of 4.8 and 3.3 ng/g respectively, and 4800 m from fish farm 2, blue mussels contained 2.2 ng/g cypermethrin. Cypermethrin was not detected in any other samples.

Deltamethrin was not detected in any of the collected samples.

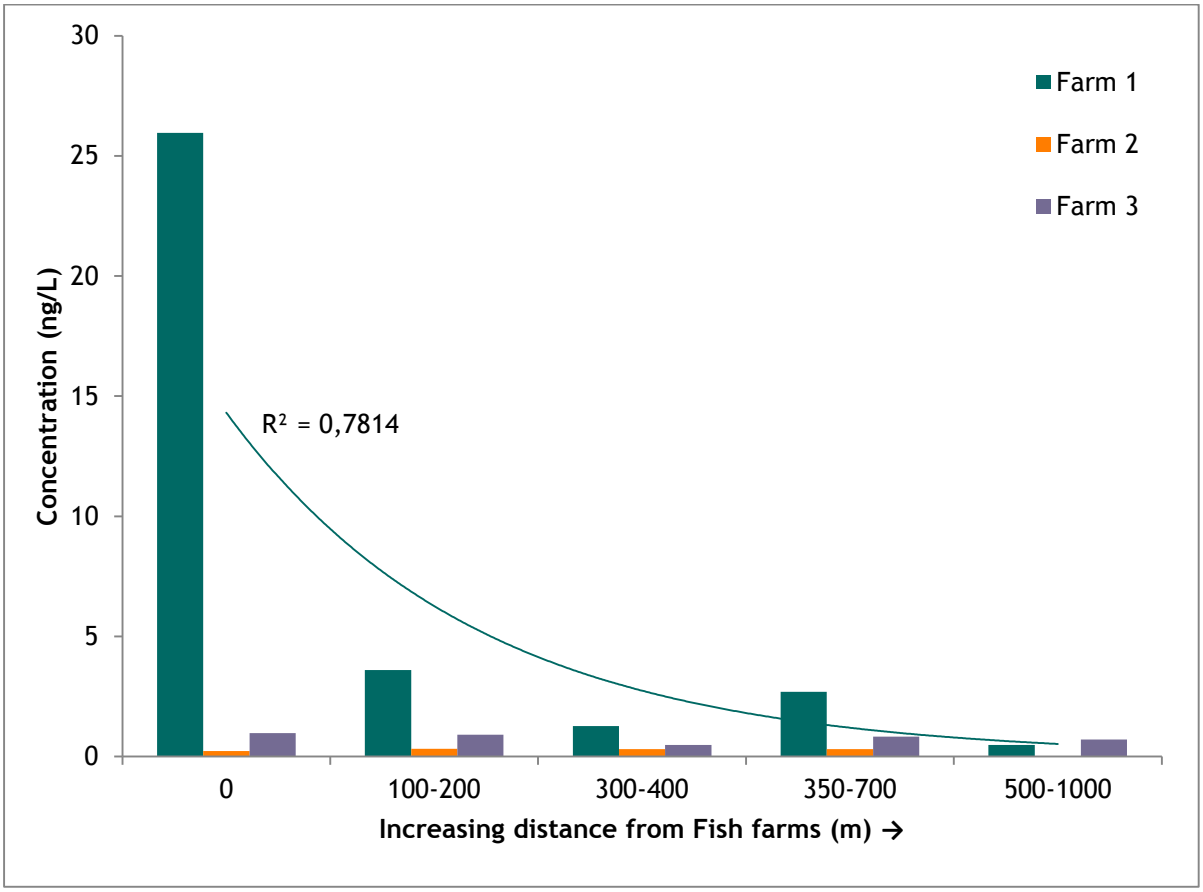


Figure 8. Concentration of azamethiphos in water

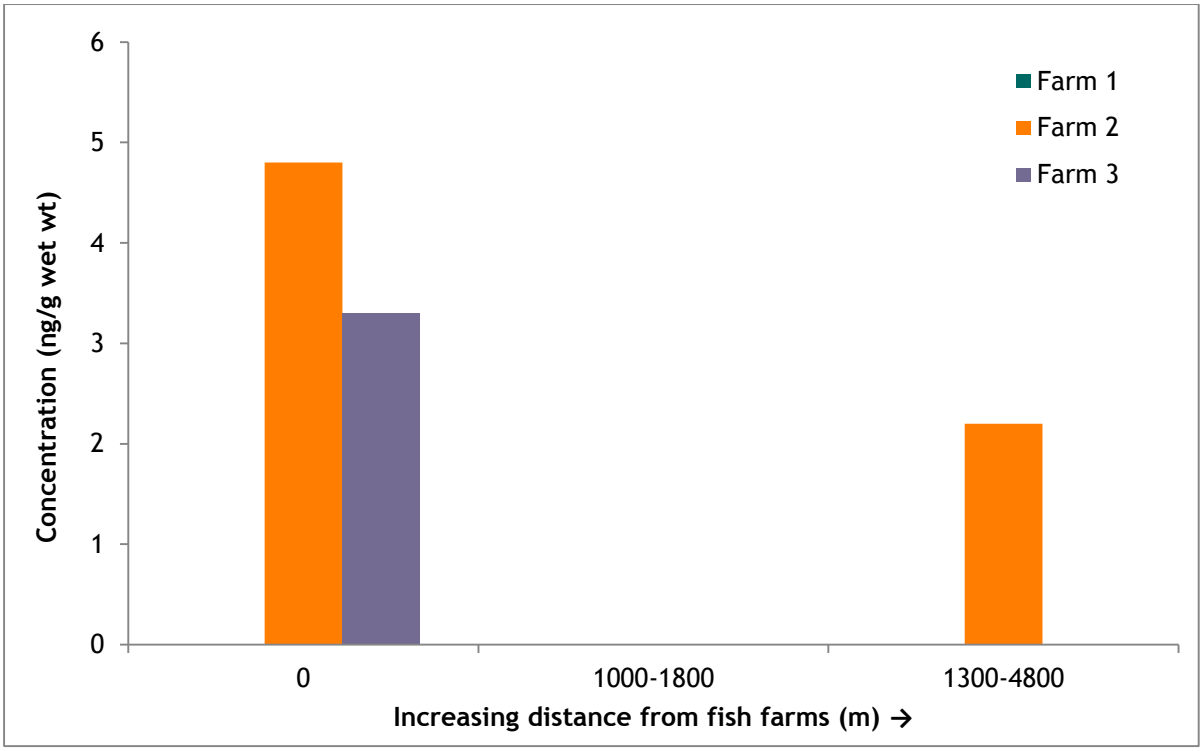


Figure 9. Concentration of cypermethrin in blue mussels

Table 4. Summary of all parameters determined
Fish Farm 1

		Azamethiphos ng/g or ng/L	Cypermethrin ng/g or ng/L	Deltamethrin ng/g or ng/L	TOC µg C/mg	DOC mg C/L	PSA % <63 µm
Water	1	26.0	<5	<1		1.5	
	2	3.6	<5	<1		1.5	
	3	1.3	<5	<1		1.5	
	4	2.7	<5	<1		1.5	
	5	0.5	<5	<1		1.6	
Particulates	1	<0.1	<50	<20			
	2	0.2	<50	<20			
	3	<0.1	<50	<20			
	4	<0.1	<50	<20			
	5	<0.1	<50	<20			
Sediment	1	<0.05	<6	<3	25.9		9
	2	<0.05	<6	<3	32.4		41
	3	<0.05	<2	<0.5	5.4		4
	4	<0.05	<2	<0.5	9.7		33
	5	<0.05	<2	<0.5	7.6		8
Blue Mussel	1	<0.1	<2	<1.5			
	2	<0.1	<1	<1.5			
	3	<0.1	<1	<1			
	4	<0.1	<1	<1			
	5	<0.1	<1	<1			
Bristle Worm	1	<0.1	<2.5	<5			
	2	<0.1	<5	<4			
	3	<0.1	<5	<4			
	4	<0.1	<5	<4			
	5	<0.1	<5	<3			

Table 5. Summary of all parameters determined
Fish Farm 2

		Azamethiphos ng/g or ng/L	Cypermethrin ng/g or ng/L	Deltamethrin ng/g or ng/L	TOC µg C/mg	DOC mg C/L	PSA % <63 µm
Water	1	0.2	<5	<1		1.4	
	2	0.3	<5	<1		1.2	
	3	0.3	<5	<1		1.3	
	4	0.3	<5	<1		1.3	
	5	<0.4	<5	<1		1.3	
Particulates	1	<0.1	<50	<20			
	2	<0.1	<50	<20			
	3	<0.1	<50	<20			
	4	<0.1	<50	<20			
	5	<0.1	<50	<20			
Sediment	1	<0.05	<3	<1	67.4		33
	2	<0.05	<6	<3	18.7		51
	3	<0.05	<3	<1	11.3		51
	4	<0.05	<3	<1	15.8		-
	5	<0.05	<3	<1	13.1		32
Blue Mussel	1	<0.1	4.8	<0.5			
	2	<0.1	<2	<0.5			
	3	<0.1	2.2	<0.5			
Bristle Worm	1	<0.1	<5	<2			
	2	<0.1	<5	<2			
	3	<0.1	<3	<1			
	4	<0.1	<5	<2			
	5	<0.1	<10	<3			

Table 6. Summary of all parameters determined
Fish Farm 3

		Azamethiphos ng/g or ng/L	Cypermethrin ng/g or ng/L	Deltamethrin ng/g or ng/L	TOC µg C/mg	DOC mg C/L	PSA % <63 µm
Water	1	1.0	<5	<1		1.3	
	2	0.9	<5	<1		1.3	
	3	0.5	<5	<1		1.3	
	4	0.8	<5	<1		1.2	
	5	0.7	<5	<1		1.3	
Particulates	1	<0.1	<50	<20			
	2	<0.1	<50	<20			
	3	<0.1	<50	<20			
	4	<0.1	<50	<20			
	5	<0.1	<50	<20			
Sediment	1	<0.05	<10	<5	18.7		31
	2	<0.05	<10	<5	37.3		54
	3	<0.05	<10	<5	58.5		39
	4	<0.05	<10	<3	49.7		30
Blue Mussel	1	<0.1	3.3	<0.5			
	2	<0.1	<1	<0.5			
	3	<0.1	<2	<0.5			
Bristle Worm	1	<0.1	<25	<10			
	2	<0.1	<20	<10			
	3	<0.1	<5	<2			
	4	<0.1	<10	<5			

4. Discussion

Azamethiphos and the pyrethroids have very different physico-chemical characteristics, and the results of this screening survey support this. Azamethiphos was detected in water samples (Figure 8) but not found to have accumulated in sediment or biota, and in contrast cypermethrin, despite not being recently used, was accumulated in a limited number of blue mussel samples (Figure 9). Deltamethrin was not reportedly used and was not detected in any samples collected.

Enzymatic and photolytic degradation play an important role in the environmental fate of pyrethroids. They are rapidly metabolised by aquatic organisms but their high log K_{ow} values will likely result in accumulation on suspended particulates and in sediment, the extent of which is related to the organic carbon content of the sediment (Maund et al., 2002); a higher organic carbon content results in pyrethroids being less bioavailable once sequestered in sediment, and organisms are less likely to be subject to chronic exposure. Acute exposure to bottom dwelling organisms may occur if the concentration in sediment reaches the equilibrium and pyrethroids desorb back into the water column. Marine sediment half-lives in different conditions were measured by (Benskin et al., 2014). Sediments were collected from the vicinity of fish farms and half-lives were measured at 4 and 10 ^{o}C, and under biotic and abiotic conditions. The sediment half-life at 10 ^{o}C was approximately 200 days and did not differ significantly in biotic or abiotic conditions. Shorter half-lives (<100 days) have been reported elsewhere at a temperature of 25 ^{o}C (Meyer et al., 2013) and with lower TOC values. For the present study, the work by (Benskin et al., 2014) is the most appropriate comparison, with more representative temperature and TOC values.

The dispersion of azamethiphos and cypermethrin has been studied elsewhere by the use of dye to track the plume (Ernst et al., 2001). The dye dispersed up to 3 km from the source and concentrations of cypermethrin were sufficiently high to cause effects on the benthic amphipod *Eohaustorius estuaris* up to 5 hours post release, despite measured concentrations being up to 3 orders of magnitude lower than the initial concentration. However, the measured concentrations reported by Ernst et al (2001) are likely to be higher than in the current Norwegian study's fish farm scenario because no fish and no nets were present, therefore no sorption would have occurred which is a highly likely mechanism in the case of cypermethrin. In addition, the toxicity tests were 48 hour exposures where as cypermethrin was only present for up to 5 hours, so the toxicity studies should be interpreted with caution. In a later study (Ernst et al., 2014) toxic effect were observed in 1 hr exposure studies with water collected up to 470 m from the fish farm cages after treatment with deltamethrin. In another study, (Fairchild et al., 2010) a 1 hr LC_{50} of 13 ng/L deltamethrin was calculated for *Eohaustorius estuaris* and no recovery was observed after transfer to clean water after exposure. Deltamethrin is more toxic than cypermethrin, which is reflected in the EQS (Environmental Quality Standard) (Table 5).

In contrast to the pyrethroid results, azamethiphos showed no toxic response to *Eohaustorius estuaris,* 20 minutes post release and the authors concluded it was a low toxic risk to receiving waters under test conditions (Ernst 2001). A similar study was undertaken (Ernst et al., 2014) and in 1 hr exposures, E*ohaustorius estuaris* showed no response to water collected post azamethiphos treatment. However, in 48 hr exposure studies, effects were observed in samples collected up to 850 m from the treatment site. In both of these studies samples were collected immediately after release of azamethiphos, however in the present study, samples were collected 1-2 weeks after treatment with azamethiphos and measured water concentrations were all under reported EQS concentrations (Table 7) (SEPA, 2008). However, due to the delay in sampling, it is not possible to conclude there was no risk associated with treatment prior to dissipation of the post treatment plume released.

Repeated short term exposures are a likely occurrence in Norway due to the number and proximity of fish farms, and as has been suggested elsewhere additive toxicity effects cannot be ruled out (Burridge et al., 2000; 2008). It is also possible that in certain scenarios, where several fish farms are treating simultaneously with different medications, that mixture effects may be observed. For example, an organophosphate (chlorpyrifos) was reported to modify the toxicity of a pyrethroid (esfenvalerate) and the authors suggested that the co-occurrence of these two types of pesticides can result in an increased risk to marine biota (Belden and Lydy, 2006) and it is possible that similar effects would be observed with mixtures of azamethiphos and cypermethrin or deltamethrin.

Table 7. Environmental Quality Standards (EQS) and maximum allowable concentrations (MAC)
Taken from the Scottish Environmental Protection Agency (SEPA, 2008)

	EQS_{water}	MAC
Azamethiphos	250 ng/L (3 hr) 40 ng/L (72 hr)	100 ng/L (72 hr)
Cypermethrin	16 ng/L (6 hr)	
Deltamethrin	6 ng/L (6 hr)	

Much of the reported toxicity data is the result of acute continuous exposure which is not truly representative of the field where treatment occurs multiple times for short periods. Pulsed exposure studies are more representative. Daily exposure of adult female marine copepods, *Acartia clause*, to 1.58 µg/L cypermethrin over a 4 day period resulted in higher egg production, as well as erratic and frantic swimming, although the behaviour returned to normal between exposure pulses (Willis and Ling, 2004). In the same study, different sensitivity was observed for different species and different life stages. *Oithona similis* was the most sensitive species tested and naulpiar was the most sensitive development stage and the 48 hr EC_{50} for naupliar *Oithona similis* was 0.14 µg/L.

Sediment dwelling organisms could potentially be exposed to pyrethroids for extended periods due to the tendency for these compounds to sorb to sediments, in contrast to azamethiphos which is unlikely to be found in sediment. Sublethal effects were observed in *Nereis virens*, a polychaete, at concentrations of deltamethrin at 11 µg/g (Van Geest et al., 2014) highlighting the potential risk when accumulation occurs. In this study however, cypermethrin and deltamethrin were not measured above detection limits in sediments or polychaetes at any of the locations sampled demonstrating limited risk with current aquaculture practices.

Considering lobster lethality as an end point, the NOEC for azamethiphos was 1.03 µg/L for adults exposed for 120 mins, and the NOEC for cypermethrin was 0.025 µg/L for adults exposed for 60 mins (Burridge et al., 2000), which is 100 and 200 times lower than the required sea lice treatment concentration. Stage IV lobster were less sensitive with a NOEC of 11 µg/L for 30 min exposure, and 0.05 µg/L for 120 min exposure, for azamethiphos and cypermethrin respectively. At a much lower concentration of azamethiphos (61 ng/L) and a longer exposure time (10 days) to simulate multiple sea lice treatments, adult male lobsters demonstrated increased stress, oxidative damage and neurotoxic effects which did not return to normal after 24 hr exposure to clean water (Couillard and Burridge, 2015). In addition, there was a significant increase in deaths relative to controls after simulated shipment indicating the occurrence of an additive stress effect.

Another study showed significant mortality of stage II juvenile lobster exposed to environmentally relevant concentrations of azamethiphos and cypermethrin (Pahl and Opitz, 1999). The 5 minute exposure LC_{50} for azamethiphos was 34 and 50 µg/L at 10 °C and 12 °C respectively, and 1.7 and 0.7 µg/L at 10 °C and 12 °C respectively for cypermethrin. While the concentrations measured in the present study are significantly lower than the LC_{50} concentrations reported, it is not possible to state that concentrations were not this high for 5 mins immediately after the treatment curtains were removed after treatment.

At the recommended dose concentration of 100 µg/L azamethiphos, juvenile lobster showed no adverse effects with respect to shelter usage in a pulsed exposure scenario however, in a continuous exposure setting, 100 µg/L azamethiphos resulted in death, although lobsters removed within 200 hours and placed in uncontaminated seawater had 100% survival. When removed from their shelter, juvenile lobsters demonstrated a decrease in delay to find shelter after exposure to 100 µg/L whereas the delay increased after exposure to higher concentrations, and the authors suggest this may be an alarm response at lower concentration compared to the inhibition of movement or orientation observed at higher concentrations (Abgrall et al., 2000). It is however unlikely that under current practices, that juvenile lobster would be exposed to the dose concentration due to the rapid dispersion of azamethiphos after treatment (Figure 8). In this study the maximum concentration measured was over 3 orders of magnitude lower than dose concentration.

Bivalves have also been exposed to the recommended dose concentration of 100 µg/L azamethiphos (Burridge et al., 1999; Canty et al., 2007). It was not lethal to bivalves (Burridge et al., 1999) but caused sublethal effects on blue mussels (Mytilus edulis) (Canty et al., 2007) at 100 µg/L. Blue mussels were also exposed to cypermethrin for 60 mins/day over 16 days (Gowland et al., 2002) and even at concentrations 200 x higher than the dose concentration, despite shell closure being effected, there was no evidence of stress effects. A change in isomeric ratio was observed indicating preferential metabolism of trans- than cis- isomers, unfortunately the concentrations and the detection frequency in the present study do not enable any observations on metabolism. The blue mussels in the study by Gowland et al. (2002) accumulated significantly higher concentrations of cypermethrin than this study, although the authors acknowledge that the exposure concentrations used were not environmentally relevant so it is unlikely that samples collected in the vicinity of fish farms in Norway will reach this concentrations using the current sea lice treatment protocols. The blue mussels reported here with positive cypermethrin detections were all in low ng/g concentration. 2 of the samples were collected from the fish farms (fish farm 2, 4.8 ng/g and fish farm 3, 3.3 ng/g) so were most likely exposed to the highest concentrations immediately after treatment as the curtains are released. A concentration of 2.2 ng/g was detected in a sample collected 4.8 km from fish farm 2 although the release of cypermethrin from other fish farms in the vicinity cannot be ruled out in this instance. All 3 samples contaminated with measurable concentrations of cypermethrin were at trace levels and it is not known whether detrimental effects will occur at such low levels. The presence of cypermethrin in blue mussels but not sediment or bristle worms indicates that dispersion of cypermethrin may be rapid, and enzymatic and photolytic degradation may occur before settlement can occur.

Blue mussel samples were collected from the structures at all 3 fish farms and these mussels were undoubtedly exposed to 100 µg/L azamethiphos immediately after sea lice treatment. The blue mussels did not accumulate any azamethiphos (tables 3-5) but it is not known whether the exposure time immediately after treatment and prior to sample collection was long enough to cause an effect. All other blue mussel samples were collected from a minimum distance of 1 km for the fish farms and were very unlikely to be exposed to 100 µg/L. Other fish farms in the area may have added to the exposure concentration although it is unlikely they reached effects concentrations. 24 hr exposure to 10 µg/L was required before impairing shell closure rate (SEPA, 1998) and as has been observed elsewhere that azamethiphos is diluted to ng/L concentrations within this time period (Ernst et al., 2014). The low log K_{ow} of azamethiphos means it is unlikely to accumulate in biota so any potential exposure will be via the water column where rapid dissipation to significantly lower than any reported effects concentrations was observed in this study.

5. Conclusions

- The concentration of selected sea lice treatments was below the UK EQS values (azamethiphos <40 ng/L, cypermethrin < 16 ng/L and deltamethrin <6 ng/L) in all of the selected samples.
- The risk to the environment from the use of azamethiphos is minimal 1-2 weeks after treatment.
- There was no reported use of deltamethrin so it is not possible to conclude anything with respect to the lack of occurrence.
- Cypermethrin used in multiple locations may accumulate in filter feeding blue mussels and the risks associated with this should be further evaluated.

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