

A review of the sea lice bath treatment dispersion model used for discharge consenting in Scotland

A report to the Scottish Aquaculture Research Forum

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Executive Summary

Introduction

- In May 2006 the Institute of Aquaculture at the University of Stirling undertook a research contract entitled “A review of the sea lice bath treatment dispersion model used for discharge consenting in Scotland”. The project was funded through a Scottish Aquaculture Research Forum (SARF) research grant, with additional financial and in-kind support from Novartis Animal Health Limited and in-kind contribution from Marine Harvest Scotland Limited.
- The project undertook to provide some supporting data and information on cypermethrin use (as EXCIS) and potential for alternate strategies in order to inform the discharge consenting requirements in Scotland. The project did not specifically include a re-evaluation of the dispersion model used for consenting, by agreement with SEPA.
- There were three key elements to the work:
 - A desk study review of the meta-data on environmental distribution of active ingredient, cypermethrin.
 - Measures of the concentration of cypermethrin during treatment and its short term distribution after release to the environment through a series of field based trials.
 - A theoretical assessment of the use of skirts as an alternative to the current tarpaulin treatment method.

Outcomes

- Using the standard treatment method it has been demonstrated that within the prescribed treatment period, there is significant uptake of cypermethrin by fish (using reduced water concentration as a proxy measure).
- This uptake appears to take place over a relatively short time during the treatment period. A treatment with no fish showed that reductions in cypermethrin concentration in the absence of fish were small, suggesting removal of cypermethrin by the net, tarpaulin and any existing biofouling was low. By contrast the treatments containing fish reduced the concentration of cypermethrin to between one third and one quarter of the nominal dose level that was applied to the cages.

- The dispersion model makes the assumption that the full treatment dose, recommended at 5µg/l, is released to the environment after the treatment is complete. With a $\frac{1}{3}$ to $\frac{1}{4}$ reduction in concentration there is, within reasonable limits, the potential to increase the permitted use of cypermethrin by a factor of 2 over current levels in order to treat a higher biomass of fish, whilst retaining a broadly precautionary approach.
- Further work is required to develop a robust method to analyse cypermethrin concentration on fish skin and then to evaluate levels of uptake directly to confirm the results.
- Cypermethrin was only occasionally identified in the surrounding environment after the release of the tarpaulin, despite prolonged and detailed assessment at 3 sites. It was observed that one reason for this may be an initial complicated dispersal, resulting from tarpaulin removal and net dropping procedures. Improvement in our understanding would benefit from a detailed study of initial dilution at the cages, to assess what impact removing the tarpaulin and dropping the net has on subsequent plume development.
- Skirt use is not currently permitted as a treatment method because of the generally perceived necessity to continually top up the treatment with cypermethrin to account for losses through the open area of the skirt. A theoretical study established key hydrodynamic parameters affecting change in concentration over time when using a skirt. In parallel, scaled flume experiments showed, for various current flow speeds, that the estimated time for the recommended concentration to reduce to $1/e$ (about 0.37) of the original value was between 1 and 18 hours. This indicates that losses during the recommended treatment period of 1 hour may be lower than previously thought.
- Whilst the project has determined that skirt use is feasible in principle, there will be a need to understand the underlying assumptions more thoroughly, through a combination of laboratory and field-based evaluation.
- In principle the theoretical evaluation combined with the considerable uptake by fish determined in this project, suggests that the use of skirts may provide a viable alternative treatment strategy, especially for larger sites in more exposed locations, where the an inability to carry out tarpaulin deployment might prohibit the use of EXCIS as a treatment option.

General Introduction

In May 2006 the Institute of Aquaculture at the University of Stirling undertook a research contract entitled “A review of the sea lice bath treatment dispersion model used for discharge consenting in Scotland”. The project was not a re-evaluation of the bath treatment dispersion model *per se* but undertook to provide some supporting data and information on cypermethrin use and potential for alternate strategies.

Within this context there were a number of specific restrictions placed on the work to be carried out, the result of comments by SEPA on our original proposal and discussion at a meeting with SEPA representatives prior to the start of the project. It was agreed that the project:

- a. would not specifically conduct a review of the sea lice bath treatment dispersion model used for discharge consenting in Scotland;
- b. would not review the way in which cypermethrin is used as a bath treatment;
- c. would not review the current Environmental Quality Standards; and
- d. would not be used to carry out tank trials.

It was, however, intended that the data generated might help inform the Scottish Environment Protection Agency (SEPA) and allow them to review their strategy and assumptions used to guide the consenting of cypermethrin (as EXCISTM).

Context

The intensive production of Atlantic salmon has the potential to increase the prevalence of disease. One such issue in Scotland is the prevalence of sea lice infestation and the impact this might have on both farmed and native fish. There are a number of treatment options available that may be used, under consent from SEPA, to reduce the prevalence of sea lice, including hydrogen peroxide, SLICE and EXCIS. .

The pharmacologically active compound in the sea lice treatment EXCIS is cypermethrin. Treatment of fish is effected by enclosing the fish cage with a tarpaulin, referred to as a bath treatment. EXCIS can be used as a primary sea lice treatment but is also used as a secondary treatment two to three weeks after SLICE has been used.

The project was developed with a specific and necessary aim; to generate data which may inform and allow SEPA to review their strategy and models used to guide the consenting of sea lice bath treatments. Taken alongside the conclusions of the Post-Authorisation Monitoring Programme report (PAMP; SAMS, 2005) and SEPA`s own data, where they investigate the occurrence of the active ingredients of sea lice treatments in sediments adjacent to marine fish farms (SEPA, 2004^a; SEPA 2004^b), this may allow for increased flexibility in development of sea lice management strategies. There were three principle outcomes designated and agreed at the start of the study. These were:

1. A review of the meta-data on environmental distribution of active ingredient.

2. An examination of the data on actual values of cypermethrin during treatment and at the point of release and short term distribution.
3. A theoretical appraisal of the implication of using skirts for treatment in terms of initial dilution of active ingredient and effect on treatment regime.

Part 1 – Review of meta-data on active ingredient, Cypermethrin, its environmental distribution and effects

Cypermethrin

Cypermethrin is a synthetically produced pyrethroid. Chemically it is described as “*α*-cyano-3-phenoxybenzyl” (Figure 1). The molecule contains two chiral centres, resulting in a number of stereo-isomers. These isomers are commonly grouped into four *cis*- and four *trans*-isomers (Jones, undated). It is the *cis*- group of isomers that are deemed to have the greatest insecticidal properties, such that the higher the *cis*- element the stronger acting is the treatment. Cypermethrin is a neuro-poison which acts on the axons of the peripheral and central nervous system of crustaceans by interfering with sodium channel transport. It acts whether adsorbed onto target species or through ingestion by that species.

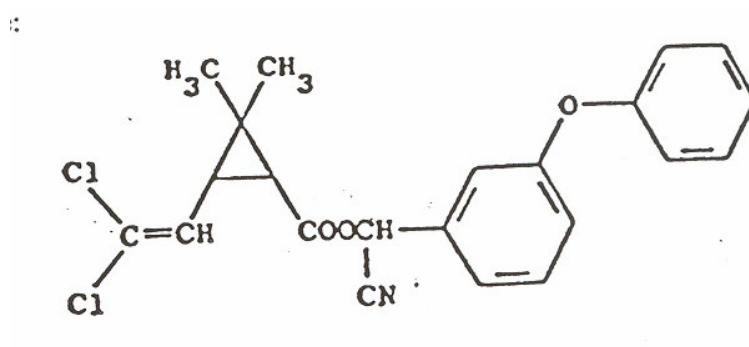


Figure 1: *Molecular structure of Cypermethrin (taken from Vericore method document CY/99/56, 2002).*

Cypermethrin is highly hydrophobic chemical with a low octanol/water partition coefficient (where $\log K_{ow} = 4.47$ to 6.6; Cole et al, 2002). This characteristic means that cypermethrin binds quickly and strongly to organic [particulate] material within the water column. It is this binding feature that makes cypermethrin suitable to treat fish in bath treatment, as it will readily adsorb to fish skin and any attached (or unattached) sea lice when they are bathed in it. Although it is also toxic to fish in sufficient concentration, products are developed to act on only target organisms, where possible, and particularly in marine systems on crustaceans, such as sea lice (see below).

EXCIS™

EXCIS was developed by Novartis Animal Health Limited (and its associated companies/predecessors, hereafter referred to as Novartis) for use as an insecticide treatment for sea lice infestation. It uses 1% w/v of the active ingredient cypermethrin (representing 1g of active ingredient per 100ml solution) in the isomeric ratio of 40:60 *cis*- and *trans*- isomers. The active ingredient is contained in an [emulsifiable] ethanolic base in liquid form for treatment. The emulsifiable base acts to lengthen the time the cypermethrin remains in solution, which for a limited time overcomes the hydrophobic nature of cypermethrin. The product is referred to as a “low-*cis* cypermethrin”, compared to other cypermethrin products, which generally increase the *cis*- ratio (e.g. 80 *cis*: 20 *trans*).

Novartis gained outline approval for use of cypermethrin as a sea lice treatment in 1997 (SEPA, 1998) although specific restriction existed which limited use at that time. The Veterinary Medicines Directorate (VMD) approval of EXCIS was granted in July 2004 in the UK (Marketing Authorisation (MA): Vm 18343/4010) and in Ireland (MA VPA 10974/22/1) and it has since become a very common method of treating sea lice infestation. It is also approved for use as an anti-sea lice treatment in Norway, the USA and Canada.

Fish are treated with EXCIS via a bath treatment, which requires that fish be treated in an enclosed system detached from the surrounding water. This is achieved by raising the net to a shallow depth and enclosing it within a large tarpaulin.

EXCIS is pre-mixed in water and then added into the tarpaulin through a number of routes, such as direct tipping, through hoses or through the leaky pipe method. The recommended treatment time is 1 hour, although lifting, deploying the tarpaulin, treatment and subsequent lowering of the net may take up to 3 hours to complete. The procedure is overseen by fish farm company Health Managers and during treatment there is careful monitoring of fish health. Water oxygen level is of particular concern given fish are corralled into a relatively small volume of water without any water exchange during treatment. Thus the process can be stressful for fish and throughout the treatment period oxygen is pumped through diffusers directly into the cages to maintain a high oxygen level.

After treatment the tarpaulin is removed as efficiently as possible. This typically involves removing 3 sides and allowing the tarpaulin to float to one side of the cage after which the net can be lowered from its 3m treatment depth to its full depth relatively quickly. Once the tarpaulin is removed the active ingredient, cypermethrin, in solution is released to the environment and here it dilutes and breaks down into less toxic products.

Discharge consent for EXCIS in Scotland

Chemotherapeutants, including cypermethrin, can have toxic effects on both target and non-target organisms and thus they have the potential to impose an environmental risk

when used. Because of this chemotherapeutants, including EXCIS, are controlled substances that may only be prescribed by a Veterinarian, under Statutory Instrument No.1646 The Medicines (Veterinary Drugs) (Prescription Only) Order 2001.

In addition to the above mentioned restrictions for anti-sea lice treatments, in order to be used in Scotland a discharge consent must be granted by the regulating authority, SEPA. SEPA are obliged to evaluate whether treatment is acceptable at a particular site, and does this by using the BathAuto (SEPA, 2007) model which predicts the likely dilution and dispersion of the chemical based on local water movement.

Approval for a particular site is granted in light of the ability of the localised environment to dilute the active ingredients so that concentrations in the environment after release remain within specified Environmental Quality Standards (EQSs). In broad terms the model uses site information (cage sizes and depth to calculate water volume), combined with the recommended treatment concentration and specific local hydrographic data, to estimate water volume into which the treatment dose will mix within a 6 hour period. It then compares the modelled concentration of cypermethrin at that time against the EQS and provided this is not breached then approval may be granted.

The model assumes the spread of chemical by a mixture of longitudinal dispersion based on mean current flow, and lateral dispersion based on a diffusion coefficient. SAMS (2005), using floating drogues, estimated lateral dispersion of between $0.049\text{m}^2\text{s}^{-1}$ and $0.014\text{m}^2\text{s}^{-1}$ at the sites they investigated. However, SEPA set the diffusion coefficient at a default value ($0.1\text{m}^2\text{s}^{-1}$) that allows for the fact that the model must remain applicable to a wide variety of water bodies that would otherwise have to be investigated in detail, which is impractical. The dispersion of the plume is, necessarily, based on relatively simple assumptions; but water movements, particularly around fish cages that act as an obstruction and alter the flow regime, mean these relatively simple model assumptions may over- or under-estimate the actual concentrations present after a particular treatment.

Prior to 2007 the EQS for cypermethrin was 16ng/l measured at the cage edge 3 hours after treatment of a single cage (SEPA 1998). During 2006/07, in light of the results presented by SAMS (2005) the time point at which the EQS applies was increased from 3 hours to 6 hours at the same concentration (i.e. 16ng/l) (SEPA, 2007). Based on the differential between the volume of water into which the cypermethrin is estimated to mix after 3 hours (based on longitudinal and lateral dispersion described above) and that which occurs using the same data after 6 hours, means this change in EQS increased the allowable quantity available for use by a factor of 2.8 and this provided a more flexible approach to the treatment regime.

Effect on crustacean species

This is not specifically a review on the actual and potential effects of cypermethrin on sea lice and other non-target species, but for completeness a brief synopsis is provided below. SAMS (2005) provide a comprehensive assessment of the potential impacts on

phytoplankton, zooplankton and benthic communities, although no specific impacts were observed during their research.

As has been identified, cypermethrin is an effective neuro-poison which acts on the axons of the peripheral and central nervous system of crustaceans by interfering with sodium channel transport. It hyper-stimulates the crustacean nervous system to induce continuous nerve stimulation, and results in mortality when the dose (i.e. synonymous with level of stimulation) is sufficiently high. Although specifically designated for use against sea lice, EXCIS acts on all crustaceans and thus will also have the same effect on non-target crustacean organisms.

Non-lethal doses on crustaceans, in general, can have the effect of inducing a range of non-lethal stimulated behaviours including increased swimming activity, increased growth in some species (Medina et al, 2004) and increased egg production in others (Maund et al, 2002). Medina et al (2002, 2004) in a study on a limited number of species details the affect of sub-lethal concentrations on the feeding behaviour of nauplii, copepodite and adult stages and fecundity in adults. Thus research on the environmental impacts of cypermethrin have shown that cypermethrin (as EXCIS) has sub-lethal effects. However, in laboratory and mesocosm studies (Barbata et al, 2002; Medina et al, 2002; Medina et al, 2004) the concentrations used are relatively high compared the environmental concentrations found after *in situ* treatment. Although sub-lethal effects are observable in the laboratory, because of subsequent dilution they are not able to be observed in the natural environment (SAMS, 2005).

Considering lethal level concentrations, Willis and Ling (2004) assessed toxicity on non-target copepod at nauplii, copepodite and adult stages and found that EC_{50} occurred in the region 0.12 to 0.24 μ g/l. These values are are larger than the EQS value of 0.016 μ g/l. In the same study, however, LD_{50} (lethal dose where 50% of the species under test die) values were below the EQS but in the environment would only be at this concentration within the bath treatment. Very short-term exposure to concentrations greater than the LC_{50} are likely to occur, especially immediately after the tarpaulin is removed. Whereas most studies on toxicity subject species to 2 hours or more exposure (e.g. Willis and Ling, 2004), SAMS (2005) showed that detectable levels of cypermethrin were not recorded 43 minutes after the release of the tarpaulin at fish farms, and therefore non-target species are unlikely to encounter lethal doses in the environment. Thus, overall, it is suggested that non-target copepods are unlikely to be effected at the concentrations they will encounter after the cypermethrin is released from the bath treatment to the wider environment. It is important that copepods are not exposed to lethal levels as they form a vital part of the food chain in marine systems, in part controlling phytoplankton growth, that may increase to bloom levels where they not consumed.

Mohaptera et al (2003) showed that cypermethrin can inhibit photosynthesis in cyanobacteria, although it remains unclear whether phytoplanktonic photosynthetic inhibition occurs. SAMS (2005) showed that repeated use of cypermethrin at a number of locations did not effect the normal seasonal cycles of phytoplankton production.

Correct treatment and dosage using cypermethrin remains important. The aim of its use is to cause high mortality in the target organism. Over-concentration potentially risks impacting non-target species. Under-concentration risks not affecting the target organism. Lyndon and Toovey (2000), for example, showed that sub-lethal doses affected sea lice reproductive capability to some extent, but where they survive, their eggs do not appear to be affected by this exposure and so would develop and allow re-infection. In practice, treated correctly, cypermethrin is successful at removing sea lice and sustaining this for a minimum of 6-8 weeks after treatment.

It remains vitally important to maintain an effective treatment regime against sea-lice infestation. Reduced sensitivity to the treatment has been identified in Scotland (Sevatdal et al, 2005), although the authors did not necessarily equate this to an increased likelihood of full resistance during their study period. Fallang et al (2005) have shown that knockdown resistance to pyrethroid insecticides in sea-lice may be caused by point mutations in the pyrethroid target site, particularly within the sodium channel of nerve membranes. A high density of host organisms, a low number of available strategies for treatment and poor treatment regimes on site are three of the overriding reasons that might promote such resistance in sea-lice (Anon, 2006).

Environmental fate

The characteristics of cypermethrin, its hydrophobicity and low octanol/water partition coefficient, means there is little or no need to investigate its long term fate when in solution. Studies have shown that cypermethrin readily binds to organic sediments over as little as 2 (Maund et al, 2000) to 6 hours (SAMS, 2005) and would at this stage be undetectable. The potential for toxic effects on water-dwelling species is significantly reduced or eliminated after this particulate binding has occurred..

Level of adsorption (attachment to particulate material in water or sediments) is highly dependent on a combination of the organic content and grain size (Maund et al, 2000) of the material cypermethrin attaches to. Once bound the toxicity is reduced and unlikely to affect organisms living within sediment at the concentrations likely to be present. The toxicity of the chemical on sediment dwelling species appears to be dependant on the equilibrium between cypermethrin concentration in the sediment, in the interstitial water and in the organism and bioavailability to sediment dwelling species may therefore be variable depending on environmental conditions (Maund et al, 2000). Effects have been noted on *Corophium* spp. (SEPA, 2004a, 2004b), for example, but at significantly higher concentrations than would be present in a standard fish farm treatment regime.

As a pyrethroid insecticide cypermethrin is not particularly persistent in the environment. In water cypermethrin breaks down relatively quickly (1-9 weeks; Cole et al, 2002) to less toxic analogues (see Jones, undated). Whilst there are no specific sediment quality standards for cypermethrin in the UK, as part of a risk assessment carried out by SEPA a Predicted No Effects Concentration (PNEC) for cypermethrin of 2.2 µg/kg has been estimated (SEPA, 1998 and references cited therein). This is based on applying a safety factor of 100 to the lowest sediment LC₅₀ of 225 µg/kg as measured for *Corophium* spp..

SEPA have conducted assessment of the presence of cypermethrin in sediment at a range of location during 2001-3 (SEPA, 2004a, 2004b) and shown that, in general, recorded levels are below the PNEC.

Part 2 – An examination of the data on actual values of cypermethrin at the point of release and short term distribution

An examination of the values of cypermethrin at the point of release and over short term distribution was carried out through a series of field studies conducted between September 2006 and March 2007. These act as a complement to a previous study conducted by SAMS (2005).

Trials were completed on three occasions using two sites. Three cages were sampled on each occasion; one without fish, one with a lower stocking density and one with a higher stocking density of salmon. During treatment, water samples were taken at each of the four cage corners throughout the 60 min treatment period and were analysed for cypermethrin concentrations. After treatment cypermethrin concentration was measured through the water column at a fixed position approximately 15 m downstream of the cage, and at the positions designated by drifting drogues that were released when the tarpaulin was removed after treatment.

Using these measurements an assessment of the fate of cypermethrin during and after treatment can be defined. In addition the results from the three treatments can be used to estimate the uptake of cypermethrin on salmon surfaces (i.e. skin), with a view to including this information in the cypermethrin dispersion models used for environmental regulation.

Methodology

Site selection

Rationale

It was originally intended that sites would be chosen to represent entirely different environmental conditions, but in what could be regarded as “typical” of Scottish loch systems. In practice there were limitations in the availability of sites for this purpose.

Suitability criteria and sites used

A number of sites were evaluated to carry out field trials and there were a number of key considerations:

- the underlying site topography and general suitability within the project context and requirements;
- site availability and access potential;
- the size of fish being stocked at a particular site at the time;

- the current SEPA discharge consent for a particular site, which had to include the use of EXCIS;
- whether there was available capacity **within** the current consent limits for the specific trial treatments to be carried out;
- whether fish had recently been treated at the site and further treatment was deemed to risk fish health;
- hydrographic and tidal considerations;
- suitable site conditions being available during daylight hours to enable safe working; and
- general fish health issues.

The outcome of these limitations was that three trials conducted were carried out at two locations only, both having relatively similar environmental conditions:

Trial 1 was carried out at Marine Harvest site Camas Glas on Loch Sunart week commencing 11th September 2006.

Trial 2 was carried out at Marine Harvest site Linnhe on Loch Linnhe week commencing 16th October 2006.

Trial 3 was carried out at Marine Harvest site Camas Glas on Loch Sunart week commencing 21st May 2007.

Site descriptions (provided by Marine Harvest (Scotland Ltd))

Camas Glas is a salmon on-growing site located in Laga Bay on Loch Sunart (Figure 1). The most recent hydrographic data (Marine Harvest, 2004) shows that the average current speeds are 7.0cms^{-1} in surface waters (depth approx 4m below water surface), 5.9cms^{-1} at net depth and 4.9cms^{-1} near to the seabed, which represent moderate current flows.

Linnhe is a salmon on-growing site located near to the Corran Narrows on Loch Linnhe (Figure 2). The most recent hydrographic data (Marine Harvest, 2005) shows that the average current speeds are 8.4cms^{-1} in surface waters (depth approx 4m below water surface), 6.8cms^{-1} at net depth and 5.1cms^{-1} near to the seabed, which again represent moderate current flows.

Mean current speeds do not vary a great deal between the two sites available for this project.

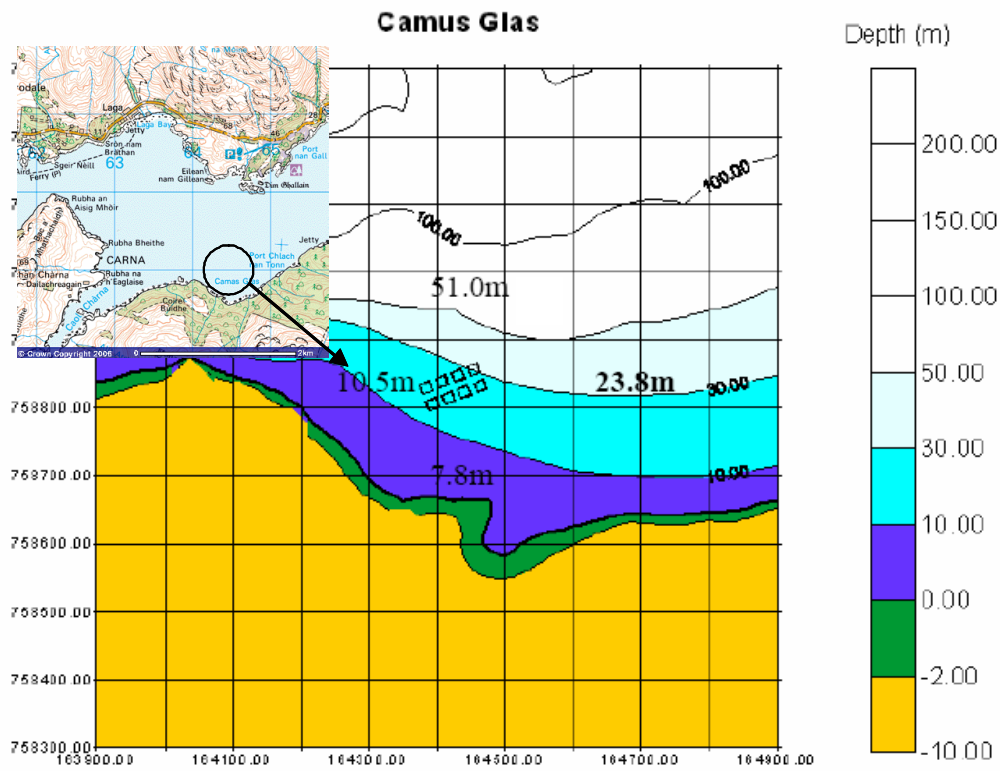


Figure 1: Representation of layout of cages, general topography and bathymetry at Camas Glas fish farm on Loch Sunart. (reproduced by permission of Marine Harvest (Scotland) Limited). Inset shows location (Crown copyright 2006)

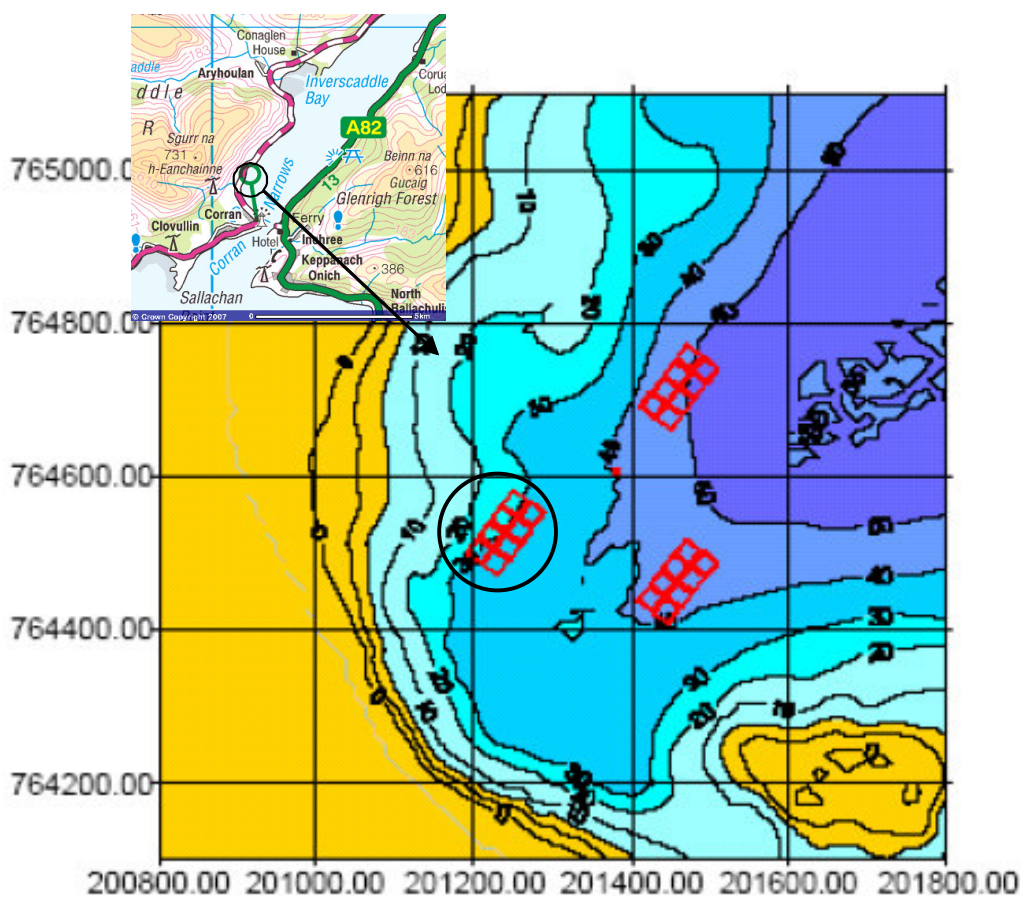


Figure 2: *Representation of layout of cages, general topography and bathymetry at Linnhe fish farm on Loch Linnhe. (reproduced by permission of Marine Harvest (Scotland) Limited). Cages used are circled. Inset shows location (Crown copyright 2006).*

Both sites consist of 24m x 24m square steel cages with adjoining steel walkways (Figure 3) and a net depth of 10m.



Figure 3: *Walkway design and net stanchions for square steel cage design (at Camas Glas fish farm on Loch Sunart).*

General Protocol

Each trial was of 1 week duration and consisted of one EXCIS dose given to each of 3 separate cages containing different biomasses of fish (the treatments), with each treatment being carried out on consecutive days.

- Treatment I – tarpaulin, nets, no fish
- Treatment II – tarpaulin, nets, low biomass of fish
- Treatment III - tarpaulin, nets, high biomass of fish

The “no fish” treatment (I) would never occur under normal conditions, but was carried out to assess whether it was possible to discern changes in concentration when no fish were present and by doing so assess the quantity of cypermethrin that might be adhering nets and associated equipment, the tarpaulin and other particulate material present in the water. Marine Harvest gained approval for the “no fish” treatment from SEPA, prior to the trials taking place.

It would have been preferable for each of the trials to have similar sized fish and stocking density, but operationally this was not possible. Thus the stocking density and total biomass of fish present at the sites varied between trials. Within each trial it would have been preferable to have a clear distinction between the “low biomass” and “high biomass” of fish present under treatments II and III. Again in practice there was some difference between the treatments, though not as large as would have been optimal for the

experiment. The stocking density data for each treatment at each trial is defined in Table 1.

Table 1: *Biomass held in treated cages during each trial under “no fish”, “low” and “high” biomass holding.*

Biomass (treatment) descriptor	Stocking density at Trial 1 (Camas Glas) kg/m ³	Stocking density at Trial 2 (Linnhe) kg/m ³	Stocking density at Trial 3 (Camas Glas) kg/m ³
No Fish	-	-	-
Low	16.1	7.6	3.5
High	21.5	7.7	4.6

- During trial 1 the fish were due to be harvested approximately one month after the trial and were therefore relatively large in the range 3-5kg, hence the relatively high biomass holding.
- Prior to trial 2 grading had been carried out at Linnhe which resulted in a similar biomass of fish in the two treatment cages. In the “low” biomass cage there was 45,000 fish at an average weight of 1066g (~48t) and in the “high” biomass cage there were 49,000 fish at an average weight of 990g (~48.5t).
- During trial 3 the fish were relatively small, 127,000 at an average weight of 210g (~26.6T) for the “high” biomass cage and 129,000 fish at an average weight of 155g (~20T) for the “low” biomass cage.

Field Methods

1) Current meter deployment

On arrival at the site a current meter was deployed, procedures conforming to standard SEPA methodology, as defined in Attachment VIII version 2.6 of the Fish Farming Manual dated 26th May 2005 (SEPA, 2007). A Doppler current meter (type: RDCP 600) was deployed on the seabed and the meter remained in the water until the final day of the trial.

2) Treatment

The administration of the bath treatments were performed within the current procedures and guidelines for that administration, as listed in the Marketing Authorisation and elsewhere (SEPA 2007). Fish were starved 24 hours before treatment took place. On the day of treatment the cage net was progressively raised to a depth of approximately 3m by site staff. During this period, and throughout the treatment, oxygen was pumped into the cage from oxygen cylinders through three equally distributed diffusers. Once complete the raised net was surrounded with an impervious tarpaulin (Figure 4) in order to isolate an identifiable volume of water for treatment. Based on the water volume enclosed sufficient EXCIS was added to give a dose equivalent to 5µg/l as recommended by the manufacturer.



Figure 4: *Tarpaulin deployment*

EXCIS was administered by Marine Harvests Health Manager and trained staff. Initially the prescribed number of 200ml bottles of EXCIS (Table 2) was added to approximately 100L of seawater in a plastic tank and mixed thoroughly. This solution was then pumped, using a petrol-operated pump, into the tarpaulin through a “leaky” pipe. The leaky pipe was not specifically designed for this project, instead supplied by Marine Harvest. The pipe (approx 8-10 m long, approx 4cm diameter, with holes perforated equi-distant around the circumference approximately every 30cm) was located on one side of the cage and laid out across the middle of the enclosed water to the other side. The holes in the pipe allowed the cypermethrin solution to leak out into the water at various locations, to ensure a reasonably even initial dispersion through the cage. Subsequent dispersion was generated through movement of water generated by the oxygen diffusers and fish swimming activity. Pumping rate was not measured during the trial but the approximately 100L of liquid took between 5 and 8 minutes to disperse. Any residual cypermethrin left in the plastic container was poured into the tarpaulin. Once all cypermethrin was added the treatment was assumed to have started and the the maximum duration of each treatment was limited to 1 hour in line with recommendations.

Fish responses to the procedures being carried out were monitored before, during and after the treatment, as fish welfare was a priority and if compromised the trial/treatment was abandoned.

Release of the tarpaulin was timed to take place approximately 2 hours after the tidal turn to ensure that water flow throughout and post-treatment was in one direction. Thus the treatments were started slightly later each day to take account of the variation in the tidal cycle.

Table 2: *Treatment dosage for each trial to achieve working dose of (approximately) 5µg/l (based on estimated water volume, assuming Tarpaulin fully charged with water).*

Trial Number	Trial Site	Tarpaulin type and size	Water volume (m³)	Treatment dose (ml)	EXCIS added (ml)
1	Camas Glas	Full cage, full depth tarp (24 x 24 x 4m)	2304	1152	1200ml (6 bottles)
2	Linnhe	½ cage, full depth tarp (24 x 12 x 3m)	864	432	500ml (2.5 bottles)
3	Camas Glas	½ cage wedge tarp (24 x 12 x 5m)	720	360	400ml (2 bottles)

3) Water sample collection

Water samples were collected prior to, during and after the treatment period as follows:

a) Pre-dose

Prior to commencing treatment, water samples were collected in open flowing water next to the cage and from within tarpaulin at two opposite corners in order to evaluate pre-dose concentrations and to act as controls. Samples were collected using a brown glass bottle attached to a rod, plunged to a depth of approximately 2m (representing ½ of the tarpaulin water depth). The water was decanted into 100ml labelled glass vials and capped with aluminium foil and lid.

At the initial trial the glassware was new. At subsequent trials the glassware and lids underwent cleaning with Decon and solvent prior to further use to avoid cross-contamination.

b) Treatment period

The start of the 1 hour treatment was deemed to have commenced once the pre-mixed solution had been completely added to the cage (= t₀), this process taking approximately 5 minutes.

Water samples were collected at all 4 corners of the enclosed cage at specific time points; 10 minutes (= t₁₀), 30 minutes (= t₃₀) and 50 minutes (= t₅₀) after the treatment start time. Duplicate samples were collected with each collection at all 4 corners being completed within 5 mins ± 1 min of the collection start time. All samples were retained on the cage site until the treatment and all collections were complete. Sample collection and decanting was as described in a) above.

c) Fixed location

After the 1 hour treatment period water samples were collected from a fixed location relative to the treatment cage. Sample collection at the fixed position started when the treatment was completed (t_{60}) and the tarpaulin removal procedure had commenced. The position and distance from the cage varied between cages and sites depending on the position of the cage within the cage block and hydrographic conditions but was within 10 – 15m from the cage edge in the direction of the prevailing current flow. A boat was moored at this distance from the cage, by tying a rope to the cage block and the boat and backing off in the required direction until the rope was under tension. The boat engine was then used minimally and only to adjust position to take account of drift.

Water samples were collected at 3 water depths; 1m, 5m and 8m in this sequence, using a 2L-volume Van Dorn water sampler. No account was taken of cypermethrin that may adsorb to the Van Dorn sampler. After return to the surface duplicate 100ml samples were decanted from the Van Dorn into labelled glass vials, with approximately 0.5 – 1L of water allowed to waste between each sample collected. Samples were capped with aluminium foil and lid. Samples were retained at the cage site until all fixed position sampling was complete.

Samples were collected in a standard sequence (1m, 5m, 8m), at 5 minute intervals and were concluded when the “plume” of cypermethrin from the cage was deemed to have passed the moored boat. The approximate time was based on current flow speed and therefore the time required to travel a known distance. On all occasions samples were collected up to a maximum of 45 minutes after the treatment was complete (from t_{60} to t_{105}).

The fixed position collections attempted to track the passage of the water containing cypermethrin (= plume) from its origin in the enclosed tarpaulin, then as it passed a known position over a range of depths; to evaluate the initial dilution experienced after release of the treatment water.

d) Drogue positions

At the time the tarpaulin was removed (t_{60}), drogues were placed into the water at the edge of the cage. The drogue depth was set at 5m and it was presumed that all water above and just below this depth was homogeneous and to have the same flow direction. Drogues were allowed to drift on the tidal current and were assumed to be tracking the cypermethrin plume front. At specific intervals after treatment samples were collected from 1m, 5m and 8m depths at the drogue locations using a Van Dorn water sampler, as described in c) above. Sampling times were 1 hour (t_{120}), 2 hours (t_{180}) and 3 hours (t_{240}) after treatment completion, where possible,

4) On-site processing

Some post-processing of the collected samples took place on-site to reduce the likelihood of sample degradation. The processing involved the removal of the cypermethrin in solution from 50 ml of seawater (the sample) and “capture” of that cypermethrin using an adsorbent material contained within a cartridge. The method utilises a technique whereby the non-polar nature of the cypermethrin (i.e. its aromaticity and methyl groups) allows cypermethrin to be selectively removed from the sample when passed through a cartridge by binding to a C₁₈ adsorbent material in the cartridge.

Labelled C₁₈ 200mg/10ml isolute cartridges (International Sorbent Technology Limited, UK) were pre-conditioned by passing through 10ml of iso-hexane followed by 4ml of methanol. 50ml of the seawater sample was accurately measured using a measuring cylinder to which was added 1 drop of orthophosphate and 2.5ml of methanol.

This mixture was slowly passed through the pre-conditioned isolute cartridges using a vacuum pump. The active ingredient, cypermethrin, was extracted from solution and retained on the cartridge, the remainder of the water discarded.

The isolute cartridge was further treated by washing with 6ml of HPLC grade water followed by 6ml of 20% v/v methanol in deionised water. The isolute cartridge was then either stored frozen (-20°C±1°C) until further analysis in the laboratory or removed from the cartridge (eluted) with 2 x 4ml of iso-hexane into a borosilicate glass tube and this was stored in a freezer (-20°C±1°C) until analysis. Extraction of cypermethrin from a number of seawater samples was conducted simultaneously using a multiple port vacuum pump.

5) Other environmental data collected:

a) Water parameters

Oxygen concentration, salinity and water temperature was measured before (pre-dose), during (treatment period) and after (at fixed position and drogue locations) using combined probes. Five-day Biological Oxygen Demand and particulate organic material in the water column was also measured initially. As there was no variation in the parameters within each trial period, or in the case of oxygen concentration was entirely driven by the oxygen pumped into the cages during treatment, the data is not reported here.

b) Fish samples

After treatments II and III (i.e. those with fish) were completed 5 specimen fish were removed from the treated cages for analysis of cypermethrin concentration on fish skin. Removal of fish from the cage was via hand net. On site, a 3cm by 3cm section of skin was removed from one side of each fish, wrapped in cleaned aluminium foil and stored frozen (-20°C±1°C) in a cleaned labeled glass vial until

further analysis in the laboratory. This procedure was carried out at the Linnhe site and at Camas Glas in May only.

Laboratory Analysis of water samples

Water samples were analysed for cypermethrin concentration using a method adapted from Vericore Limited method MCY/99/56 (Vericore, 2002), using Gas-Chromatography with an Electron Capture Device (GC-ECD) (TRACE GLC, Thermo Finnegan, Thermo, USA).

For commercial reasons outside the confines of this project Vericore were unable to provide the on-site processing, laboratory processing and analysis of all water samples, as agreed at the outset of the project. This requirement was therefore carried out by agreement, by the Institute of Aquaculture. It was assumed that the standard method for analysis of cypermethrin used by Vericore Limited could be incorporated within Institute of Aquaculture procedures without the need for further validation.

In the event the specific analytical equipment, including column and detector specifications and carrier gases used at the Institute of Aquaculture was different from the defined procedure. Although the method was similar to that defined in MCY/99/56 (Vericore, 2002) these differences necessitated further detailed method development and validation. This was incorporated as part of the project.

The TRACE GLC with ECD at the Institute of Aquaculture used a standard polar column (Column Ttx.5-ms from Thames Restek, Buckingham, UK) being 30m x 0.025 ID x 0.25µm. Run-time for the procedure occurred in stages up to an operating temperature of 300°C. The carrier gas was helium under constant flow. Injection of the sample was splitless at 250°C. Although variations to the Vericore procedure, these are standard methods for the analytical equipment used.

As part of the development and validation the linearity of detector response was examined, over the range of approximately 1ng/ml to 100ng/ml (= 1 – 100µg/l). Regression analysis was performed using Microsoft Excel software giving a correlation co-efficient (R^2) of 0.9908 (Figure 5).

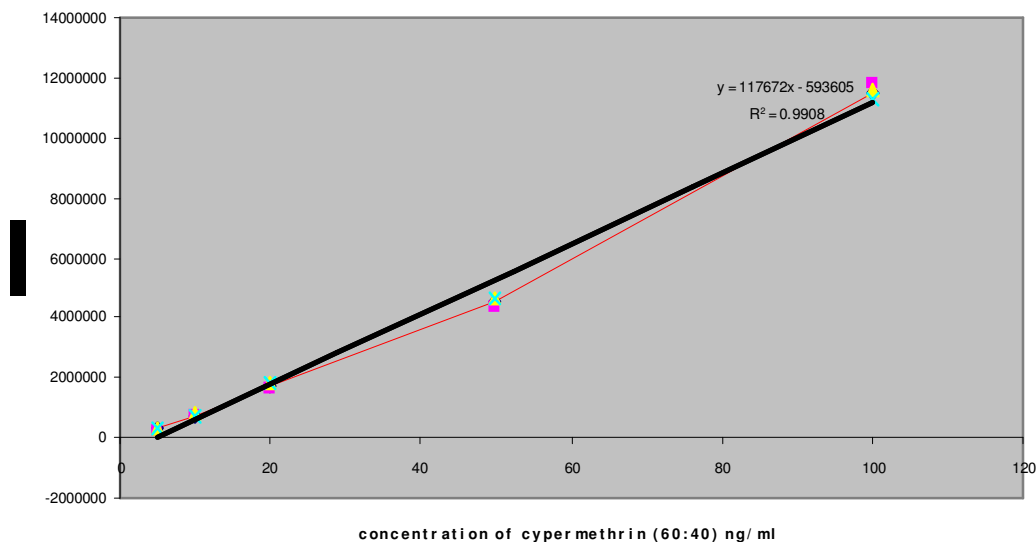


Figure 5: *Linearity of detector response for cypermethrin (EXCIS) over the range 1 to 100ng/ml using a TRACE GLC with Electron Capture Device.*

The accuracy and reproducibility of the procedure were determined by analysing seawater samples spiked with known quantities of cypermethrin with results expressed in percentage recovery (Table 3). Percentage mean recovery is then applied to all subsequent samples analysed. The three levels of fortification (spiking) examined were nominally 25pg/ml, 50pg/ml and 100pg/ml in seawater relating to actual measured levels of 5ng/ml, 10ng/ml and 20ng/ml. All fortification levels are as defined in Vericore procedure MCY/99/56 (2002).

Method development was complicated between trials 2 and 3, during which there was an attempt to analyse fish skin samples (see below). Analysis of fish skin contaminated the equipment and reduced the sensitivity of the detection. This in turn affected the limits of quantification (LOQ) and Limits of Detection (LOD) on water samples analysed after the fish. Whilst account has been taken of this in samples that contained significant quantities of cypermethrin (i.e. the dosing samples taken in the tarpaulin during treatment), the contamination affected the validation process, with insufficient resources within this study to provide a complete validation of the method.

Through the development process the revised method enabled the delineation of 4 primary cypermethrin isomers present rather than an overall concentration (Figure 6); and the limit of detection was not compromised as a result of changing the defined method.

Table 3: Accuracy and reproducibility data for 40:60 cis:trans cypermethrin in seawater.

LCC Concentration in seawater (pg/ml)	% Recovery	% Mean Recovery
25	85.7 94.9 107.6 68.5 88.9 96.3	91.8
50	78.6 90.6 65.9 89.3 91.1 87.6	83.9
100	87.9 78.3 86.1 90.4 81.8 73	82.9

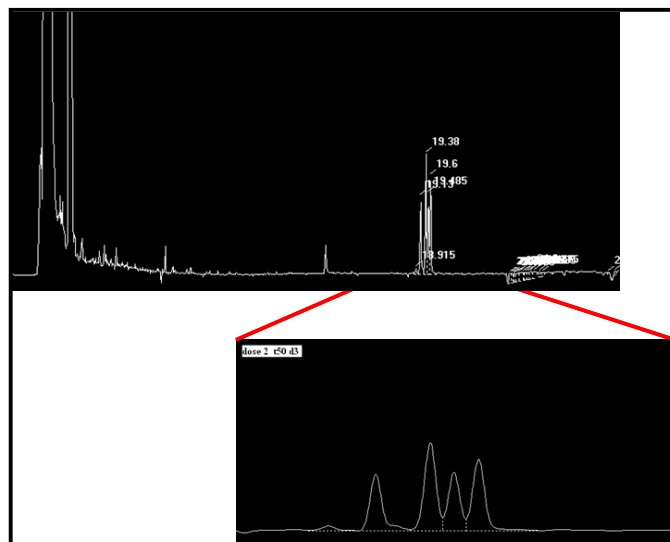


Figure 6: Graphical representation of the 4 isomers of cypermethrin analysed through GC-ECD

Conversion to concentrations

After the samples are injected through the GC-ECD and results produced, calibration standards (Figure 5) are used to quantify field sample concentrations through the allocation of actual concentrations to the detected levels. Once the concentration is determined the data is interpreted, based on the signal to noise ratios within the samples. This means the detected amount (i.e. the signal) is interpreted with the background variations of the baseline (i.e. the noise). This, combined with the percentage recovery from the spiked samples (Table 3) enables calculation of the actual concentration value along with a confidence interval, in each sample.

Laboratory analysis of fish samples

It was the intention of this project to analyse cypermethrin concentration in fish skin, using a method provided by Vericore Limited. Cypermethrin on fish skin is more difficult to analyse than cypermethrin in water and thus requires additional processing. In removing the cypermethrin through boiling in solvent a number of other “contaminants” are also removed (e.g. lipids). The procedure (Vericore MCY/94/24R1 dated Nov 1996) incorporates additional “clean-up” steps prior to the final extraction to remove these contaminants.

Fish skin samples were boiled in a hexane/acetone solvent mixture and then homogenized. The homogenate was then re-boiled in further hexane/acetone solvent to remove contaminants. The solvent/cypermethrin mixture was decanted to a pre-cleaned glass test tube. The liquid (solvent and cypermethrin) was boiled to dryness under nitrogen gas in order to concentrate the cypermethrin and finally 1ml of solvent was added to solubilise the concentrated cypermethrin prior to analysis.

Despite repeated trials, the Vericore method was not successful in completely removing the contaminant lipid fractions, although this could not be discerned before analysis using the TRACE GLC. During TRACE GLC analysis, both the column and Electron Capture Device were severely contaminated with lipid and no reliable results for cypermethrin concentration were obtained.

Whilst a cleaning procedure was used to remove the lipid residue on the detector, subsequent sensitivity on the analysis was compromised as described above.

Results

Hydrographic measurement

Deployment of the current meters at Loch Linnhe and Camas Glass fish farms showed the current speed and direction during the trials period. Full data is available but is summarized here as scatter plots and cumulative vector plots for each site. These give an

indication on the speed and direction of flow and the residual speed and direction of the currents and indicate the main direction of dispersal of the cypermethrin from the sites. The hydrographic data collected confirms that the fixed position at which samples were collected were in the correct orientation.

Loch Linnhe

The scatterplot and cumulative vector plot for the currents at 5 m and 8 m depth are given in Figures 6 and 7 respectively.

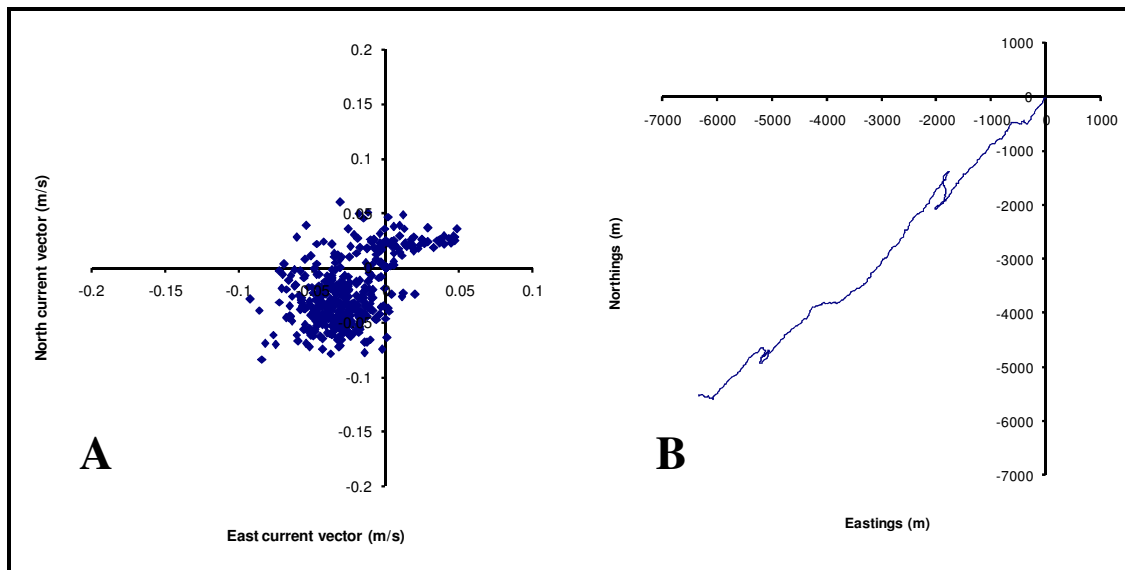


Figure 6 Scatterplot (A) and cumulative vector plot (B) of water flow at 5 m near the cages at Loch Linnhe (see Figure 2 for deployment position)

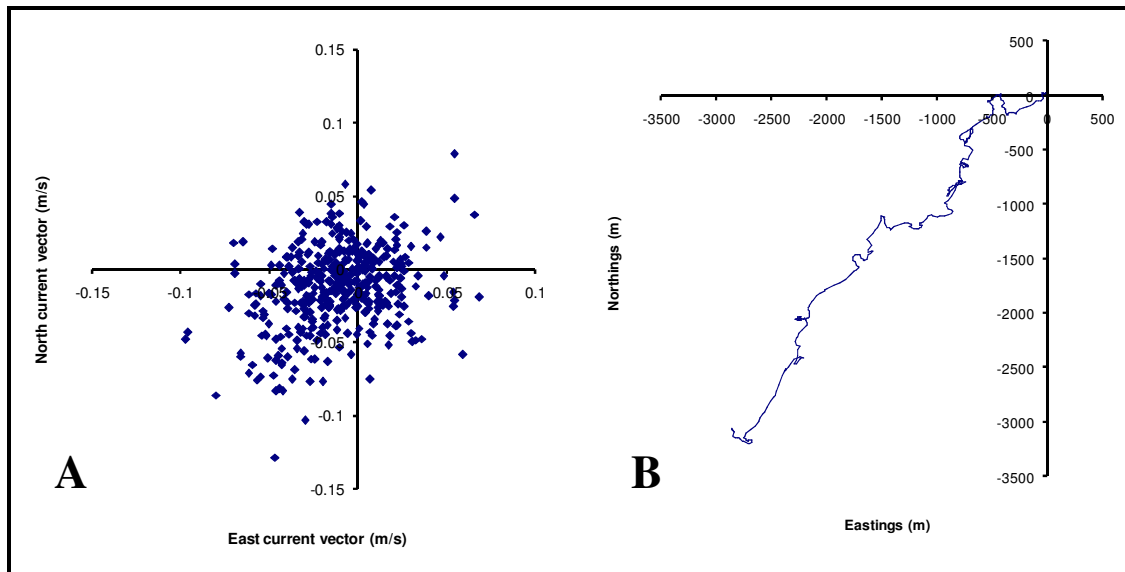


Figure 7: Scatterplot (A) and cumulative vector plot (B) of water flow at 8 m near the cages at Loch Linnhe (see Figure 2 for deployment position)

The mean current speed at 5 m depth in Loch Linnhe was 0.048 m/s and at 8 m depth was 0.035 m/s. Though the currents were apparently indistinct in terms of speed and direction over the three days of the trial (Figures 6a and 7a), there was a very distinct residual direction to the south-west (Figure 6b and 7b). The current direction and residual flow at Linnhe does not specifically indicate the complicated flow structure at this site. Over repeated days during the trial the natural topography of this location caused variable water flow in and around the cages, which resulted in limited drogue data and difficulty in identifying a specific point for the fixed point sample collection. Despite the fact that the tarpaulin was dropped some two hours after the tide had turned, initial movement in a south-east direction, as indicated by the residual flow (Figures 6b and 7b), was followed immediately by movement back and forth from the cages which affected the reliability of the data collected at the drogue positions.

Camas Glas

The scatterplot and cumulative vector plot for the currents at 5 m and 8 m depth are given in Figures 8 and 9 respectively.

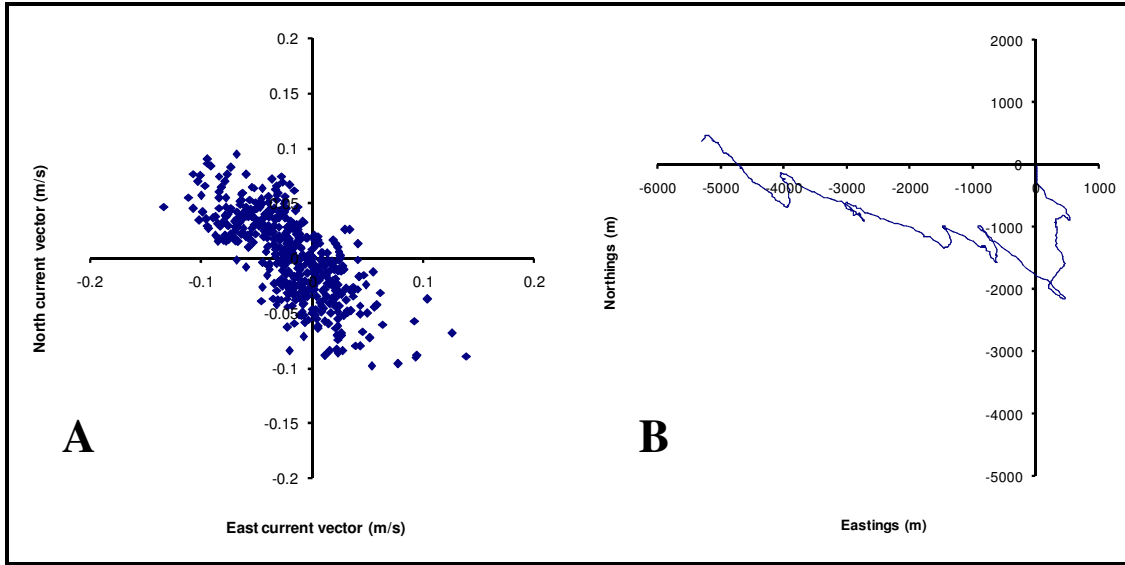


Figure 8 Scatterplot (A) and cumulative vector plot (B) of water flow at 5 m near the cages at Camas Glas (see Figure 1 for position of deployment)

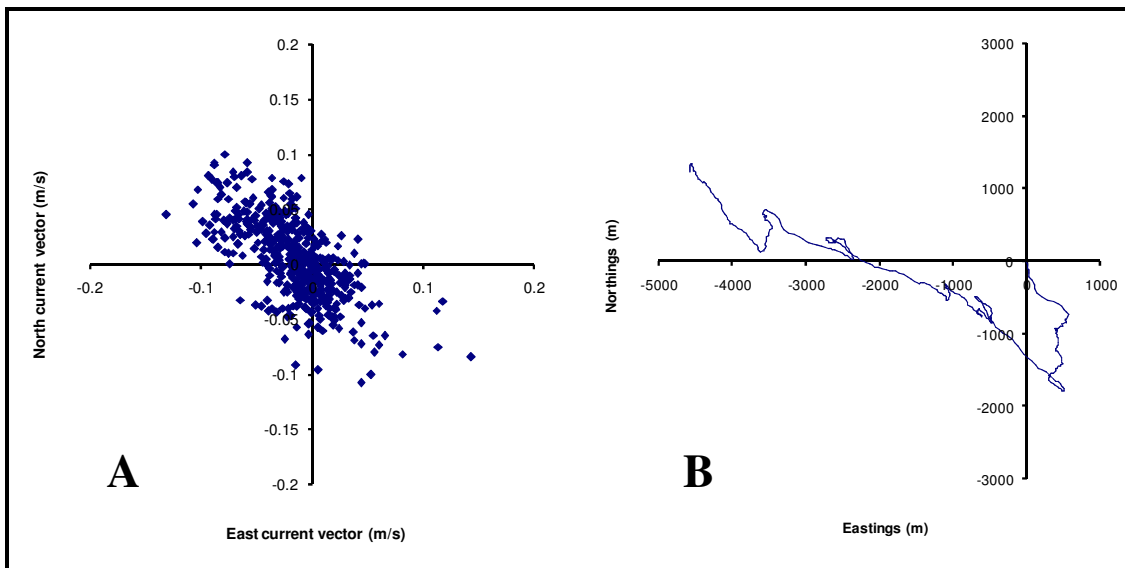


Figure 9 Scatterplot (A) and cumulative vector plot (B) of water flow at 8 m near the cages at Camas Glas (see Figure 1 for position of deployment)

The scatter plots in Figure 8a and 9a shows a distinct north-west/south-east current flow with a mean current speed of 0.049 m/s at 5 m and 0.045 m/s at 8 m. The cumulative vector plot shows a main residual direction at both depths in a north-westerly direction.

The trials and sample collection took place during the South-easterly flow during the ebb tide.

Cypermethrin concentrations

Trial One: Camas Glas

Trial 1 at Camas Glas was conducted using large fish near harvest weight. Treatment 1 (no fish) was successful and samples were collected, along with fixed position and drogue samples. There was a technical problem with the application of the tarpaulin at the site during the second day, when the low biomass treatment (II) was being assessed. Prior to the application of the dose fish were observably stressed and their welfare became a concern. It was agreed that the treatment would be abandoned, the tarpaulin was removed and the net dropped to its full depth. After detailed discussions with the company Health Manager it was agreed that treatment III, on a larger biomass of fish the following day, should be abandoned. Samples collected during the **no-fish** treatment, were used for analytical testing and method development only.

Trial Two: Linnhe

Treatment I: No fish

It was not possible to be absolute about the volume of water enclosed within the tarpaulin, although following the standard treatment method, with a well-hung tarpaulin and no billows, the presumed nominal applied dosage was 5.78 $\mu\text{g/l}$ (based on the criteria in Table 2). Initial measured concentration ranged between 3.62 and 9.92 $\mu\text{g/l}$ at each of the four corners after 10 minutes, and reduced slowly until the final mean concentration was 5.508 $\mu\text{g/l}$ at 50 minutes after treatment (Table 4).

The inequity of measures at the four corners during the early stages of the treatment resulted from a lack of full dispersal throughout the tarpaulin. As there were no fish present during this treatment the amount of water movement was limited to that generated by the oxygen diffusers. During the initial stages “hotspots” of cypermethrin at higher concentration were therefore recorded. Variability between the replicate samples reduced with time and based on the measured values at 50 minutes, full dispersal throughout the tarpaulin was likely to have taken up to 1 hour or longer.

Presuming that the water volume was as identified in Table 2, then it is assumed that the difference (0.27 $\mu\text{g/l}$) was due to uptake by the net and tarpaulin, both of which were present during treatment. There is currently no method to assess cypermethrin concentration on nets and tarpaulin, so specific uptake could not be verified. The reduction in the water concentration of cypermethrin was not excessive and the fish (in subsequent treatments) can be assumed to have received a correct dose.

Table 4: *Corrected concentration of active ingredient, Cypermethrin before and after treatment with EXCIS. Trial 2, Linnhe site, loch Linnhe conducted 16th – 19th October 2006. Treatment I (no fish). Treatment time 1 hour.*

Time post treatment start (mins)	Corner	Replicate no. (mVolts)		Mean (area units)	Conc. in seawater µg/l
		1	2		
predose inside cage	1	75082	119626	97354	0.023
predose inside cage	3	211403	35274	123338.5	0.029
predose outside cage		95827		95827	0.022
10	1			42865798	9.921
10	2			39434666	9.127
10	3			15647956	3.622
10	4			24989228	5.784
10 min Mean Conc					7.113
30	1			37261425	8.624
30	2			48148126	11.144
30	3			26104883	6.042
30	4			21773501	5.039
30 min Mean Conc					7.712
50	1			28887313	6.686
50	2			26862596	6.217
50	3			14988240	3.469
50	4			24465779	5.662
50 min Mean Conc					5.508

Nominal concentration applied to the cage was 5.78µg/l (500ml EXCIS in 864m³ of seawater)

Treatment II: Low biomass of fish

Although referred to as low and high stocking density treatments the fish size in both cages was similar.

During treatment II with “low” biomass of fish the mean concentration of cypermethrin within the tarpaulin did not vary significantly between the time points (One-way ANOVA F=0.110; df=3; p=0.897), with mean concentrations of 1.174µg/l after 10 minutes, 1.204µg/l after 30 minutes and 1.050µg/l after 50 minutes of treatment (Table 5). A Kruskal-Wallis Oneway Analysis of Variance on Ranks shows there is a significant difference between the initial dose in Treatment I (no fish) and the dose measured after 50 min treatment in Group II (H=9.066; df=3; p=0.028).

It would appear that during the treatment period there is significant uptake of cypermethrin by fish over a relatively short time period, such that the concentration in the water does not reach the actual applied dose level at any time point during the treatment. This may be important for modelling purposes if the assumption is that after the tarpaulin is released the concentration in the water is 5µg/l. The quantity released to the environment post treatment is significantly lower than this.

Table 5: *Corrected concentration of active ingredient, Cypermethrin before and after treatment with EXCIS. Trial 2, Linnhe site, loch Linnhe conducted 16th – 19th October 2006. Treatment Group II (low biomass of fish). Treatment time 1 hour.*

Time post treatment start (mins)	Corner	Replicate no. (mVolts)		Mean (area units)	Conc. in seawater µg/l
		1	2		
predose inside cage	1	39975	27077	33526	0.008
predose inside cage	3	57378	60594	58986	0.014
predose outside cage		25268	58481	41874.5	0.010
10	1			8426011	1.950
10	2			516269	0.119
10	3			4750190	1.099
10	4			6591925	1.526
10 min Mean Conc					1.174
30	1			5101205	1.181
30	2			3675712	0.851
30	3			5558873	1.287
30	4			6463980	1.496
30 min Mean Conc					1.204
50	1			4856443	1.124
50	2			3525913	0.816
50	3			4239124	0.981
50	4			5516586	1.277
50 min Mean Conc					1.050

Nominal concentration applied to the cage was 5.78µg/l (500ml EXCIS in 864m³ of seawater)

Treatment III: High biomass of fish

The results for the treatment III, with higher stocking density of fish are given in Table 6. There is a clear decrease in the concentration of cypermethrin in the cages from the nominal calculated treatment dose (5.78µg/L). The concentrations decreased rapidly in the first 10 min of the trial to a mean of 1.98µg/L in the four corners and continued to decrease, giving a final dose at the end of the trial (at 50 minutes) of 1.181µg/L. A Oneway ANOVA showed that there was a significant decrease in concentration between 30 and 50 min into the treatment period (F=9.619; df=2; p=0.006). A Kruskal-Wallis Oneway Analysis of Variance on Ranks showed that there was a significant difference between the initial concentration (assumed to be the Treatment I 10 min concentration) and the cypermethrin concentrations after 30 and 50 mins (H= 12.64; df= 3; p=0.005).

Table 6: Corrected concentration of active ingredient, Cypermethrin before and after treatment with EXCIS. Trial 2, Linnhe site, loch Linnhe conducted 16th – 19th October 2006. Treatment Group III (high biomass of fish). Treatment time 1 hour.

Time post treatment start (mins)	Corner	Replicate no. (mVolts)		Mean (area units)	Conc. in seawater $\mu\text{g/l}$
		1	2		
predose inside cage	1	59083	48446	53764.5	0.012
predose inside cage	3	41139	38553	39846	0.009
predose outside cage			41314	41314	0.01
10	1			9234817	2.137
10	2			10251688	2.373
10	3			8339101	1.93
10	4			6393748	1.48
10 min Mean Conc					1.980
30	1			5660082	1.31
30	2			6146371	1.423
30	3			7101090	1.644
30	4			4409378	1.021
30 min Mean Conc					1.350
50	1			5490933	1.271
50	2			5237105	1.212
50	3			4479191	1.037
50	4			5206175	1.205
50 min Mean Conc					1.181

Nominal concentration applied to the cage was $5.78\mu\text{g/l}$ (500ml EXCIS in 864m^3 of seawater)

Fixed position and drogue data

Throughout the trial at Linnhe there was a disparity between the expected current flow (as measured in 2004 - Marine Harvest, 2004) and confirmed through currents readings during the trial (see above); and the observed flow regime as it affected the drogues.

The drogues were initially observed to drift away from the cages in the expected direction. Almost immediately they returned to the opposite direction and bobbed in the water close to the cages. This was followed by a continuing movement away from and towards the cages. As a result it was not possible to discern where the plume front was and it was subsequently difficult to identify the correct position to take the fixed point samples on the day. However, the drogues remained in the water and they did eventually move away from the cages and were moved by the current flow

The fixed position (determined from past hydrography rather than movement on the day) was 10m from the cage in a south-easterly direction coincident with the expected residual flow. Samples analysed showed that cypermethrin was encountered at 1m depth at t_{60} (just after tarpaulin release), then not at t_{65} and t_{70} but again at t_{75} and t_{80} . This disparity appears to confirm the observed in/out movement of the water as described above.

At the fixed position the maximum concentration at 1m depth was $1.42\mu\text{g/l}$ at t_{60} , this concentration being similar to that measured at t_{50} within the tarpaulin. At t_{80} the

concentration had decreased to 0.62µg/l. Overall, there was insufficient data to provide any defined correlation in time or distance travelled. During the same collection no cypermethrin was measured at lower depths.

As a result of the drogue movement in and out, there was no confidence that the plume front could be discerned and no samples were collected at drogue positions.

Trial Three: Camas Glas

Treatment I: No fish

The results for treatment I are given in Table 7. Mean concentrations varied from 3.98 to 3.48µg/L. A Oneway ANOVA showed that there was no significant difference between the concentrations in water over the treatment period (F=1.674; df=2; p=0.24). Though this appears to be considerably less than the calculated treatment dose of 5.56 µg/L (no statistical comparison possible).

Table 7: *Concentration of active ingredient, Cypermethrin before and after treatment with EXCIS. Trial 3, Camas Glas site, Loch Sunart, conducted 22nd – 24th May 2007. Treatment Group I (no fish). Treatment time 1 hour.*

Time post treatment start (mins)	Corner	Replicate no. (mVolts)		Mean (area units)	Conc. in seawater µg/l
		1	2		
predose inside cage	1	16799	5696	11248	0.028
predose inside cage	3	9724	6312	8018	0.02
predose outside cage			9527	9527	0.024
10	1			1476358	3.731
10	2			1602118	4.049
10	3			1709087	4.319
10	4			1520998	3.844
10 min Mean Conc					3.986
30	1			1675327	4.234
30	2			1456867	3.682
30	3			1022381	2.584
30	4			1385453	3.502
30 min Mean Conc					3.501
50	1			1268765	3.207
50	2			1349287	3.41
50	3			1487896	3.76
50	4			1397743	3.533
50 min Mean Conc					3.478

Nominal concentration applied to the cage was 5.56µg/l (400ml EXCIS in 720m³ of seawater)

Treatment II: Low biomass of fish

The results for the treatment II (low biomass of fish) are given in Table 8. The concentrations varied from 1.69 to 1.35 µg/L and a Oneway ANOVA showed there was no significant difference in concentrations during the treatment period (F=0.570, df=2;p=0.585). As in the previous trial at Loch Linnhe, an ANOVA showed there was a significant difference between the cypermethrin concentration illustrated by the Treatment I 10 min concentration (no fish) and all measured concentrations during the Treatment II trial with a low biomass of fish (F=31.946;df=2;p=<0.001).

Table 8: *Concentration of active ingredient, Cypermethrin before and after treatment with EXCIS. Trial 3, Camas Glas site, Loch Sunart, conducted 22nd – 24th May 2007. Treatment Group II (Low biomass of fish). Treatment time 1 hour.*

Time post treatment start (mins)	Corner	Replicate no. (mVolts)		Mean (area units)	Conc. in seawater µg/l
		1	2		
predose inside cage	1	8665	9837	9251	0.023
predose inside cage	3	6998	20189	13594	0.034
predose outside cage		5671	7987	6829	0.017
10	1			654045	1.653
10	2			79810	0.202
10	3			816772	2.064
10	4			651890	1.648
10 min Mean Conc					1.392
30	1			675890	1.708
30	2			689927	1.744
30	3			588720	1.488
30	4			722148	1.825
30 min Mean Conc					1.691
50	1			567787	1.435
50	2			595665	1.505
50	3			561934	1.42
50	4			409827	1.036
50 min Mean Conc					1.349

Nominal concentration applied to the cage was 5.56µg/l (400ml EXCIS in 720m³ of seawater)

Treatment III: High biomass of fish

The results for the Treatment III (with higher biomass of fish) are given in Table 9. The concentrations during the treatment period varied from 1.30 to 1.12µg/L. A Oneway ANOVA showed that there was no significant difference between the cypermethrin concentrations during the treatment, though a Oneway ANOVA indicated that there was a significant difference between the initial treatments (indicated by the Treatment I 10 min concentration) and all concentrations found during the Treatment III trial (F=40.339;df=3;p=<0.001).

Table 9: *Concentration of active ingredient, Cypermethrin before and after treatment with EXCIS. Trial 3, Camas Glas site, Loch Sunart, conducted 22nd – 24th May 2007. Treatment Group III (High biomass of fish). Treatment time 1 hour.*

Time post treatment start (mins)	Corner	Replicate no. (mVolts)		Mean (area units)	Conc. in seawater µg/l
		1	2		
predose inside cage	1	7502	11961	9732	0.025
predose inside cage	3	21003	21274	21139	0.053
predose outside cage		2587	5412	4000	0.01
10	1			654045	1.653
10	2			475487	1.202
10	3			90867	0.23
10	4			790876	1.999
10 min Mean Conc					1.271
30	1			490765	1.24
30	2			548790	1.387
30	3			379865	0.96
30	4			638178	1.613
30 min Mean Conc					1.300
50	1			367259	0.928
50	2			453467	1.146
50	3			518972	1.312
50	4			438765	1.109
50 min Mean Conc					1.124

Nominal concentration applied to the cage was 5.56µg/l (400ml EXCIS in 720m³ of seawater)

Fixed position and drogue data

Fixed position and drogue position samples were collected during treatment II and treatment III and these have been analysed on the TRACE GLC but the information was not post-processed to estimate measured concentrations.

Comparing trials

Given the differences in biomass between the treatments and between trials it would be inappropriate to directly compare the trials on the basis of fish biomass and stocking density. O'Shea et al (2006) provide a conversion factor to calculate salmon skin surface area from a measure of weight. Using the data available (notes to Table 1) the surface areas of the fish in treatment II and III in trials two and three were estimated (Table 10) and these were used to evaluate whether there were differences between treatments containing designated high and low biomass. Given the similarity between the treatments at each trial (biomass) and the fact that there is no consistency in the concentration per surface area of fish between the trials no specific relationship was identified (Figure 10).

Table 10: *Estimated surface area of all fish contained in treatment cages during trials one at Linnhe on 16th October 2006 and Camas Glas on 21st May 2007. Surface area based on O'Shea et al 2006 where $SA=14.93W(g)^{0.59}$ for Atlantic salmon.*

Trial	"biomass" designation	Ave fish weight (g)	Area of one fish (cm ²)	no. fish	total surface area m ²
Two (Linnhe)	low	155	292.7	129000	3775.2
	high	210	350.1	127000	4446.0
Three (Camas Glas)	low	1066	912.9	45000	4108.2
	high	990	873.9	49000	4282.3

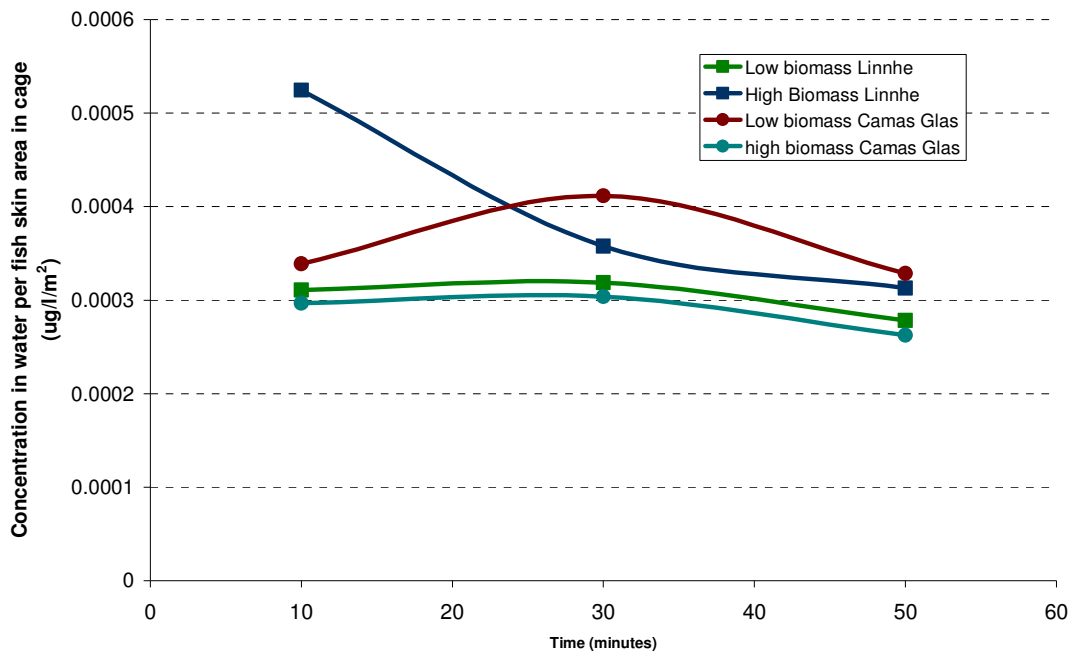


Figure 10: **Change in concentration of cypermethrin in water over time taking into account variation in fish skin surface area.**

Discussion

Practical limitations

There was considerable alteration between the documented number of samples within the original proposal and the number of samples taken at the sites, which lead to restrictions in the ability to process all samples. There were practical limitations within the project funding in relation to the transfer of analysis from Vericore to the Institute of Aquaculture. This resulted in more focus on processing samples for method development and treatment dose concentrations prior to the tarpaulin being released, with less resource devoted to post-release concentrations.

Pre-dose concentrations

Within each of the three treatments at each trial there is little variation between the pre-dose samples taken within the tarpaulin over the three days of use within each trial (Tables 4 – 9). Although cypermethrin is expected to be adsorbed onto the tarpaulin (and net) during successive treatments, based on the data presented, uptake is relatively low compared to the overall volume of cypermethrin used.

Consequently under normal treatment conditions, where the same tarpaulin is used over consecutive days, such use does not appear to increase the overall concentration found in subsequent treatments. Although not specifically measured (no protocol available) any cypermethrin that does bind to the tarpaulin either remains bound when the tarpaulin is subsequently used or remains bound in the short term only (a matter of hours). No analysis was carried out of the breakdown products of cypermethrin, to know whether these were present in the water prior to each treatment.

Dose concentrations and fish uptake

The treatment regime during each of the trials conducted shows that the dose part of the treatment is readily controlled and detailed data can be generated. SAMS (2005), using a similar technique to that applied here, showed that the concentration of cypermethrin in the treatment water in the tarpaulin at the end of the treatment period (but prior to tarpaulin removal and release to the environment) was 3.25 and 2.96 $\mu\text{g/l}$ at two trials. This is approximately 75-50% higher than any data recorded here, where the mean was 1.349 $\mu\text{g/l}$ after 50 minutes in trial two at Camas Glas (Table 5) and 1.124 $\mu\text{g/l}$ in trial three at Camas Glas (Table 9).

In comparing concentrations, SAMS (PAMP, 2005) assumed a standard nominal concentration of 5 $\mu\text{g/l}$ during their trials and their report contains no information regarding tarpaulin volume measured or assumed, or calculation of actual nominal concentrations. Speculatively, it might be possible that the nominal starting concentrations during the PAMP trial were higher than identified (due to a low water volume, perhaps), making the apparent loss (attachment to fish, nets tarpaulin and so on)

appear lower than they actually were. There may have also been a differential in the organic load in the water or attached to nets and tarpaulin that may also account for some of the difference. There is, however, no specific explanation for the difference.

There was a differential between the final measured concentrations under treatment I (no fish) between trials 2 (Linnhe) and 3 (Camas Glas), despite similar calculated nominal concentrations. This is important as it informs the extent to which error can be determined within the estimated reductions in concentration due to fish uptake. Within the context of the project standard methods were used, in which it was not possible absolutely to determine water volumes. A conservative estimate will be the nominal concentrations were within $\pm 30\%$, which is the difference between nominal concentration and the measured concentration in treatment 1 in trial 3. However, from a practical viewpoint it must be recognised that measuring water volume precisely, when using a tarpaulin, will not be possible.

Given that (1) the no-fish concentration remained similar to the expected treatment dose applied and (2) the two remaining treatments showed a significantly lower maximum concentration; this indicates that through the treatment there is significant uptake of cypermethrin by fish. The concentration in the water does not reach the “applied dose” concentration at any time point in the treatments containing fish (Treatments II and III). There was no significant difference between the concentration at each of the time points (10, 30 and 50 minutes) within each of the treatments. There was, however, a significant difference between the initial treatments as indicated by the Treatment I 10 min concentrations and all concentrations found during the Treatments II and III, which provides evidence of the high fish uptake.

This information by itself does not suggest that the treatment period should change, but it remains possible that in the event the tarpaulin has to be removed earlier than the recommended 1 hour treatment, on fish welfare grounds for example, fish may continue to receive the required concentration on their skin tissue.

Recognition that a lower concentration will be released to the environment at the end of treatment has implications for the modelling parameters applied by SEPA to give consent to discharge. At present the AutoBath model used by SEPA to estimate the mixing zone and concentrations after a given time period indicates how much cypermethrin can be approved for use by farmers and assumes that $5\mu\text{g/l}$ leaves the tarpaulin after treatment. This project has shown that the measured concentration leaving the cage is 3.7 to 4.4 times lower than this and therefore offers the potential for a change in one of the model assumptions.

It might be argued that the assumption that the dose level leaving the cage provides an in-built safety factor in the environmental quality standard. However, reducing the expected concentration leaving the cage to, for example $2.5\mu\text{g/l}$, retains some degree of safety factor within the assumption. This factor takes into account the variability in water volume discussed above. Such a change might allow an increase in cypermethrin use by a factor of 2.0 in order to be able to treat more fish biomass, whilst remaining within the

EQS. Such an increase would be over and above the revised EQS issued by SEPA in 2006 which increased the permitted use by a factor of 2.8 at that time. However, SEPA is more generally committed to reducing the amount of chemical released into the environment and it should be recognised that these results also suggest that the initial treatment dose concentration might be reduced, thus allowing a greater biomass of fish to be treated with less cypermethrin.

A recommendation that does come out of the work conducted is a need to undertake a focused short-term project to confirm 1) the method for removing contaminants (e.g. lipid) from the fish skin samples before analysis and 2) to investigate uptake on fish surfaces, as it relates to a reduction in the concentration in the water, through focused tank trials. An attempt was made to determine whether biomass in the cage had an effect on concentrations released to the environment. It would appear that biomass *per se* does not impact the resulting concentration in the water during treatment with cypermethrin. Logically there should be a correlation between concentration and skin surface area, but this was not specifically identified during this project. O'Shea et al (2006) provide a conversion factor to calculate salmon skin surface area from a measure of weight but this does not take account of gill area, for example, to which cypermethrin will also attach. The relationship between concentration and surface area also does not take into account other factors; such as lice load, organic content in the water and on surfaces; which act to further confound any readily identifiable relationship. However, such a study will enable a more definitive assessment of the concentration remaining in the treatment water that will then be released to the environment. Although it should be recognised that other factors such as fish movement and the homogeneity of the treatment dose within the overall treatment volume will also affect the final discharge concentration.

Post-treatment concentrations

The samples taken post-release did not show any specific uniformity or correlation to collection time after release, which limited the validity of that data to provide any new meaningful information on concentrations in the water after treatment was completed. No additional information on dilution and dispersal of the cypermethrin is available, therefore, to complement the data provided by the PAMP study (SAMS, 2005).

During each of the three trials there was necessary variation in protocol (e.g. distance for fixed point collections) to take account of:

- the differences in duration and extent of the tarpaulin removal procedure,
- the variability in apparent hydrography and the observed situation at the time of sampling,
- variation in the time the treatment took place after the tidal swing and
- practical limitations in treatment regime applied by the different sites.

Such variability could not have been foreseen and whilst all of the samples have been analysed (and are available for post-processing should funding become available) only a limited number of samples (mainly dosing samples) have been post-processed.

Further observations

During the trials it was observed that the removal of the tarpaulin itself created significant water movement and disturbance, both on the surface and throughout the tarpaulin depth as fish movement increased. Immediately after this the situation was further complicated by the dropping of the net to its full depth, as an immediate requirement to improve the water quality and available space for the fish after treatment. Surface and sub-surface structures at fish farms can also have a bearing on water movement surrounding cage sites (Innoue, 1990; Beveridge, 2005).

The combination of these two practical activities appears to result in very turbulent water being generated at the cages. In this context it remains important to more fully understand the initial, localised movement of the cypermethrin-containing water at the cages. For example a future study might focus specifically on the concentration changes close to the cages, as it is influenced by the combination of tarpaulin removal and net dropping. A better understanding of changes and concentrations at the cages during the early phases of tarpaulin release and net drop would help inform models of plume development.

Such a study should also have equipment available that simultaneously takes water samples from two or three different depths (e.g. perhaps using a series of three adapted Van Dorn water samplers linked via sequential messengers). In this study water samples collected “at the same time point” were actually collected over a two minute period, sequentially at 8m, 5m and 1m depths using a single Van Dorn water sampler, rather than at a single simultaneous time point at all depths. As a result it was never clear at the time of the trials whether the plume was being “captured” at all, something which only became clear after the samples had been analysed and were shown to contain no cypermethrin.

Finally, given the limited data generated by this project and the limited measures generated by the PAMP project using the same techniques (SAMS, 2005) there has to be some concern about whether drogue use at fish farms is sufficiently robust to give a reasonable approximation of the subsequent movement of an idealized plume of water containing cypermethrin. It may be possible to better understand the plume using dye studies, where the “concentration” can, to some extent, be observed. In order for this to be successful there is a need to integrate dye within the cypermethrin matrix, but to ensure the fundamental characteristics of cypermethrin, such as its low octanol/water partition coefficient remain unchanged.

Part 3 – A theoretical appraisal of the implications of using skirts for treatment.

Introduction

Complete enclosure of a volume of water using a leak-proof tarpaulin is one of two methods authorised for treating fish with the anti-sea lice chemical cypermethrin. The second method uses treatment in wellboats, licensed by the Fisheries Research Service, under part II of the Food and Environment Protection Act 1985. The latter method is not discussed further here.

It is generally acknowledged that the use of the tarpaulin method can be limited at sites that maintain high current flow, where adding what is essentially a large square of material under and around a cage is physically difficult and can require a considerable time to deploy. This procedure can increase fish stress and affect their welfare and so does not provide a viable alternative under these circumstances. In addition, deploying a large tarpaulin in extreme weather conditions may also be restrictive, limited to favourable weather condition only at certain more exposed sites. These limitations leave certain sites in Scotland vulnerable to a heavy reliance on SLICE as the only treatment method available. As discussed previously reliance on one method of treatment can lead to sea lice strain resistance, which leaves the potential for no viable means of treating for sea lice at certain sites.

One alternative to the fully enclosed tarpaulin is the use of skirts. These are ribbons of weighted tarpaulin that surround the cage to a depth of approximately 8-10m but do not have an enclosed bottom and are therefore open to water exchange (bottomless-bath treatments). In certain circumstances skirts are used, where a veterinarian has prescribed treatment on fish welfare grounds but a tarpaulin cannot be deployed. Thus, although they are not a sanctioned method, skirts are used in Scotland in certain circumstances.

The study of treatment using skirts has not been carried out previously and there was a need to investigate the implications of using this technology. The concern arises that skirt use perhaps necessitates increased use of the active ingredient, cypermethrin, in order to maintain the recommended treatment dose of 5µg/l, to overcome the likely losses from water exchange. This would require the use of a larger quantity of cypermethrin than can currently be approved for discharge consent by the regulator, which may lead to non-treatment, even where it is deemed to be required.

A practical investigation of skirt treatment in the field did not form part of this project. Instead a theoretical analysis of the potential to use skirts was conducted, through an assessment of available metadata and a limited number of flume tank trials.

Background

The study was undertaken to assess the hydrodynamics of fish cage facilities adapted for skirt treatment through the application of therapeutic agents such as cypermethrin. The motivation for such a study arises on the one hand from the SEPA requirement¹ that no bath treatment chemical should be discharged without having been applied within a fully contained enclosure and, on the other hand, from the common practice of employing partial enclosure (skirt treatment) in Norway and other countries. The SEPA concern is that treatment with partially-enclosed skirt configurations will result in the use of much larger quantities of chemical than with full enclosure because of the necessity to achieve optimum working concentration while compensating for the dilution due to water exchange through the exposed cage openings. SEPA condition A1.1 recognises that, in exceptional circumstances, skirt treatments may be approved if the discharger can demonstrate that (i) a more efficient treatment can be achieved with this arrangement (i.e. less need for repetitive treatments and less overall compound use) than with the enclosed tarpaulin deployment and (ii) compliance is maintained with EQS restrictions.

Literature searches and informal enquiries reveal that no modelling investigations have been carried out on the hydrodynamics of skirted cages containing confined volumes of active fish. Likewise, no field investigations can be found in the open literature in which the explicit focus is placed upon the entrainment of the therapeutants into the water column and cross current. There is evidence² from sampling data that cypermethrin and deltamethrin falls through the bottom of the skirted cage during treatment and can be detected at depths of 10 - 15 m, depending upon site and conditions. Similarly, there is anecdotal evidence (but no available documentation) indicating that the diluted cypermethrin within the treated part of the cage has a small negative buoyancy with respect to the surrounding sea water, such that, in the undisturbed state, the chemical will gradually sink under gravity into the water column below the treated volume.

Skirt treatments operate in various forms². Ideally, the skirt will totally surround the fish cage and the net holding the fish will be raised so that the skirt goes several meters below the bottom of the cage for treatment (example would be fish in net raised to 5 m depth and skirt being 8 m deep). In practice, several variations of this are used: the cage is not lifted and the fish are fed so they come to the surface. The skirt only partially surrounds the cage 50% or 75% and this is often against the direction of the prevailing current so the skirt partially goes under the cage (if lifted). Typical cage sizes are 20m x 20m square, 24m x 24m square, 60m, 80m (very common), 90m, 100m 120m and now 160m circumference circles. The cages typically hold biomasses pre-harvest of 150 tons to 800 tons of fish per cage. Treatments are usually diluted with seawater to several tens or hundreds of litres for administration to ensure a more homogeneous dispersion of the active compound throughout the overall treatment volume. These are administered by water cannon, bucket, or leaky hose methods. Often a top-up treatment is used part way through the treatment period. For bigger pens (90 m - 120 m in circumference)

¹ http://www.sepa.org.uk/pdf/guidance/fish_farm_manual/attachments/A1.pdf

² personal communication, John Marshall, Novartis

containing several hundred tons of salmon it is held to be difficult (and risky) to operate a totally closed tarpaulin³. In such cases the skirt method may be applied; the pen volume is reduced by raising the pen bottom until a fish density of 60 - 90 kg/m³ and an open tarpaulin (without bottom) is mounted along the sides of the pen. This method is deemed to have the same oxygen requirements as the closed tarpaulin methods. All the mentioned methods can be used with the different active agents made for lice bath treatments.

Modelling

Hydrodynamic parameters

The idealised configuration considered for the study is shown in Figure 11. The fish cage has lateral dimension L and is totally surrounded by a skirt of length H . The portion of the cage open to the surrounding water is assumed to be horizontal and to lie at a depth h below the free surface of the nominally-uncontaminated water column. The skirted cage is placed in a cross current U that is assumed here to be steady and uniform over the water column. The initial concentration (i.e concentration at time $t = 0$) of uniformly-distributed therapeutant within the cage is C_0 . (The uniform distribution of chemical is assumed to be achieved by the turbulence generated by the moving fish and the circulation resulting from any artificial aeration applied externally). The mesh size of the cage is always sufficiently small compared with the overall dimensions of the cage that it is not considered as a crucial parameter in the exchange process (see below).

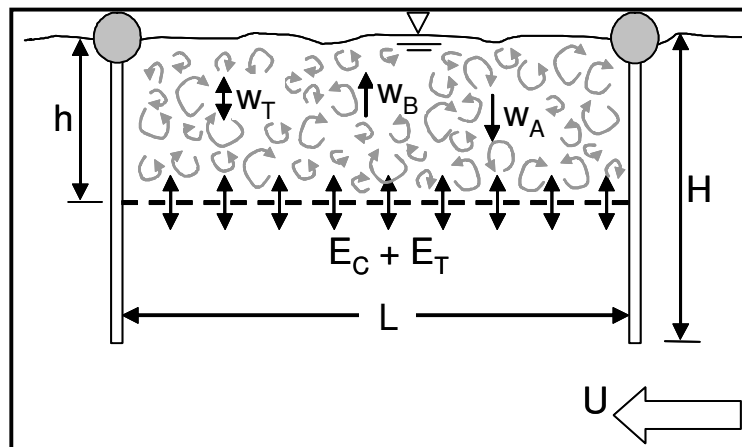


Figure 11: *Schematic representation of the hydrodynamic processes within the skirted cage.*

³ (<http://www.ecoserve.ie/projects/sealice/caligus2.html>)

The important hydrodynamic processes governing the time-dependent concentration $C(t)$ of the therapeutant in the skirted cage are assumed to be the following: (i) entrainment into the cage of initially-uncontaminated water outside the cage (but within the skirts) by the turbulence generated by the contained, moving fish, (ii) entrainment into the cross current of the whole trapped water volume within the skirted region, (iii) the generation of a mean circulation within the skirted cage by the action of (a) the cross current and (b) the external aeration and (iv) a downward flux (with velocity $w_A(t)$) of therapeutant due to differential buoyancy. In Figure 11, E_C and E_T represent the entrainment velocities associated with the cross-current and the fish-induced turbulence respectively; the velocities w_T , w_B and w_A represent typical instantaneous values of the components associated with the turbulence field, the external aeration and the negative buoyancy of the chemical solution respectively. The time-dependent dilution $S(t)$ of therapeutic chemical within the cage is defined by the ratio $C(t)/C_0$.

For the purposes of the present investigation, it is not necessary to distinguish between the two entrainment mechanisms and, in what follows, both will be combined into a single process, with entrainment velocity E . Likewise, unless specified otherwise, the circulation established within the volume enclosed by the skirt will not be treated separately, other than through its effect upon the overall entrainment process discussed above. For the purposes of this theoretical study, where the aeration and negative buoyancy contributions to the dilution within the cage are not specifically under evaluation, these will be assumed to be negligible.

The parameterisation of the turbulence field generated by the moving fish within the cage is a serious challenge! In previous hydrodynamic modelling studies of turbulent entrainment, particularly those conducted within the laboratory with the aim of quantifying turbulent entrainment coefficients, the preferred archetypal system for the generation of a turbulence field that represents well the turbulence fields in nature is the oscillating grid. There is a huge body of work on this topic cited in the literature but most of it is irrelevant to the present flow configuration. The oscillating grid system is deemed unsuitable for the cases considered here where the three-dimensional turbulent stirring of the fluid is accomplished by an array of arbitrarily-directed and random moving disturbances. An empirical approach will be followed in this aspect of the parameterisation

Modelling approach

The first intention was to base estimates of entrainment upon a desk study of published work. However, most of the existing and relevant literature (Armfield & Debler, 1993; Debler & Armfield, 1997; McGrath et al, 1997; Strang & Fernando, 2004) is concerned with turbulent entrainment from flows in which density effects are significant. For such cases there are well-established relationships (see, for example, Fernando & Hunt, 1997) between entrainment coefficients and the Richardson number Ri , though, unfortunately, such relationships are invalid for the cases ($Ri = 0$) under consideration here.

Instead, in view of the absence of any theoretical modelling framework for this problem, the approach taken has been (i) to design and carry out some new, simplified, idealised laboratory model experiments in order to obtain qualitative information on the flushing of the treated volume by the process of turbulent entrainment and (ii) to attempt to scale the results of these experiments by exploiting an analogy between the present flow type and others for which large scale experimental data have been obtained.

Laboratory model

The experiments were carried out in a small laboratory flume of width 80 mm and length 6 m, through which water could be passed at a steady prescribed discharge rate Q . The water depth in the flume could be varied by the insertion of suitable control structures and, in the cases to be described, a constant water depth of 240 mm was used. A model skirted cage arrangement was constructed from plastic mesh attached to rigid impermeable curtains (skirts) to resemble the arrangement shown schematically in Figure 11. The cage structure was attached to the flume and the position of the base of the cage could be adjusted to allow runs to be made with different values of h/H (see Figure 11). The turbulence produced by moving fish was simulated in the experiments by pairs of small electric motors fitted with submerged paddles operated in torsional oscillation mode. Such an arrangement produced an agitated flow field within the cage without mean circulation and the degree of turbulent agitation could be increased or decreased by control of the motor output speed. A schematic view of the arrangement is shown in Figure 12. Fluorescent dye was introduced into the “cage” to represent the dosing with therapeutic solution and the whole system was illuminated against a white background in order to gauge the intensity of the dye. The progressive dilution of the dye field by the entrainment processes described in above could be followed and measured by videotaping the system from the side. The videotape could then be analysed by means of the image processing option in the *DigImage* system to provide a time history of the dilution within the model fish cage as a function of (i) the cross current U and (ii) the ratio h/H (see Figure 11). In principle, the other dependent parameter here was the frequency of torsional oscillation of the motor paddles, though cases were considered only when the vigour of the agitation was sufficient to provide a turbulent motion field within the cage).

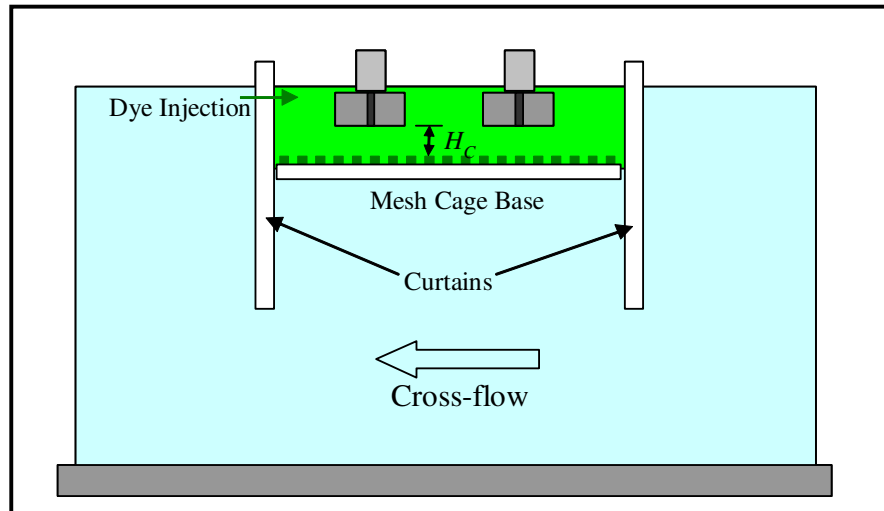


Figure 12: *Schematic representation of experimental set-up for the evaluation of flow regime and exchange using skirts as an alternative to full tarpaulin bath treatments.*

Dye dilution experiments

Five trial runs were to investigate the factors affecting the temporal variation in dye concentration within the model cage. The temporal variation in light intensity was averaged over a large area of the fish cage and it was assumed that the transmitted light intensity correlated linearly with the dye concentration. (Because of the qualitative nature of the experiments, no attempt was made to quantify the above correlation, particularly since the measured intensity was also affected by external factors such as changes in ambient light conditions within the lab). Thus, the experiments merely illustrated qualitatively the temporal change in dye concentration, although more detailed experiments within a controlled environment would clearly provide quantitative information.

Figure 13 shows the results for the case with no cross-flow within the flume (Run 1). The plot shows that the light intensity I increases (i.e. dye concentration decreases) with time t , while the rate of change is shown to decrease with time. This plot confirms that the dye concentration within the cage becomes increasingly and gradually diluted as a result of the turbulent diffusion of dye through the mesh of the base of the cage. The decrease in concentration with time is seen to be well represented by an exponential decay, with an e-folding time of about 250 -300s.

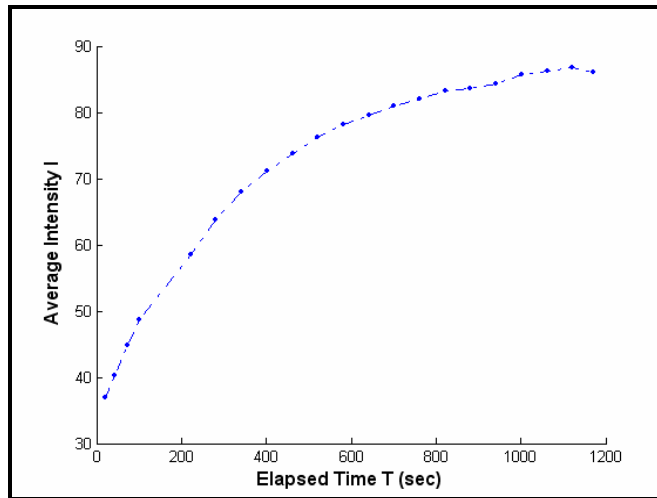


Figure 13: *Temporal variation in light intensity within cage with turbulent agitation but no cross-flow ($U = 0$).*

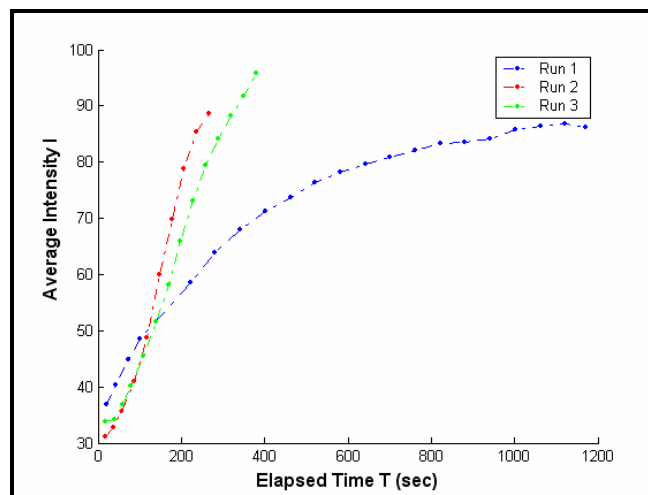


Figure 14: *Temporal variation in light intensity within model fish cage for Run 1 (no cross flow, $U = 0$), Run 2 ($U = 4.1 \text{ cm}\cdot\text{s}^{-1}$) and Run 3 ($U = 2.6 \text{ cm}\cdot\text{s}^{-1}$) under otherwise identical conditions.*

Figure 14 shows a comparison between runs with relatively high and low cross-flow values. The data from Figure 13 – the case with no cross flow – are superimposed for information. Although none of the runs was continued until the dye was completely flushed from the cage, the removal of dye by the action of the cross flow in both cases (runs 2 and 3) is seen to be markedly more rapid than observed in the reference case (Run 1) for which turbulent agitation alone only was applied. The plots on Figure 14 indicate that the time taken for dye to be flushed from the cage is almost an order of magnitude lower for cases with cross currents than with quiescent water conditions. The plots confirm also that the decrease in dye concentration (i.e. increase in light intensity) for the

higher cross-flow condition (run 2) was more rapid than for the lower cross-flow condition (run 3), suggesting that the entrainment of the dye at the bottom boundary was greater for larger values of the cross-flow current U , as expected.

It should be noted that the above mentioned runs were not repeated because the accuracy of the flow meters and the digital image processing software used to measure the dye intensity and the cross-current respectively were deemed to be sufficiently high (less than 1%) that reliance could be placed on results from single runs. Though the dye intensity values (I) determined digitally from pixel values recorded by the video camera were not calibrated absolutely, conditions of illumination and background lighting were unaltered between runs. In consequence, the differences in I values between runs are significant and real because the same calibration would apply to all. The tendency shown, namely the reduction in flushing time caused by increasing cross-flow velocity, is therefore significant. The properties of the flow demonstrated by Figure 14 that (i) a non-zero cross-velocity has a dramatic effect compared with the no-flow case and (ii) where a cross-flow is present, the magnitude of the cross-flow is less influential on the flushing are consistent with the generation within the cage of a large, cage-scale circulation that is relatively unaltered in form as the cross-flow is increased and is absent when no cross-flow is present.

The entrainment characteristics of the cross current are illustrated in Figure 15 by typical frames from the video image for a case with the base of the cage at almost the same depth as the bottom of the skirt ($h/H \approx 1$). In particular, the side views show how dye initially within the cage is entrained by the cross flow to form a shear layer containing relatively large, vortical coherent structures that scoop the dye from the cavity and into the cross current.

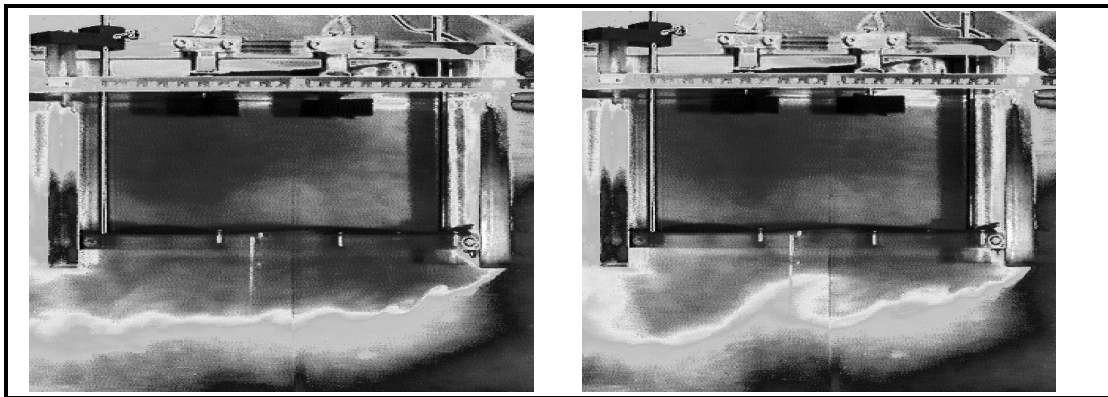


Figure 15: *Example images showing side view of the entrainment of dyed fluid from bottom of fish cage by cross-flow (direction shown). Base of “cage” corresponds to horizontal black line in the middle of the frame*

Figure 16 shows the influence of the turbulent agitation on the decrease in dye concentration within the fish cage, comparing runs 4 (without agitation) and 5 (with agitation) for constant cross-flow conditions. Although there were clearly different scales over which the light intensity changed within these two runs, it was observed that the run with agitation took slightly longer to reach a quasi-steady dilute dye concentration within fish cage. This slightly surprising result suggests that the turbulence field generated within the cage aids the retention of dye (i.e. therapeutic) within the cage, as compared to the case of a cross current alone.

In these runs the representation of the disturbance field by small electric motors is, of course, a gross oversimplification of the potential turbulent field. The low form drag associated with the motion of an individual fish means that flow separation downstream of it is unlikely to occur if such a fish is unconfined and swimming in isolation. Difficulties arise in practice, when groups of fish are present within a finite volume, each swimming in arbitrary directions with respect to the neighbouring fish and each experiencing collisions and penetrations of the surface of the water column. The resulting surface motion likely to be generated by a confined fish population then at least resembles a field of turbulence, which the use of motors is trying to replicate. This property of turbulence acting to increase dye retention would warrant further investigation.

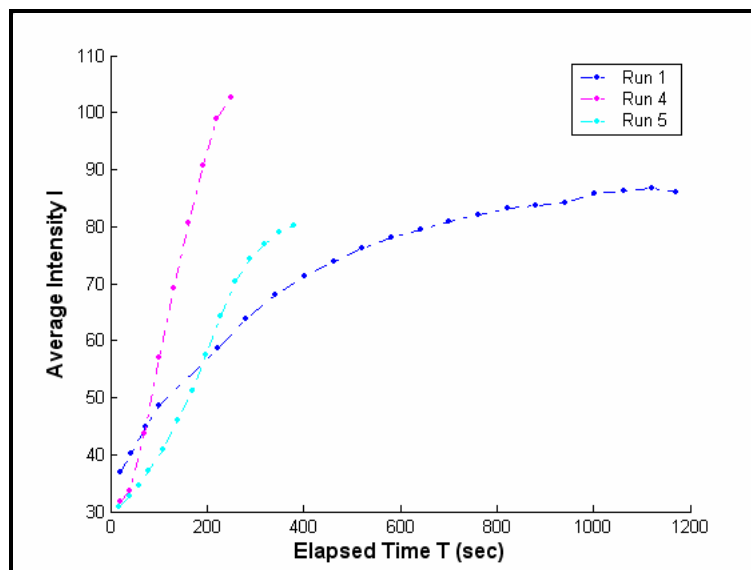


Figure 16: Temporal variation in light intensity within model fish cage for Run 1 (no cross flow, $U = 0$), Run 4 ($U = 2.6 \text{ cm.s}^{-1}$; with agitation) and Run 5 ($U = 2.6 \text{ cm.s}^{-1}$; no agitation) under otherwise identical conditions.

The above exploratory experiments were run with values of h/H almost unity, such that the base of the skirt and the base of the cage were almost coincident (see Figure 15). Three further experiments were carried out to determine whether differences in depth

between cage and skirt affect significantly the flushing of dye from the cage volume. In the notation of Figure 11, the ratio of h/H was varied between values of 0.3 to 0.7. In each case, dye fluid was introduced into the cage with the oscillating paddles in operation and a cross-flow established in the channel. The time taken for the dye concentrations to be removed from each cage configuration is shown in Figure 17 below. This figure indicates that the dye concentration within the cage reduces more quickly with the cage above the skirt. The flushing time is seen to decrease systematically with decreasing values of h/H .

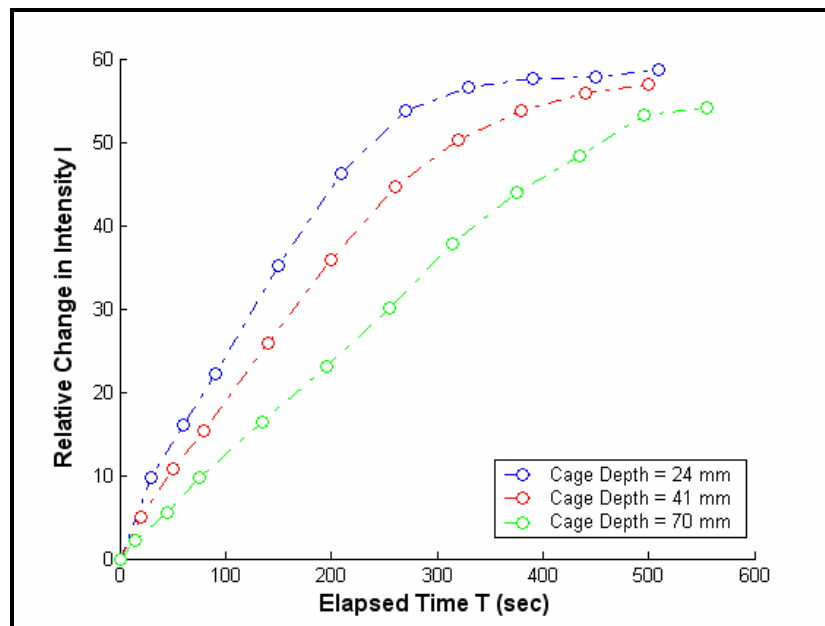


Figure 17: Temporal variation in light intensity within model fish cage for cage depths of 24 mm ($h/H = 0.3$), 41 mm ($h/H = 0.5$) and 70 mm ($h/H = 0.7$). ($U = 2.6 \text{ cm.s}^{-1}$).

Summary of dye experiments

The main result of the experiments is that the presence of a cross current has a strong influence upon the time taken for a contaminant to be flushed from a cage in which a turbulence field is maintained. The other main factor affecting the dilution of the contaminant is the depth of the cage with respect to that of the bottom of the skirt, such that, on the basis of the exploratory model experiments above, the quickest time for flushing is to be expected with the combination a strong cross current and a cage lifted significantly above the base of the skirt. The explanation for this finding is that the cross current sets up a strong circulation within the volume enclosed by the skirt (see below). Note, however, that caution should be exercised in applying the above results to field predictions. Firstly, the experiments that have been conducted have been for 2-dimensional flows; i.e there is no flow *around* the skirt. This means that the results are not directly applicable to round skirted cages in unconfined waters, where the incident

current can flow around as well as under the skirt. In the opinion of the author, however, these differences in geometry are unlikely to affect the main findings summarised above, not least because the shear produced at the base of the skirt (and not the flow around the suspended skirt itself) provides the dominant entrainment mechanism.

Secondly, the question of scale has not been investigated thoroughly. In order to apply the laboratory scale results to field scale installations it is necessary to identify the relevant non-dimensional parameter governing the flow structure. Because free surface and buoyancy effects are negligible here, the only relevant parameter to consider is the familiar Reynolds number Re , suitably defined for these cases as $Re = UH/\nu$, where ν ($\sim 1 \times 10^{-6} \text{ m}^2 \cdot \text{s}^{-1}$) is the kinematic viscosity of water. In the model experiments, the range of non-zero values investigated was $3.1 \times 10^3 - 5 \times 10^3$.

Re values appropriate for fish cages are difficult to obtain, not least because to the variability of the current systems and the large variations between spring and neap conditions. For example, Perez et al (2002) report measurements of velocities at an unnamed fish cage site of $<0.001 < U \text{ (m} \cdot \text{s}^{-1}) < 0.160$ and $<0.001 < U < 0.02$ for spring and neap conditions respectively, with mean values of $U = 0.076 \text{ m} \cdot \text{s}^{-1}$ and $0.003 \text{ m} \cdot \text{s}^{-1}$ respectively. For a typical skirt length of $H = 8 \text{ m}$ (see Hydrodynamic parameters section), the corresponding mean Re values for spring and neap conditions would be 6.1×10^5 and 2.4×10^4 respectively. (Note that, though these values are typically at least one order of magnitude greater than in the laboratory experiments, the consequences are not necessarily important since flow structures at sufficiently high Reynolds number do not generally show significant dependence upon Re).

Related flows

As stated above, the dominant entrainment mechanism for the flushing of therapeutant from a skirted fish cage is provided by (i) the mixing layer initiated at the upstream face of the skirt and the production and shedding of relatively large vortices within this region and (ii) the interactions between these shed vortices and the cellular flow structures generated within the cavity formed by the skirt. The large vortical structures seen downstream of the leading face of the skirt (see Figure 14) are associated directly with the localised shear associated with the blockage of the incident flow by the sharp-edged solid barrier provided by the skirt. As such, they are very similar in character to the vortical structures seen in the shear regions formed in rivers by the presence of groynes. Save for the differences in orientation, the structural characteristics of the two flows are identical. For example, Figure 18 shows an image of dye being entrained into a cross current flow from the so-called dead zone formed between a pair of groynes placed transverse to the flow.

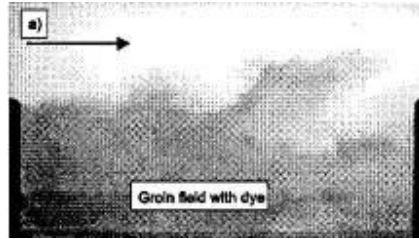


Figure 18: *Plan view of dye entrainment (shown dark) from a model cavity formed by two groynes (black strips at Right and Left edges of frame). Cross current direction indicated.*

Figure 19 shows the associated velocity structure of the model flow, with pairs of images inverted to facilitate direct comparison with Figure 15. Because there are no buoyancy effects to consider, differences in orientation of the gravitational acceleration vector between the two types of flow are irrelevant. Free-surface effects are also negligible, so the two types of flow are directly comparable (except for the absence in the groyne flow of the additional turbulence associated with the fish in the enclosed cage). The analogy between the flows can be exploited for the purposes of interpreting the skirted cage hydrodynamics because (i) the groyne flows have already been studied in relatively great detail (e.g. Weitbrecht et al, 2006; Hinterberger et al, 2007) and (quantitative estimates of entrainment have already been obtained for this configuration. A key set of observations that explain many of the aspects of the entrainment process driven by the cross current deformation by the barrier (skirt or groyne) is illustrated in Figure 20.

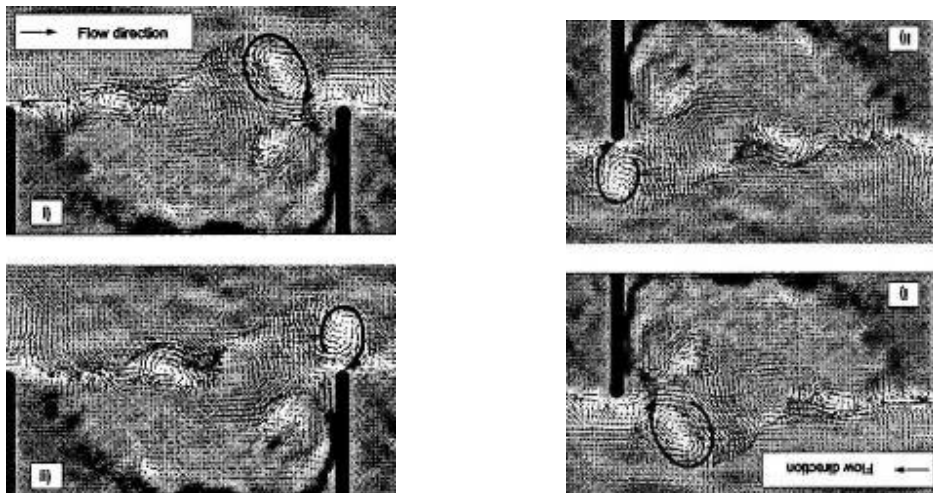


Figure 19: *Plan views of coherent structures within the shear region generated by the presence in a uniform river flow of pairs of transverse barriers (groynes). Original image (left column) shows plan view of the flow field; right column shows the image reversed for comparison with Fig 12. From Weitbrecht et al (2006)*

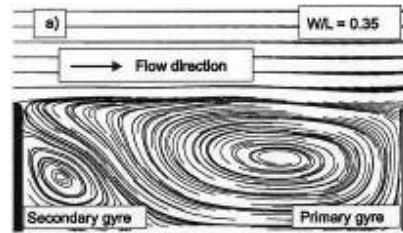


Figure 20: *Schematic representation of the flow structure generated by the interaction of a uniform cross current with pairs of transverse barriers having aspect ratio W/L (corresponding to H/L in Fig 1) = 0.35. From Weitbrecht et al (2006).*

In particular, the schematic diagram in the figure demonstrates clearly the generation of an internal gyre circulation within the fluid cavity in question and the dependence of the form of the gyre system upon the aspect ratio H/L of the enclosed volume. Note from the discussion in Section 1 that this aspect ratio is typically about 0.1 (and always less than unity) for the skirted cage cases.

Theoretical aspects

The conservation of mass $M(t)$ of dye (theraputant) in the model cage can be expressed as:

$$dM/dt = -ELB C \quad (1)$$

where E is the entrainment velocity (see Section 2.1) into the cross current, L is the dimension of the cage in the flow direction (see Figure 10), B is the width of the cage transverse to the current and $C(t)$ is the concentration of dye (theraputant). A common entrainment hypothesis adopted for this type of flow is that the entrainment velocity E is proportional to the cross current velocity U , viz

$$E = kU \quad (2)$$

where k is a proportionality constant to be determined. The mass M of dye within the cage can be expressed as

$$M = BHL C \quad (3)$$

so equations (1)-(3) can be combined to give the following:

$$dC/dt = - (kU/H) C \quad (4)$$

This equation describes the (exponential) change in dye or therapeutant concentration C with time in terms of the entrainment coefficient k , the skirt length H and the cross current velocity U . More specifically, the quantity T given by

$$T = (kU/H)^{-1} \quad (5)$$

represents a characteristic dilution time scale for the dye within the cage – formally, the time taken for the dye (therapeutant) concentration to reduce by a factor of $1/e$ through the action of entrainment.

In order to calculate the dilution time scale T it is necessary to estimate a characteristic value for the entrainment coefficient k . The work of Weitbrecht et al (2006) with groynes showed that the value of k varied significantly with the aspect ratio H/L of the cavity. However, for values of H/L comparable with the skirted fish cage structures, namely order 0.1, a typical value of k for $H/L = 0.16$ was 0.02. Note that this value was obtained from experiments in which the Reynolds number Re had a value of 2.3×10^5 , a value comparable with the value expected for a typical skirted cage (see Section *Laboratory model*). Inserting typical values of $U = 0.076 \text{ m.s}^{-1}$ and 0.003 m.s^{-1} respectively and $H = 8 \text{ m}$ (see Section *Laboratory model*), the corresponding mean values of T for spring and neap conditions are 1.46 hrs and 37 hrs respectively

Conclusions

In the above analysis of therapeutant dilution from a skirted fish cage, a large number of simplifications and assumptions have been used to parameterise the entrainment of therapeutant from the open mesh bottom of the fish cage and into the cross current existing in the ambient water column. The results of simplified idealised experiments demonstrate that this shear-induced entrainment is the dominant mechanism responsible for the dilution of the therapeutant, being modified only slightly by the ambient turbulence generated by the moving fish within the cage itself. The importance of shear entrainment into the cross current is confirmed by studies of a similar hydrodynamic system, namely the river/groyne system. The only significant modifying influence upon this dominant mechanism is shown to be the ratio h/H of the cage depth h to the skirt length H , with entrainment being increased by a factor of about 2 when the cage is raised significantly above the base of the skirt (see Figure 19). Such a modification is evidently associated with the effect of the turbulent portion of fluid within the cage upon the circulating gyre structure formed within the whole skirted cavity (see Figure 20)

On the basis of the above, estimated characteristic flushing times for skirt-treated fish cages in the typical sea lochs characterised in Perez et al (2002) would be between 1 and 18 hours for spring and neap conditions. Of course, these values do not represent the times for all therapeutant to be completely flushed from the skirted cage, merely the estimated e-folding time – that is the time for the initial concentration to reduce to $1/e$ (about 0.37) of the original value. Note, however, that these estimates are based upon steady, mean velocity values for the current U , whereas the velocity fields within which

the skirted cages are placed during treatment are tidally-driven and inherently unsteady (Perez et al, 2002).

Note also that, for reasons provided under the modelling parameters and approach, above, the definition of the problem under consideration is not well-posed. The estimates of dilution times should therefore be treated with caution until more exhaustive investigations of the individual processes described above are undertaken.

Part 4 – Conclusions and recommendations

Many reports and articles (see text) note the hydrophobic properties of cypermethrin but do not acknowledge the difficulty of studying the chemical in the environment. Cypermethrin is a particularly difficult substance to evaluate, simply because it adsorbs to available surfaces over a very short period. It adsorbs onto particulate material, nets, tarpaulins, boat hulls, and other non-target structures in the water at the time of treatment, particularly as the majority of such surfaces will be covered in organic material. Thus partitioning the subsequent fate of the cypermethrin is extremely difficult to determine, and especially so under field conditions.

This report documents research designed to assess the fate of cypermethrin used in tarpaulin bath treatment regimes for sea lice infected salmon in fish cages. In practice the results suggest that more detailed experimental work will be required in order to inform the parameterisation of the regulatory model used to consent cypermethrin discharges.

There is a presumption within the AutoBath dispersion model that the concentration of cypermethrin leaving the cage after treatment is set at the treatment concentration of $5\mu\text{g/l}$. It is, however, intuitive that some proportion of the active ingredient will adsorb to fish and other surfaces and thus release concentrations will be lower. The question is how much lower? Previous studies (SAMS, 2005) have shown that the concentration remaining in the cage after treatment is less than $3.5\mu\text{g/l}$. This study showed, over repeated treatments and using the same sampling and measurement techniques used within the PAMP study (SAMS, 2005), that remaining concentration after a 1 hour treatment was significantly less than the SAMS (2005) value; being less than $1.5\mu\text{g/l}$.

Given the differential between the no fish treatment, where uptake by other surfaces (e.g. biofouling, nets, tarpaulin) was seen to be relatively low, and the two with-fish treatments where uptake was higher (shown by the reduction in water concentration of cypermethrin), it must be presumed that there is a higher adsorption onto fish skin than is prescribed by the model parameterisation. Much of this reduction appeared to take place in the first minutes after the treatment was added. This is counter to what is currently presumed (SAMS, 2005) and it is therefore necessary to have more focused trials designed specifically to evaluate fish skin uptake over variable durations and under tightly controlled conditions. In such a study it would be important to measure fish skin concentrations directly alongside reduction in water concentration, to avoid the need of discerning uptake using water concentration as a proxy. This would require development of a more robust method of measuring fish skin concentration reliably in order to overcome the interference from lipids and other contaminants in the analytical process, as observed in this project.

Pending further trials, this project indicates that there is the potential for more leeway to be given in the modelling that determines permissible treatment doses. Presuming all other parameters remain the same, by reducing the “treatment concentration” parameter in the model to half its current level (say to $2.5\mu\text{g/l}$), this would increase permitted use by a factor of 2 above current levels. The robustness of this evaluation is dependant on better

clarification of the water volume into which the EXCIS is added, which could only be presumed in this project, based on a simplified calculation from tarpaulin dimensions. Within the tank trials suggested water volume is readily measurable, but in practice the treatment regime will always retain a margin of error when applied in the field. It is therefore reasonable to assume a degree of conservatism within the model framework. That said, based on the project results, the potential for increased use denoted above takes this into account.

If a comprehensive study was possible it would also be pertinent to evaluate the extent to which the treatment concentration varies over the first few minutes in treatment to confirm the results identified in this study and whether a steady-state point is reached in terms of the binding capability into fish. There is a discrepancy between the perceived uptake in the first few minutes in this project, compared to the notable concentrations in sediment in the first few hours in the PAMP project (SAMS, 2005), but this is not comparing like with like, the latter requiring particulate-bound cypermethrin to settle into the seabed. Whilst it may never be possible to fully evaluate the full fate of cypermethrin in the environment, further evaluation of fish uptake, and the divide between particulate-bound materials and likely other binding points, such as biofouling, and more detailed scrutiny of changes in concentration over the short-term (minutes) and medium-term (hours) would enable a more comprehensive understanding of environmental fate.

Transport of cypermethrin was assessed using drogues but while the theory behind the use of drogues to track water movement is sound, in the highly complex hydrographic environment around fish farms they may be inappropriate. Significant difficulties arose during this study, where deployment and movement of drogues were compromised by the presence of the cages and sub-surface mooring lines, which interfered with the smooth passage of the drogues. Immediately they were compromised the plume was lost and could not be re-identified. Although the diffusion coefficient was not measured during this study, other studies concluded that the standard coefficient applied by SEPA would appear appropriate. Future studies might consider alternative methods of assessing plume dispersion, such as using dyed cypermethrin, although this would require some product development to ensure that the dyed cypermethrin acts, in the same way as the standard product.

Of vital importance to the dispersal of cypermethrin after treatment, would appear to be the extent to which initial dilution occurs when the tarpaulin is removed and the net dropped. This project proved inconclusive, but SAMS (2005) in general showed that the measured concentration was always below the level predicted by the model outcome. It is possible that considerable mixing and turbulence is generated by the release procedures and that this influences the subsequent dilution with the water flow. Also possible is that the process of dropping the net after treatment entrains the water containing cypermethrin and drags it down to depths below that traditionally measured in studies such as this. Certainly, samples analysed in this project shows there is considerable patchiness in cypermethrin concentration present in the out-flowing water. A future study, conducted more intensively inside and outside the cages might enable a better understand the mixing processes that occur and by doing so improve estimates of subsequent plume dispersal.

This project included a theoretical assessment of the use of skirts for treatment alongside some initial flume-based evaluations of likely impacts of their use. Skirts are not currently permitted a treatment method because of the generally perceived necessity to continually top up the treatment with cypermethrin to account for losses through the open skirt (SEPA 2007^b). The theoretical investigation carried out during this project made some assumptions regarding such losses, but showed, through a series of calculations and flume studies that losses, under various current flow regimes, may be less than presumed. The skirt study indicated that the estimated time for the initial concentration to reduce to $1/e$ (about 0.37) of the original value was between 1 and 18 hours after treatment. However, the field study (Part 2) showed that the treatment concentration is reduced much more quickly than this as a result of cypermethrin being taken up by fish. Combined results suggest that the use of skirts, without the need to apply more cypermethrin, may be a viable alternative strategy to complete enclosure with tarpaulins. Skirts have the advantage of being able to be deployed much more quickly than tarpaulins, which is likely to reduce fish stress and improve welfare during the procedure; and because of the ease of deployment can be used in more adverse weather conditions and faster flow regimes. Further research would be required to evaluate the effects of density differences between EXCIS and seawater and what effect this has under differing flow regimes.

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