Distribution of pyrethroids in salmon cages and well boats-direct quantification and tracer methods

F. Finne-Fridell*[1], E. Wilkinson[1], K. Steinsvoll Rikardsen[1], E. Aksnes[1], R. Stigum Olsen[2], E. Høy[3], F. Oppedal[4], B. Bjøru[5] and R. N Grøntvedt[5]

[1] PHARMAQ AS, Oslo, Norway [2] Novartis Animal Health, Bergen, Norway
[3] SINTEF, Trondheim, Norway [4] Institute of Marine Research, Matre, Norway
[5] Norwegian Veterinary Institute, Trondheim, Norway

Introduction

The focus on distribution of therapeutic used for sea lice treatment in salmon (*S. salar*) cages and well boat has increased significantly in the recent years. Homogeneous distribution of the therapeutic throughout the whole treatment volume during exposure ensures efficient clearance of sea lice, and reduces the development of resistance. Pyrethroids such as deltamethrin and cypermethrin have been used for several years for bath treatments against sea lice. The therapeutic concentration is 2µg/L of deltamethrin when using ALPHA MAX® (PHARMAQ) and 15 µg/L of cypermethrin when using Betamax Vet. (Novartis Animal Health). In order to document the distribution in a certain water volume; the pyrethroids can be directly quantified or a tracer method can be used. In the present TOPILOUSE project, two indirect methods have been used: synthetic DNA tracer and fluorescein tracer. The aim of the study was to examine and compare the different methods for volume and distribution control in commercial scale cages and well boats.

Methods

Tank, well boat and cage studies were performed in order to evaluate different methods used for evaluation of distribution of a therapeutic in commercial scale. DNA tracer or fluorescein were added in the mixing tank before distributing into the treatment volume (tarpaulin or well). At different time point post distribution water was sampled in both glass bottles (Borosilicate3.3) for direct quantification or in eppendorf tubes for DNA analysis. The fluorescein was measured by a Seapoint Chlorophyll Fluormeter.

DNA tracer

Synthetic DNA tracers was used as a tracer. Each water sample was purified with MinElute PCR purification Kit, post Q-PCR analysis with specific primers and probe. Dilutions were performed and $2.17x10^9$–$2.17x10^1$ DNA molecules per µl corresponding to C_T values from 7.03 to 33.92. C_T values<40 were considered reliable. The Ct values were transformed to DNA copies, and normalized to ppb corresponding to either ALPHA MAX or Betamax Vet concentrations.

Direct analysis

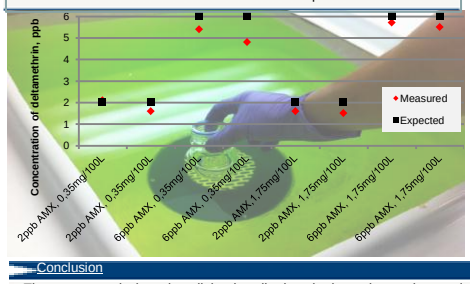
Deltamethrin samples (50ml) were prepared by a liquid-liquid extraction with dichloromethane followed by an GC method equipped with an electron capture detector, LOD=0.3 ppb (RPC laboratories, Canada). Cypermethrin samples were prepared by triplicate liquid-liquid partitioning in hexane which, following evaporation and quantified by GC/MS (Covance Laboratories, UK.). LOD=0.5 ppb.

Fluorescein

Fluorescein was added to the mixing tank using about 1,75 kg before distributing into the cages (estimated volume 22000m3) . Prior to and during treatment distribution of dye was continuously measured by two or three profiling fluorescence probe (Seapoint Chlorophyll Fluormeter) mounted on a ctd's (SAIV204. www.saivas.com).

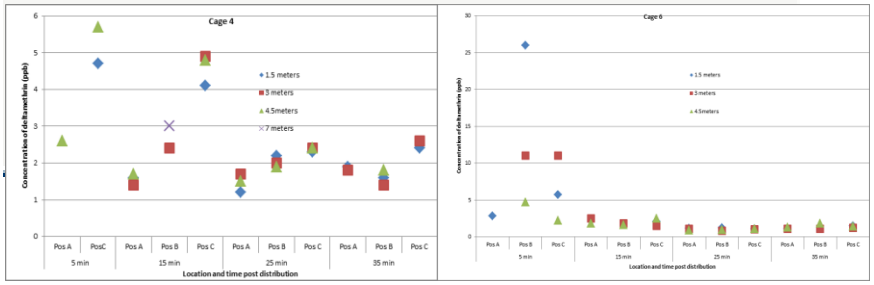
Fluorescein vs direct quantification

In order to examine if Fluorescein influenced on the direct analysis of pyrethroids, a tank study was performed. Different concentrations of deltamethrin and fluorescein were mixed in a tank, and deltamethrin was quantified. The fluorescein did not influence the analytical result as all the deltamethrin concentrations were as expected..



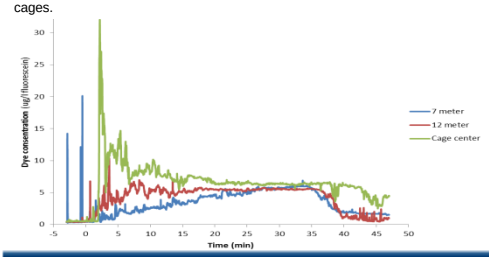
Results, distribution of ALPHA MAX and Fluorescein in large scale cages

In the present full-scale study treatment with ALPHA MAX and fluorescein were performed in cages with closed tarpaulin. The therapeutic and tracer were administered at the surface. Both direct analysis of deltamethrin and analysis of fluorescein were performed in order to evaluate distribution dynamics in the treatment volume. The results of both methods indicate that it takes at least 15 minutes in order to obtain homogeneous distribution of the therapeutic in the treatment volume. NB: The sampling points for deltamethrin and fluorescein were not equal.

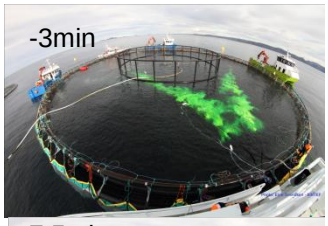


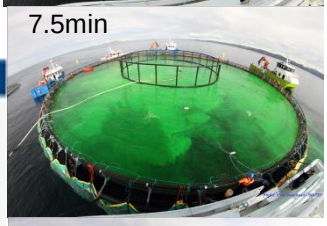
The figure above show the distribution of ALPHA MAX (measured as amount of deltamethrin) at different depths and positions in cages with closed tarpaulin. Pos A were in the middle of the cage and Pos C where close to the boat.

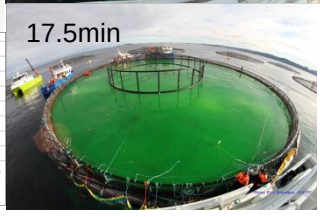
The figure below show the distribution and logging of fluorescein at different times post distribution (Time 0). Green, Blue and Red indicate thee different positions in the same cages. The graph below represent average values from four different cages.



Pictures of the cages at different times post finishing of distribution

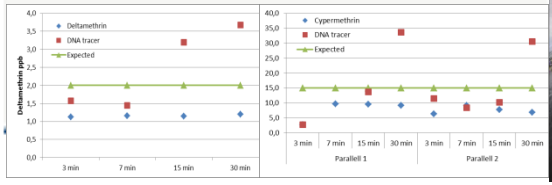






Results, distribution of pyrethroids and DNA tracer in well boat

In two well boats, with and without fish, both ALPHA MAX and Betamax vet was mixed together with a synthetic DNA tracer in a mixing tank before distribution. In all studies parallel samples were analyzed by the direct method and Real Time PCR for the DNA tracer. The graphs below show the result from selected treatments. The expected concentrations were not obtained for either ALPHA MAX nor Betamax vet in the two well boats. The results have initiated a discussion regard to pyrethroids affinity to selected surfaces in well boats, and it is recommended to monitor the concentration in every boat used for sea lice treatment with pyrethroids.



Conclusion

The present work show that all the described methods can be used as tools for evaluation the distribution in a water volume. Direct analysis is easy to analyze and gives relevant information of the reactive substances with regard to affinity to organic and non organic substances. The challenges related to the pyrethroids affinity to different surfaces, also the sampling bottles, cause optimizing in the whole analysis chain from sampling, storage and rinsing of the bottles with solvent at the lab. Due to the high analytical cost these, the number of samples are limited, and the method give a more rough picture of the distribution. Both DNA tracer and Fluorescein is quite optimal for frequent or continuously monitoring of the dynamics in a certain water volume, but they does not describe the through for a certain medicine product. Due to the sensitivity of the DNA tracer and Fluorescein methods it is important to avoid contamination from one treatment to another.

The main conclusions from the project is: It is challenging to distribute a therapeutic in the large cages, and optimized equipment is needed. In these well boats the time to get homogeneous distribution were short, but the absolute concentration, for bot ALPHA MAX and Betamax Vet, were only 50% of the expected concentration. More research is needed for understanding of the dynamic between pyrethroids and surfaces.

The studies were founded by Norwegian Research Council, Norwegian Seafood Research Fund and industrial partners, in the project: Topilouse: A multi-disciplinary effort to improve topical treatments in salmon louse control