Deltamethrin resistance; a hard nut to crack

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Background: Over the last decades, salmon lice (*Lepeophtheirus salmonis*) have developed resistance towards many of the compounds used in the salmon farming industry, including the pyrethroid deltamethrin (DMT). The mode of action of DMT is believed to be neurotoxic as DMT binds to voltage gated sodium channels (VGSC) and prolong the electrical signal in nerves. Consequently, a continuous excitation results in paralysis and possibly death of the organism. Resistance against pyrethroid has been studied in crop pests and malaria carrying mosquitoes and several mechanisms are attributed DMT resistance in these arthropods. The following have been suggested as resistance strategies in arthropods and may be possible resistance mechanism in the salmon louse:

1) Knock down resistance, i.e. mutation in the VGSC
2) Reduced cuticle penetration
3) Increased efflux of DMT from the cytosol through ABC-transporters (p-glycoproteins)
4) Increased metabolism by e.g. cytochrome P450s and carboxylesterases

These points will be further discussed below in relation to the salmon louse, either by existing knowledge or ongoing experiments.

1) Knock Down Resistance: Pyrethroid resistance have been linked to knock down resistance (kdr) in the voltage gated sodium channel. Knock down resistance is a mutation in the gene for the target protein of the substance when the mutation gives a protective phenotype against the effect of the substance. Three genes for the voltage gated sodium channel has been identified in *L. salmonis* (Figure A) and the expression level of the three genes has been found in several strains (Figure B) (Helgesen 2015). The most highly expressed gene, namely the $Na_v1.1$, was further investigated for mutations in hot spots identified in other arthropods.

Results: No mutations were found in the $Na_v1.1$ gene (Helgesen 2015).

Figure A

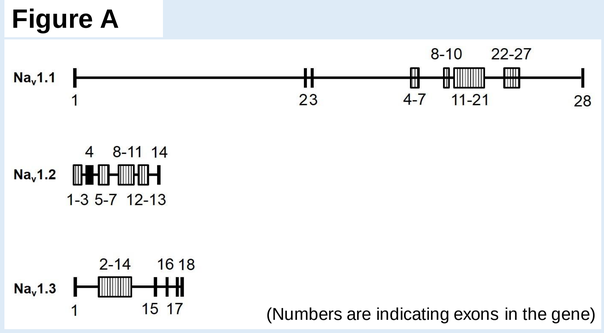
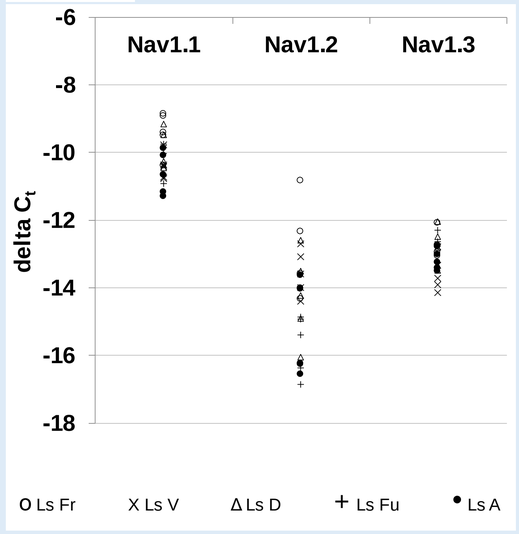


Figure B



Helgesen, K.O., 2015. Monitoring of drug resistance and resistance development mechanisms in salmon lice (*Lepeophtheirus salmonis*). PhD thesis. Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences, Oslo.

2) Cuticle penetration: Thicker cuticle and altered cuticle protein content have been shown to reduce DMTs ability to penetrate the cuticle in other arthropods. The result is that less DMT enter the organism and the effective exposure dose is decreased. To investigate the uptake of DMT in resistant and susceptible salmon lice, ^{14}C-labelled DMT was used in a 30 minutes exposure regime with 2 ppb. Lice were collected immediately after exposure and were washed with dichloromethane to remove DMT adhering to the outside of the cuticle. Two adult female were pooled and digested in Soluene® prior to addition of liquid scintillation cocktail (Hionic Fluor). The counting took place in a Tri-Carb liquid scintillation analyser (1900CA, Packard) with a build-in quencher standard.

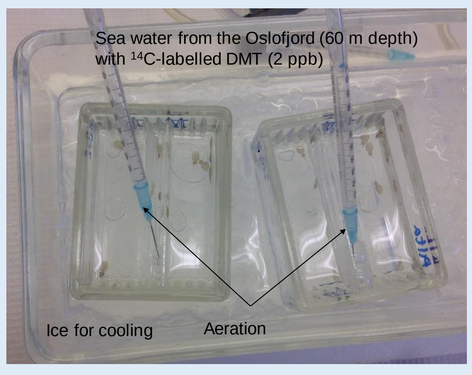
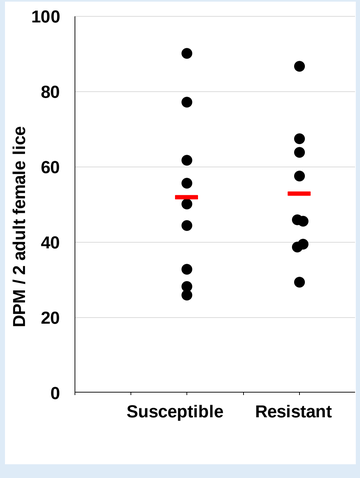


Figure C

30 minutes exposure



Results: There were no differences between susceptible and resistant lice directly after exposure to ^{14}C-DMT (Figure C). The result indicates that there is no difference in uptake through the cuticle between susceptible and resistant salmon lice.

(Each data point represent two adult female lice.
Red lines indicate the geometric mean of the group.)

3) ABC-transporters (p-glycoproteins): The involvement of ABC-transporters in resistance towards other substances, such as emamectin benzoate, has been suggested. Carmona-Antonanzas et al. (2015) give an overview of these transporters in the salmon louse but their possible involvement in DMT-resistance have not been investigated. Preliminary results from analysis in our group indicate no differences in the expression level between a DMT susceptible and DMT resistant strain.

Carmona-Antonanzas, G., Carmichael, S.N., Heumann, J., Taggart, J.B., Gharbi, K., Bron, J.E., Bekaert, M., Sturm, A., 2015. A Survey of the ATP-Binding Cassette (ABC) Gene Superfamily in the Salmon Louse (Lepeophtheirus salmonis). Plos One 10(9). DOI: 10.1371/journal.pone.0137394

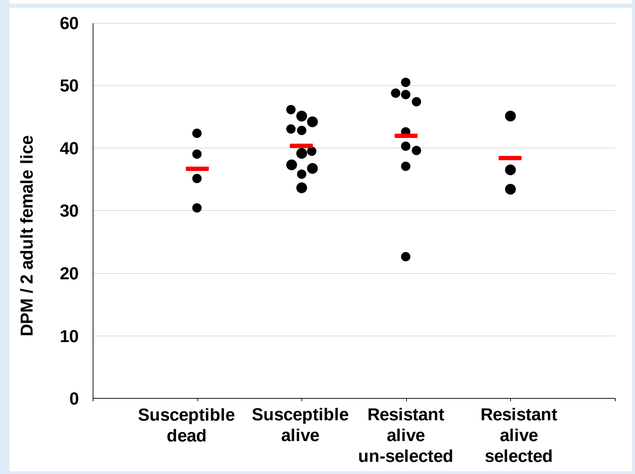
4) Increased metabolism: DMT metabolism includes many groups of enzymes and many metabolites, according to knowledge from vertebrates and insects. Monoxygenases (includes the group of P450s) have been implied to participate in the DMT metabolism in the salmon louse (Sevatdal et al., 2005), but no other studies have been published on DMT metabolism in the salmon louse. To evaluate the excretion of DMT and its metabolites, lice from a susceptible and a resistant strain was used. The lice were exposed to ^{14}C-labelled DMT (2 ppb) for 30 minutes, whereupon they were transferred to fresh sea water with continuous aeration where they were kept for 24 hours. The lice were collected as either alive or dead (only susceptible lice died), rinsed in sea water and subjected to scintillation counting as described under paragraph 2, cuticle penetration.

Results: No significant differences in radioactivity was found between any of the susceptible or resistant groups 24 hours after DMT exposure (Figure D).

However, this study does not evaluate the internal metabolism of DMT and does not discriminate between radiolabeled DMT and radiolabeled metabolites.

Figure D

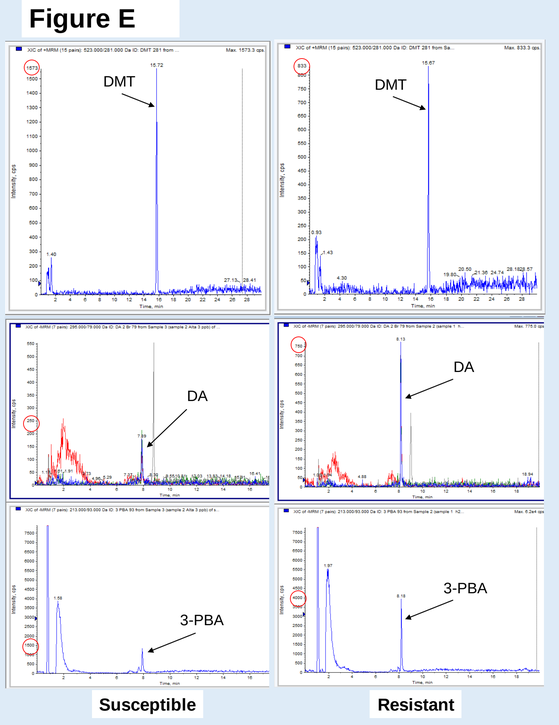
30 minutes exposure + 24 hours in fresh sea water



To further investigate the DMT metabolism, two main metabolites were chosen for analysis, the deltamethric acid (DA) and 3-phenoxybenzoic acid (3-PBA). Lice from a susceptible and a resistant strain was exposed to DMT (3 ppb, Alphamax®) for 30 minutes whereupon they were transferred to fresh sea water with continuous aeration where they were kept for 24 hours. The contents of DMT and the metabolites were measured on LC-MS after extraction with acetonitrile.

Preliminary results: The levels of both metabolites seem to be lower in susceptible lice compared to resistant lice (Figure E). *However, more analysis are required to verify these results.*

Figure E



Sevatdal, S., Fallang, A., Ingebrigtsen, K., Horsberg, T.E., 2005. Monooxygenase mediated pyrethroid detoxification in sea lice (Lepeophtheirus salmonis). Pest Management Science 61, 772-778.

Conclusions: Of the suggested resistant mechanisms, knock down resistance and reduced cuticle penetration seem to be less likely in the salmon louse. The contribution of the ABC-transporters in protection against DMT needs further attention. We have results that indicate that excretion of DMT and its metabolites prior to death is of little significance. However, preliminary results indicate that there is an increased metabolism of DMT in resistant lice, resulting in less potent metabolites such as DA and 3-PBA. Further studies are needed to clarify which enzymes that are involved in the DMT metabolism and the extent of the metabolic protection against DMT. In addition, the genetic marker for the potential metabolic DMT resistance needs to be identified.

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