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Drug resistance in sea lice: a threat to salmonid aquaculture

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Sea lice are copepod ectoparasites with vast reproductive potential and affect a wide variety of fish species. The number of parasites causing morbidity is proportional to fish size. Natural low host density restricts massive parasite dispersal. However, expanded salmon farming has shifted the conditions in favor of the parasite. Salmon farms are often situated near wild salmonid migrating routes, with smolts being particularly vulnerable to sea lice infestation. In order to protect both farmed and wild salmonids passing or residing in the proximity of the farms, several measures are taken. Medicinal treatment of farmed fish has been the most predictable and efficacious, leading to extensive use of the available compounds. This has resulted in drug-resistant parasites occurring on farmed and possibly wild salmonids.

Massive technological progress above and below sea level

Terrestrial food production represents the main protein source in the industrialized world. Due to a rapidly growing human population, and thus a deficiency of traditional protein resources, alternative ways to provide for the increase in nutrient demand are being sought. In 2006, the world aquaculture production of fish contributed to 47% of the world's food fish supply, mainly carps and other cyprinids (59%) but also salmonids. Approximately 7% of the world's fish production in aquaculture comes from salmonid farming [1]. Fish farming in sea water possesses vast potential for protein processing [2], with the most heavily industrialized production being farming of Atlantic salmon. Developing from small-scale production to a massive industry within 40 years, the optimal techniques for fish farming are probably yet to be identified. Several pathogens are compromising salmon production, most of which are being addressed through vaccines and other precautions. However, caligid copepods have proved to be a major constraint to biological sustainability. In 2002, Denholm *et al.* [3] reviewed the development of sea lice resistance towards available compounds. Intensive research has been conducted with several sea lice species since then, catapulting the knowledge about parasite–fish interactions and molecular mechanisms to the next level.

A state of constant increase

Commercial farming of salmonids was initiated in mid-Norway in the late 1960s (see Store Norske leksikon

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Glossary

Acetylcholine (ACh): a neurotransmitter present in cholinergic synapses.
Acetylcholine esterase (AChE): an enzyme involved in inactivation of acetylcholine.
Azamethiphos (AZA): an organophosphate targeting acetylcholine esterase, used as a delousing compound applied as bath treatment, and has been used since ~1994.
Benzoyl ureas: a class of chemical compounds that inhibit molting in several parasite or pest species. These compounds inhibit the synthesis of chitin.
Cypermethrin (CYPER): a compound within the class of pyrethroids; it is derived from the organic compound pyrethrin.
Deltamethrin (DELTA): a compound from the group of pyrethroids. It is structurally similar to cypermethrin.
Diflubenzuron (DIFLU): a compound from the group of benzoyl ureas.
Emamectin benzoate (EMB): a chemical compound within the class of avermectins. This compound acts mainly as activator of chloride channels in membranes.
Fitness cost: the loss of a feature or a favorable metabolic pathway through changes in genetic material to avoid the effects of xenobiotics.
Hydrogen peroxide (H_2O_2): a disinfectant with insecticidal and ovicidal properties.
γ-Aminobutyric acid (GABA): a neurotransmitter present in both vertebrates and invertebrates.
Glutamate (GLU): a proteinogenic amino acid and neurotransmitter.
Glutathione S-transferase (GST): a class of isoenzymes with a range of functions, including metabolism of xenobiotics.
Integrated pest management (IPM): a theory for extirpation of pest organisms through a combination of complex measures.
Mixed function oxidases (CYP): metabolic enzymes of the cytochrome P450 class.
Organophosphates (OPs): a class of chemical compounds that have been utilized as insecticides in agriculture for more than 50 years. These compounds inhibit acetylcholine esterase, leading to paralysis.
P-glycoprotein (P-gp): a protein of the cell membrane responsible for the efflux of a range of substances from the cell. P-gp is also known as multidrug resistance protein 1.
Pyrethroids (PYRs): a class of compounds that includes the substances cypermethrin and deltamethrin.
Refugia: parasites not treated with medicinal compounds to let them spread their genetic material to the population of reduced sensitivity. In theory, refugia will slow down resistance development.
Teflubenzuron (TEFLU): a compound from the group of benzoyl urea compounds.
Therapeutic index: a mathematic factor describing the medicinal dose toxic to salmon/dose used to remove sea lice off fish.
Voltage-dependent sodium channels (VDSC): channels specific to sodium ions, opened by depolarisation of the membrane.
Xenobiotics: foreign chemical substances present in living organisms.

(https://snl.no/fiskeoppdrett)) with the seawater cultivation phase conducted in floating net pens, allowing for the exchange of water and its contents with the environment. Fish farms are considered a major factor for spreading sea lice to wild salmonids in Europe and North America [4]. When the critical parasite abundance level is exceeded, the host could eventually suffer from osmotic stress and secondary infections, leading to mortalities [5]. The interplay of farmed salmonids, parasites, and wild salmonids is, however, a complex matter. Species-dependent ability to reject the parasite, interaction between the parasite and the host immune system, self-imposed delousing in rivers, and fish size are all important factors determining the outcome for the host (reviewed by Torrisen *et al.* [6]). Thus, sea lice have varying impacts on wild salmonid populations [6,7].

Sea lice levels in salmon farms are under comprehensive surveillance throughout the sea water production period. Medicinal treatments have historically been the most predictable measures to prevent the occurrence of high sea lice abundance. The possibility of infecting vulnerable wild salmonids residing in the proximity of fish farms is a major concern behind the monitoring of sea lice levels. Altogether, sea lice parasites constitute a serious threat to sustainable salmon farming in the main producing countries, Canada, Chile, the Faroe Islands, Ireland, Norway, and Scotland, as the total production of Atlantic salmon has almost doubled from 1.1 billion tons in 2002 to 2.1 billion tons in 2012 (FAO Cultured Aquatic Species Information Programme Salmo salar (http://www.fao.org/fishery/culturedspecies/Salmo_salar/en)). Spillover from medicinal treatments to remove sea lice is potentially harmful to other organisms such as lobsters and shrimps [8]. Extensive use of medicines has resulted in an inevitable drift towards resistance. This imposes a huge threat for fish health and welfare, the environment, the economy in salmonid production, and for seafood production in general. In summary, these parasites represent a massive economical and biological obstacle to fish farming companies in all salmon-producing countries.

Life cycle and current combat strategies

In Europe and Canada, the most frequently occurring sea louse is *Lepeophtheirus salmonis* (Krøyer), whereas *Caligus rogercresseyi* (Boxshall and Bravo) is its counterpart in the Southern Hemisphere. A third species, *Caligus elongatus* (Nordmann), is to some degree prevalent in Europe and Canada, affecting a variety of salmonid and non-salmonid species. *C. elongatus* is only sporadically subject to research because of its low prevalence and limited influence on fish morbidity. All three copepods are crustaceans, and their life cycle consists of eight stages; three of these stages are planktonic whereby the third stage (copepodid) infects the fish, and five of these stages are parasitic instars. Regarding *L. salmonis*, two instars (chalimus I and II) attach to the fish by a protein filament, whereas the following three (pre-adult I, II, and adult) are able to move on the host while grazing on mucus and blood. The *Caligus* species also comprise five parasitic instars: the first four (chalimus I–IV) attach to the host by a protein filament, with the latter molting to the adult stage [9]. The developmental time from the extrusion of egg strings to adults is temperature dependent. For *L. salmonis*, it is approximately 40 days for males and 50 days for females at 10°C, but shorter for *C. rogercresseyi* (reviewed by Costello [10]).

Apart from chemical intervention through bath treatments and medicated feed, several non-chemical methods are utilized to remove or reduce the number of parasites attaching to farmed fish. Fallowing, synchronized treatments within geographic zones, cleaner fish, delousing laser, and plankton shielding skirts are already in use, with others such as snorkel cages and enclosed cages on the verge of commercial introduction. Despite the use of alternatives to chemical treatment, extensive use of medicinal compounds combined with limited access to effective chemical compounds has led to widespread resistance towards the most applied medicinal products. The evolving reduced sensitivity in sea lice is a good example of microevolution, and shows how humans can influence nature considerably in a relatively short time frame.

Addressing a prevailing parasite in fish farming, this text will review current knowledge of resistance against chemical treatment agents in *L. salmonis* and *C. rogercresseyi*. The emergence of more accessible molecular methods has already contributed to a rapid increase in knowledge regarding this issue.

General definitions of resistance

The World Health Organization expert committee in 1957 defined resistance to insecticides as the 'development of an ability, in a strain of insects, to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species' [11]. The degree of reduced susceptibility needed to survive an antiparasitic treatment differs between agricultural pests and parasites attached to a vertebrate host. Successful treatments of agricultural pests are mainly dependent on the dose, whereas for parasitic pests, the maximum applicable amount is determined by the host's ability to withstand the toxicity as well as the parasite's susceptibility to the compound. For several anti-sea lice medicines the therapeutic index (see Glossary) is small; thus, only minor reductions in sea lice susceptibility may present as clinical resistance in the field [12–14]. Resistance development is driven by the survival and reproduction of the less sensitive individuals [15].

The expression "resistant sea lice" is mainly applied for populations showing a high degree of reduced sensitivity in bioassays. A therapeutic failure in the field could be caused by a reduced sensitivity to the applied medicinal compound, but also by suboptimal treatment regimens, such as insufficient drug dispersal, poor medicine management, or badly adjusted feeding procedures.

Several genetic resistance mechanisms have been identified in arthropods. These include point mutations in the chemical's target gene rendering the protein insensitive to the chemical, upregulation of genes for detoxifying metabolism of the chemical by the parasite, and upregulation of efflux pumps in the parasite's intestine leading to decreased uptake of chemicals given to the fish as medicated feed. Similarly, changes in genes coding for cuticle thickness or