Agenda item 6.2
For information

Council

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Drug resistance in sea lice and integrated lice management strategies
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Sea lice (*Copepoda*: *Caligidae*) are ectoparasitic crustaceans feeding on the mucus and skin tissues of wild and farmed marine fish. Sea louse infections constitute a major disease problem during the marine phase of Atlantic salmon (Salmo salar) culture [1]. In the Northern hemisphere, most caligid infections of farmed salmon are caused by the salmon louse (*Lepeophtheirus salmonis*), which requires salmonid hosts to complete its life cycle. In addition, the smaller species *Caligus elongatus* can occur on farmed salmon in the North Atlantic. In Chile, sea louse infections on salmon farms are cause by the species *Caligus rogercressyi* [2].

During their life cycle, sea lice go through host-associated and free living stages. Adult females produce a series of paired egg strings, which remain attached to the female until the hatching of eggs. The larval development initially passes through three non-feeding planktonic stages, of which the third needs to find and successfully settle on a host fish to survive. While the first host-associated stages are attached to the skin of the host through a frontal filament, subsequent pre-adult and adult stages can move freely over the body surface of the fish. Mating occurs between adult males and freshly moulted females and is preceded by the formation of pre-copula pairs. Over their life-span females can produce up to 11 egg strings [3]

Effects of sea louse infections on the host fish include stress, reduced growth and suppression of immune function. At high levels skin lesions and secondary infections may occur, leading to severe disease and, if left untreated, potentially death. Control of sea lice in salmon mariculture is key to assuring the health and welfare of farmed fish, and preventing potential impacts of farm-origin parasites on wild fish populations [1]. The global costs of caligid infections to the salmon farming industry have been estimated to exceed €300 million per annum, which mainly accounts for treatment costs, but further includes negative impacts of infections on growth rates and as a consequence of downgrading of the product [4].

In the salmon farming industry, sea louse control is maintained through integrated pest management strategies, which employ a broad range of tools to achieve control. Farm management measures consist of single-year class stocking, the regular fallowing of sites and area management agreements. Salmon delousing can be achieved by the use of licenced veterinary drugs, which are applied as topical bath treatments or through medicated feeds. In addition, a number of non-medicinal control strategies are available and are currently at different stages of commercial implementation. Such alternative approaches include biological control using cleaner fish, modified cage designs lowering infection rates, removal of lice by mechanical or laser technologies, use of semiochemicals and deterrents to disrupt host detection by infective larvae, and approaches aiming at lowering the susceptibility of host fish for infection through vaccination, administration of immunostimulants, or selective breeding [1].

A key non-medicinal approach to sea louse control, which is now widely applied commercially, involves the co-culture of salmon with wrasse or lumpfish (“cleaner fish”), which remove ectoparasites [5]. Initially, different wrasse species sourced from wild fisheries were used for sea louse control. Recently, methodologies for the intensive farm production of Ballan wrasse have been developed, allowing the deployment of cleaner fish at large scale. In addition, current

research efforts are focused on developing aquaculture of lumpfish, a species showing superior feeding activity at low ambient temperature compared to Ballan wrasse.

Veterinary drugs licensed for use as salmon delousing treatments comprise bath and in-feed treatments. Compounds administered as medicinal baths include the organophosphate azamethiphos (Salmosan ®), the pyrethroid deltamethrin (AMX ®) and the non-specific disinfectant hydrogen peroxide (Paramove ®), whereas the avermectin emamectin benzoate (SLICE ®) is available for oral treatment. An inherent problem of chemical control strategies is that the target species can develop drug resistance, a process known to be driven by the continual use of the same control agents with limited or no rotation between compounds having distinct modes of action. The ability of sea lice to develop resistance to chemical treatments is well documented. Losses of efficacy of drugs targeting *L. salmonis* have been reported, at least locally or temporally, for organophosphates, hydrogen peroxide, pyrethroid and avermectins [6].

To ensure optimal farmed fish health care and disrupt potential resistance formation in sea lice, it is critical to base treatment choices on reliable knowledge of the drug susceptibility status of the parasite population causing the infection. This is currently achieved through so-called bioassays, which are small scale treatments of sea lice in Petri dishes [7]. However, bioassays require large numbers of sea lice of specific developmental stages. Moreover, they are sensitive to interfering factors and show limitations regarding their sensitivity of resistance detection. In insects, genetic diagnostic tests based on the detection of specific resistance mechanisms have proven advantageous in drug susceptibility assessment. Such tests, which have started to become available in sea lice [8], will provide important tools supplementing traditional bioassays.

At present, comparatively little is known about the molecular mechanisms of drug resistance in sea lice. In contrast, insecticide resistance in terrestrial arthropods is well understood, and typically involves either or both of two main mechanisms. First, mutations of molecular targets can affect the binding of the chemical, and second, mutations enhancing the efficiency of detoxification pathways can reduce internal exposure to the insecticide [9]. Taking into account the fact that crustaceans share evolutionary origins with insects, it may be hypothesised that similar molecular mechanisms are involved in sea louse resistance against chemical control agents.

A number of studies have investigated whether genes known to be relevant in insecticide resistance play roles in the resistance of sea lice to control agents. Recent results obtained by the group of Tor Horsberg show that azamethiphos resistance in *L. salmonis* is determined by a single non-synonymous mutation in the sequence of a gene encoding acetylcholinesterase, known to represent the target site for organophosphates [8]. Very similar missense mutations have been found in organophosphate-resistant populations of different insect species. Together, these findings provide an impressive example of parallel evolution in response to the same selection pressure, exposure to toxic organophosphates [9].

A number of further potential resistance factors have been studied with regard to their involvement in resistance of sea lice against control agents. In particular, gene sequences of voltage-gated sodium channels and glutamate-gated sodium channels have been analysed in order to find mutations related to resistance against pyrethroids and avermectins, respectively [10, 11]. Similarly, enzymes and transporters involved in detoxification pathways have been

investigated in sea lice showing resistance to emamectin benzoate and deltamethrin [12, 13]. However, these studies have so far not led to the identification of resistance mechanism.

In contrast to the candidate gene approach, which focuses on gene(s) suspected to be involved in a biological function of interest, broad scale genomic and transcriptomic studies make no *a priori* assumptions of mechanism. Instead they consider the entirety of gene or transcript sequences, as far as is possible using the specific methodology employed. Different research teams are currently applying genomic methodologies to sea lice, and it can be expected that in the near future a wider array of genetic resistance markers will become available. Such markers will allow the systematic testing of parasite populations from salmon farms in order to optimise sea louse control and avoid resistance formation.

Successful resistance management relies on the use of measures to reduce selection pressure for resistance development [6, 14]. This can be achieved first by reducing the overall number of treatments through increased use of farm management and other non-medicinal control approaches. However, where treatments are required, it is important to avoid under-treatments, as these can favour the enrichment of partially resistant parasites in the population. Moreover, rotation between drugs showing distinct modes of action should be applied. Finally, refuges where parasites remain unexposed to control agents, such as populations parasitizing wild fish, play a key role in keeping non-resistant genotypes in the gene pool. The greater number of wild as compared to farmed salmonids in marine systems of the Canadian West coast as compared to the North Atlantic is likely to be one factor explaining the few resistance problems reported from sea lice affecting salmon farms in this region.

In summary, effective sea louse control is an essential element of environmentally sensitive, sustainable salmon farming. Traditionally, sea louse control has relied strongly on the use of veterinary drugs; however, the potential of sea lice to develop resistance against chemical control agents is a potential threat to this approach. Recently, a number of non-medicinal control approaches have been developed far enough to allow their wide industrial implementation. Current research by different scientific groups focuses on resolving the molecular mechanisms of drug resistance. First, genetic tests to detect resistance have been developed, and more diagnostic tests can be expected to become available in the near future. The increased use of non-medicinal control strategies, combined with a targeted and restricted use of chemotherapeutants, supported by resistance monitoring using novel tests, will contribute to reducing the environmental impacts of salmon farming and improving the sustainability of this industry.

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