Management of Sea Lice on Farmed Salmon with Veterinary Medicines and Biological Control Strategies

Synopsis
In 2012 salmon farming reached a worldwide production of over 2,100,000 MT. However, growth of the industry is slowing. Between 1991 and 2001, production of Atlantic salmon, the dominant species grown, grew 328%, but only grew 62% between 2001 and 2011. Infestations with sea lice have emerged as a dominant factor limiting growth of the industry. Initially, the first line of defence against sea lice was the use of veterinary medicines. With the emergence of resistance to organophosphates in the early 1990s, several new compounds from different chemical classes were progressively developed for sea lice control. Despite these efforts, and increasing use of integrated pest management strategies to improve treatment outcomes, resistance has developed to these compounds, with a few notable exceptions. Multiple resistance, now present in several regions, exacerbates this situation. This presents a significant problem for farmers, veterinarians and the pharmaceutical sector given that, on average, it takes 15 to 20 years for the emergence of a new chemical class that might be considered a good candidate for parasite control. Further, it is estimated that 7 to 10 years is required to register new drugs for veterinary use. With no new sea lice control products in the development pipeline, the need to use integrated pest management strategies is more urgent than ever. Within this context, current sea lice management strategies are discussed in relation to current trends in the development of alternative control strategies that depend less on veterinary medicinal products.

Introduction
Commercial salmon farming in the ocean was first developed in the 1960s, building on the success of early pioneering enhancement projects to raise salmon in hatcheries to repopulate declining wild stocks. The transition from enhancement aquaculture to commercial aquaculture was realised in the 1960s with coho salmon in the Pacific Northwest and with Atlantic salmon in Norway. By 2012 global production of farmed Atlantic salmon exceeded 2 million metric tonnes, representing 66% of all salmon species used for human consumption (wild and farmed). Atlantic salmon are now farmed in twelve countries around the world, with Norway, Chile and Scotland being the top three producers, accounting for 87% of the total farmed Atlantic salmon production. Norway, by far the world’s largest producer, currently accounts for 60% of the world’s total at 1.2 million metric tonnes.

FAO statistics indicate that between 1991 and 2001, production of Atlantic salmon grew 328%, but only grew 62% between 2001 and 2011, suggesting that early gains in production volume are beginning to level off. Hoping to reverse this trend in order to meet the ever-increasing global demand for seafood and economic opportunities, salmon farming companies and regulatory development agencies are looking to expand the production of farmed salmon. However, one of the main impediments to the future growth of the industry is the ongoing and future management of infestations of copepod parasites, more commonly known as sea lice. For companies like Marine Harvest, the world’s largest producer of farmed salmon, sea lice is their number one material issue from a corporate and stakeholder perspective.

Sea Lice
Not long after the farmers in Norway and Scotland began to scale up production, sea lice emerged as a new clinically important pathogen. Problems with sea lice subsequently developed in other farming regions, notably Ireland, Canada and Chile, due to the ubiquitous nature of sea lice as parasites of wild salmon and other marine fishes. Sea lice include *Lepeophtheirus salmonis* (the “salmon louse”), in the Northern Hemisphere and several species of *Caligus* (found in Northern and Southern Hemispheres), the most notable of which is *Caligus rogercressyi* in Chile. Left untreated, sea lice numbers can quickly escalate on farmed salmon, resulting in significant losses. More insidious, perhaps, are the effects sea lice have on growth performance and predisposition of fish to infection from other pathogens (such as bacteria or virus), all of which amount to a significant fish welfare issue if left unchecked.

Sea lice have also been linked to declines in wild salmon. While this link appears more pronounced in some regions such as the North Atlantic, in others such as the North Pacific where Atlantic salmon are farmed amidst an abundance of Pacific salmon, the discussion is more controversial. As a result, the management of sea lice on salmon farms must also take into consideration the effects on, and protection of, wild salmon that migrate in the vicinity of salmon farms. This has led to legislation in most regions requiring routine auditing of lice and development of “trigger” levels, maximum numbers of lice allowed before actions must be taken to bring lice numbers down.

Because of their ubiquity in the marine environment, sea lice cannot be avoided by salmon grown in net pens, and therefore must be managed by farmers. The cost from direct losses (mortality), indirect losses (growth and secondary infection) and fish health management costs (treatments and labour to administer them) is significant and estimated in the millions of dollars/euros. A summary of cost estimates derived an average cost of USD $0.22/kg (€0.19/kg), suggesting at the time (circa 2000, but based on 2006 production figures) a global cost of USD $480 million (€305 million). However, in reality the number is probably greater given the recent emergence of resistance resulting in increased lice burden and the need to increase treatment frequency. Efforts to model treatment scenarios more reflective of current practices suggest the cost (in Norway) could be as high as USD $0.32/
kg (€0.28/kg)\textsuperscript{12}. Applying this average to current (2012) production volumes would suggest that the global cost could be as high as USD $742 million (€660 million). This cost will certainly rise without new approaches to sea lice control and is not sustainable if the industry is to grow.

**Veterinary Medicines**

Initially, the first line of defence against sea lice was through the use of veterinary medicines. Sea lice control products have been the subject of numerous reviews over the years\textsuperscript{12 - 17}. Chemical classes used for sea lice control include organophosphates, pyrethroids, and peroxide (as both treatments) and avermectins, and benzoylureas (as in-feed treatments). While representatives from each of the chemical classes were either in development or use by the mid-1990s, all compounds were not available in all regions at the same time. This led to overuse of some compounds where product rotation would have been preferred. The first documented case of resistance came about in Scotland following reports of reduced sensitivity by clinicians\textsuperscript{18}. This is not surprising since Scottish growers were limited to the use of a single compound (dichlorvos). In most instances, the sequential development of compounds combined with preference to use the most efficacious or easiest to use (at the time) contributed to successive development of resistance. [Some products are not effective against all sea lice developmental stages; and bath treatments are much more difficult to administer than in-feed treatments.] Resistance is now widespread and found in almost all regions to all chemical classes of compounds with the exception of benzoylureas – which are difficult to assess\textsuperscript{19}. In fact, we now have strains of lice that are resistant to several classes of compounds\textsuperscript{19}. This should not be too surprising, given our current understanding of resistance in crops, and was even reported in the 1960s for the freshwater fish louse, Argulus, following repeated exposure to lindane, an organochlorine pesticide\textsuperscript{20}. Interestingly, resistance to avermectins, the only compound available for use on the Pacific Coast of Canada has, to date, not been observed\textsuperscript{21}. Whether this is due to the relatively low production density (over the region), treatment frequency, or the large population of lice that exists on wild salmon, is unknown. Genetic studies suggest that lice populations from wild and farmed salmon on the Pacific coast are panmictic, allowing free flow of genes over a very large geographic area\textsuperscript{22}. As a result, untreated wild lice prevent increases in the frequency of resistance genes in lice on farmed fish, a so-called “hedgerow effect”.

At present, there are no new compounds or classes of compounds in the development pipeline for sea lice control. Other classes of compounds that might hold promise include the milbemycins (close relatives to the avermectins) due to high toxicity to lice\textsuperscript{23} or the more recently developed isoaxazolines\textsuperscript{24, 25} as evidenced by the recent commercialisation of NexGard\textsuperscript{®} (afloxolane) and Bravecto\textsuperscript{®} (furalaner).

However, it is likely that the biggest gains in sea lice chemotherapy will come from determining how best to apply veterinary medicines within an integrated pest management (IPM) strategy. This includes strategies such as the use of single-year class stocking, fallingow, treatment timing (winter vs spring), coordinated treatments (within a defined region) and maximum lice thresholds\textsuperscript{26 - 28}. Alternative approaches might also include synergistic use of existing veterinary medicines\textsuperscript{29}, or synergists such as piperonyl butoxide commonly found in a wide range of commercial products. For example, when piperonyl butoxide is combined with the pyrethroid resmethrin, toxicity to lice is increased\textsuperscript{30}. Alternatively, polymers, for the delivery and release of veterinary medicines (and vaccines), represent a vast area of biotechnology that is virtually untapped for aquatic veterinary applications. One example (of many) includes the dewormer LONGRANGE\textsuperscript{®} that incorporates eprinomectin (avermectin) with a slow release polymer\textsuperscript{32}.

The case of synergistic application of licensed products is particularly interesting since it presents a regulatory dilemma if, for example, it requires applying the compound in a manner that is not consistent with an approved label claim. Doing so would effectively invalidate the label claim for animal, food, and environmental safety (amongst others). Such approaches would have to be supported by product manufacturers or licensees and additional data submissions – effectively supporting a new data claim.

A major constraint to the use of veterinary medicines has been the difficulty, and more importantly time, to obtain full product registration/marketing authorisation. The regulatory situation is not expected to change. Current estimates by the International Federation of Animal Heath indicate that it takes 7-10 years to develop a new animal drug at a cost of $100M\textsuperscript{33}. Further, regulatory review times and costs to meet requirements have been steadily increasing. Since 1991 the time to develop and register a new veterinary medicine for a major livestock species has increased 7.5 years in Europe with a 229% increase in cost\textsuperscript{34}. In short, it takes longer and costs more to register veterinary medicines.

**Biological Control**

When the idea of biological control with cleaner fish was first developed in the mid-1980s, it may have seemed a bit odd\textsuperscript{35}. Cleaner fish comprise several species of wrasse from the family Labridae, including the goldsinny, cokwing, rock cook, cuckoo and juvenile ballan wrasse. This discovery led to extensive research studies in Norway, Scotland and Ireland to fine-tune the use of cleaner fish, optimising stocking ratios and how best to protect wrasse within cages through the use of refugia, as well as assessing disease risks\textsuperscript{36}. Efficacy of wrasse decreases as salmon get larger for a variety of causes\textsuperscript{36}, resulting in renewed interest in using larger adult ballan wrasse\textsuperscript{5, 37}.

Today, with reduced efficacy of veterinary medicines and ongoing issues with resistance management, the use of wrasse has become a critical component of IPM. So much so, that in Norway over 16 million wrasse were stocked in salmon pens in 2013, with a value of €18M (USD$21M)\textsuperscript{38}. This renewed interest in wrasse has made them a victim of their own success as they are sourced from wild fisheries. In Norway, the fishery is unregulated/lightly regulated, leading to concerns over exploitation\textsuperscript{39}. This has led to renewed interest
in commercial culture of wrasse to supply the demand\textsuperscript{45–49}. Additional concerns include the risk of introductions and transfers of wild fish over long distances. While many studies have shown that wrasse are relatively disease-resistant, the movement of wrasse in Scotland came to a halt in the late 1990s follow an outbreak of infectious salmon anemia virus\textsuperscript{50}. More recently, concerns over the welfare of wrasse in net-pens has led to the establishment of welfare indices for stocked wrasse\textsuperscript{51} as wrasse are prohibited under the Royal Society for the Prevention of Cruelty to Animals (RSPCA) welfare standards for farmed Atlantic salmon\textsuperscript{52}.

Comparable species of wrasse have yet to be identified in the North West Atlantic off the coast of North America or the Pacific off the coasts of Canada or Chile; however, research on the lumpfish Cyclopterus lumpus suggest they function as an alternative cleaner fish\textsuperscript{53–56}; although feed preference findings suggest more research is required\textsuperscript{57}. Interestingly, the geographic range of lumpfish covers the Atlantic on the East Coast of Canada and the North Eastern United States\textsuperscript{58} making the lumpfish a possible candidate cleaner fish in those regions.

Functional Feeds

Functional feeds represent another fish health management control tool. While not as immediately effective as veterinary medicines, they have been in use in aquaculture for many years. Broadly speaking, functional feeds are feeds/feed additives that include prebiotics and probiotics, including synergistic mixtures of the two, immunostimulants and more recently masking compounds. They are defined as feeds or feed ingredients that confer benefits beyond nutrition requirements. Due to the direct selection pressure that chemotherapeutants put on pathogenic organisms limiting their long-term utility, functional feeds hold promise within an IPM framework as key fish health management resources for fish farmers.

Immunostimulants are essentially dietary nucleotides that include beta glucans derived from yeast. Compounds derived from alginates, plant extracts, micro and macro algae also act in immunostimulatory ways\textsuperscript{57–59}. Examples of commercial products included MacroGuard\textsuperscript{60}, Optimum\textsuperscript{61}, Ergosan\textsuperscript{62}, Boost\textsuperscript{63}, and Protec\textsuperscript{64}, to name a few. In general, immunostimulants increase resistance to disease by affecting inflammatory processes, usually indicated by immunological proxy measures involved in humoral and cellular responses\textsuperscript{65}. While a significant body of work has looked at effects on immunological processes, growth rates, and feed conversion, resistance to disease has largely focused on bacterial and viral pathogens.

There are relatively few reports demonstrating effects on salmon lice. Several studies have shown that beta glucans and prebiotics (such as mannan oligopoly saccharides (MOS)) have been used to reduce sea lice infections\textsuperscript{50, 51}. A similar result was reported with diets containing plant proteins and MOS\textsuperscript{52}. More recently, diets supplemented with beta glucans, MOS, and two different plant extracts, were compared (separately) with the plant extracts conferring the largest reduction in lice (20\%)\textsuperscript{53}. These results are interesting in that they suggest that much is to be learned on what compounds may be best suited as feed additives for sea lice control. While encouraging, further study is needed under farm conditions before any conclusion can be made from a clinical perspective, stressing the need for and relevance of field studies.

Probiotics are microorganisms (primarily lactic acid bacteria and certain Bacillus spp.) with beneficial attributes that assist with the prevention and management of disease. The main focus of research has been the effect on the gut and gut tissues\textsuperscript{48, 54}. Similar to research on immunostimulants, studies focus on immune response, growth and feed conversion, with a few looking directly at control of infectious pathogens. Benefits in fish are largely achieved though better feed utilisation, competition with other harmful bacteria, improved immunological/haematological responses, production of inhibitory compounds and down regulation of virulence expression\textsuperscript{55}. Disease work to date has largely focussed on bacterial disease, which tend to be more systemic in nature. Of interest from a sea lice/ectoparasite perspective are the results from Pieter et al.,\textsuperscript{55} who demonstrate a positive effect from the application of probiotics (via IP injection) to reduce infections of the protozoan parasite Ichthyophthirius multifilis. However, the difficulty with probiotics, from a commercial application perspective, is administration and keeping them viable if incorporated into commercial diets.

As noted above, prebiotics have been demonstrated to confer some level of protection against sea lice as well as other protozoan ectoparasites\textsuperscript{56}. Prebiotics, which also have a long history in aquaculture, are largely carbohydrates that stimulate the growth of beneficial indigenous bacterial in the gut flora. They are principally oligosaccharides sugars such as mannan oligosaccharides, fructooligosaccharides, glucooligosaccharides and trans-galactooligosaccharides\textsuperscript{56–57}. Similar to immunostimulants, prebiotics also have immune-modulatory effects that confer a certain level of disease resistance, as well as improve biological performance of the host. Commercial products include Previdal\textsuperscript{60}, Grobiotic\textsuperscript{65}, and Prebiosal\textsuperscript{60}, to name a few.

Not surprisingly, combining pre and probiotics can result in a synergistic effect\textsuperscript{57, 58, 59}. Many of the same immunological and haematological parameters show increased indices indicative of improved immune and overall biological performance. From an ectoparasitic perspective, studies have shown that application of prebiotic/probiotic combinations resulted in increased mucous production\textsuperscript{58} or resistance to fungus\textsuperscript{60}, indicating an interesting line of research for sea lice control.

We know that Atlantic salmon show a higher susceptibility to sea lice than several Pacific salmon species\textsuperscript{61}. However, our understanding of the mechanisms behind this requires more research. Attempts to capitalise on this concept have resulted in the development of “masking” compounds that interfere with the proper recognition of hosts by sea lice, thereby reducing infections. One such commercial product, “Robust\textsuperscript{65}”, marketed in several jurisdictions, purports to do
just this; however, it’s difficult to evaluate the effect of such compounds as much of the research to develop such products is proprietary and can take a long time to reach the literature.

Vaccines
Efforts to develop a sea lice vaccine represent a Herculean task for the pharmaceutical sector. Compared to bacteria and viruses, ectoparasitic copepods are structurally and immunologically complex, with huge genomes. While the challenge is immense, so is the reward. Currently sales of smolts in Norway are 314 million fish/year. At current vaccine prices this represents a potential market value of approximately USD $75 million (€66 million). Considering the rest of the world and the high demand such a product would have, an effective sea lice vaccine would have a potential global market value of USD $100 - $125 million (€88 - €111 million). To date, vaccines for parasites remain elusive. There are no commercially available (i.e. fully licensed) vaccines for parasitic diseases in humans - despite the toll on society from diseases such as malaria and the resources that are currently being applied (although several are in large-scale clinical trials). Interestingly, several parasitic vaccines have been developed for veterinary applications including the cattle tick and the cattle lung worm.

Inspired by the cattle tick model, initial attempts to evaluate potential antigens involved targeting gut-associated antigens from purified whole lice homogenates. However, these early attempts met with little success. The issue here is the nature of the antigen target, a gut membrane-bound protein that is not “seen” by the immune system, otherwise referred to as a concealed antigen. As a result, large amounts of antigen need to be injected into cows to stimulate the production of sufficient antibodies, making the vaccine difficult to use for the farmer. Other antigen targets considered for further development have included recombinant vitellogenin-like, louse secretory/excretory, adhesion and cuticle binding and akrin proteins. While several attempts to evaluate these targets have been undertaken, much of this work remains unpublished. Limitations to advancing this work further include optimising expression systems to produce sufficient antigen for evaluation and access to laboratory lice challenge models and facilities. While several laboratories have developed capacity to engage in lice studies with and without fish, the facilities and model developed by the Sea Lice Centre in Bergen being a notable example, continued development and evaluation of veterinary products for aquatic animals is constrained by a lack of dedicated facilities. The results reported by Carpio et al. are noteworthy, but need to be validated in the laboratory with larger sample sizes and replicates to allow a more accurate assessment of efficacy.

It is clear that a more targeted approach is needed to identify critical antigens if the development of a vaccine will be successful. The field genomics may therefore prove to be a game changer for the development of a sea lice vaccine. Through the collaboration of several groups, it is expected the genome for the salmon louse (Lepeophtheirus salmonis) will be published in the very near future as more and more sequence data are updated to public databases such as GenBank.

Expressed sequence tags (ESTs) have been used to address questions of population dynamics in sea lice as well as biological function attributes. Gene microsatellites/single nucleotide polymorphisms (SNPs) are now commonly used to study gene function to great effect. The use of RNA interference (RNAi) is now proving to be a valuable tool for assessing gene function and for screening putative gene targets for potential vaccines. DNA libraries can now be scanned for putative antigens for vaccine targets, and functionality more accurately assessed using RNAi. This is a very powerful tool when considering the future development of sea lice vaccine. Use of RNAi technology is now being applied as an oral vaccine for bees for the control of Israeli Acute Paralysis Virus (IAPV), which is linked to colony collapse disorder (CCD). Interestingly, RNAi has been found to transfer from hosts to pathogens (insects, nematodes and mites), suggesting that vaccinating fish directly with short interfering RNA (siRNA) may be possible.

While such discoveries as RNAi vaccines may be realised in the not too distant future, commercialisation of such products can be a long process, as noted above, due to product development research, the need to develop cost-effective manufacturing processes and, more importantly, demonstrating target animal and environmental safety requirements. Nonetheless, such hurdles can be overcome, as evidenced by the licensing of the first commercially available DNA vaccine for the control of Infectious Haematopoietic Necrosis virus in salmon in 2005 in Canada and subsequently in the United States. Since sea lice first appeared as a pathogen of farmed salmon, a great deal has been learned about their biology and control. Given its economic importance, some would say that it’s the most studied fish parasite in the world. It is clear that significant benefits have been made through our understanding of sea lice biology that have enabled continued management of this economically important pathogen by extending the usable shelf-life of veterinary medicines through the application of integrated pest management.

Veterinary medicines are valuable and necessary tools for animal health management, but they are not long-term solutions. Recent advances in biological control and functional feeds will most likely be the focus of future research and control efforts. In the long term, a better understanding of the lice genome and its functionality will, it is hoped, lead to the development of a vaccine and a more integrated, preventative sea lice management paradigm that will support the production and growth of salmon aquaculture.
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