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	Genetic and Other Innovative Strategies to Reduce Sea Lice	**Agenda Item: 5(a)**

Genetic and Other Innovative Strategies to Reduce Sea Lice

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The United States Department of Agriculture-Agricultural Research Service (USDA-ARS) National Cold Water Marine Aquaculture Center (NCWMAC) in Franklin, Maine has been supporting the United States cold water marine aquaculture industry since 2003 by developing genetically improved North American (NA) Atlantic salmon and conducting research to address industry issues. In the past six years, the NCWMAC has begun two programmes to help manage sea lice on marine net pen farms. The first programme incorporates sea lice resistance into the existing selective breeding programme and the second is the development of lumpfish culture techniques and broodstock for use as cleaner fish in marine net pens. Both selective breeding and the use of cleaner fish are management options that can be used by the domestic industry as part of their existing integrated pest management programmes (IPMP).

Breeding Programme

The NCWMAC utilises the St John River (SJR) strain because of fast growth, certification of NA origin and widespread utilisation by industry. Since 2015, the primary objectives of the salmon selection programme have been to improve commercially important traits including: carcass weight, fillet colour, fat content and sea lice resistance. Recently, the programme started to evaluate and validate the usefulness of incorporating genomic based selection techniques into the salmon breeding programme. Culture of Atlantic salmon in the breeding programme and research facility is based on life stage and separation of year class (Figure 1).
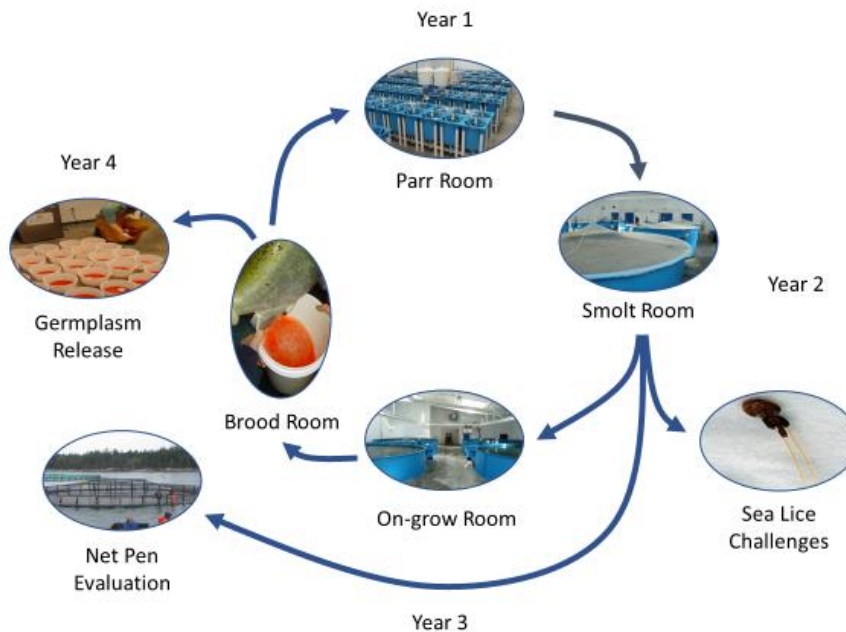

Figure 1. *Overview of the 4-year cycle for the breeding programme at the U.S. Department of Agriculture's National Cold Water Marine Aquaculture Center.*

An incubation system is used for eggs and newly hatched fry before first feeding, parr tanks are used for first feeding fry to 30 – 40 g salmon, the smolt tanks are for 30 – 100 g pit-tagged salmon, on-grow tanks are for 100 g to 1.0 kg salmon in their second year, 3-yr old broodstock tanks are for 1.0 kg to 3.0 kg salmon and four 4-yr old broodstock tanks are for growing salmon 3.0 to 8.0 kg when they will be spawned. Up to 234 families of Atlantic salmon can be cultured in the breeding nucleus and approximately 250 eggs are saved from each family mating and reared. The remaining eggs are distributed to industry and used as their next generation of brood to provide eggs for commercial production. Of the fish from the 250 eggs that remain in the programme, typically 30 – 40 smolts per family are saved as the nucleus of fish (broodstock) for the breeding programme and cultured in biosecure tanks at the NCWMAC. An additional 30 – 40 smolts per family are cultured and transferred to an industry collaborator for stocking into net pens for growth performance evaluations. Additional smolts are also kept for various studies involving measurement of traits such as sea lice resistance. Selection of 4-year old fish for spawning occurs when fish are moved from 3-year old broodstock tanks into the 4-year broodstock system prior to the spawning season.

Sea Lice Selection

Since 2015, the NCWMAC has evaluated three of the four year classes per generation and continues to evaluate and select the offspring of those year classes. The programme utilises a challenge model based on multiple small challenges to evaluate 70 – 120 families for lice resistance. Approximately 16 pit-tagged fish from each of these families are set aside for challenge trials. One fish from each family is stocked into a 1 m^3 tank, setting up a series of 16 replicate tanks.

Sea lice egg strings are collected from commercial farms and then brought back to be cultured in the laboratory to the infective copepodid stage. Challenge fish are infested via a 4-hour bath challenge as 2 – 4 day old copepodids are available. Lice are allowed to develop to the 2nd chalimus stage, approximately 10 – 14 days post infection (dpi). Fish are euthanised by an overdose of MS-222 in saltwater and then transferred to a freshwater staining bath of neutral red for 20 minutes before being individually bagged. Fish are kept on ice until the lice are counted, fish weighed, fork length measured and fin clips are preserved in 95 % Ethanol. Lice counts are standardised by lice density (LD; Gjerde *et al.* 2011) and lice per cm^2 of surface area. Surface area is calculated from the model in Frederick *et al.* (2017). Estimated breeding values for each family are generated using the programme MTDFREL.

The evaluations to date have revealed heritability values within our population of between 0.20 and 0.31 that are consistent with those published in the literature. Looking at the average lice densities for each family in each year reveals a similar pattern with a few families showing resistance and a gradual increase towards highly susceptible families (Figure 2). Efforts to date have focused on utilising the most resistant families to select a few highly resistant families, while simultaneously ensuring that most families which are selected based on growth are families not within the most susceptible 30 % of families.
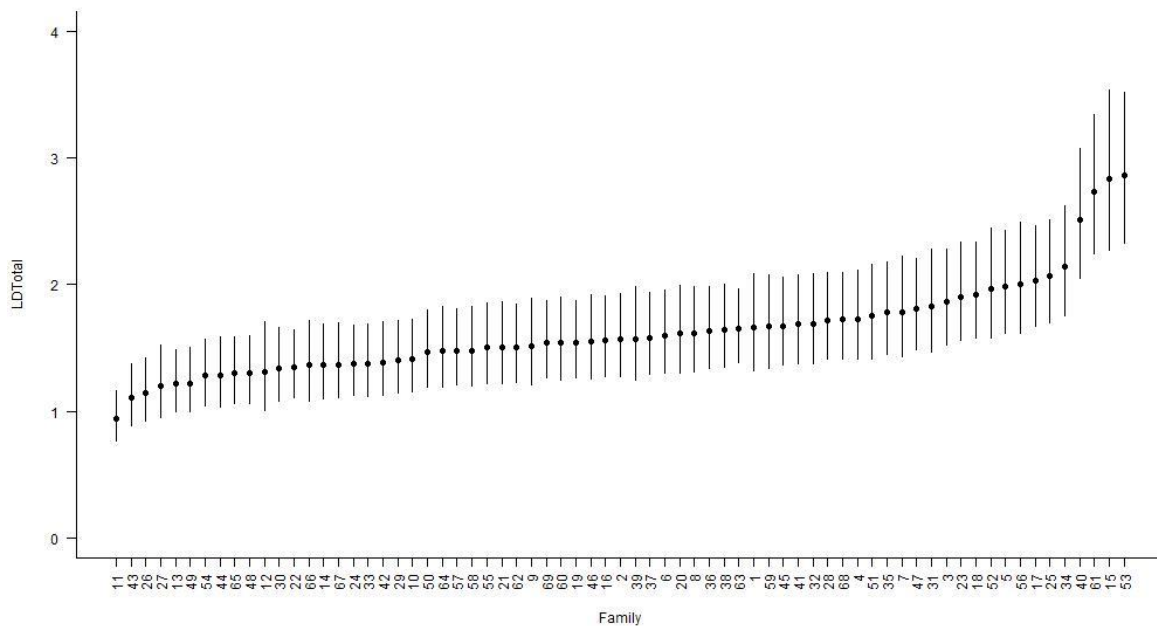
Figure 2. *Typical graph illustrating average lice density (LDTotal) per family with 95 % confidence intervals. Families exhibiting lower lice density are more resistant.*

Genomic Based Selection

Genomic based selection has made significant advances over the past 20 years since the development of the use of genome-wide dense marker maps (Meuwissen *et al.* 2001). In combination with the reduction in genotyping costs, this new technique has allowed for improved selection accuracy. This is accomplished by using a combination of traditional phenotypic based estimated breeding values (EBV) and genomic data to generate genomic estimated breeding values (gEBV) which account for within family variation in addition to between family variation. The techniques were pioneered in dairy and beef cattle and other major terrestrial livestock, but recently are more widely being used in fish for multi-locus traits such as growth and disease resistance to some pathogens (Vallejo *et al.* 2017; Garcia *et al.* 2018).

Over the past several years the programme has been collaborating with USDA-ARS scientists at the National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia. These efforts have included constructing a reference genome for North American Atlantic salmon of the SJR strain and developing a publicly available 50K single nucleotide polymorphism (SNP) chip for genotyping salmon of NA origin (Gao *et al.* 2020). The goal is to implement a genomic selection programme for sea lice in November 2021. This represents a significant step forward in selective breeding for sea lice resistance.

Lumpfish

Cleaner fish have been used in European salmon farms for over 30 years (Pike 1989). However, the wrasse species that have been primarily used are not native to the western North Atlantic Ocean. More recently the lumpfish (*Cyclopterus lumpus* L.) has become a popular option for use as a cleaner fish. Lumpfish are native to the eastern coast of North America (Cox and Anderson 1918). As a result, the use of cleaner fish has only been adopted within the past 5 years or so with the commercial industry on the North American side of the Atlantic Ocean. Despite the disparate adoptions in the use of cleaner fish between the two sides of the Atlantic, the recent surge in use of lumpfish is common to both and the development of a lumpfish

farming industry is rapidly developing in both locations. This rapid development presents a range of research potential and knowledge gaps for the species (Brooker *et al.* 2018; Powell *et al.* 2018).

The wide range of research coming out on lumpfish can be grouped into four broad areas. The first is the development of captive reared broodstock populations. The reliance on wild caught animals to provide eggs has been identified in several key reviews as a critical knowledge gap (Brooker *et al.* 2018; Powell *et al.* 2018). However, this situation is rapidly evolving as development of captive brood is constantly occurring in major lumpfish rearing countries. This research area is evolving to incorporate potential selective breeding, manipulation of spawning timing and improving reproduction (Imsland *et al.* 2016a; Imsland *et al.* 2019). This is one of the primary areas of focus for our lumpfish programme. In collaboration with the University of Maine, current efforts are focused on development of captive brood populations derived from local populations. Likewise, the U.S. Lumpfish Consortium spearheaded by NCWMAC, University of New Hampshire, the University of Maine and industry partners are also investigating the manipulation of spawning and interested in the potential for selective breeding.

The second broad area of research involves optimising the effective husbandry of all stages of lumpfish care both in hatcheries and in sea cages. There is a wide range of issues and topics being explored under this area. Work in this area is coming out in both peer reviewed and trade literature but is also being conducted within facilities and companies on a private basis. On the hatchery side, research into optimum water quality, temperature, nutrition and other husbandry issues are being conducted (D'Arcy *et al.* 2020; Dahle *et al.* 2020; Mortensen *et al.* 2020; Pountney *et al.* 2020). Optimising hatchery husbandry, including the maintenance and nutrition of captive broodstock populations, is the other primary focus of the NCWMAC in collaboration with the University of New Hampshire. On the net pen side, nutrition, lumpfish recapture and housing are areas of interest (Imsland *et al.* 2015a; Imsland *et al.* 2018a; Imsland *et al.* 2018b; Imsland *et al.* 2020). Feeding techniques, such as the use of gel blocks or submersible feeders to keep lumpfish from eating salmon feed are also being examined. One of the more interesting trends that has occurred with the development of lumpfish culture is that not only is there interest in optimised husbandry as demonstrated above, but there is interest in animal welfare research and how to improve this research simultaneously (Gutierrez Rabadan *et al.* 2020).

The third major area of research revolves around disease. The development of any new aquaculture species is accompanied by many new areas for disease research as it is difficult to study disease in wild populations. As might be expected, new occurrences of bacterial, viral, parasitic and mycotic pathogens are being observed (Einarsdottir *et al.* 2018; Ellul *et al.* 2018; Rouleau *et al.* 2018; Scholz *et al.* 2018; Pietrak and Rosser 2019; Stagg *et al.* 2020). Research into lumpfish immunity, pathogen treatment and other areas to improve overall health is also rapidly developing (Erkinharju *et al.* 2018; Pietrak and Backman 2018; Haugland *et al.* 2019; Jacobsen *et al.* 2019; Eggestøl *et al.* 2020; Klakegg *et al.* 2020). One new area of health research that is, and will become more, prominent is the ecology of pathogens in these polyculture systems (Murray 2017). Large scale western aquaculture has traditionally been a monospecies approach and the benefits of polyculture systems has focused on multi-trophic systems rather than multiple fish species. The increased co-culture of cleaner fish can complicate disease management on farms given the ability of some pathogens or strains to remain asymptomatic in one species cultured, but potentially cause outbreaks the other.

Finally, the last major area of research is arguably the most important and that is documenting and optimising the efficacy of using lumpfish as cleaner fish. Early efforts focused on demonstrating the effectiveness of lumpfish by comparing sea lice levels in cages with and

without lumpfish, and with varying densities of lumpfish and other factors (Imsland *et al.* 2014a; Imsland *et al.* 2014b; Imsland *et al.* 2015; Imsland *et al.* 2016b; Imsland *et al.* 2018c). However, while there is a consensus that lumpfish can help manage sea lice populations, it has been observed that not all lumpfish exhibit cleaning behaviour. A pressing topic is understanding what genetic, environmental, seasonal or life history factors are influencing cleaning behaviour (Imsland *et al.* 2016a; Imsland *et al.* 2016b; Eliasen *et al.* 2018; Leclercq *et al.* 2018). By starting to understand the various factors influencing cleaning behaviour, studies can start to help refine and optimise the most appropriate situations for utilising lumpfish (McEwan *et al.* 2019).

Summary

Significant advances are being made in the development of selective breeding for sea lice resistance and in the use of cleaner fish, especially lumpfish. However, neither of these technologies likely represents a silver bullet to managing sea lice populations on either a local or global scale. Both technologies can be important tools and components in effective IPMP as salmon farmers move from IPMP that primarily use drug-based management strategies to IPMP that use non-drug-based strategies. It is important to remember that no specific system of control strategies will fit all regions, water bodies or even companies. Rather, systems need to be individually tailored to each farm. With the proliferation of non-drug management tools in IPMP, it is critical that more research be focused on rigorously determining what strategies and technologies are effective at the local level and defining the economic costs of each strategy.

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