

MOLECULAR EPIDEMIOLOGY OF AMOEBIC GILL DISEASE CAUSING *NEOPARAMOEBA PERURANS* IN ATLANTIC SALMON AQUACULTURE



¹ B. A. Robertson*, ¹ L. A. Covington, ^{1,2} Bachar Cheaib, ¹ M. S. Llewellyn

*Email: Brendan.Robertson@glasgow.ac.uk

¹ School of Biodiversity, One Health and Veterinary Medicine, Graham Kerr Building, University of Glasgow, Glasgow

² Department for Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University Hospital, Heidelberg, 69120

Introduction

- Amoebic Gill Disease (AGD) → Caused by *Neoparamoeba perurans*
- Symptoms include:
 - Pale lesions → raised white mucoid patches on the gill
 - Lethargy → Lack of appetite → Respiratory distress → Death
- AGD affects a variety of marine fish and cleaner fish not only Atlantic salmon
 - Downstream effects of such high levels of AGD infection and resultant suppressed immune function can lead to greater susceptibility to other marine pests, such as salmon lice
- Predicted to cost the UK salmon farming industry **£124 million in 2023 in losses**
- Ascertaining the transmission and evolutionary pathways of this amoeba is of great importance to prevention efforts.

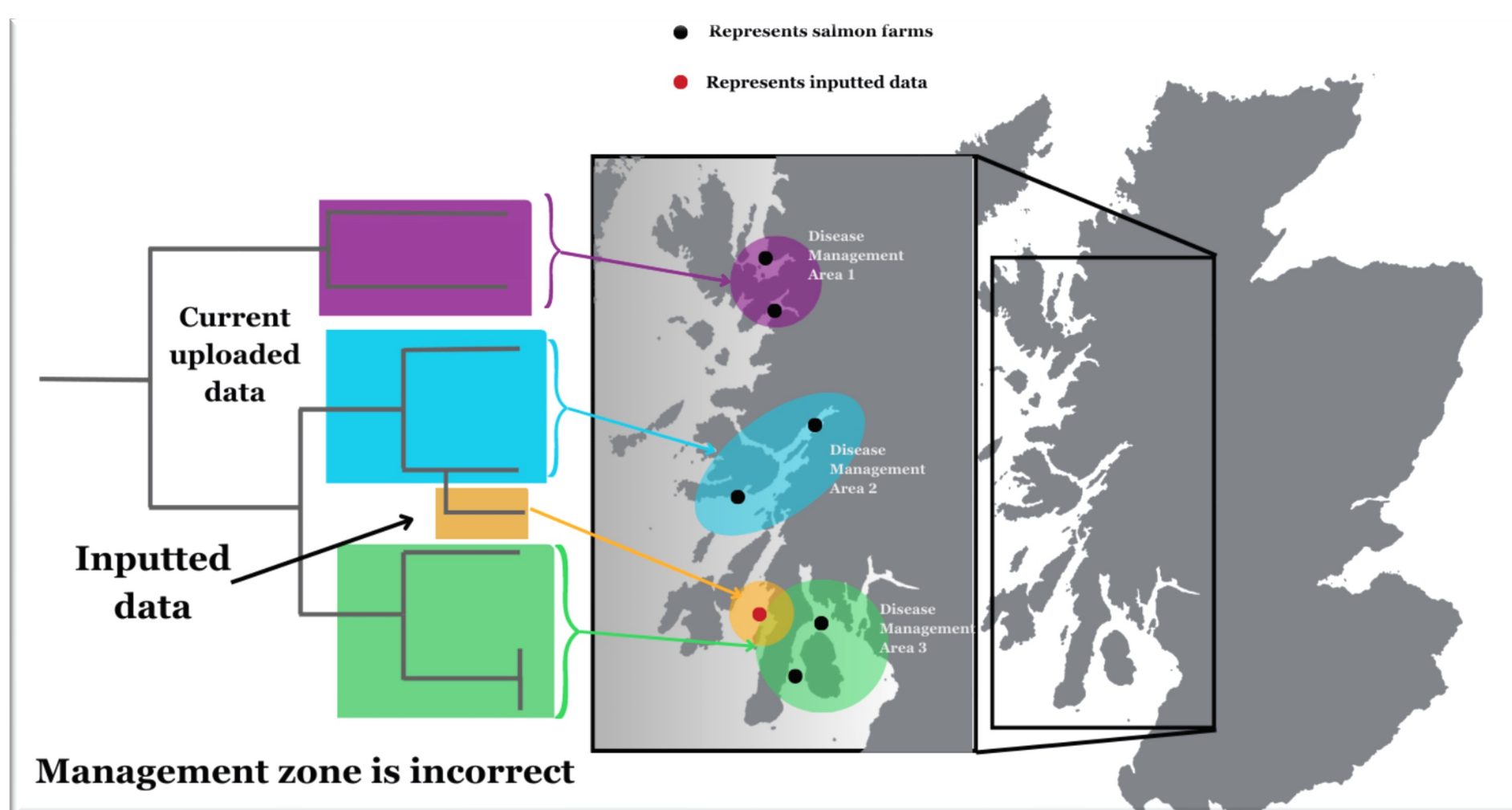
Aim

Using the Llewellyn lab's draft genome of *N. perurans*, develop a amp-seq SNP panel to genotype the parasite and enable near real-time information to inform disease control management plans including when are where to fallow for Atlantic salmon aquaculture.

Methodology

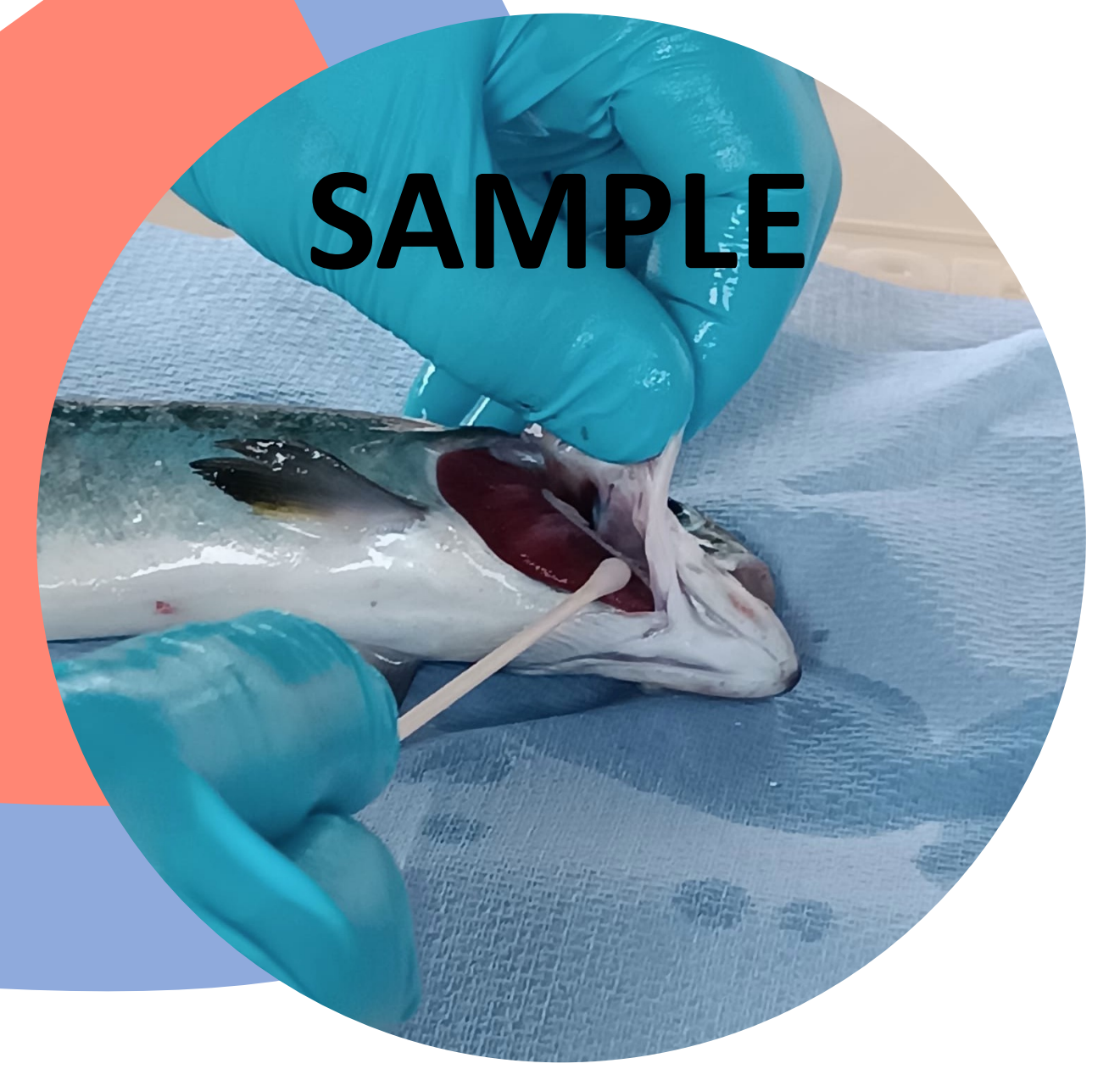
- Gill swab collection
- DNA extraction and Quantification (DNAeasy & Qubit)
- RT-qPCR – confirm the presence (ng/μl) & aid selection of positive samples for sequencing
- Multiplex PCR
 - 96 experimental primers, mapped against *N. perurans* draft genome in the Llewellyn lab
 - Success in amplifying up to 48 primers at a time
- Sequencing
 - Nanopore MinION
 - Demultiplexed and trimmed
 - IGV used to view primer pile up plots
 - Aligned using MUSCLE (Jalview)
- Molecular Epidemiology
 - Phylogenies built-in MEGA11
 - Trees refined in FigTree

UNDERSTAND EPIDEMIOLOGY



SEQUENCE

Multiple Regions
Nuclear +
Mitochondrial



Results

- Primers clearly amplify *N. perurans* genetic material.
- Cyclical reintroduction and reinfection of the same amoeba strains and genetic material year over year may occur.
- Phylogenetic analysis indicates **significant genetic mixing and reinfection year over year in the Scottish and Irish marine environment**

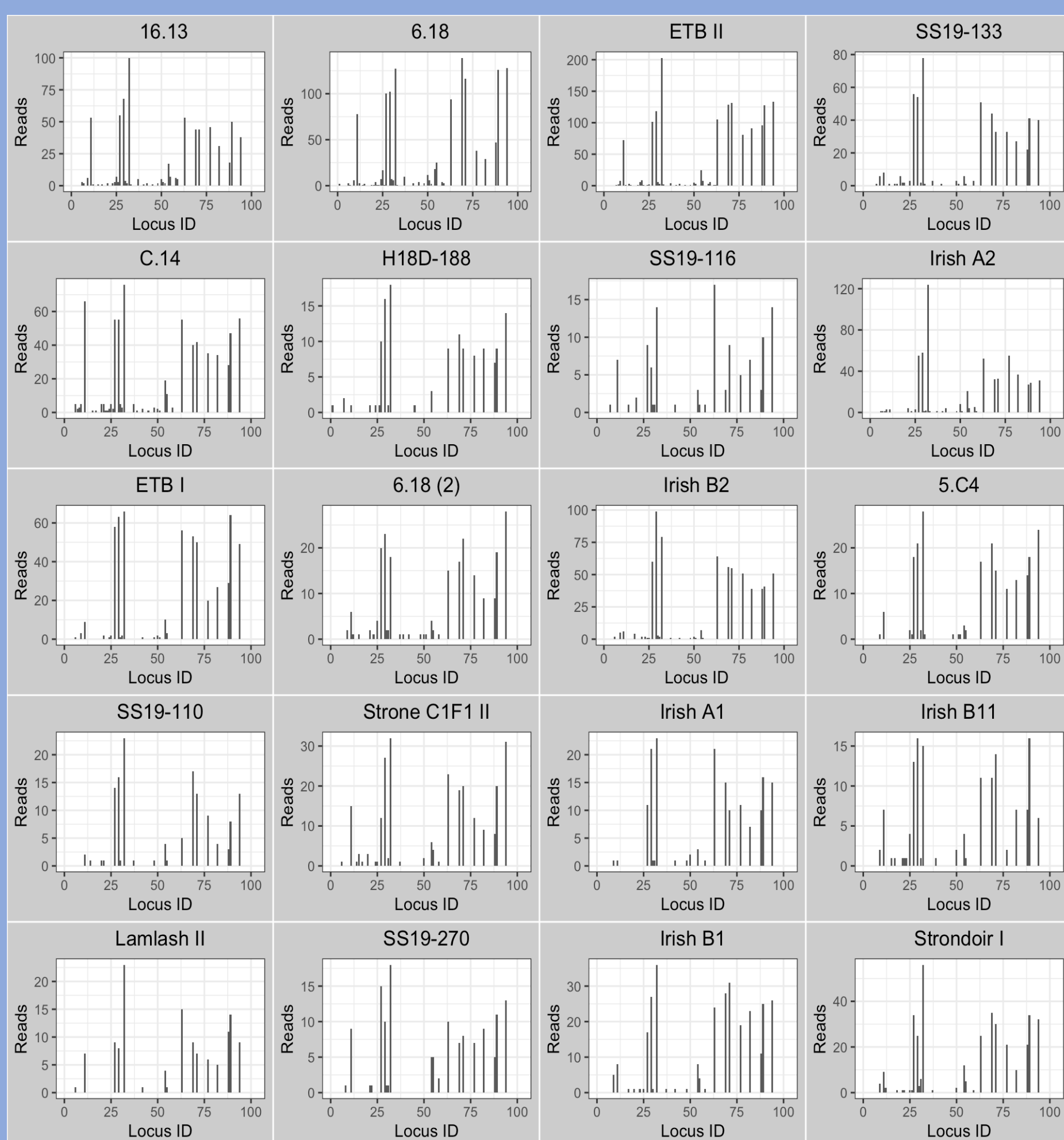


Figure 1: Bar graphs visualizing the number of reads per primer loci (labeled 1-96) as recorded via Nanopore MinION sequencing for each gill swab sample sequenced

Symbol	Location/Year
Blue Box	Scotland
Green Box	Ireland
Orange Highlight	2019 sample
Pink Highlight	2022 sample
Yellow Highlight	2023 sample

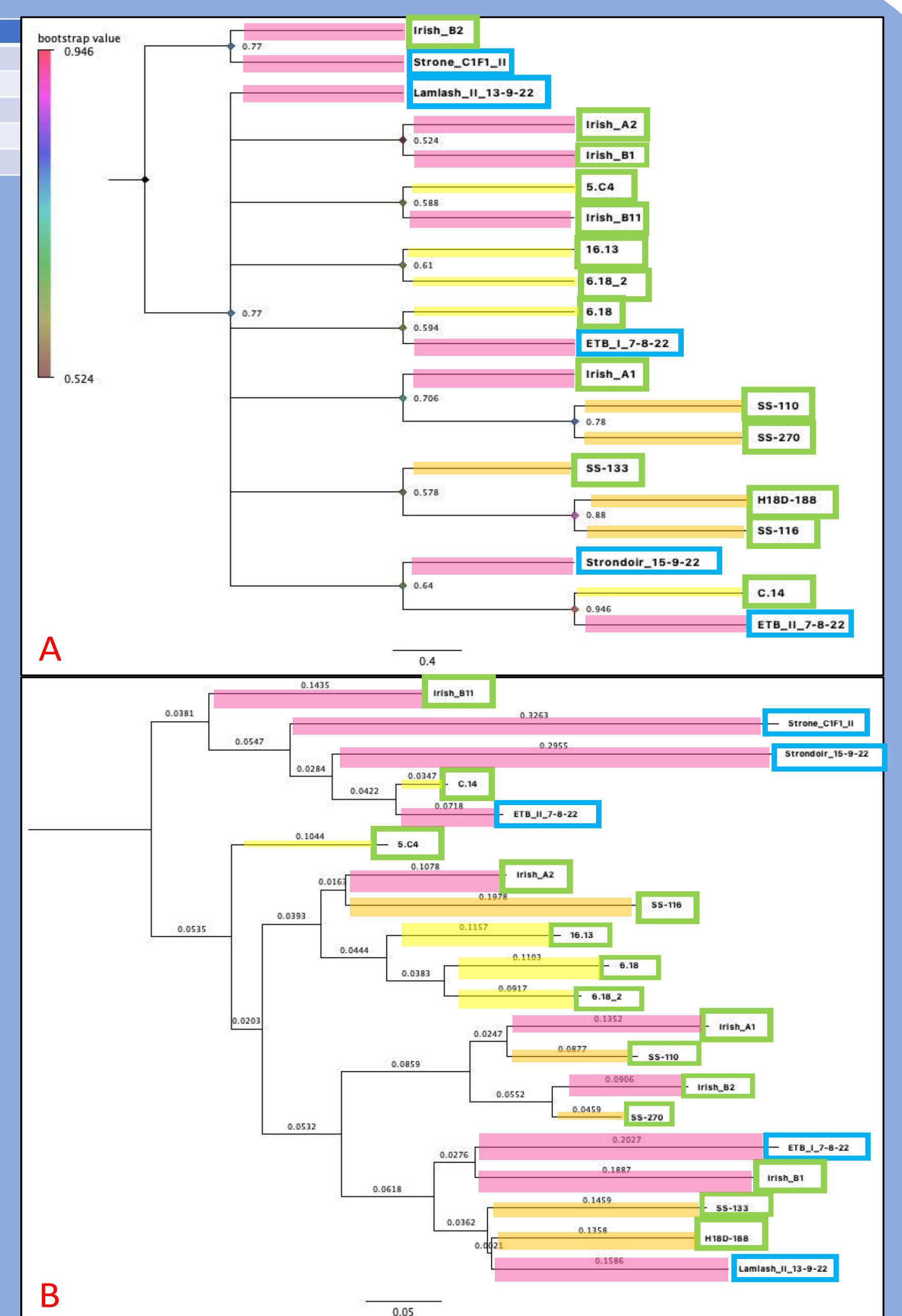


Figure 2: Evolutionary trees of *N. perurans* using a bootstrapped Neighbor-Joining method (A) and a Maximum Likelihood (ML) method (B) where bootstrap values and branch lengths are displayed respectively. Key top left.

Further work

- Further research on primer design to discover why many of the primers aren't amplifying, and which ones are working sporadically
- Test GLST AmpSeq approach using 202 experimental primers on tissue and gill swab samples from different years and sites across 3 countries (Scotland, Ireland, & Norway) to understand spread and genomic variation over time.
- Sequencing using Illumina → more suited for small fragments of DNA
- Development of a web-based data visualisation tool to track genotypes, spread over time, and new more virulent lineages of *N. perurans* for Atlantic salmon farming sites (potential to be used worldwide) can enable targeted and timely approaches to AGD outbreaks.

Take Home Message(s)

- Phylogenetic analysis of trees created from useable consensus sequences indicate reinfection and significant genetic mixing and similarity year over year in the Scotland and Ireland marine environment
 - Which would negatively affect the efficacy of prophylactic treatment strategies for AGD and recommend practices centered around freshwater fallowing and general cage hygiene
- Appears to be strong evidence that AGD treatment in aquaculture should remain focused on cage hygiene and freshwater fallowing as needed.
- Information obtained from the GLST AmpSeq panel on *N. perurans* dispersal can be relayed back to the individual farm managers of affected sites (and potentially other site managers nearby) in near real-time to allow appropriate decisions to be made i.e. treatment protocols (freshwater bathing), emergency harvest, and fallowing.