Using the microbiome to discriminate production method in turbot (*Scophthalmus maximus*): wild versus farmed.

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Introduction

Turbot (*Scophthalmus maximus*) is a flatfish with a high economic value. The largest production of turbot is obtained from aquaculture, which is ten times higher than that obtained by catch[1] , but wild turbot is more appreciated by the consumers. Since 2013, in the European Union is mandatory to indicate the production method on the labelling of fishery products [Regulation (UE) Nº 1379/2013]. Hence, it becomes necessary to use traceability methods, as molecular traceability techniques, that allow the production method verification. Currently, high-throughput sequencing (HTS) technologies have been applied successfully in host-associated microbiome studies to establish geographical patterns since microbiota is affected by numerous factors as: environmental variables, human manipulation, or geographical origin[2].

Objective

The aim objetive of this study was to analyze microbiome associated with gill, mucus, and gut from wild and aquaculture turbot to evaluate their ability to discriminate the production method employing a 16S rRNA metabarcoding approach (Figure 1).

Material and Methods

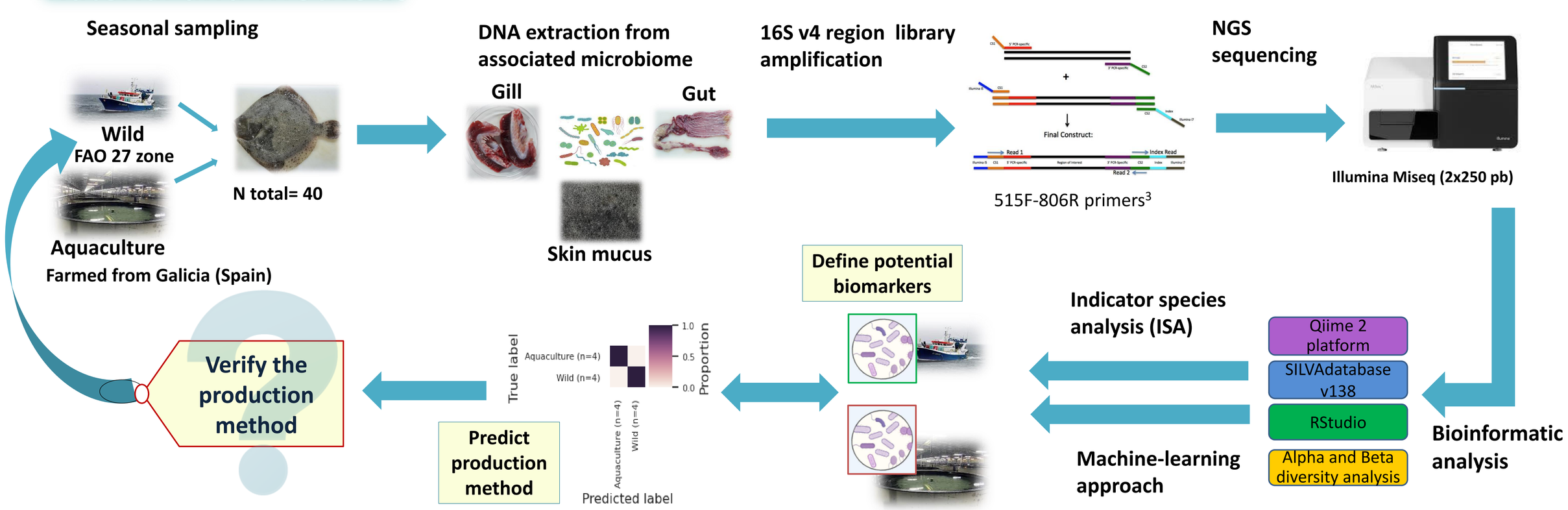


Figure 1. Workflow of the turbot production method prediction based on metabarcoding technology

Results

Sequencing results were adquate, with an average number of reads per sample of 54,765, which allowed the normalization of data **maintaining a reliable representation of the diversity of the microbiome communities associated to the turbot tissues**.

Alpha Diversity

Alpha diversity between production methods was not significant (Simpson index) in the case of mucus and gut associated microbiomes, even considering the seasonality. However, significant differences were found for wild turbot in gill microbiome due to the community found in autumm that was very different as can be seen in Figure 2.

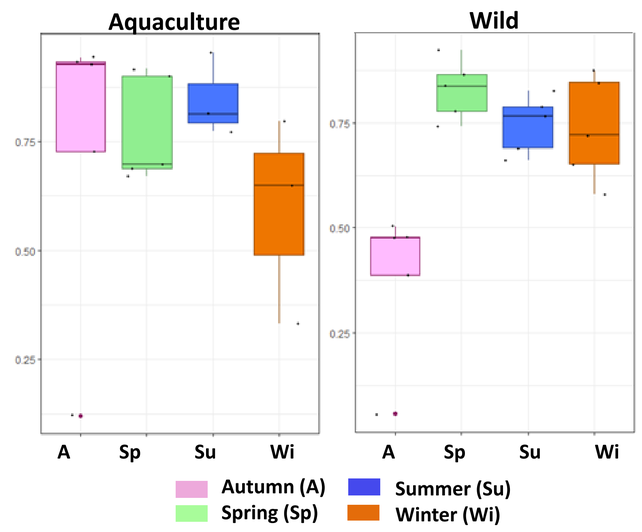


Figure 2. Seasonal alpha diversity boxplot for gill microbiome community.

Beta Diversity

Clear clustering pattern, dividing wild and aquaculture samples was observed (Anosim test, $p < 0.001$), specially for gill simples (Figure 3), demostrating a different comunity (structure and composition) between the microbiome communities that come from different production method.

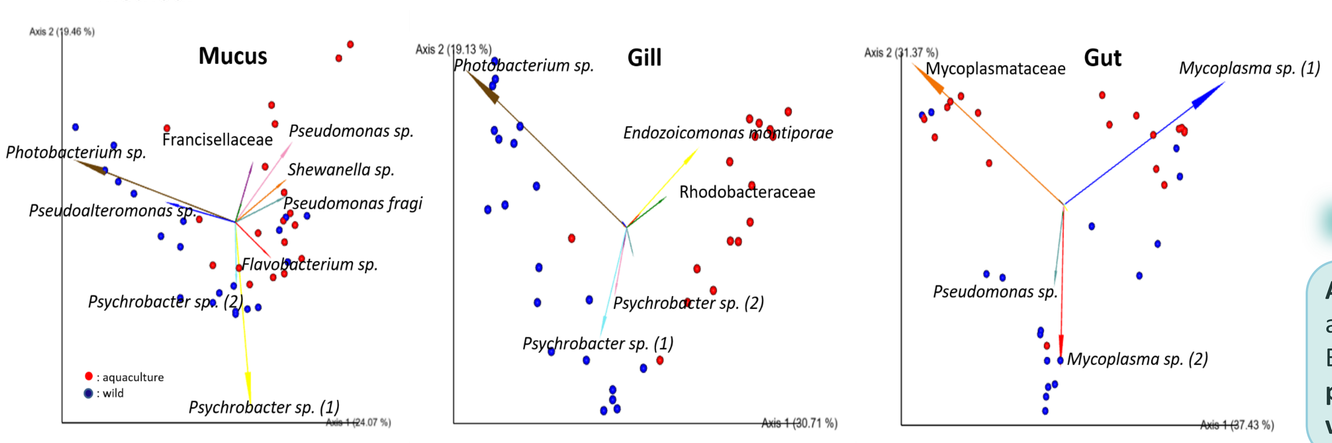


Figure 3 PCOA biplot for mucus, gill and gut dataset. The most influential taxa (species level) in the clustering pattern are represented.

Microbiome Community Composition

The most representative genus in mucus and gill samples are Photobacterium and Phychrobacter followed by Shellanella, Pseudomonas and Flavobacterium in mucus profile and Endozoicomonas in gill samples (Figure 3). Otherwise, Mycoplasma genus was only found in gut microbiome profile. The microbiome composition found is in accordance with that found by other authors for this type of tissues, fish and environment[2,4].

Machine learning analyses: Production Method Prediction

Based on the microbiome data obtained for each of the three sample types, a prediction model of the turbot production method was developed using a machine learning approach in which the 80 % of the samples were used as training samples. A good overall accuracy percentage was obtained for the mucus and gill models (88 %) for the test samples (20 % of samples). This percentage of prediction was lower for the model based on gut samples (75 %).

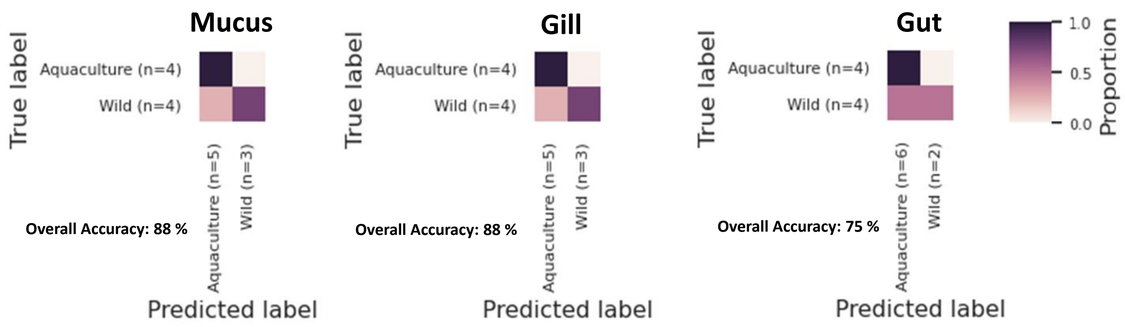


Figure 4 Sample prediction models

Biomarkers from ISA analysis

Taxa that were represented in a percentage equal o greater than 1 % in each dataset and with a presence equal o greater than 75 % of the samples were selected with the aim of identifying the best biomarkers to assign production method. Table 1 shows the taxa that were significantly representative (p-value <0.01) of the wild and farmed turbot and therefore susceptible to being used as production method biomarkers.

Table 1. Potential biomarkers for predicting turbot production method

	Aquaculture	Wild
Mucus	*Shewanella sp.*	-------
Gill	*Rubritalea sp., Endozoicomonas montiporae*	*Photobacterium sp., Psychrobacter sp.* (strain 1 and 2)
Gut	*Mycoplasma sp.* (strain 1)	*Mycoplasma sp.* (strain 2)

Conclusions

Aquaculture and wild turbot samples showed different microbiomes, with some taxa more associated to one specific production method and without significant influence of seasonality. Based on that, **we show some potential biomarkers in mucus and gill microbiomes and a prediction model for identification** with acceptable accuracy percentages, **that can be used to verify the production method of the turbot**.

References

1. FAO. 2024. FAO Fisheries and Aquaculture FishStatJ Software for Fishery and Aquaculture Statistical Time Series. In FAO Fisheries and Aquaculture Division. Rome; **2**. Ruiz-Rodríguez, M., et al. 2020. Microb. Ecol. 80: 212-22; **3**. Caporaso, J., et al. 2012. PNAS. 108: 4516-22; **4**. Piredda, R., et al. 2023. LWT. 186-115264.

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