IT'S IN THE BLOOD: USE OF BIOMARKERS AND MACHINE LEARNING TO RAPID DETERMINE THE SMOLTIFICATION STATUS IN ATLANTIC SALMON (*Salmo salar*)

Saad Zah[1,2], Eric Bendiksen[1], Ioannis Vatsos[2], André Madsen[1], Kjetil Korsnes[1,2]

[1] Aquapredict AS, Bodø, Norway

[2] Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

Presenting author: saad.zah@aquapredict.no

Introduction

Seawater tolerance is a fundamental and critical aspect that salmon must acquire when transferred from hatcheries to growing locations in the sea. The fish acquires this tolerance during smoltification, which is characterized by profound behavioral, biochemical, physiological and morphological changes. Insufficiently smoltified fish will exhibit osmotic problems when transferred to sea and are likely to die. Current techniques to determine the smolt status rely on measuring the plasma chloride level in seawater-challenged fish or the genetic expression analysis of Na+K+ATPases enzymes in the gill. The current methods are not the most effective in terms of being robust and cost-efficient.

Aims of study: Identify biomarkers suitable to determine smoltification status using point-of-care blood testing and machine learning for objective assessment as basis for rapid analysis on-site.
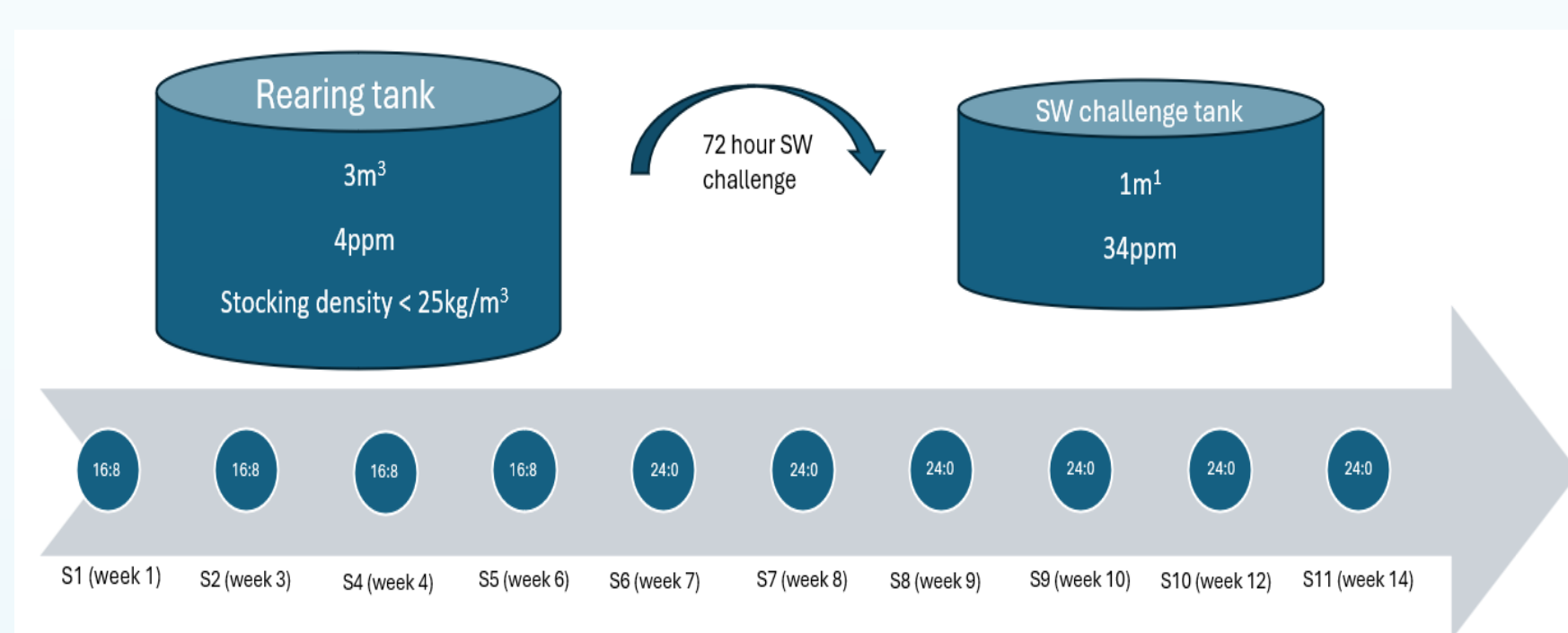
Methodology

Figure 1 : experimental design of the smoltification trial including the sampling timeline and the light regime.

Blood samples were harvested, and plasma levels of 11 biomarkers were quantified (creatine, blood urea nitrogen , phosphorus, Mg2+, K+, Ca2+, Na+, Cl-, glucose, lactate, total CO2) using instrument SMT-120VP (Chengdu Seamaty Technology Co. Ltd.) Point of Care system. For the SW challenged group, plasma chloride levels were additionally measured using a M926S Chloride Analyzer (Sherwood Scientific Ltd.). Gill expression levels of NKA1a and NKA1b, from gill RNA, were obtained by qPCR analysis (Nilsen et al. 2007) for both groups. The biomarkers were used to establish supervised machine learning (ML) models to predict smolt status and outcome of a qPCR NKA1a/NKA1b analysis.

Results

A receiver operating characteristics (ROC) curve analysis conducted on the individual analyte obtained by the point of care system indicated that plasma chloride exhibited the most significant predictive value in terms of discriminating smolt and non-smolt fish (AUC = 0.88, p < 0.05 (Mann-Whitney U)). Furthermore, calcium, sodium, glucose, and potassium also provided predictive values and exhibited significant differences between the groups. The compounded predictive values of all applicable biomarkers were deployed as independent feature variables for training a xgboost ML model in the statistic software R, and its output predictions corresponded to a ROC-AUC of 0.98. A challenger ML regression model was used to predict the qPCR index (0-100 scale), and it achieved a root mean square error (RMSE) of 0.11. Increased plasma chloride levels in seawater-challenged fish indicated a transient de-smoltification during the experiment.
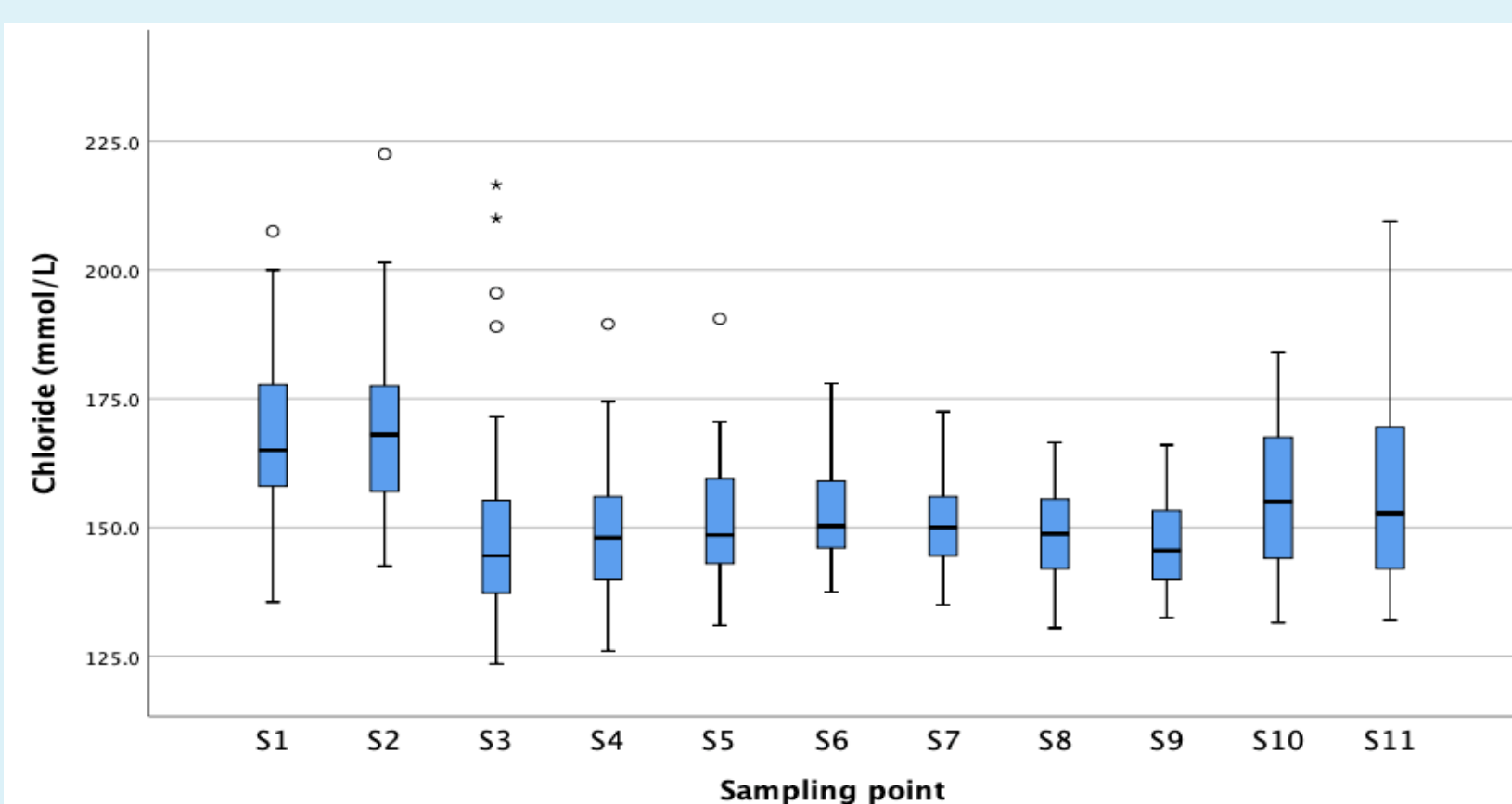

Figure 2 : Plasma chloride levels of seawater challenged fish.

Table 1: Comparison of fingerlings and smolts based on the blood profile and gene expression.

Characteristic	Fingerling	Smolt	P-value
Weight (g)	24.3	91.5	0.000
Length (cm)	12.16	19.27	0.000
Chloride (mmol/L)	130.4	141	0.000
Calcium (mmol/L)	2.48	2.87	0.000
Potassium (mmol/L)	5.16	4.02	0.000
Sodium (mmol/L)	142.7	148.8	0.000
Magnesium (mmol/L)	0.97	0.76	0.000
Phosphorus (mmol/L)	5.36	5.34	0.218
Creatinine (umol/L)	143.2	83.8	0.000
Blood urea nitrogen (mmol/L)	1.79	1.55	0.019
tCO2 (mmol/L)	12.62	12.78	0.308
Glucose (mmol/L)	5.36	6.51	0.000
Lactate (mmol/L)	4.7	4.19	0.013
NKA1a (Cq)	14.72	17.33	0.000
NKA1b (Cq)	14.49	12.47	0.000
Delta Ct	-0.23	-4.86	0.000
Index	1.56	35.35	0.000

Conclusion

- **Correlation between the actual and predicted delta Cq based on the blood profile suggests that blood testing coupled with machine learning may optimize seawater transfer.**
- **Chloride analysis of SW challenged fish and genetic analysis show conflicting results.**
- **Blood testing can be used on-site to provide farmers with instant biological information.**
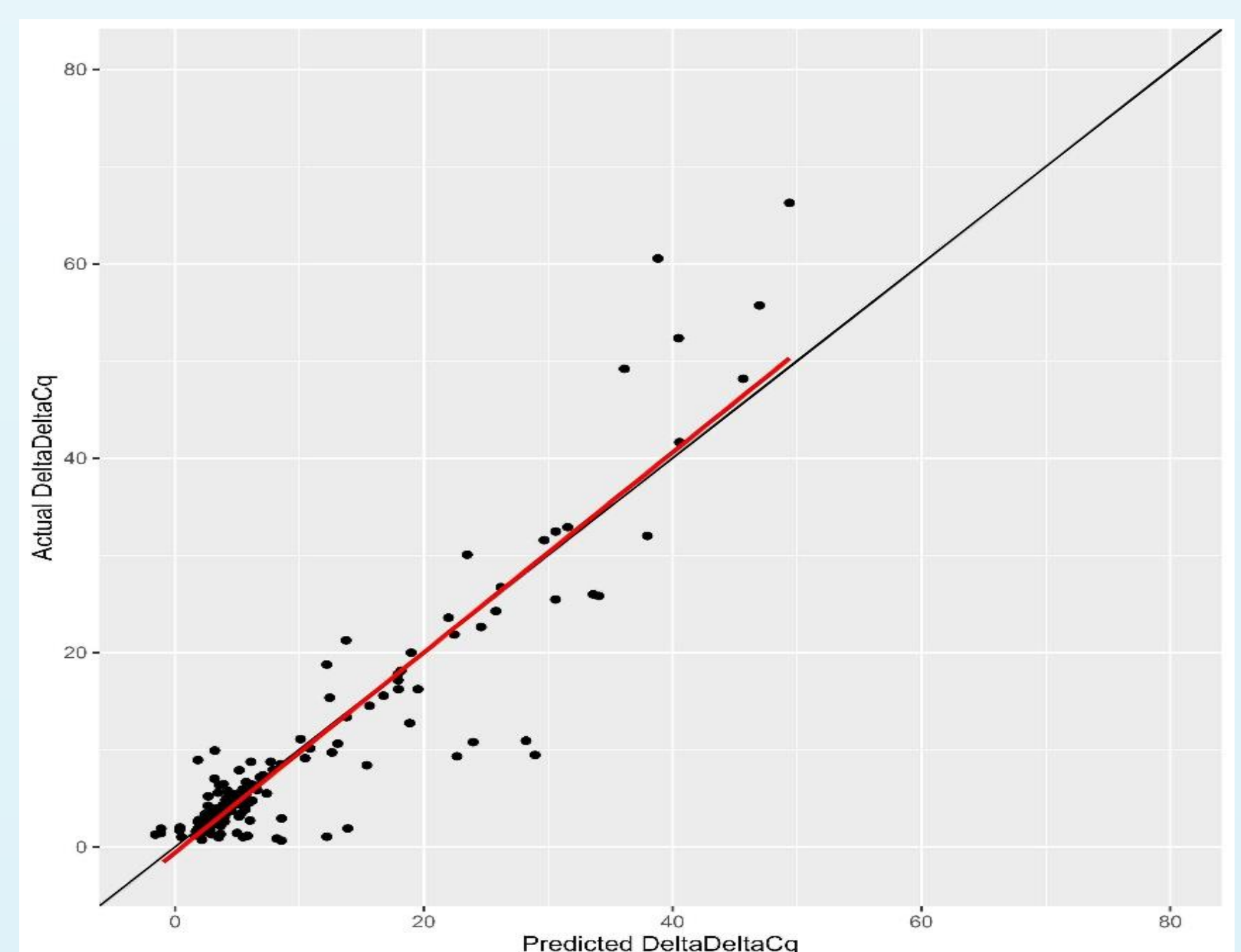- **The current Machine Learning models are under validation through real-world testing.**

Figure 3: Correlation between predicted and expected DeltaDeltaCq of NKA alpha1a and alpha1b in a supervised machine learning model trained on data from experiment.

Acknowledgements

References

Nilsen, TO, Ebbesson, LOE, Madsen, SS, McCormick, SD, Andersson, E, Björsson, BTH, Prunet, P, Stefansson, SO. 2007. Differential expression of gill Na+, K+-ATPase α- and β-subunits, Na+, K+, 2Cl- cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon Salmo salar. The Journal of Experimental Biology 210:2885-2896