# ABSTRACTS

# SESSION 3: GENOMIC PREDICTION

# DEVELOPMENT OF COST-EFFICIENT AND HIGH-THROUGHPUT GENOTYPING PLATFORMS FOR GENOMIC SELECTION IN SCALLOP BREEDING

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**Abstract**

Genomic selection (GS) is an efficient method for estimating breeding values of quantitative traits using dense markers covering the whole genome. Despite GS can greatly increase the genetic gain and shorten the generation intervals, its application in aquaculture animals is usually limited by the lack of efficient and economical genotyping platforms and corresponding statistical models for genomic prediction. Our group have developed Multi-isoRAD, an upgraded genotyping method of 2b-RAD, which allows the preparation of five concatenated restriction site-associated DNA tags for Illumina paired-end (PE) sequencing. The configuration of the five concatenated tags can be flexibly designated to meet various research purposes. When using methylation-dependent restriction enzymes, the genotyping method can also profile genome-wide epigenetic variations. Along with the accumulation of genetic and phenotypic information of scallop, targeted genotyping become one of the next steps in scallop breeding. Therefore, we developed a high throughput diverse marker (HD-Marker) genotyping platform that can investigate genome-wide site-specific variants. This platform is designed with scalable multiplex assays and different kinds markers, including SNP, CNV, and INDELs. Through evaluating the real datasets of scallop, we demonstrated that these genotyping methods are applicable to GS with sufficient power and accuracy. To improve genomic prediction accuracy and algorithmic efficiency, we combined GWAS and GS in a stepwise linear mixed regression model (StepLMM). This model divides the breeding values into two parts: major effects from significant variants and minor effects from non-significant variants. These two parts are fitted separately with different distributions. By doing so, the model exhibits superiority in both the GWAS and GS, since fitting QTL effects increases the genomic prediction accuracy, while identification of polygenic effects improves mapping precision of GWAS. The proposed flexible genotyping methods and efficient genomic prediction model provide feasible solutions for scallop-breeding programs and genetic studies.

*Scallop, Genomic Selection, Genotyping*

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# LESSONS FROM THE APPLICATION OF GENOMIC SELECTION TO RAINBOW TROUT AQUACULTURE

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**Abstract**

Selective breeding programs to improve performance in economically important traits such as growth, disease resistance, slaughter traits and flesh quality are key to the success and sustainability of maturing aquaculture industries. The rate of genetic progress per generation has traditionally been impaired for traits like disease resistance and fillet characteristics that cannot be measured directly in the selection candidates, as fish breeders have typically relied on phenotype records from siblings of the breeding candidates. This has limited progress to family-based selection that does not exploit within family variation. Recent advances in molecular biology technologies coupled with sophisticated bioinformatics and statistical modeling have facilitated the use of molecular information in selective breeding programs to take advantage of within family variation and accelerate the rate of genetic gains. The most widely used approaches in salmonids aquaculture are marker assisted selection and whole-genome enabled selection, or genomic selection in short. The efficacy of genomic selection approaches to enhance genetic and economic gains is dependent upon the level of additive genetic variation and the genetic architecture of the trait in the specific breeding population. In addition, factors that can impact the accuracy of genomic predictions include the size of the training population, the marker density of the genotyping panel, the effective population size, the effective number of chromosome segments and the degree of relationship between training and testing animals. Examples from recent rainbow trout breeding research with nucleus populations from Troutlodge, Inc. and the USDA/ARS National Center for Cool and Cold Water Aquaculture will be presented, and a systematic research approach that is based on those empirical examples will be described and discussed.

*Keywords: selective breeding, genomic selection, GWAS, rainbow trout, bacterial cold water disease, disease resistance*

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# GENOMIC PREDICTION FOR A PRECOCIOUS PHENOTYPE IN THE TIGER PUFFERFISH (*TAKIFUGU RUBRIPES*).

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**Abstract**

The tiger pufferfish, *Takifugu rubripes*, is one of the most valuable aquaculture fish species in Japan. While its ovary is highly poisonous, the testis (shirako) is edible but also the most expensive among the edible parts of the species. In most individuals, the testis reaches market size (> 100g) in late January or February (~22 months old), but some 20% of individuals have testis larger than 100 g in early December (~20 months old), also a time when prices are highest. Hence, precociousness is an important economic trait for the species. In this study, we attempted genomic prediction of a precocious phenotype by means of Genomic BLUP. In total, 501 individuals were sampled in early December and genotyped at approximately 3,000 SNP loci by means of custom amplicon-sequencing (Ampliseq). Estimation of heritability and prediction of genomic breeding values (GEBV) were done for total length (TL) and body weight (BW) using all individuals and for precociousness, i.e. gonad weight (GW) and gonad-somatic index (GSI), using males (n = 237). Prediction accuracies were calculated by cross validation. Estimated heritability for TL, BW, GW and GSI were 0.523, 0.477, 0.408 and 0.443, respectively. Prediction accuracy for these traits were 0.476, 0.335, 0.390 and 0.410, respectively. These relatively high heritability and prediction accuracy suggest the possibility of prediction of GEBV for precociousness as well as body size in the tiger pufferfish.

*Keywords: genomic prediction, precociousness, the tiger pufferfish*

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# IMPLEMENTATION OF MODERN BREEDING TECHNIQUES IN ATLANTIC SALMON

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**Abstract**

Genomic selection and marker assisted selection have the potential to increase accuracy of selection and per generation genetic again, which in turn can significantly increase profitability. We present a study conducted in collaboration with Cermaq Canada Ltd. to identify markers significantly associated with early maturation in Atlantic salmon. In addition, based on the same dataset, we tested the feasibility of the use of imputed genotypes to generated genetic breeding values(GEBVs) for this trait. Two-hundred and forty phenotyped fish and their parents were genotyped using 130K Axyom genotyping array. Genome-wide association analysis was conducted by fitting both phenotypic and genotypic data using a mixed linear model. A subset of 50,000 high quality SNPs were selected to test the feasibility of using that density for genomic selection. In addition, 3,000 SNPs evenly distributed across the 29 Atlantic salmon chromosomes were sub-sampled to evaluate accuracy of imputation of ~47,000 missing genotypes using the parental high-density data as a reference set for haplotype estimation. Seven SNPs were identified as significantly associated with early-maturation and a multi-variate linear model fit of these had an r-squared of 0.40. A five-fold cross validation of GBLUP-estimated GEBVs had an average accuracy of 0.523.

Keywords: Atlantic salmon, GWAS, Genomic Selection, Maturation, Imputation

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# USE OF DNA POOLING IN GENOMIC SELECTION FOR A DISEASE RESISTANCE TRAIT IN ATLANTIC SALMON *(SALMO SALAR)*

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**Abstract**

Genomic selection has a great potential in aquaculture breeding; however, its implementation in this species has been hindered by high genotyping cost due to many individuals to genotype. DNA pooling is a strategy for reducing genotyping cost. In this study we demonstrated its potential as genomic selection tool using *in silico* pooling technique. 4115 salmon post-smolts were challenged with pancreases disease (PD) and 914 individuals with extreme phenotypes (435 alive and 479 dead) were genotyped with ~55k SNPchip. The data was divided into reference dataset (589 samples) and validation dataset (325 samples). The effect of number of pools were investigated by grouping individuals into 1, 2, 4, 10, …, 200 pools per phenotype group (the respective number of fish per pool is given in Table 1). Given the genotype of individuals in each pool, allele frequencies were calculated by sampling with replacement. Effect of sequence coverage on accuracy of selection was studied by varying the sampling times to 40X and 100X. SNP effects from the pool data were estimated based on allele frequencies calculated from the pools. Genomic breeding values (GEBVs) were predicted for the validation individuals as sum of SNP effects. The accuracy of selection was calculated as the correlation between predicted GEBVs and phenotypes and weighted by the square root of the heritability ($h^2$).

Accuracy of prediction increased from 0.574, when only single pool is used per phenotype group, to 0.976 when number of pools increased to 200 (Table 1). Similar trend also observed for the correlation between GEBV of pools and individual genotypes, it increased from 0.84 to 0.976. The accuracy of prediction for individual genotypes was 0.712. Limited effect of sequence coverage on correlation of GEBVs and accuracy was observed (Table 1). Results showed that large number of pools are required to achieve as good prediction as individual genotypes; however, alternative effective pooling strategies should be studied to reduce the number of pools without reducing prediction power. Nevertheless, it is demonstrated that pooling of reference population can be used as a tool to optimize between cost and accuracy of selection.

Table 1: Accuracy of prediction

| # pools per enotype group | /pool | Accuracy | |
|---|---|---|---|
| | | 40X | 00X |
| 1 | 295 | .575 | .574 |
| 2 | 147 | .574 | .575 |
| 4 | 74 | .575 | .575 |
| 10 | 30 | .576 | .576 |
| 20 | 15 | .581 | .579 |
| 40 | 7 | .592 | .588 |
| 100 | 3 | .640 | .636 |
| 150 | 3 | .668 | .687 |
| 200 | 1.5 | .676 | .691 |

*Keywords: Atlantic salmon, accuracy of prediction, genomic selection, pooling, in silico pooling*

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# GENOME-WIDE ASSOCIATION STUDY (GWAS) REVEALS GENES ASSOCIATED WITH THE ABSENCE OF INTERMUSCULAR BONES IN TAMBAQUI (*COLOSSOMA MACROPOMUM*)

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**Abstract**

The presence of intermuscular bones in fisheries products limits the consumption and commercialization potential of many fish species, including the tambaqui (*Colossoma macropomum*). These bones have been reported in medical emergencies, and also are an undesirable characteristic for fish farming because their removal is labor-intensive during fish processing. Despite the difficulty in identifying genes related to the lack of intermuscular bone in diverse species of fish, discovery of intramuscular boneless individuals of Neotropical freshwater characiform fish tambaqui would help elucidate the genetic mechanisms underlying the pathways of intramuscular bone formation. In this study, we carried out a genome-wide association study among boneless and wild-type tambaqui populations to identify markers associated with lack of intermuscular bone. After analyzing 11,423 SNPs in 360 individuals (12 boneless and 348 bony individuals), we reported 675 significant ($P_{adj} < 0.003$) associations for this trait. Of these, 13 associations were located around candidate genes related to the reduction of bone mass, promotion of bone formation, inhibition of bone resorption, central control of bone remodeling, bone mineralization, and other related functions. To the best of our knowledge, for the first time, we have successfully identified genes related to lack of intermuscular bones using GWAS in a non-model species.

*Key words; cachama negra; intermuscular bones; GWAS; GBS.*

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# GENOMIC APPROACHES TO SELECTIVE BREEDING FOR DISEASE RESISTANCE IN PACIFIC OYSTERS (*CRASSOSTREA GIGAS*)

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**Abstract**

Pacific oyster (*C. gigas*) is one of the most important aquaculture species in the world, but sustainable production is hampered by outbreaks of Ostreid herpes virus (OsHV-1), which can cause high mortality rates. Control by biosecurity and vaccination is generally not possible, but there is substantial host genetic variation in resistance within farmed stocks. Therefore, improvement of survival rates via selective breeding has the potential to form a major component of disease prevention. Selective breeding programs for shellfish are underway in several countries, and genomic tools such as SNP arrays can potentially expedite genetic gain for target traits via genomic selection. A medium density (~50K) SNP array for oysters was developed, combining SNP assays targeted to Pacific oyster and European flat oyster (*Ostrea Edulis*) on the same array. This array was successfully applied to generate genome-wide SNP marker data for several oyster populations across the world. Subsequently, the SNP array was applied to genotype resistant and susceptible oysters from OsHV-1 challenge experiments in both the UK and New Zealand. Genetic parameters for host resistance to OsHV-1 were estimated, and genome-wide association studies were performed to assess the genetic architecture of resistance and to map individual resistance QTL in the Pacific oyster genome. A high density linkage map was created to facilitate orientation of significant markers with respect to the ten pairs of chromosomes, revealing several candidate genes of interest. Genomic prediction approaches to breeding value estimation were tested using morphometric and disease resistance traits. Accuracy of breeding value prediction was notably higher than using pedigree-based prediction. Further, for several traits, reduction of SNP density had little impact on prediction accuracy, even when reduced to below 1,000 SNPs. These data highlight the possibilities for incorporating genomic data into selective breeding decisions for production of oysters with improved disease resistance. Strategies for cost-effective use of genomic data to enhance selective breeding in oysters will be discussed.

*Keywords:* oyster, genomic selection, SNP array, disease resistance

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# HERITABILITY OF TOLERANCE TO GILL-ASSOCIATED VIRUS UNDER EXPERIMENTAL CHALLENGE IN THE BLACK TIGER SHRIMP (PENAEUS MONODON)

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**Abstract**

Gill-associated virus (GAV) is a lethal pathogen that causes significant production losses in the Australian shrimp farming industry. Gill-associated virus is highly prevalent in both wild and farmed *Penaeus monodon* and is transmitted through vertical and horizontal routes. Selective breeding for tolerance to GAV may be a good option for managing the risk of disease in farming provided there is significant additive genetic variation. In this study, heritability of tolerance to GAV infection under experimental challenge conditions was estimated from 1717 shrimp from 72 full- and half-sib families that were spawned and reared under communal conditions. Individual pedigree was determined using DNA parentage analysis. Shrimp were challenged using intramuscular injection of a weight standard dose of GAV. The challenge period lasted 15 days and total cumulative mortality across all families reached 37%, but ranged from 0% to 71% among families. Variance components were estimated for mortality using binomial and Cox's proportional hazards models. From the survivors of the challenge test, GAV load was measured to determine its utility as an indirect measure of GAV tolerance. Variance components of GAV load were estimated using a linear mixed animal model. Heritability estimates for mortality ranged from 0.11 ± 0.03 using the binomial model and 0.14 using the Cox's model that takes into account time of death. Correlations of family breeding values estimated from each model were highly correlated, $r > 0.95$. Heritability of GAV load was 0.23 ± 0.08, however, correlations of family breeding values estimated for mortality and GAV load were not significantly different from zero.

This is the first study that demonstrates the use of communal family rearing and DNA parentage analysis for large scale disease challenge testing. Using this challenge design significant additive genetic variation was observed for mortality; thus selective breeding could be used to improve tolerance to GAV. However, further studies are needed to confirm the genetic relationships with natural challenge /outbreaks to GAV, and genetic relationships with all major production traits need to be understood.

*Keywords: Black tiger shrimp, disease tolerance, heritability, gill-associated virus*

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# JAPANESE FLOUNDER: GENOME TO GENOMIC SELECTION BREEDING

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**Abstract**

Japanese flounder (*Paralichthys olivaceus*) is an economically important cultured marine flatfish, which has the most extreme asymmetric body morphology of vertebrates. To study the role of genomic architecture in asymmetry development during flatfish metamorphosis, we produced a high-quality reference genome (546 Mb) of the Japanese flounder, based on 52.6 Gb of high-quality Illumina sequencing data. The contig and scaffold N50 sizes was 30.5 kb and 3.9 Mb, respectively. Comparative genomics between flounder and Chinese tongue sole (*Cynoglossus semilaevis*), and transcriptomic analyses during metamorphosis revealed that thyroid hormone and retinoic acid signaling, as well as phototransduction pathways may play a role in metamorphosis. We demonstrated that retinoic acid is critical in establishing asymmetric pigmentation and, via cross-talk with thyroid hormones, in modulating eye migration. Moreover, the unexpected expression of the visual opsins from the phototransduction pathway in the skin translates illumination differences and generates retinoic acid gradients that underlie the generation of asymmetry. In aquaculture of Japanese flounder, diseases caused by bacteria and virus occur frequently and resulted in huge economic loss. Genomic selection is a promising technique for selective breeding with superior strains. To perform the genomic selection in the Japanese flounder, we have constructed approximately 300 families. A total of 931 individuals from 90 families were selected and challenged with *Edwardsiella tarda*, a major pathogenic bacteria, in 2013-2015 and their survival rates were recorded as the phenotypic traits. We performed genome resequencing for these individuals (931) and their parents (71), and obtained 1,934,475 SNP markers. Two different algorithms, BayesCπ and GBLUP, were used for calculation of GEBV (genome estimated breeding value), respectively. According to the survival rates after infection, families were divided into high and low resistant groups, respectively. GEBV of different groups had a significant difference (*p-value* < 0.05), and high resistant group had a higher mean GEBV. In the selection candidates, Pearson's correlation between TBV (true breeding value) and GEBV calculated by BayesCπ and GBLUP was 0.706 and 0.795, respectively. To summarize, we have established the genomic selection method and applied it in selection of Japanese flounder with enhanced disease resistance.

*Keywords: genome sequencing, metamorphosis, genome selection, disease resistance, Paralichthys olivaceus*

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