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PII: S0044-8486(26)00913-0

DOI: https://doi.org/10.1016/j.aquaculture.2026.744530

Reference: AQUA 744530

To appear in: *aquaculture*

Received date: 4 May 2026

Revised date: 1 September 2026

Accepted date: 2 September 2026

Please cite this article as: Z.W.J. Poon, N.T. Vu, X. Shen, et al., Influence of infection route and population origin on survival and genomic prediction of scale drop disease resistance in barramundi (Lates calcarifer), *aquaculture* (2026), https://doi.org/10.1016/j.aquaculture.2026.744530

Influence of infection route and population origin on survival and genomic prediction of scale drop disease resistance in barramundi (*Lates calcarifer*)

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Abstract

Scale drop disease virus (SDDV) is a major threat to barramundi (*Lates calcarifer*) aquaculture, causing mass mortality and severe economic losses. While laboratory challenge models based on intraperitoneal (IP) injection based on Singaporean stocks have been explored for genomic selection, the extent to which these results generalise across alternative infection routes and different population backgrounds remains poorly understood. This study evaluated the influence of infection route (IP injection versus cohabitation) and population origin (Singapore versus Malaysia) on survival, genetic parameter estimation, and genomic prediction performance for SDDV resistance using 1,709 individuals genotyped using the ThermoFisher MyDesign Axiom barramundi 70k SNP array in a within-tank mixed -route and -origin challenge design. Malaysian fish exhibited significantly higher survivability than Singaporean fish under IP injection (49.1% vs 25.6%), whereas no significant population differences were detected under cohabitation. Heritability estimates were moderate, ranging from 0.12 to 0.44, and were consistently higher for survival time (continuous trait) than for survival status (binary trait). Censoring-aware Cox-GBLUP analysis confirmed that the ranking of breeding values for survival time was robust to right censoring (r = 0.97 with linear GBLUP). High genetic correlations (r_g = 0.74 to 0.92) and consistent clinical signs indicate that both infection routes are biologically valid models with substantial shared genetic control for laboratory SDDV resistance. Within-dataset genomic prediction accuracies were comparable (accuracy = 0.19 to 0.54) between survival time and survival status; however predictive ability was consistently higher for survival time and under IP infection (r = 0.21 to 0.33), likely reflecting a stronger genetic signal and more synchronous disease progression than survival status and cohabitation exposure. Across-dataset analyses showed positive cross-scenario prediction between infection routes (accuracy 0.18 to 0.41), but poor transferability between origins (accuracy 0.10 to 0.15). These results indicate that survival time under IP injection provides the most reliable signal for genetic evaluation and is the preferred trait for genomic selection of SDDV resistance under laboratory conditions, with population origin representing a key factor influencing resistance expression and implementation.

Keywords

barramundi; Scale drop disease; Genomic prediction; disease resistance; genomic selection

1. Introduction

Infectious diseases represent a major constraint on the sustainability and profitability of global aquaculture (Maezono et al., 2025), particularly in tropical production systems where high stocking densities and frequent stock movements increase the risk of transboundary spread and disease outbreaks (Leung & Bates, 2013; Rowley et al., 2024). For many economically important pathogens, especially viral agents, effective treatments are unavailable or impractical at commercial scale, and management strategies are largely restricted to biosecurity, functional feeds, husbandry practices, and stock replacement following outbreaks (Cain, 2022; Chattaraj et al., 2022; Wright et al., 2023). As a result, improving host genetic resistance has emerged as a key long-term approach for disease control in aquaculture breeding programmes.

Selective breeding for resistance has proven to be effective for disease prevention in several key aquaculture species (Moss et al., 2012; Robinson et al., 2023; Sciuto et al., 2022). The adoption of genomic selection has further progressed breeding efficiency by improving the prediction of breeding values using genome-wide marker information compared to traditional pedigree-based methods (Song et al., 2023; Yáñez et al., 2023). This is particularly advantageous for disease resistance traits, in which statistical models can be developed on challenge-tested relatives rather than high-valued selection candidates themselves. However, the effectiveness of genomic selection depends on the quality, biological relevance, and consistency of resistance phenotypes (i.e. survival time or binary survival after pathogen exposure) used to train prediction models. Phenotypes that incorporate substantial non-genetic or context specific variation may inflate environmental noise, bias selection responses and reduce prediction accuracy (Biscarini et al., 2016). Hence, careful consideration of how phenotypes representing disease resistance are collected is essential for effective genetic improvement in breeding programmes.

As natural disease outbreaks are unpredictable and difficult to replicate experimentally, resistance phenotypes in aquaculture are mostly commonly derived from controlled laboratory challenge tests. Such challenge models have been widely applied in immunity-focused applications such as testing of vaccines (Jose Priya & Kappalli, 2022), therapeutic treatments (Nakai & Park, 2002), and genetic selection

strategies (Ødegård et al., 2011). Laboratory challenge models offer several advantages, including controlled pathogen exposure, defined observation periods, and the ability to standardise environmental conditions, thereby minimising confounding factors and facilitating the estimation of genetic parameters.

However, the design of laboratory challenges varies among studies, particularly with respect to pathogen exposure method, infectious dose, and disease progression dynamics. Among these factors, infection route represents a fundamental component of challenge design that can strongly influence disease trajectory, survival outcomes, and the expression of host resistance (Demars et al., 2019). In aquaculture, intraperitoneal (IP) injection and cohabitation exposure are the two most widely applied infection routes. IP injection delivers a pathogen dose directly into the body cavity, bypassing natural defence barriers such as skin and mucus, ensuring all individuals are exposed simultaneously at a standardised load (Hudson & Nowak, 2021). This uniform exposure theoretically facilitates clearer genetic analyses by limiting variation in infection timing and dose; however, there have been concerns regarding if IP is accurately reflecting natural resistance to a pathogen as several components of the fish's immune system are bypassed (e.g mucosal antibodies and skin layers, gut epithelium etc). In contrast, cohabitation challenges rely on natural pathogen transmission from infected "shedders" to naïve fish through the water column and physical contact, thereby more closely resembling infection dynamics under natural farm conditions (Hudson & Nowak, 2021; Nordmo, 1997). However, cohabitation exposure inherently introduces heterogeneity in infection timing and dose, as individual fish may differ in contact rates, susceptibility, and behavioural interactions. Importantly, because IP injection bypasses mucosal barriers, it may place greater emphasis on resistance mechanisms acting after viral entry, whereas cohabitation challenges may additionally capture barrier immunity, behavioural responses, and transmission-related components of resistance. As a result, selection based solely on a single infection method may bias genetic improvement towards resistance pathways that do not generalise into on-farm exposure, highlighting the importance of understanding how infection route influences the genetic expression of disease resistance.

Comparative studies across fish species have demonstrated that infection route affects mortality dynamics and disease progression (Meza et al., 2019; Nordmo et al.,

1997; Pulpipat et al., 2020). However, it remains unclear to what extent resistance measured under different infection routes reflect shared genetic control versus infection route specific mechanisms. To date, few studies have explicitly investigated this, with one study reporting no major differences in genetic analyses between experimental IP injection and cohabitation for *Streptococcus agalactiae* resistance in Nile tilapia (*Oreochromis niloticus*) (Joshi et al., 2021). However, disease pathology and host immune responses are highly pathogen specific, and results from one host-pathogen system may not be directly transferable to others. Hence, major economically important pathogens should be investigated separately for breeding goals. Addressing this knowledge gap is therefore critical for the development of challenge protocols that are both biologically relevant and practically feasible for routine implementation in selective breeding programmes.

Barramundi (*Lates calcarifer*) is a high-value marine finfish species cultured across the Asia-Pacific region due to its fast growth, and tolerance to a broad range of temperatures and salinities (Domingos et al., 2021; Jerry, 2013; Vij et al., 2020). In recent years, the emergence of scale drop disease virus (SDDV) has posed a major threat to barramundi aquaculture, causing acute mortality events characterised by clinical symptoms including scale loss, dry skin, and abnormal “zombie” swimming behaviour (de Groof et al., 2015; Gibson-Kueh et al., 2012). SDDV has had substantial economic impacts on the barramundi industry particularly during the juvenile phase in sea-cage production, where outbreaks commonly result in mass mortalities upwards of 40%, resulting in production losses severe enough to lead to farm closures (The-Strait-Times, 2023). Since its initial identification, SDDV outbreaks have been reported in multiple countries such as Singapore, Malaysia, Thailand and Indonesia (Nurliyana et al., 2020; Senapin et al., 2019), and more recently in yellowfin seabream (*Acanthopargrus latus*) (Fu et al., 2021) and silver pomfret (*Pampus argenteus*) (Zhou et al., 2026) in China. Given the absence of commercial vaccines or treatments, genetic improvement for resistance represents a promising and sustainable approach for mitigating the impacts of SDDV.

Selective breeding programmes in aquaculture commonly source founder populations originating from different geographic regions, each with distinct genetic backgrounds and breeding histories to maximise diversity (Abwao et al., 2023; Khang et al., 2018; Loughnan et al., 2016). Such population origin effects may influence baseline survival,

immune responsiveness, and the amount of genetic variance available for selection. SDDV has been reported in farmed barramundi in both Singapore and Malaysia; however, mortality during natural outbreaks appears to differ between the two origins, with higher severity reported in Singaporean populations (Gibson-Kueh et al., 2012; Nurliyana et al., 2020). This observation suggests that barramundi populations from different origins or breeding lines may vary in their level of genetic resistance to SDDV. As aquaculture breeding programmes increasingly aim to enhance disease resistance, understanding how resistance is expressed across populations is essential for evidence-based decisions on founder population establishment and the strategic introduction of new genetic material into existing nuclei.

Our previous genomic studies of SDDV resistance in barramundi have demonstrated moderate heritability and high genomic prediction accuracy under controlled IP injection challenges, supporting the feasibility of incorporating genomic selection into commercial breeding programmes (Poon et al., 2026b). Furthermore, strong genetic correlations between laboratory-measured resistance and field survival have further suggested that resistance expressed under controlled conditions can be informative for performance during natural outbreaks (Poon et al., 2026a).However, these studies were based exclusively on Singapore-origin barramundi challenged via IP injection, limiting our understanding of whether resistance is consistent across different exposure routes and between barramundi populations of different population background. Consequently, it remains unclear whether the same genetic factors underpin resistance across infection scenarios or whether route-specific resistance mechanisms exist. Addressing this knowledge gap is critical for breeding programme design, as reliance on a single challenge model may bias selection towards resistance pathways that are not fully representative of natural infection dynamics.

In this study, we provide the first systematic comparison of IP and cohabitation challenge models for SDDV resistance in barramundi using two farmed populations originating from Singapore and Malaysia. The objectives of this study were to: (i) compare survival outcomes between infection routes and populations, (ii) estimate heritability of survival traits under each method, (iii) evaluate the genetic correlation between traits and across infection routes, and (iv) assess within- and across-dataset genomic prediction accuracy performance across different infection route- and origin-specific datasets. Together, these analyses aim to provide empirical guidance on the

suitability of alternative challenge designs for genomic selection, supporting the development of robust breeding strategies for disease resistance in aquaculture.

2. Methods

2.1 Ethics statement

This study was carried out at the Animal Research and Teaching Facility (ARTF) at James Cook University Singapore (JCUS), in accordance with approval granted by the Institutional Animal Care and Use Committee (IACUC; reference number 2023-A027).

2.2 Fish material

This SDDV challenge study compared barramundi from Singapore and Malaysia. The Singapore-origin was produced from a mass-spawn using a commercial operators broodstock located at the Marine Aquaculture Centre (MAC), Singapore, from a total of nine broodfish (four males and five females). Fertilised eggs were transported to ARTF, where larvae were reared in 2,000 L tanks under controlled conditions. Following larval and nursery phases, juveniles were transferred to an 8,000 L holding tank at approximately 20 g average body weight. The Malaysian-origin fish consisted of juveniles imported from a commercial hatchery (6.18°N; 100.26°E) located in Kedah, Malaysia. Broodstock were mass spawned involving approximately 25 fish (1:1 sex ratio). Prior to importation, the fish were screened and tested negative for SDDV, Iridovirus (Megalocytivirus), *Lates calcarifer* Herpes Virus (LCHV) by a diagnostic laboratory, Lab-Ind Resource Sdn. Bhd (Selangor, Malaysia). Upon arrival in Singapore, these Malaysian fish (77.0 ± 21.4 g) were housed in an 8,000 L tank operated in recirculating aquaculture system (RAS) at ARTF and acclimated for 2 weeks prior to the commencement of the disease challenge trial.

All fish were fed a commercial pelleted diet (Grobest Winner Marine Fish Sinking #3) twice daily to apparent satiation. Water quality parameters including dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), pH, and salinity were monitored daily using a SpinDisk® FF Salt Water Series photometer (LaMotte, USA). Fish from the two origins were maintained separately through the acclimation period and only mixed during the subsequent disease challenge.

2.3 SDDV challenge

A controlled challenge experiment was conducted to evaluate resistance to SDDV under two infection methods, IP injection and cohabitation. Fish from the two origins were evenly assigned to four groups: i) Singapore-IP, ii) Singapore-Cohab, iii) Malaysia-IP, iv) Malaysia-Cohab (n = 240 fish per group). All groups were replicated across two 8,000 L tanks operated in RAS, with all four groups mixed within each tank, giving a stocking density of 950 fish per tank (approximately 11.5kg/m^3 at challenge) and 1,920 individuals in total. Within each tank, 50% of the fish were IP-injected with SDDV and served as actively infected “shedder” fish, providing a continuous source of viral exposure for the cohabitation groups via horizontal transmission, giving a fixed shedder to naïve fish ratio of 1:1 (480:480) in both replicate tanks. The shedder population within each tank comprised 240 Singapore-origin and 240 Malaysia-origin fish, a balanced 1:1 origin ratio applied identically to both replicate tanks be design. Consequently, all cohabitation fish were exposed to the same pooled, mixed-origin shedder population rather than to origin-matched shedders, such that Singapore- and Malaysia-origin recipients experienced an identical viral exposure environment. Minor deviations from these numbers in the final analysed dataset (**Table 1**) arose from the exclusions described below and did not alter the balance of the design.

For the challenge, four phenotyping stations operated concurrently, with fish processed sequentially through standardised procedures. Each fish was sedated with Aqui-S® at 20 ppm, weighed (± 0.1 g), measured (± 1 mm), and tagged with a Passive Integrated Transponder (PIT) tag individually using Biomark equipment (Electronic Measuring Board; Ohaus Defender Scale; MK25 PIT Tag implanter). Caudal fin clips (~ 0.5 cm^2) were collected and placed in 96 x 2 mL deep-well plates (Eppendorf) containing 1.5 mL of 95% ethanol and stored at 4 °C for subsequent genotyping. All equipment was used for this experiment were disinfected with a 100 ppm bleach solution before, during, and after use. Finally, the fish in the IP groups were infected via IP injection with 0.1 mL of 1 x 10^7 copies/mL of cultured SDDV diluted with L-15 Medium (Merck). Fish under the cohab groups were injected with 0.1 mL of L-15 Medium without SDDV. A single batch of cultured SDDV inoculum was used for all IP injections across both populations and both replicate tanks, and all injections were

performed within the same session. A fixed volume of 0.1 mL was administered to every fish, and the dose was not normalised to individual body weight. Because Singapore-origin fish were heavier than Malaysia-origin fish at challenge (**Table 1**), this fixed dose corresponded to a marginally higher viral dose per unit body weight in Malaysia-origin fish, which is conservative with respect to the higher survival subsequently observed in that population. The fish were then recovered and placed in into one of the replicate 8,000 L challenge tank.

During the challenge, dead fish were removed twice daily, and any moribund fish euthanised with an overdose at 50 ppm of Aqui-S®. Fish were fed with Grobest Winner Marine Fish Sinking #3 twice daily to satiation. Two disease resistance traits were defined: survival status and survival time. Survival status was recorded as a binary trait, where fish that were moribund or dead during the challenge period was coded as 0 and fish alive at the end of the challenge were coded as 1, with this coding applied consistently across all genomic analyses. Survival time was defined as the number of days from challenge initiation (day 0) to the day of death or euthanasia for moribund fish. The challenge trial lasted 28 days. Both replicate tanks were maintained on a shared RAS with common biofiltration, temperature control, and water quality management, ensuring consistent environmental conditions across replicates throughout the challenge. Water quality parameters across both tanks were: DO 6.8 ± 1.3 mg/L, temperature 30.0 ± 0.8 °C, pH 7.8 ± 0.1, salinity 29.2 ± 0.8 ppt, NH_3 1.2 ± 0.9 mg/L, NO_2 2.5 ± 0.8 mg/L, and NO_3 90.0 ± 27.7 mg/L.

Fish that died within the first three days after injections (n = 4) were removed from subsequent analyses, as the cause of these mortalities were considered unrelated to SDDV and likely attributable to handling stress or other non-infectious factors. Additionally, fish removed from the challenge for other sampling purposes or had missing information were excluded (n = 121) from the analyses in this study.

To confirm SDDV infection, kidney samples were collected from clinically affected and apparently healthy fish (n = 55; 28 from IP-injected groups and 27 from cohabitation-exposed groups) during the challenge period and analysed by quantitative PCR (qPCR) using primers 5'-AATGACCGAAATACGACCGAGAAC-3' and 5'-GCGGGGATCAAATGTCGTTTTG-3' (Sriisan et al., 2020). Infection status was

confirmed separately for each infection route, and SDDV viral load was quantified by qPCR and expressed as viral copies per 10 mg of kidney tissue.

2.4 Genotypes, quality-control, and population structure

Fin clips collected from day 0 (n = 1,797 individuals with complete phenotypes) were genotyped on the ThermoFisher MyDesign Axiom barramundi 70k SNP array platform at Neogen Australasia Pty Ltd, Australia. Raw genotyping data (CEL files) were processed in Axiom Analysis Suite v5.3 using the Best Practices Genotyping Analysis Workflow on default settings. Using the custom probe library, 54,934 SNPs were retained from the initial 67,716 markers. Additional quality control was conducted in the ASRgenomics package (Gilmour et al., 2021) in R v4.4.1(R-Core-Team, 2021) for excluding markers with call rate < 0.95, individual call rate < 0.90, minor allele frequency (MAF) < 0.05, heterozygosity > 0.9, and inbreeding coefficient (Fis) > 0.9, which excluded 88 individuals from further analyses. Genotype imputation was not performed due to low proportion of missing data (< 1%). The remaining 52,319 SNPs from 1,709 individuals were used for downstream analyses in this study.

For the population structure analyses, the SNP genotypes were further pruned for linkage disequilibrium using a 50-SNP window, a 10-SNP step, and an r^2 threshold of 0.2, retaining 5,818 SNPs. Principal component analysis was performed in PLINK v2.0 (Chang et al., 2015), and ancestry proportions were estimated using ADMIXTURE (Alexander et al.). Genetic differentiation between origins was quantified on the quality-controlled SNP set using Hudson's F_{ST} (Bhatia et al.) and Weir and Cockerham's F_{ST} (Weir & Cockerham, 1984), and the mean absolute allele-frequency difference between origins was calculated.

2.5 Survival analysis

Survival analysis was performed using Kaplan-Meier estimators to compare differences between infection methods, populations, and replicate tanks. Fish that survived until the end of the challenge period were treated as right-censored observations. Survival probabilities were estimated using the "*survfit()*" function in the R package *survival*. At each time event t_i, the survival function was given by:

$$\hat{S}(t) = \prod_{t_i \leq t} (1 - \frac{d_i}{n_i})$$

where n_i is the number of fish at risk at time t_i, and d_i is the number of mortalities observed at that time. Differences in survival curves between groups were tested using the non-parametric log-rank test.

To estimate relative risks, Cox proportional hazards model were fitted using the "*coxph()*" function in the R package *Survminer*. The hazard function was expressed as:

$$h(t|x) = h_0(t) \exp(\beta x)$$

where $h_0(t)$ is the baseline hazard function, x is the vector of covariates (e.g., infection route, population origin), and β is the vector of regression coefficients. Exponentiated coefficients ($\exp(\beta)$) were interpreted as hazard ratios between groups.

2.6 Genomic statistical analysis

Two disease resistance traits (survival time and survival status) were analysed across subsets of data defined by infection route(s) and population origin(s).

Variance components of the two SDDV resistance traits were estimated using the GBLUP model. The linear mixed model used in GBLUP was specified as:

$$y = Xb + Za + e$$

where *y* is the vector of observed phenotypic values, *b* is the vector of fixed effects (including tank, infection route, population origin, and body weight as a covariate), *a* is the vector of random additive genetic effects, *X* and *Z* are the corresponding incidence matrices, and e is the vector of residuals. The additive genetic effects were assumed to follow a normal distribution, $a \sim N(0, G\sigma_g^2)$, where G is the genomic relationship matrix (GRM) generated by VanRaden method (VanRaden, 2008) from the QC-ed SNPs, and σ_g^2 is the genomic additive genetic variance. Variance components and genomic estimated breeding values (gEBVs) were estimated by restricted maximum likelihood (REML) using ASReml-R 4.2 (Gilmour et al., 2021).

The SNP heritability (h^2) of survival status and survival time was estimated using univariate models. Analyses were performed both on the full dataset (combining origins and infection routes) and separately within each origin-method combination. In all cases, h^2 was calculated as a ratio of the additive genetic variance (σ_a^2) to the total phenotypic variance ($\sigma_a^2 + \sigma_e^2$) where σ_e^2 is the residual variance:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

Statistical significance of heritability was assessed using likelihood ratio tests (LRT) (Wilson et al., 2010). The test statistic was computed as twice the difference in log-likelihoods between the full model and a reduced model that excluded the random additive genetic (animal) effects. Since the null hypothesis constrains the additive genetic variance to the boundary of the parameter space (σ_g^2 = 0), p-values were obtained from a 50:50 mixture of χ^2 distributions with 0 and 1 degrees of freedom.

To account for the right-censored nature of survival time, where fish surviving to the end of the challenge have incomplete time-to-event records, heritability of survival time was additionally estimated using a censoring-aware mixed-effects Cox proportional hazards model (Cox-GBLUP) as a sensitivity analysis. The GRM was fitted as a random effect on the log-hazard scale using the *coxme* package in R (Therneau, 2015) with the same fixed effects as the linear GBLUP, and fish surviving to day 28 treated as right-censored. Heritability on the underlying frailty scale was approximated as $h^2 = \frac{V_g}{V_g + \frac{\pi^2}{6}}$, where V_g is the genomic (frailty) variance and $\frac{\pi^2}{6}$ is the variance of the standard extreme value distribution assumed for the residual term on the log-hazard scale. Whereas the linear GBLUP estimates heritability from observed phenotypic variance components, the Cos-GBLUP is semi-parametric and estimates heritability on a latent log-hazard scale. The two values are therefore not directly comparable In magnitude and should be interpreted as complementary rather than interchangeable. Agreement between the approaches was accordingly assessed by Pearson correlation coefficient (Pearson, 1895) between full-data gEBVs for survival time from the linear GBLUP and Cox-GBLUP models, which reflects consistency in the ranking of breeding values rather than in the absolute magnitude of variance components.

Genetic correlations (r_g) between survival status and survival time, as well as genotype-by-environment (GxE) effects between fish infected via IP injection and cohabitation were estimated using bivariate models. The genetic correlations were calculated as:

$$r_g = \frac{cov(a_1, a_2)}{\sqrt{\sigma^2_{a1}} \times \sqrt{\sigma^2_{a2}}}$$

where $cov(a_1, a_2)$ is the additive genetic covariance between the traits or infection route, and σ^2_{a1} and σ^2_{a2} are the corresponding additive genetic variances. For comparisons between survival status and survival time within the same individual, the residual covariance was defined as positive. For comparisons between infection routes, each individual was exposed to only one route; therefore, residual covariance was constrained to zero. Significance of genetic correlations was evaluated using LRT by comparing full and reduced models, as described for heritability.

2.7 Prediction accuracy, bias, and cross-prediction scenarios

The predictive performance of the univariate models were evaluated using five-fold cross-validation (training-testing) repeated 10 times, resulting in a total of 50 validation (testing) sets. Within each set, predictive ability was assessed by correlating the gEBVs with the observed phenotypes using the Pearson correlation coefficient (r) (Pearson, 1895) for both survival time and survival status. Pearson correlation coefficient was calculated as $r = corr_{y,\hat{y}}$, where $\hat{y}$ are the gEBVs, and y are the actual phenotypes. Model accuracy was then calculated as $accuracy = \frac{r}{\sqrt{h^2}}$, with the heritability being from the corresponding full-data univariate model. The corresponding accuracy values of each model were then averaged over all 50 validation sets. To ensure consistency in comparisons among statistical methods, the same 50 training and validation subsets were used across all models. For survival status, the area under the receiver operating characteristic curve (AUC) was additionally computed as a threshold-independent measure of binary classification.

Bias in the predicted gEBVs was assessed by regressing observed phenotypes on predicted gEBVs within each validation set. An unbiased model is expected to yield a regression coefficient (ß) of 1. Deviations from this value indicate bias: coefficients less than 1 suggest over-dispersion (inflation), whereas values greater than 1 indicate under-dispersion (deflation).

The regression coefficient was calculated as:

$$ß = \frac{cov(y, \hat{y})}{var(\hat{y})}$$

where $\hat{y}$ are the predicted breeding values y are the actual phenotypes. The final bias estimate was obtained by averaging the regression coefficients across the 50 validation sets.

To directly evaluate the transferability of resistance phenotypes, explicit cross-scenario validation was performed in addition to the within-dataset cross-validation described above. Models were trained on one scenario and gEBVs were predicted on the other for three contrasts: infection route (train on IP, predict cohabitation, and the reverse), population origin (train on Singapore, predict Malaysia, and the reverse), and replicate tank (train on tank 1, predict tank 2, and the reverse). For consistency, the pooled heritability of the corresponding trait was used for computing accuracy.

3. Results

3.1 SDDV challenge and survival outcomes

At day 0, fish of Singaporean origin were larger than Malaysian-origin across both infection routes, with higher mean body weight (106.44 – 108.48 g vs 83.66 – 84.17g) and total length (195.35 – 195.57 mm vs 179.37 – 180.64 mm) (**Table 1**).

Principal component analysis separated the Singapore and Malaysia origins into two clearly distinct clusters along the first principal component, with PC1 and PC2 explaining 50.9% and 10.7% of the total genetic variance, respectively (**Figure 1**). The Singapore population showed genetic sub-structuring along PC2, whereas the Malaysia population formed a single cluster. Genetic differentiation between the two origins was marked and near-identical across estimators (Hudson's F_{ST} = 0.167, Weir and Cockerham's F_{ST} = 0.167), with a mean absolute allele-frequency difference of 0.22 between origins. ADMIXTURE-based ancestry estimation supported the same separation, with the two origins resolving into distinct ancestral clusters (**Supplementary Figure S1**)

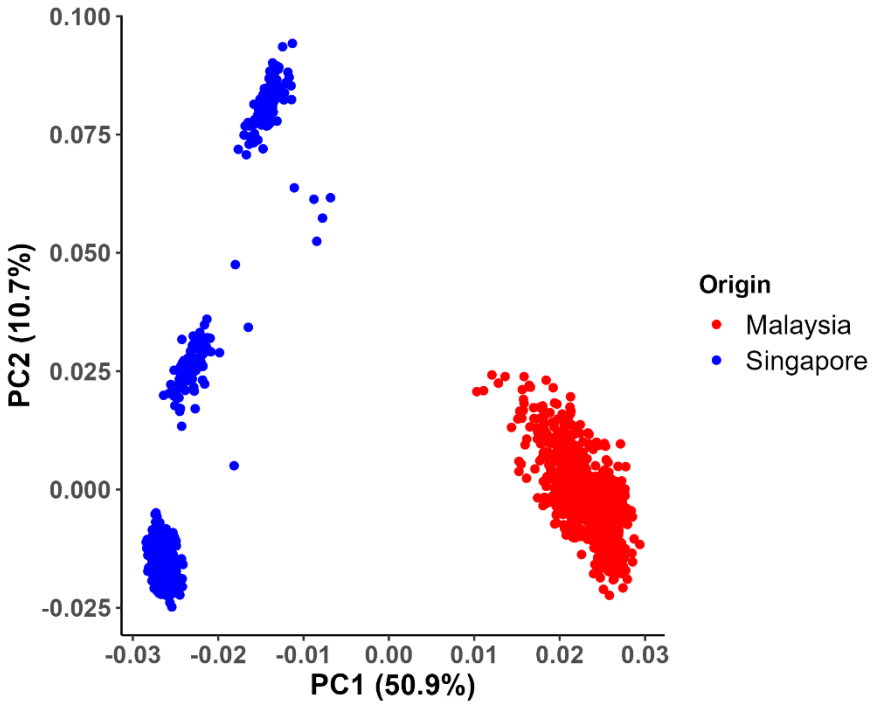

Figure 1. Principal component analysis of 1,709 barramundi (Lates calcarifer) from the Singapore (n = 833) and Malaysia (n = 876) populations, based on 5,818 genome-wide SNPs after quality control and linkage disequilibrium pruning. Each point represents one individual, coloured by population of origin (blue, Singapore; red, Malaysia).

The SDDV challenge lasted 28 days, during which clinically affected fish displayed symptoms consistent with SDDV infection, including scale loss, abnormal swimming behaviour, and dry skin. Molecular screening by qPCR confirmed SDDV infection in fish from both infection routes. Viral load peaked earlier in IP-injected fish, reaching a mean of 12,646 ± 8,680 copies per 10 mg kidney at day 10, than in cohabitation-exposed fish, which peaked at day 20 with a comparable mean of 12,157 ± 10,820 copies per 10 mg kidney. The similar peak magnitudes between routes confirm successful cohabitation exposure, while the later peak under cohabitation is consistent with the delayed mortality onset observed in this group. Mortality onset occurred earlier in fish infected via IP injection compared to naïve fish exposed through cohabitation, whereas no clear differences in the timing of mortality onset were observed between fish of Singaporean and Malaysian origin (**Figure 2**).

Table 1. Descriptive statistics of barramundi (*Lates calcarifer*) from the SDDV challenge.

Origin	Route	n	Weight (g) @ D0	Total length (mm) @ D0	Survival time (days)	Survival rate @ D28
Singapore	IP	399	106.44 ± 15.58	195.35 ± 10.81	16.86 ± 7.27	25.6%
	Cohab	434	108.48 ± 15.58	195.57 ± 10.46	24.86 ± 4.32	56.9%
Malaysia	IP	454	83.66 ± 18.84	179.37 ± 14.17	20.42 ± 8.12	49.1%
	Cohab	422	84.17 ± 17.65	180.64 ± 13.94	24.63 ± 4.92	58.8%

IP represents fish infected via the intraperitoneal route

Cohab represents fish infected via the cohabitation route

"n" indicates the number of fish, weight, length and survival time are presented as mean ± SD at day 0 (D0), and survival rate is reported for day 28 (D28).

Across both infection methods, fish of Malaysian origin consistently exhibited higher survivability than those of Singaporean origin. Under IP injection, Malaysian fish had a higher survival rate at day 28 than Singaporean fish (49.1% *vs* 25.6%; **Table 1**; **Figure 2**) and 45% lower risk of mortality (hazard ratio, HR = 0.55, $p < 0.001$; **Table 2**). When data from both infection routes were combined, origin remained a significant predictor of survival, with Malaysian fish having a lower HR than Singaporean fish (HR = 0.72, $p < 0.001$; **Table 2**).

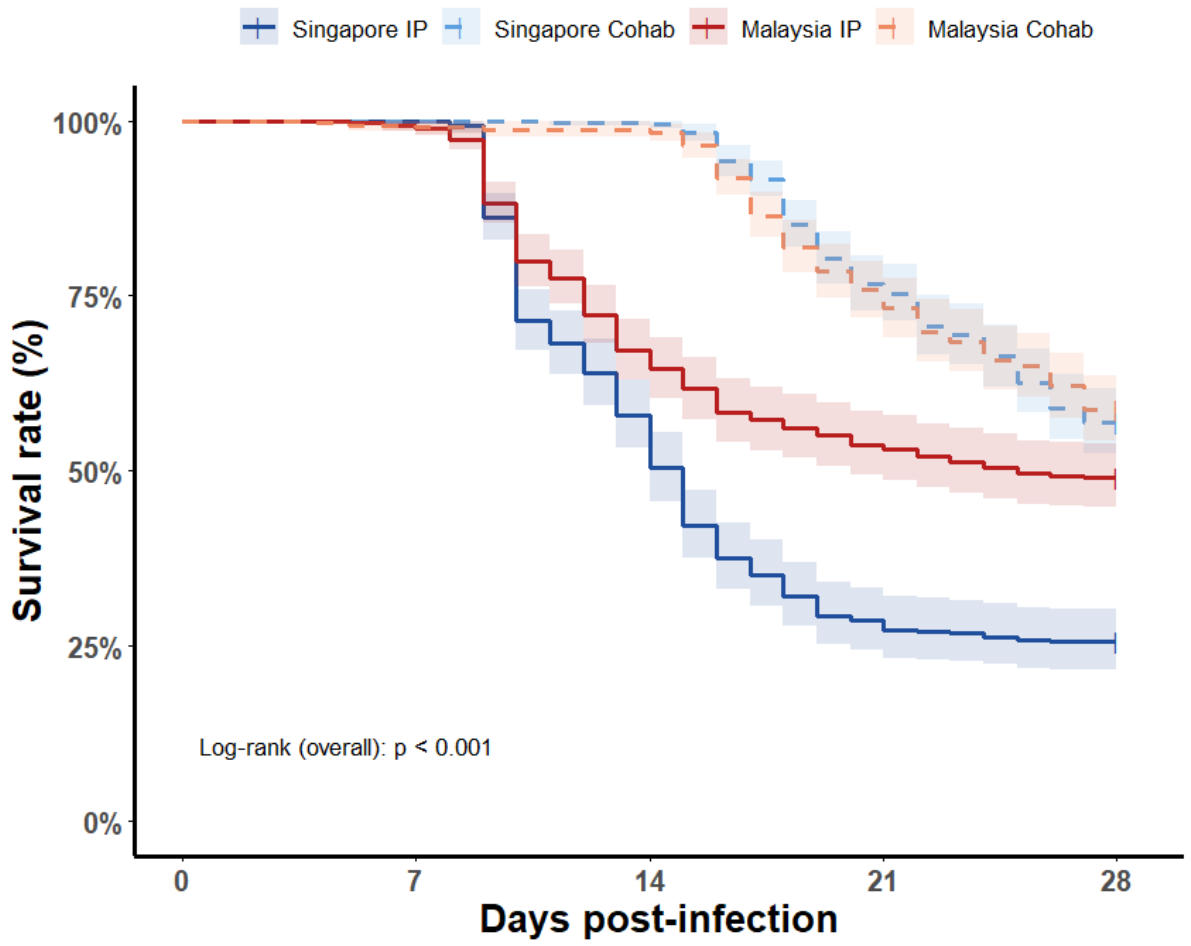

Figure 2. Kaplan-Meier survival curves for barramundi (Lates calcarifer) challenged with scale drop disease virus, by population origin (Singapore, Malaysia) and infection route (IP injection, cohabitation) over the 28-day challenge. Shaded bands are 95% confidence intervals. Vertical tick marks indicate right-censored individual. Singapore is shown in blue and Malaysia in red, with IP injection as solid lines and cohabitation as dashed lines. The overall log-rank test across the four groups is shown on the figure ($p < 0.001$). Pairwise log-rank tests: IP versus cohabitation $p < 0.001$ within both Singapore and Malaysia; Singapore versus Malaysia $p < 0.001$ under IP injection and $p = 0.795$ under cohabitation.

Infection route had a strong effect on survival across origins, with cohabitation-exposed fish having a lower mortality risk than those that were IP-injected (HR = 0.44, $p < 0.001$; **Table 2**). This was similarly found within both origins, where cohabitation significantly reduced hazard relative to IP injection for Singaporean fish (HR = 0.29, $p < 0.001$) and Malaysian fish (HR = 0.61, $p < 0.001$).

Table 2. Cox proportional hazards analysis of mortality across intraperitoneal injection (IP) and cohabitation infection routes and Singapore and Malaysia population origins.

Dataset	Covariate	Reference	n	β ± SE	HR	HR^{-1}
IP	Origin	Singapore	853	-0.59 ± 0.09	0.55*** [0.47 - 0.66]	1.80
Cohab	Origin	Singapore	856	-0.03 ± 0.11	0.97 [0.79 – 1.19]	1.03
IP + Cohab	Origin	Singapore	1709	-0.32 ± 0.07	0.72*** [0.63 - 0.83]	1.38
IP + Cohab	Route	IP	1709	-0.83 ± 0.07	0.44*** [0.38 - 0.50]	2.30
IP + Cohab	Origin + Route	Singapore	1709	-0.44 ± 0.07	0.65*** [0.57 - 0.74]	1.55
		IP		-0.89 ± 0.07	0.41*** [0.36 - 0.47]	2.43
Singaporean	Route	IP	833	-0.22 ± 0.09	0.29*** [0.24 - 0.35]	3.40
Malaysian	Route	IP	876	-0.50 ± 0.10	0.61*** [0.50 - 0.74]	1.65

Notes: β is the regression coefficient of the Cox proportional hazards model, SE is the standard error.

HR (hazard ratio) represents the relative risk of mortality for the tested category compared with the specified reference category, with 95% confidence intervals shown in brackets [ ]. HR > 1 indicates increased mortality risk, whereas HR < 1 indicates reduced risks. HR^{-1} is the reciprocal hazard ratio (1/HR) and represents the relative mortality risk of the reference category compared with the tested category.

The covariate column indicates the factor being evaluated within each dataset (e.g. origin or route), and results are interpreted relative to the stated reference category.

*** represents $p < 0.001$.

Interestingly, no significant difference was observed in survival between origins under the cohabitation groups (HR = 0.97, p > 0.05), with similar survival rates at the end of the 28-day challenge for Malaysian and Singaporean fish (58.8% and 56.9% respectively; **Figure 2; Table 1**).

3.2 Genetic parameters

Heritability (h^2) estimates for survival traits were significant across all origins and infection routes based on likelihood ratio tests, with significance levels indicated in **Table 3**. Within each infection route, heritability estimates were generally higher for Singaporean fish than Malaysian fish for both traits, although standard errors overlapped (e.g. survival time for IP: Singapore 0.44 ± 0.09 vs Malaysia 0.30 ± 0.08; survival time for cohabitation: Singapore 0.36 ± 0.09 vs Malaysia 0.23 ± 0.08). Across infection routes, heritability estimates were higher in fish infected via SDDV IP injection compared to those exposed by cohabitation (e.g. survival time for Singapore: IP 0.44 ± 0.09 vs cohab 0.36 ± 0.09; survival time for combined origins: IP 0.38 ± 0.06 vs cohab 0.32 ± 0.06).

Table 3. Variance components and heritability for SDDV survival traits for Singaporean and Malaysian population barramundi (*Lates calcarifer*) infected by intraperitoneal injection (IP) and cohabitation.

Trait	Origin	Route	N	Va	Ve	GBLUP h^2 ± SE	Cox-GBLUP h^2
Survival time	Singapore	IP	399	30.82	39.60	0.44 ± 0.09***	0.47
		Cohab	434	8.58	15.61	0.35 ± 0.09***	0.35
		IP + Cohab	833	13.84	29.49	0.32 ± 0.07***	0.49
	Malaysia	IP	454	19.67	46.47	0.30 ± 0.08***	0.35
		Cohab	422	5.72	18.92	0.23 ± 0.08***	0.21
		IP + Cohab	876	13.27	33.47	0.28 ± 0.05***	0.41
	Singapore + Malaysia	IP	853	25.44	41.78	0.38 ± 0.06***	0.44
		Cohab	856	7.70	16.66	0.32 ± 0.06***	0.28
		IP + Cohab	1709	14.90	31.76	0.32 ± 0.04***	0.47
Survival status	Singapore	IP	399	0.07	0.16	0.31 ± 0.09***	-
		Cohab	434	0.05	0.23	0.19 ± 0.10*	-
		IP + Cohab	833	0.06	0.20	0.22 ± 0.06***	-
	Malaysia	IP	454	0.07	0.19	0.26 ± 0.08***	-
		Cohab	422	0.03	0.21	0.12 ± 0.07*	-
		IP + Cohab	876	0.05	0.20	0.21 ± 0.05***	-
	Singapore + Malaysia	IP	853	0.08	0.17	0.31 ± 0.06***	-
		Cohab	856	0.03	0.22	0.12 ± 0.05**	-
		IP + Cohab	1709	0.05	0.20	0.21 ± 0.04***	-

Va, additive genetic variance; Ve, residual variance; GBLUP h^2, linear genomic heritability; Cox-GBLUP h^2, frailty-scale heritability for survival time only. Significance of the additive genetic variance component was assessed by likelihood ratio test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Dashes denote not applicable.

Across all corresponding comparisons, heritability estimates for survival time (0.23 ± 0.08 to 0.44 ± 0.09) were consistently higher than survival status (0.12 ± 0.07 to 0.31 ± 0.09). For survival time, h^2 was highest in Singaporean fish under IP injection (0.44 ± 0.09) and lowest in Malaysian fish under cohabitation (0.23 ± 0.08). Similarly, for survival status, the highest heritability was observed in Singaporean fish under IP injection (0.31 ± 0.09) and lowest in Malaysian fish under cohabitation (0.12 ± 0.07).

Tank effects were largely non-significant across datasets, reaching significance only for survival time in the Singapore IP-injection dataset (Wald $p = 0.045$) and the pooled

IP dataset (p = 0.006), and were non-significant in the remaining models (Supplementary Table S1). Additionally, body weight was included as a covariate in all models but was non-significant in every dataset (Supplementary Table S1).

Censoring-aware Cox-GBLUP heritability estimates for survival time were largely consistent with the linear GBLUP estimates, falling within their standard errors in most datasets (**Table 3**). The exceptions were the three mixed-route (IP + Cohab) datasets, namely Singapore, Malaysia, and the combined population, where Cox-GBLUP estimates were higher than the corresponding linear estimates (0.32 to 0.49, 0.28 to 0.41, and 0.32 to 0.47, respectively). The full-data correlation between linear GBLUP and Cox-GBLUP gEBVs for survival time was 0.97, indicating that the ranking of breeding values was robust to the modelling of censoring, and that both approaches are suitable for this trait.

3.3 Genetic correlations

Within the same infection route, genetic correlation for survival time and survival status was r_g = 1.00 ± 0.00 for both IP-injected and cohabitation-exposed fish (**Table 4**). For the same trait across different infection routes, correlations ranged from r_g 0.74 ± 0.12 to 0.83 ± 0.16, indicating low to moderate genotype-by-infection route effects between IP injection and cohabitation. Interestingly, the genetic correlation between different survival traits across infection routes was high (r_g = 0.92 ± 0.16). All correlations were statistically significant based on LRT.

Table 4. Genetic correlations (below diagonal) and heritability estimates (diagonal) for survival time and survival status across intraperitoneal injection (IP) and cohabitation, estimated using bivariate GBLUP models (estimates ± SE).

		Survival time		Survival status	
		IP	**Cohab**	**IP**	**Cohab**
Survival time	**IP**	*0.40 ± 0.05*			
	Cohab	0.77 ± 0.11	*0.32 ± 0.06*		
Survival status	**IP**	1.00 ± 0.00	0.74 ± 0.12	*0.34 ± 0.06*	
	Cohab	0.92 ± 0.16	1.00 ± 0.00	0.83 ± 0.16	*0.14 ± 0.05*

Bivariate heritability estimates were consistent with the corresponding univariate estimates, with values falling within the respective standard errors (**Table 3**; **Table 4**). For instance, heritability for survival time for fish under cohabitation was 0.32 ± 0.06 in both univariate and bivariate analyses, while survival status for IP injection was 0.31 ± 0.06 in univariate and 0.34 ± 0.06 in bivariate models.

3.4 Within-dataset prediction performance

Across all datasets, predictive ability (Pearson correlation) was consistently higher for survival time than survival status (**Table 5**). For the combined population, predictive ability for survival time was 0.31 ± 0.01 under IP injection and 0.23 ± 0.01 under cohabitation, compared with 0.30 ± 0.01 and 0.13 ± 0.01, respectively, for survival status. When standardised to accuracy, values for the two traits were more similar, because the lower heritability of survival status increases its standardised accuracy relative to its raw predictive ability.

Table 5. Within-dataset genomic prediction predictive ability (Pearson), accuracy (Pearson/√h²), AUC, and bias (ß) for survival time and survival status by population origin and infection route (mean ± SE).

Trait	Origin	Route	Pearson ± SE	Accuracy ± SE	AUC ± SE	ß ± SE
Survival time	Singapore	IP	0.33 ± 0.01	0.49 ± 0.02	-	1.02 ± 0.06
		Cohab	0.21 ± 0.01	0.36 ± 0.02	-	1.07 ± 0.09
		IP + Cohab	0.21 ± 0.01	0.37 ± 0.02	-	0.93 ± 0.05
	Malaysia	IP	0.29 ± 0.01	0.53 ± 0.03	-	1.12 ± 0.08
		Cohab	0.23 ± 0.01	0.49 ± 0.02	-	1.19 ± 0.10
		IP + Cohab	0.28 ± 0.01	0.54 ± 0.02	-	0.99 ± 0.04
	Singapore + Malaysia	IP	0.31 ± 0.01	0.51 ± 0.02	-	1.00 ± 0.04
		Cohab	0.23 ± 0.01	0.40 ± 0.02	-	0.99 ± 0.06
		IP + Cohab	0.25 ± 0.01	0.44 ± 0.01	-	0.90 ± 0.03
Survival status	Singapore	IP	0.25 ± 0.01	0.45 ± 0.03	0.66 ± 0.01	1.13 ± 0.09
		Cohab	0.08 ± 0.01	0.19 ± 0.03	0.55 ± 0.01	1.23 ± 0.18
		IP + Cohab	0.13 ± 0.01	0.29 ± 0.02	0.58 ± 0.01	1.02 ± 0.06
	Malaysia	IP	0.27 ± 0.01	0.53 ± 0.03	0.65 ± 0.01	1.17 ± 0.09
		Cohab	0.15 ± 0.01	0.42 ± 0.03	0.58 ± 0.01	1.46 ± 0.17
		IP + Cohab	0.24 ± 0.01	0.53 ± 0.02	0.64 ± 0.01	1.06 ± 0.05
	Singapore + Malaysia	IP	0.30 ± 0.01	0.53 ± 0.02	0.67 ± 0.01	1.11 ± 0.05
		Cohab	0.13 ± 0.01	0.37 ± 0.03	0.57 ± 0.01	1.46 ± 0.17
		IP + Cohab	0.22 ± 0.01	0.48 ± 0.01	0.63 ± 0.01	1.05 ± 0.04

AUC is reported for survival status only. Dashes denote not applicable.

Malaysian fish exhibited higher genomic prediction accuracies than Singaporean fish under both IP injection (0.53 ± 0.03 vs 0.49 ± 0.02) and cohabitation (0.49 ± 0.02 vs 0.36 ± 0.02). However, these higher accuracies were accompanied by greater deviation of ß from unity in Malaysian fish, with ß = 1.12 ± 0.08 for IP-injected fish and ß = 1.19 ± 0.10 under cohabitation, compared with ß = 1.02 ± 0.06 and 1.07 ± 0.09 in

Singaporean fish, respectively. Greater deviation from unity in the cohabitation group reflects greater non-genetic variation in phenotypes, leading to deflation (under-dispersion) of breeding value estimates and further supporting IP injection as a more stable challenge method. When data from both origins were combined, prediction accuracies were intermediate, with estimates of 0.51 ± 0.02 for IP, 0.40 ± 0.02 for cohabitation, and 0.44 ± 0.01 when both infection routes were analysed jointly.

Prediction accuracies were consistently higher for IP-injected fish than fish exposed through cohabitation across both traits and origins. For survival time, accuracies ranged from 0.49 ± 0.02 to 0.53 ± 0.03 for IP injection, compared to 0.36 ± 0.02 to 0.49 ± 0.02 for cohabitation. This similar trend was observed for survival status, with higher accuracies under IP injection (0.45 ± 0.03 to 0.53 ± 0.02) than under cohabitation (0.19 ± 0.03 to 0.42 ± 0.03). For survival status, AUC was consistently higher under IP injection (0.65 ± 0.01 to 0.67 ± 0.01) than under cohabitation (0.55 ± 0.01 to 0.58 ± 0.01). Bias was generally smaller for IP injection, with β closer to unity, particularly for survival status (e.g. Singapore IP: 1.13 ± 0.09 vs cohabitation: 1.23 ± 0.18; Malaysia IP: 1.17 ± 0.09 vs cohabitation: 1.46 ± 0.17), indicating less deflation of predicted breeding values. In addition, β values closest to unity were observed when infection methods were analysed separately as their own dataset, for both IP (β = 1.00 ± 0.04) and cohabitation (β = 0.99 ± 0.06).

3.5 Across-dataset prediction performance

Cross-scenario validation showed that the transferability of genomic predictions varied across the three contrasts (**Table 6**). Predictions performed best between replicate tanks for both traits (survival time accuracy 0.33 ± 0.04 to 0.37 ± 0.03; survival status 0.32 ± 0.05 to 0.38 ± 0.05), demonstrating consistency within the shared environment. Transfer between infection routes was lower but positive (survival time Pearson 0.20 ± 0.04, accuracy 0.32 ± 0.06 to 0.35 ± 0.02; survival status Pearson 0.10 ± 0.06 to 0.14 ± 0.01, accuracy 0.18 ± 0.10 to 0.41 ± 0.03). In contrast, transfer between population origins was poor for both traits (survival time Pearson 0.05 ± 0.02 to 0.08 ± 0.02, accuracy 0.10 ± 0.04 to 0.15 ± 0.04; AUC 0.53 ± 0.01 to 0.54 ± 0.01), indicating that breeding values estimated in one origin had limited predictive value in the other.

Table 6. Across-dataset genomic prediction predictive ability (Pearson), accuracy (Pearson/$\sqrt{h^2}$), and AUC for survival time and survival status, for models trained on one scenario and validated on the other across infection method, population origin, and replicate tank (mean ± SE).

Trait	Cross-prediction type	Scenario	Pearson ± SE	Accuracy ± SE	AUC ± SE
Survival time	Infection method	Cohab -> IP	0.20 ± 0.04	0.32 ± 0.06	-
		IP -> Cohab	0.20 ± 0.01	0.35 ± 0.02	
	Origin	MY -> SG	0.08 ± 0.02	0.15 ± 0.04	-
		SG -> MY	0.05 ± 0.02	0.10 ± 0.04	-
	Tank	Tank 1 -> Tank 2	0.21 ± 0.02	0.37 ± 0.03	-
		Tank 2 -> Tank 1	0.19 ± 0.02	0.33 ± 0.04	-
Survival status	Infection method	Cohab -> IP	0.10 ± 0.06	0.18 ± 0.10	0.56 ± 0.03
		IP -> Cohab	0.14 ± 0.01	0.41 ± 0.03	0.58 ± 0.01
	Origin	MY -> SG	0.06 ± 0.02	0.12 ± 0.04	0.53 ± 0.01
		SG -> MY	0.07 ± 0.02	0.15 ± 0.04	0.54 ± 0.01
	Tank	Tank 1 -> Tank 2	0.17 ± 0.02	0.38 ± 0.05	0.60 ± 0.01
		Tank 2 -> Tank 1	0.15 ± 0.02	0.32 ± 0.05	0.58 ± 0.02

AUC is reported for survival status only. Dashes denote not applicable.

4. Discussion

This study evaluated the effects of infection route (IP injection and cohabitation) and population origin (Singapore vs Malaysia) on phenotypic survival, genetic parameter estimations, and genomic prediction for SDDV resistance in barramundi under controlled laboratory conditions. Genetic correlations between resistance expressed under IP injection and cohabitation were moderate to high, indicating substantial shared genetic control but with partial re-ranking of genotypes across infection routes. Population origin also influenced survival outcomes and genetic parameters, highlighting that population background is a critical consideration for the interpretation of challenge test results and for the selection of appropriate populations when implementing genomic selection programmes. Together, these results demonstrate that both infection route and population origin are key experimental factors that must be carefully considered when generating resistance phenotypes for selective breeding programmes.

A key feature of this study is the adoption of a within-tank, mixed-route challenge design, in which fish infected via IP and cohabitation were maintained in the same rearing environment. Rather than evaluating IP injection and cohabitation in separate tanks, this design minimises confounding from tank-specific environmental effects when comparing differences between infection routes. To our knowledge, this represents one of the first shared-environment frameworks for assessing laboratory-based disease resistance. In this system, IP-injected fish served as viral shedders, generating exposure for cohabitation recipients, such that cohabitation survival reflects recipient resistance under a pooled transmission environment determined by the combined shedder population. As described in the Methods, exposure was not origin-segregated, so cohabitation recipients of both origins experienced the same pooled transmission environment, in which viral pressure varied over the course of the challenge. Any origin-specific differences in viral shedding would therefore have affected both recipient groups equally rather than confounding the between-origin comparison of cohabitation survival. Lower heritability and prediction accuracy under cohabitation should therefore be interpreted as the combined outcome of recipient resistance and this heterogeneous, time-varying exposure, rather than as a purely

biological route effect. While this design increases biological realism to what fish faces under farming conditions, cohabitation challenges introduce additional complexities whereby survival may be influenced not only by individual resistance, but also by genetic variation among infectivity of shedder fish, affecting viral shedding load and timing (Anacleto et al., 2019; Dorfman et al., 2024). Variation in transmission dynamics and exposure timing among recipients is therefore expected to contribute to increased non-genetic variance in cohabitation phenotypes, which may partially explain the lower heritability estimates observed under cohabitation relative to IP injection, as well as the genetic correlations between infection routes. In contrast, IP injection standardises exposure dose and timing, producing phenotypes more strongly governed by within-host resistance mechanisms. Collectively, these results indicate that while within-tank cohabitation challenges may better reflect natural infection dynamics and offer practical relevance for selective breeding programmes, explicit consideration of shedder composition, shedder-to-recipient ratios, and transmission dynamics will be essential to optimise experimental design and genetic evaluation under cohabitation-based challenge systems in future studies.

A limitation of this study is that waterborne viral load was not quantified. Quantification by environmental DNA in tank water was attempted but did not yield conclusive results and is therefore not reported. While dead and moribund fish were removed during the challenge, which could in principle alter shedding dynamics, the intended transmission source was the live IP-injected shedder population rather than the removed carcasses, and effective cohabitation exposure was confirmed by the comparable peak viral loads observed in cohabitation-exposed fish. Both replicate tanks were further maintained on a shared recirculating system, and tank effects were non-significant in the majority of models (Supplementary Table S1), indicating minimal environmental differences between replicates. Nevertheless, standardised monitoring of waterborne viral load represents an important refinement for future cohabitation-based challenge studies.

Consistent with previous findings of SDDV resistance in barramundi (Poon et al., 2026b), a substantial additive genetic component was detected for laboratory-measured resistance, with heritability estimates falling within comparable ranges (0.12 to 0.44). Across all corresponding datasets, heritability estimates were consistently

higher for survival time than survival status, reiterating the advantage of this trait as a genetically informative resistance phenotype (Poon et al., 2026a). This is further supported by the near-unity genetic correlation between survival time and survival status within infection routes, indicating that both traits reflect the same underlying genetic architecture. This observation further supports the use of survival time as a genetically informative proxy for resistance. Censoring-aware Cox-GBLUP analysis supported this, yielding a high correlation between linear and Cox-GBLUP breeding values (r = 0.97) and indicating that the ranking of breeding values was robust to the treatment of censoring. Heritability estimates from the two models agreed within standard errors for most datasets, although Cox-GBLUP estimates were higher for the mixed-route datasets. Because the linear and Cox models estimate heritability on different scales, this does not imply that censoring had no effects on the variance components, and the magnitude of heritability estimates for survival time should therefore be interpreted with reference to the scale of the model. Within infection routes, Singaporean fish generally exhibited slightly higher heritability estimates than Malaysian fish for both traits, which may be attributed to greater phenotypic variance, but also variation in family composition, allele frequencies, or historical selection pressures leading to potential differences in genetic variance structure (Falconer, 1960; Walsh & Lynch, 2018). Practically, this indicates that genetic gain may be potentially higher when using Singaporean fish. Although the relative natural performance of Malaysian and Singaporean fish during SDDV outbreaks should first be properly evaluated under commercial farming conditions to establish a baseline resistance level, anecdotal evidence by Malaysian farmers indicate that SDDV outbreaks are not as severe as those observed in Singapore. Such information would be particularly relevant when establishing a founder population or when introducing new genetic material into existing breeding programmes.

Across infection methods, heritability estimates and genomic prediction accuracy were consistently higher under IP injection than cohabitation exposure, likely reflecting stronger genetic signal associated with controlled infection conditions, reduced environmental variance, and more synchronous disease progression under IP challenge, where all injected fish receive a uniform viral dose (Hudson & Nowak, 2021). Typically, cohabitation was characterised by delayed mortality onset and lower cumulative mortality across both populations, consistent with more gradual and

heterogeneous exposure dynamics. This likely reflects lower and variable effective viral doses and the requirement for viral entry across mucosal barriers prior to systemic infection, which may allow greater opportunity for early immune responses to partially control infection, thereby delaying mortality (M. Monte et al., 2016; Meza et al., 2019). In contrast, IP injection bypasses these initial defence barriers, leading to more rapid and severe disease progression. Together, these differences in exposure dynamics are expected to increase environmental and non-genetic variance under cohabitation, thereby reducing the precision of genetic parameter estimation relative to IP challenge.

This is supported by bivariate analyses, revealing genetic correlations ranging from 0.74 to 0.92 between infection routes, indicating partial re-ranking of individuals and the presence of genotype by infection route interactions. Interestingly, a high genetic correlation was observed between survival traits across infection routes (survival time IP and survival status cohab; r_g = 0.92), demonstrating that resistance across exposure scenarios is largely governed by shared genetic mechanisms. Similar findings have been reported for resistance to *S. agalactiae* in Nile tilapia, where a large-scale challenge study comparing IP injection and cohabitation across ~2,600 genotyped fish using univariate and bivariate GBLUP models identified high genetic correlations (~0.9) and comparable prediction accuracies between infection routes (Joshi et al., 2021). Consistent with the present study, these findings indicate that resistance under different exposure pathways is largely governed by shared genetic mechanisms, while still allowing for route-specific differences in genetic signal and expression. Together, these results indicate that despite infection route specific effects, SDDV resistance is underpinned by both generalised host defence mechanisms as well as infection route-specific components, consistent with expectations for fish innate immunity responses to viral exposure (Leiva-Rebollo et al., 2024; Ortega-Villaizan et al., 2022). Genetic correlations of 0.74 to 0.83 indicate 20% to 30% route-specific genetic variation, likely linked to mucosal immunity and behavioural resistance mechanisms unique to cohabitation exposure. Long-term breeding programs may combine IP-based selection with limited cohabitation validation to improve field performance.

These findings have important implications for breeding programme design. While IP injection provides a stronger genetic signal, reliance on a single infection route may

bias selection towards resistance mechanisms that are not fully representative of natural exposure scenarios. In this study, a direct comparison of IP- and cohabitation-derived resistance against farm performance was not assessed and is therefore not possible from these data. A strong laboratory-to-field genetic correlation has, however, been established previously for IP-derived SDDV resistance (Poon et al., 2026a), making IP injection the route with the better empirically supported link to field survival to date. The high genetic correlations between routes observed here further suggest that resistance expressed under cohabitation is largely governed by the same genetic mechanisms, such that IP-derived breeding values are expected to remain informative for field performance. Whether the additional barrier, behavioural, and transmission-related components captured under cohabitation improve field prediction beyond that achieved with IP-derived resistance remains untested and warrants direct validation against farm survival. Nevertheless, because standardised IP exposure minimises environmental variance and maximises the accuracy of estimated breeding values, it remains the primary method successfully driving commercial genomic selection. For example, long-term family-based selection programmes in rainbow trout have successfully utilised IP injection challenges to evaluate and significantly improve resistance to bacterial cold water disease over multiple generations (Leeds et al., 2010; Silverstein et al., 2009). Although cohabitation challenges theoretically may capture additional biological components of resistance relevant under farm conditions, their added value relative to IP challenges requires further investigation. Future studies should therefore evaluate whether incorporation of cohabitation phenotypes enhances long-term selection response beyond that achieved using IP-based challenges alone.

Genomic prediction accuracies obtained in this study varied across traits, populations, and infection routes. Predictive ability for survival time consistently outperformed that for survival status, mirroring patterns observed for heritability, as documented in simulation studies showing that prediction accuracy generally increases with increasing heritability (Behmaram et al., 2013; Yin et al., 2024). Survival time is expected to capture a greater proportion of additive genetic variation owing to its continuous nature compared with the binary survival status. When predictive ability was standardised to accuracy, values for the two traits were more similar, reflecting the lower heritability of survival status, which increases its standardised accuracy

relative to its raw predictive ability. To enable a consistent comparison across traits, Pearson correlation was reported for both traits, with AUC additionally reported for survival status; AUC was modest but consistently higher under IP injection. This interpretation, however, remains theoretical and requires empirical validation in future studies, although it is currently supported by the bias estimates, which are independent of correlation metrics and show greater deviation of ß from unity for survival status predictions.

The genomic prediction accuracies obtained in this study (0.19 to 0.54) were substantially lower than our previous study for IP-based SDDV challenges (0.51 to 0.91; Poon et al. (2026b)). Genomic prediction accuracies are affected by multiple factors such as experimental design, population structure, trait architecture, and family composition (Liu et al., 2018; Widener et al., 2021; Zhao et al., 2023). In aquaculture species, genomic prediction accuracies for disease resistance traits typically range from ~0.3 to 0.8 depending on the species and study design. For example, accuracies of 0.34 to 0.61 have been reported for sea lice resistance in Atlantic salmon (*Salmo salar*), 0.41 to 0.71 for bacterial cold water disease resistance in rainbow trout, and 0.31 to 0.63 for *Francisellosis* resistance in Nile tilapia (reviewed by Song et al. (2023)). Within this context, the accuracies obtained in the present study fall within the lower to moderate range of published estimates, particularly for cohabitation-based traits. However, pedigree information was unavailable in both the current and our previous study, and therefore could not be incorporated, which may have assisted in disentangling family effects and improving prediction accuracy. Hence, the lower accuracies observed here are likely attributable to differences in experimental design and increased environmental and transmission-related variance, rather than reduced genetic control of the traits. This is supported by comparable heritability estimates for IP injection relative to our previous study and is consistent with the stronger genetic signal observed under controlled infection conditions.

The cross-scenario validation provides direct evidence on the transferability of resistance phenotypes. Genomic predictions transferred well between replicate tanks and, to a lesser extent, between infection routes, the latter consistent with the high genetic correlations observed between IP injection and cohabitation. However, transferability between population origins was poor, with breeding values trained in one origin showing little predictive value in the other. This pattern aligns with the clear

genetic differentiation between the Singapore and Malaysia populations, where decreased genetic relationships have been reported to result in a decrease in genomic prediction performance (Fraslin et al., 2022; Palaiokostas et al., 2019; Poon et al., 2026a). Overall, these results indicate that, while resistance phenotypes generalise reasonably across infection routes within a population, origin-specific reference populations are required for effective genomic selection across populations of different geographic backgrounds.

In this study, Malaysian fish exhibited higher survivability than Singaporean fish under IP injection, consistent with field observations that SDDV outbreaks are generally more severe in Singapore (Gibson-Kueh et al., 2012; Nurliyana et al., 2020). Because IP injection bypasses early barrier defences and establishes systemic infection directly, this difference may reflect population-specific variation in intrinsic antiviral resistance mechanisms expressed after infection has been established. Such differences are plausible given the distinct genetic backgrounds of the two populations, which were confirmed by clear separation in principal component analysis and marked genetic differentiation between origins (Hudson's and Weir and Cockerham's F_{ST} = 0.167; Fig. 1). The SNPs used here were derived from a commercial genotyping array, and ascertainment bias arising from the marker discovery process may influence allele-frequency spectra and estimates of population differentiation, so the F_{ST} values should be interpreted with this consideration in mind. Barramundi breeding programmes in Singapore typically maintain highly biosecure, unexposed nucleus environments, rendering these populations largely naive to SDDV. In contrast, hatcheries in Malaysia frequently source broodstock from grow-out farm survivors, a practice that may have inadvertently driven indirect selection for SDDV resistance or general robustness following natural field exposure. Population-specific variation in disease susceptibility has been documented across aquaculture species, often reflecting local adaptation, historical pathogen interactions, and inherited resistance (Garcia de Leaniz et al., 2007). For example, significant differences in viral resistance have been demonstrated among strains of common carp (*Cyprinus carpio*) exposed to Koi Herpesvirus (Shapira et al., 2005) and population level differences among Atlantic salmon challenged with infectious pancreatic necrosis (IPN) virus (Moen et al., 2009). Because population origin is inherently confounded with source, broodstock composition, and rearing

history in this study, origin differences in survival are best interpreted as population-level differences that may reflect both genetic and non-genetic factors rather than differences in genetic resistance alone. In this study, however, no significant population differences were detected under cohabitation. The experimental challenge was terminated at 28 days, prior to complete stabilisation of survival curves in the cohabitation groups due to logistical limitations. This earlier termination may have reduced phenotypic variance and prevented population-specific divergence in survival from becoming fully apparent.

The reduced phenotypic variance under cohabitation may additionally have contributed to the lower heritability estimates and genomic prediction accuracies observed for cohabitation compared with IP injection. Nevertheless, the present results, together with findings from previous SDDV challenge studies, support the suitability of IP injection as a robust and efficient laboratory challenge model for evaluating genetic resistance to SDDV. IP injection enables a shorter experimental duration, more rapid disease onset, which are advantageous for reducing the cost of large-scale challenge testing (Meza et al., 2019). Most importantly, clinical signs characteristic of SDDV were consistent across both infection routes, supported by molecular confirmation of SDDV infection in affected fish, and high genetic correlations between routes. Collectively, these findings indicate that both IP injection and cohabitation are biologically valid models for studying host resistance to SDDV under controlled conditions. Hence, where feasible, a combined modelling strategy incorporating data from multiple infection routes may improve robustness of genetic evaluations to balance experimental efficiency and biological realism for improved long-term selection response.

Finally, while this study demonstrates the suitability of both IP injection and cohabitation challenges for evaluating SDDV resistance across two barramundi populations, only a single population per origin was examined. Given the wide geographic distribution of barramundi and differences in stock history (e.g. originating from selected vs non-selected stocks) (Grey, 1987; Yue, 2025), evaluation of a broader range of populations will be necessary to establish baseline resistance levels and support informed decision making in selective breeding programmes. Furthermore,

although Malaysian-origin fish showed higher survivability under IP injection, this study did not investigate the genomic basis underlying these differences. Future work incorporating genome-wide association studies (GWAS) could identify specific resistance loci, an approach that has substantially improved disease control of IPN in Atlantic salmon (Houston et al., 2008; Moen et al., 2015). Integrating GWAS with transcriptomic may also further elucidate host–pathogen interactions and candidate resistance mechanisms (Bai et al., 2022; Pan et al., 2025). In addition, genetic correlations among infection routes, population origins, and economically important traits (e.g., growth or fillet yield) should be evaluated to improve applicability in breeding programmes. This is particularly important to avoid unintended selection responses, as unfavourable genetic correlations between growth and disease resistance have been reported in species such as coho salmon (*Oncorhynchus kisutch*) (Yáñez et al., 2016). Finally, as this study focused on a single viral pathogen, the extent to which these findings generalise to other pathogen classes remains uncertain. Resistance mechanisms may differ for bacterial or parasitic infections, particularly where infection route plays a stronger role in shaping host–pathogen interactions, highlighting the need for pathogen-specific validation of challenge models and selection strategies.

5. Conclusion

This study demonstrated that resistance to SDDV in barramundi is a heritable trait with stable genetic signals across infection routes and population origins. Moderate heritability estimates and high genetic correlations suggest that relative genetic performance is largely consistent under different exposure scenarios. While high genetic correlations indicated that the two infection routes had substantial overlapping genetic mechanisms for resistance, the highest heritability estimates and genomic prediction accuracies were consistently obtained when modelling survival time under IP injection, indicating that this approach provides the most informative and reliable phenotype for genetic evaluation. In contrast, cohabitation challenges yielded lower heritability and prediction accuracy, likely reflecting increased non-genetic variation associated with transmission dynamics. Collectively, these results support the use of survival time under IP injection as the preferred approach for SDDV resistance breeding under the present laboratory challenge conditions, rather than necessarily under natural farm exposure, for which its performance relative to cohabitation remains to be validated. The high genetic correlations observed between infection routes nonetheless suggest that IP-derived breeding values are likely to remain informative for field performance, while population origin remains an important factor influencing resistance expression and should be considered in breeding programme design.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Highlights

- Comparison of IP injection and cohabitation challenge models across Singapore and Malaysia populations for SDDV resistance.
- Moderate heritability estimates for survival traits across both infection routes and population origin.
- High genetic correlations between infection routes indicate substantial shared genetic control.
- Genomic prediction accuracy was highest when SDDV resistance modelled using intraperitoneal injections and survival time.