Genome-wide association study (GWAS) analysis identifies genetic factors associated with acute high temperature-induced cortisol levels in olive flounder (*Paralichthys olivaceus*)

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Abstract

The Republic of Korea leads the world in farm-raised olive flounder production, supplying nearly half the global output. However, the aquaculture industry in Korea faces significant challenges, including acute temperature elevation during transportation, the amount and form of dissolved oxygen, etc. These temperature-related stresses profoundly impact olive flounders' survival, growth, and immunity. To address these challenges, our study investigated the correlation between cortisol levels, indicating the high-temperature acute stress in olive flounders, using Genome-Wide Association Studies (GWAS) and genomic prediction strategies. By identifying genetic markers associated with stress resilience, we aim to develop acute high-temperature stress tolerance olive flounder traits and mitigate the adverse effects of temperature fluctuations in the Korea aquaculture industry. Therefore, in this study, 384 healthy fish were exposed to 29 °C, and blood was collected at 30 min and 1 h after the exposure for cortisol level measurement. Also, fin tissues were collected from all the individuals for the gDNA extraction. A high-quality 70K SNP chip was utilized to extract 57,638 high-quality SNPs from a cohort of 376 individuals. After the GWAS analysis, significant SNPs were identified based on the Bonferroni cutoff. Moreover, various genomic prediction methods were employed to determine the best model for analyzing the genomic estimated breeding values (gEBVs) within the study population. Our findings collectively indicate that genes significantly associated with high-temperature acute stress conditions and diverse prediction models hold promise for enhancing genetic diversity in flounder breeding within the aquaculture industry, paving the way for future genomic breeding programs.

Background & Objectives

The application of genome-wide association studies (GWAS) in aquaculture research has grown significantly in recent years. Olive flounder, alongside other commercially important species, has benefitted from this approach. Researchers have utilized high-density SNP markers in GWAS to investigate a variety of traits in these fish, including growth performance (Omeka et al., 2022), resistance to viral hemorrhagic septicemia virus (VHSV) (Liyanage et al., 2022), and tolerance to thermal stress (Udayantha et al., 2023).

This study employs a GWAS approach to identify genetic variants associated with acute, high temperature-induced cortisol levels and chronic high-temperature thermal tolerance in olive flounder. We hypothesize that specific polymorphisms within the olive flounder genome will influence individual variability in cortisol regulation and thermal stress tolerance. By analyzing a large population of flounder for both their genetic makeup and their cortisol response to controlled high-temperature exposure and thermal tolerance ability, we aim to identify Single Nucleotide Polymorphisms (SNPs) significantly associated with cortisol levels and fish survival following high-temperature exposure, elucidate the potential biological pathways underlying the association between these SNPs and cortisol regulation and inform the development of selective breeding programs for olive flounder with enhanced thermal stress tolerance.

References

Liyanage, D.S., Lee, S., Yang, H., Lim, C., Omeka, W.K.M., Sandamalika, W.M.G., Udayantha, H.M.V., Kim, G., Ganeshalingam, S., Jeong, T., Oh, S.-R., Won, S.-H., Koh, H.-B., Kim, M.-K., Jones, D.B., Massault, C., Jerry, D.R., Lee, J., 2022. Genome-wide association study of VHSV-resistance trait in Paralichthys olivaceus. Fish Shellfish Immunol. 124, 391–400. https://doi.org/10.1016/j.fsi.2022.04.021

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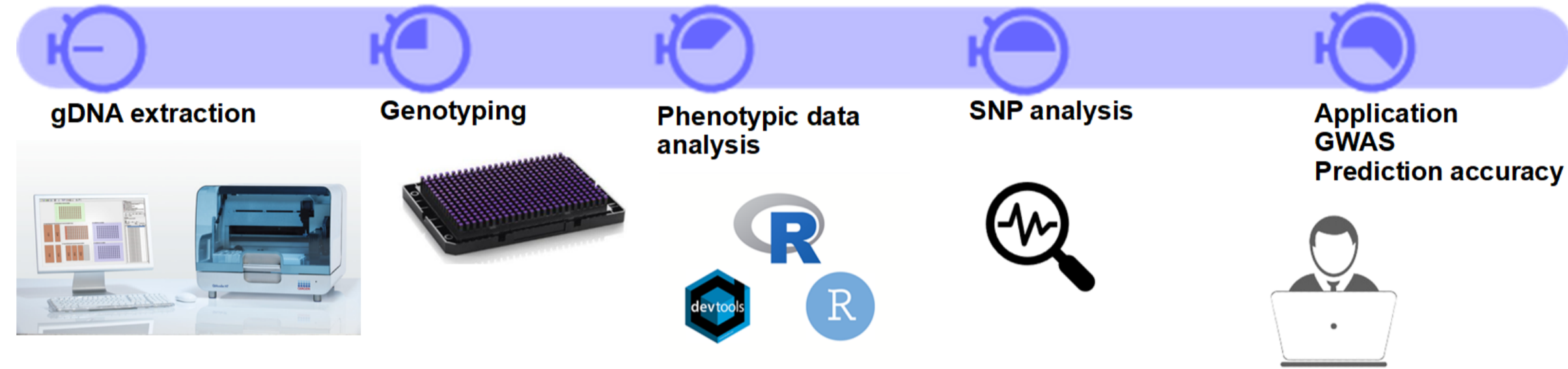
Udayantha, H.M. V, Lee, S., Liyanage, D.S., Lim, C., Jeong, T., Omeka, W.K.M., Yang, H., Kim, G., Kim, J., Lee, Jihun, Nadarajapillai, K., Ganeshalingam, S., Park, C.-U., Lee, Jiwon, Oh, S.-R., Gong, P., Jang, Y., Hyun, J., Park, A., Koh, H.-B., Kim, M.-K., Jones, D.B., Massault, C., Jerry, D.R., Lee, Jehee, 2023. Identification of candidate variants and genes associated with temperature tolerance in olive flounders by Genome-Wide Association Study (GWAS). Aquaculture 576, 739858. https://doi.org/https://doi.org/10.1016/j.aquaculture.2023.739858

Materials & methods

Field work Time Frame

2022.11 Breeding at Pyoseon

2023.05 dF0 fish at Pyoseon

2023.06 Tagging, Measuring & Blood collection

2023.11 High temperature chronic stress

2024.02 ELISA

Analysis Time Frame

gDNA extraction

Genotyping

Phenotypic data analysis

SNP analysis

Application GWAS Prediction accuracy
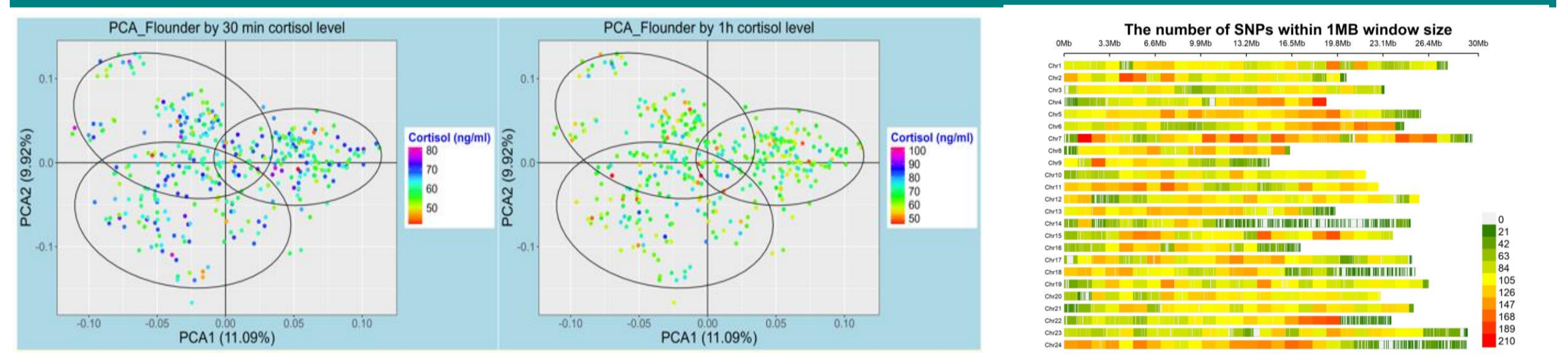
Results

Fig 1: PCA plot for the Cortisol level measurement at (A) 30 min (B) 1 h acute high temperature exposure time

Fig 2: SNP distribution along the flounder genome

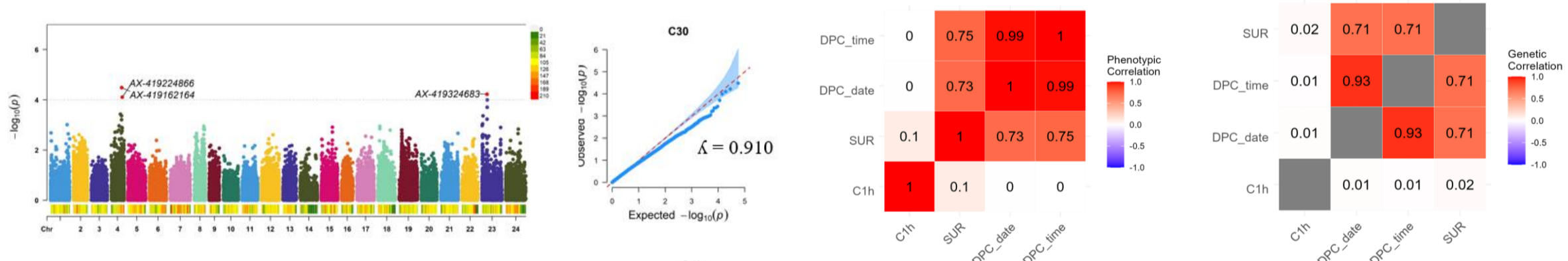| Chr	SNP ID	Position (bp)	A1	A2	Freq (A2)	P-value	Significance	VarP (%)	Associated gene
19	AX-419312234	4330372	C	T	0.614361702	8.69E-12	Genome-wide	7%	Myosin heavy chain, fast skeletal muscle-like, transcript variant X2 (myhc)
19	AX-419312236	4344315	G	A	0.635809565	2.48E-11	Genome-wide	6.7%	NLR family CARD domain containing 5 (nlrc5)
19	AX-419194856	4248358	A	C	0.542553191	2.58E-11	Genome-wide	6.7%	HYDIN, axonemal central pair apparatus protein (hydin)
18	AX-419305596	12631578	G	A	0.554521277	9.23E-08	Genome-wide	4.5%	Glucose-fructose oxidoreductase domain-containing protein 1 (gfod1)
19	AX-419197913	14012485	T	C	0.543882979	5.37E-07	Genome-wide	4%	S-phase cyclin A associated protein in the ER (scaper)
18	AX-419292991	2390583	G	A	0.634308511	1.61E-06	Suggestive	3.6%	Catenin delta-2-like, transcript variant X4 (ctnnd2)
10	AX-419296956	3777074	C	T	0.63368984	1.99E-06	Suggestive	3.6%	RNA binding protein fox-1 homolog 2-like (rbfox2)
18	AX-419291193	7299387	A	G	0.537533512	9.39E-06	Suggestive	3.1%	Chromosome unknown C3orf58 homolog, transcript variant X2 (cunh3orf58)
15	AX-419263188	14331018	T	C	0.66	1.55E-05	Suggestive	2.9%	Ephrin B2, transcript variant X2 (efnb2)
19	AX-419195929	15640134	G	A	0.5625	1.91E-05	Suggestive	2.9%	Cadherin-related family member 5-like (cdhr5)
11	AX-419250528	3479233	A	C	0.865691489	2.20E-05	Suggestive	2.9%	TSC22 domain family protein 2-like (tsc22d2)
11	AX-419178439	2265643	A	G	0.825333333	2.40E-05	Suggestive	2.9%	Ribosomal protein S15 (rps15)
4	AX-419162164	15473804	C	T	0.77393617	3.28E-05	Suggestive	3.3%	Urotensin-2 receptor-like (uts2r)
19	AX-419312138	13866568	C	T	0.613031915	3.65E-05	Suggestive	2.7%	Talin 2, transcript variant X6 (tln2)
19	AX-419275573	7107648	T	C	0.545576408	4.05E-05	Suggestive	2.7%	PET100 homolog (pet100)

Table1: Annotation of associated SNPs

Fig 3: Manhattan and qq plots of the trait associated ($p < 1 \times 10^{-5}$) SNPs

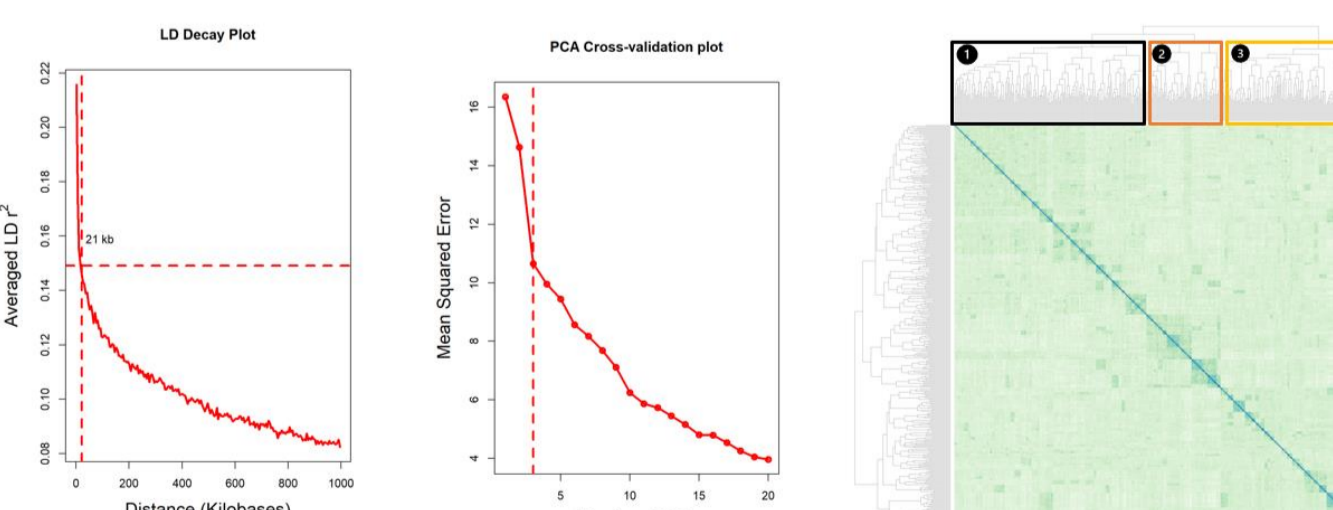Fig 5: Phenotypic and genetic correlation of the traits
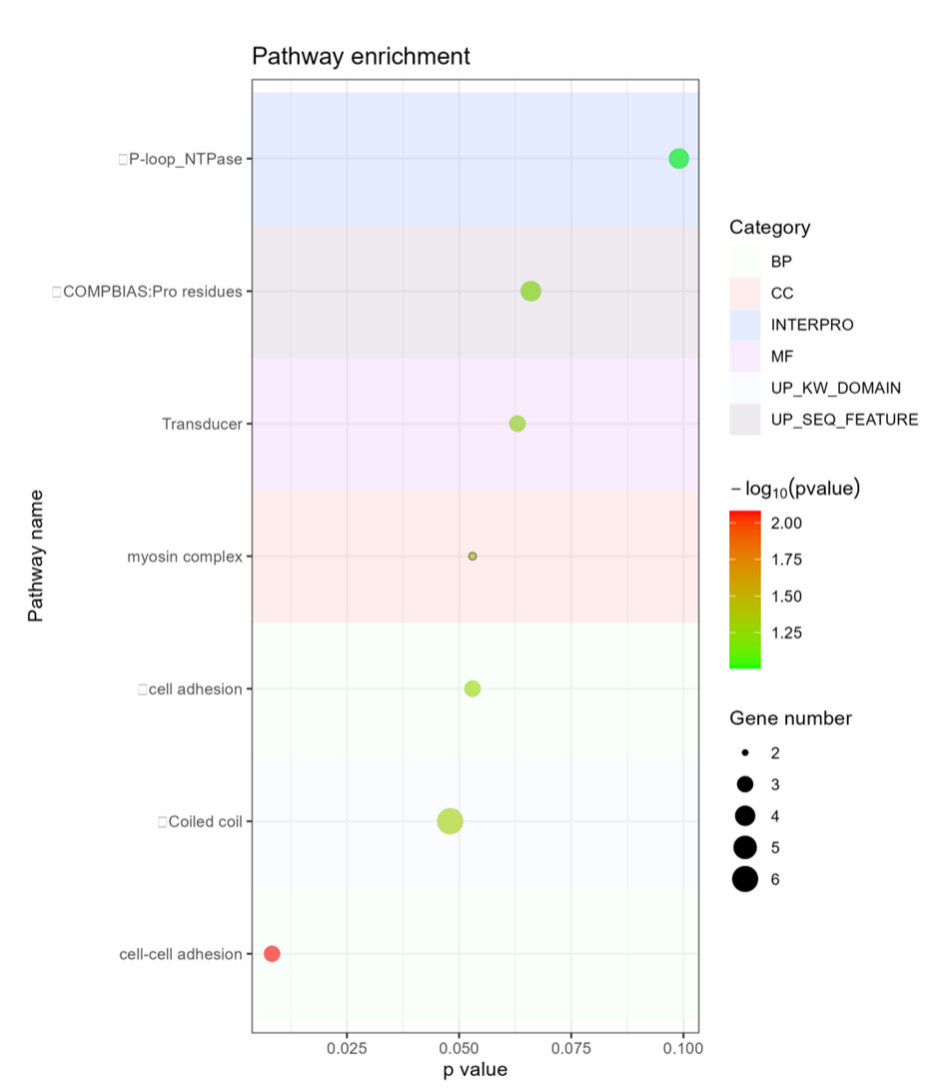

Fig 4: Structure of the study population

Fig 6: DAVID analysis of prominently associated ($p < 1 \times 10^{-4}$) SNP harboring genes

Conclusion

This study is the first to investigate acute and chronic high-temperature stress resistance in olive flounder using GWAS approach. The GWAS identified significant and suggestive SNPs associated with thermal stress resistance. We found 34 SNPs linked to various genes potentially involved in direct or indirect thermal tolerance and physiological stress maintenance during high-temperature exposure. DAVID analysis results further confirmed significant SNPs association with thermal stress resistance. Additionally, the observed moderate heritability for stress cortisol levels and high heritability for fish survival in chronic stress conditions highlight the potential of these significant SNPs for marker-assisted selection. This approach could be instrumental in developing stress regulation and thermal tolerance in olive flounder aquaculture programs.