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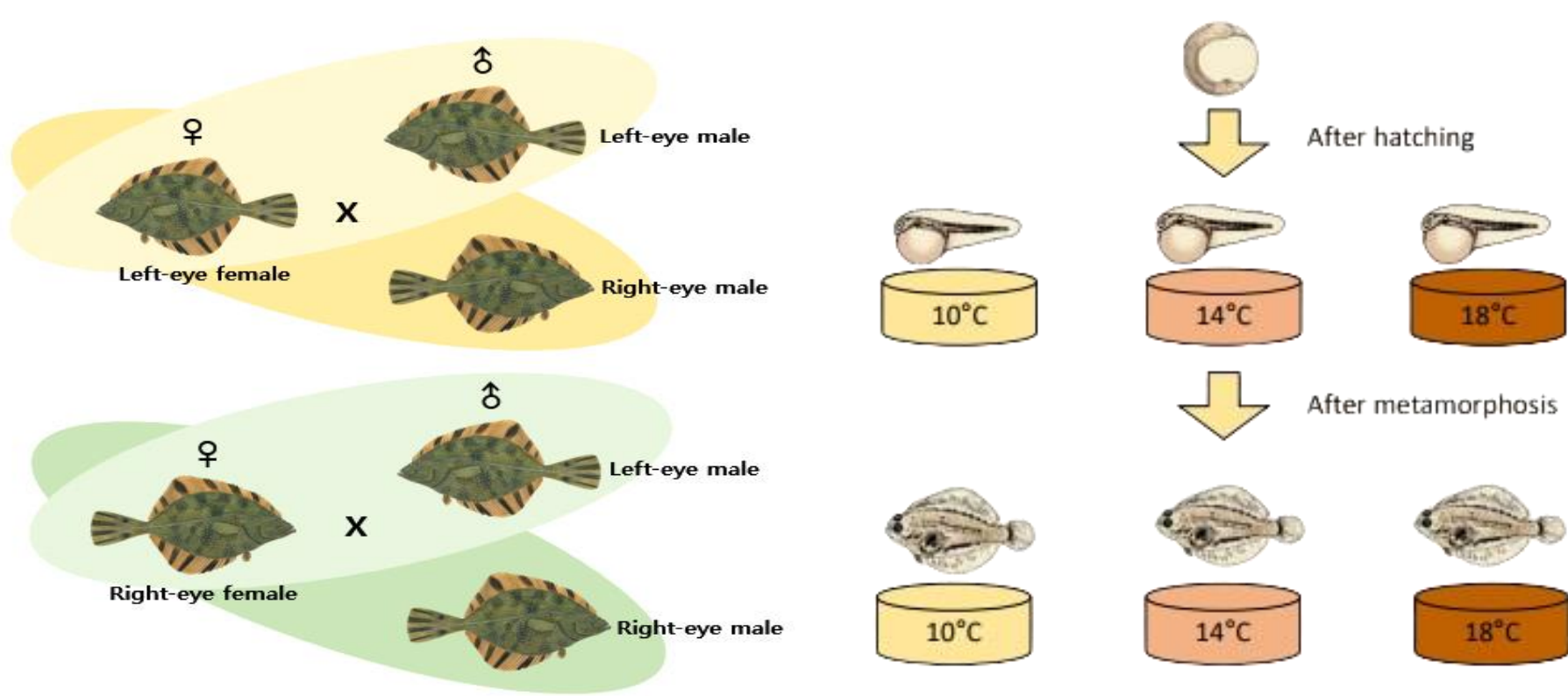
## Abstract

The direction of eye movement of Pleuronectiformes is species-specific. In farmed flounder, deviations in eye direction can reduce their marketability. Therefore, this study aims to explore the effect of genetic or environmental factors on the direction of eye movement in starry flounder (*Platichthys stellatus*) to address this economic challenge. In the mating experiment, four pairs of female and male starry flounder broodstock, with eyes positioned to the right or left, were selected and used((A: Right ♀ × Right ♂, B: Right ♀ × Left ♂, C : Left ♀ × Right ♂, D: Left ♀ × Left ♂). For the water temperature experiment, experimental groups were established with breeding seawater temperatures set at 10, 14, and 18°C. Whole-body samples of the juvenile were collected to analyze the expression of genes related to eye movement and direction. The results of the mating experiment showed no significant differences in abnormal eye movement. In the water temperature experiment, the rate of abnormal eye movement was significantly higher at 32.19±1.33% in the 18°C group. Genetic analysis of eye movement related to water temperature revealed that the expression of eye migration genes was significantly higher at 10° C. Moreover, no significant difference was observed in the mating experiment. Mating did not affect the eye movement of starry flounder, whereas water temperature was found to have an effect.

Key word:Starry flounder, Eye migration, Eye direction

## Material & Method

### Experiment design



### Primers for qRT-PCR

Purpose	Gene	Primer(5'~3')
qRT-PCR	Bmpr1B	F: TGAAGGGTCGGTAGGAAC TG
		R: GTCTCGTC CAGAACCTCTGG
	FGF7	F: TTGTAGGTTTCTCTCGCCTTG
		R: AGTCGGTGGCATCTTGAGTC
	Six1	F: TTGAGGACGCTCTCGTTCTT
		R: CCACTTGC TCCTCGGTAAG
	TgfBr2	F: CTCGTCC TGTGGAGATCAT
		R: AGCATGAAGGACAGGTTCT
	Dio1	F: AGGCTACGCTGAGATTTG
		R: AGCAGTTATAGACGGAGGT
	Dio2	F: CTGGTGCCAGGATGCGAAAT
		R: AAGTCCTCAACCAACTGTG
	Dio3	F: GGATTTC TTGTGCATCAGGA
		R: GGGACTCCAAGGTGAACATC
	Otx2	F: CTGCATGCAGAGGTCCTACC
		R: GGTAAGAGCCG CAGTCCATG
	Wnt1	F: CCAAGAACGTGCAGTTGGTC
		R: GCCACTTGCACTCCTTTATG
	GAPDH	F: CCAGAACATCATCCAGCTT
		R: GGCCTTCACAACCTTCTTGA

## Results

### Eye deformity rate

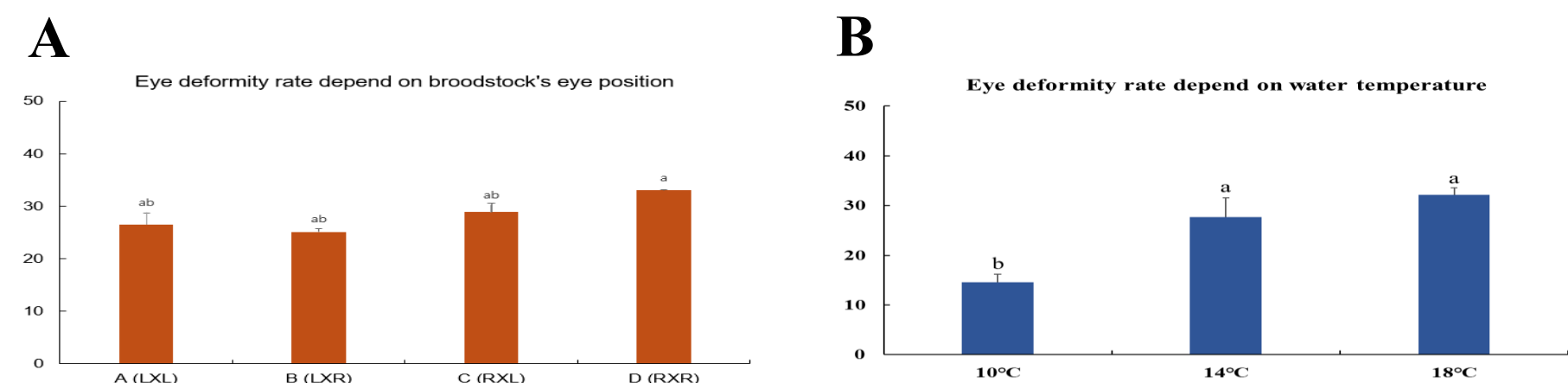
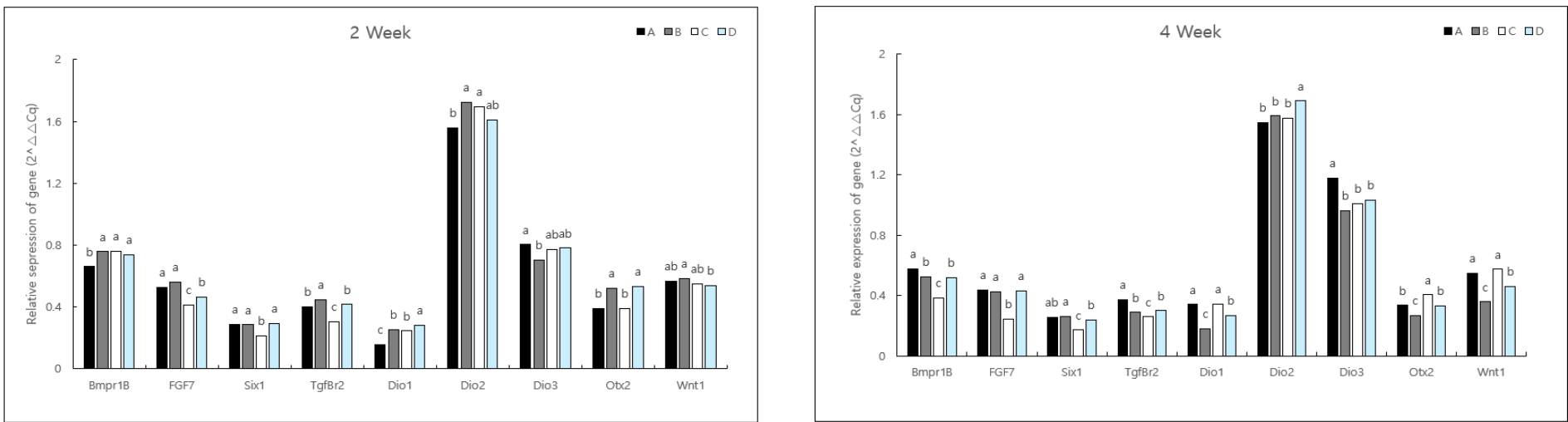
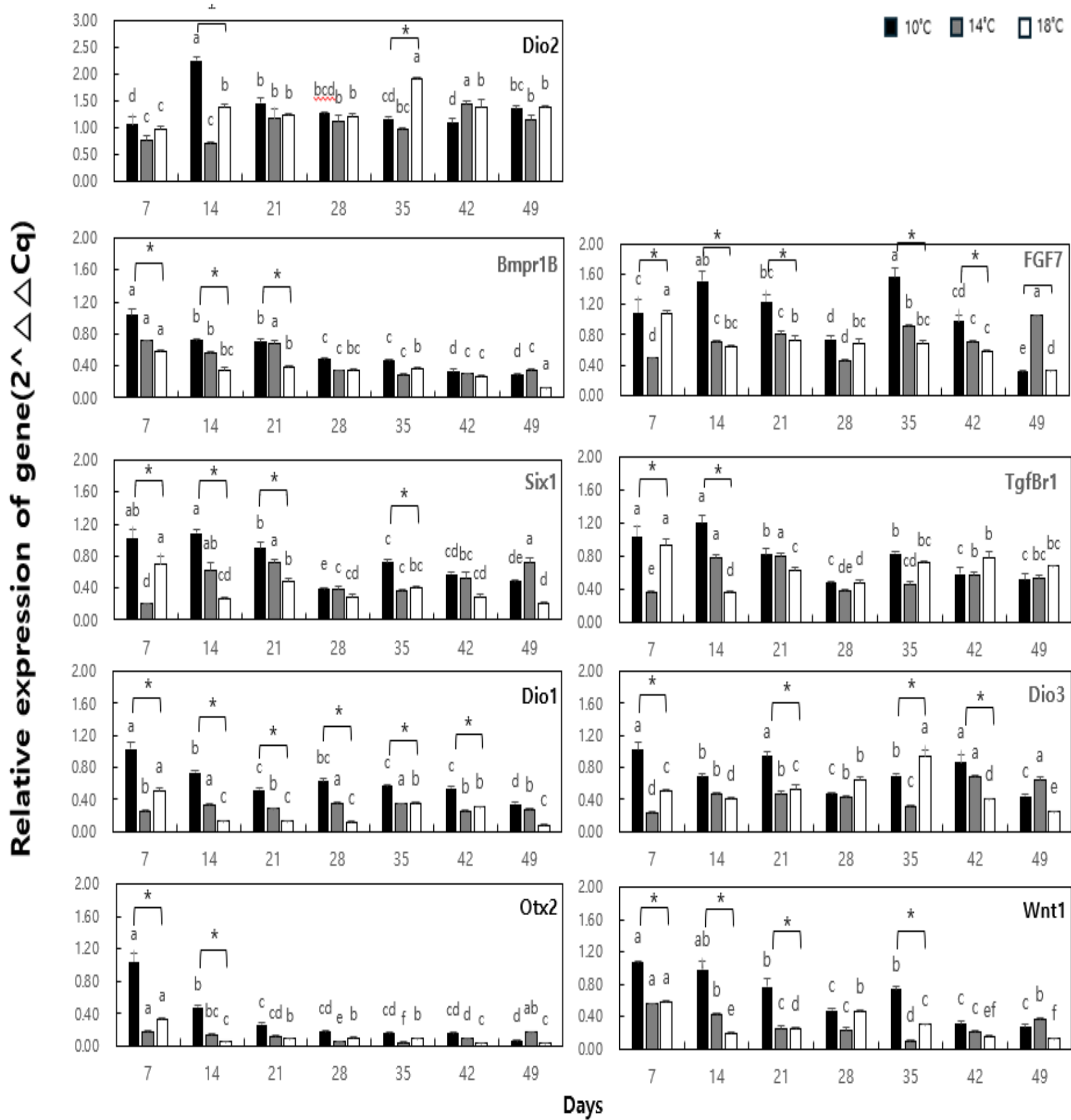


Figure 1. (A) The eye deformity rate in F1 juveniles varies based on the eye position of the broodstock ; (B) The eye deformity rate in F1 juveniles as influenced by different water temperatures. Different letters represent a significant statistical difference ( $p < 0.05$ ).

### Expression of transcripts determined by qRT-PCR



**Figure 2.** Relative expression of genes in different breeding experimental groups (A, B, C, D) at 2weeks and 4 weeks, focusing on genetic factors influencing eye direction. The genes analyzed include *Bmpr1B*, *FGF7*, *Six1*, *TgfBr2*, *Dio1*, *Dio2*, *Dio3*, *Otx2*, and *Wnt1*. Different letters represent a significant statistical difference ( $p < 0.05$ ).



**Figure 3.** Relative expression of eye migration gene normalized to GAPDH in starry flounder at different temperatures (10°C, 14°C, 18°C) over 49days. Different letters indicate significant differences based on ANOVA followed by Duncan’s multiple ranged test ( $P < 0.05$ ). Asterisks indicate significant statistical differences between experimental groups on the difference temperature (\*,  $P < 0.05$ ).

## Conclusion

The eye migration rate water temperature experiment was highest in the 18°C . There was no significant difference in other experiments. In addition, mating experiment *Fgf7* and *Six1* gene expression levels was the lowest in C group, was no significant difference. Water temperature experiment, In all the gene expression levels was the highest in the 10°C.

We found no effect on eye migration on mating, it has been found that there is an effect on the water temperature. Therefore, in the formed flounder the appropriate water temperature must be maintained to reduce eye movement.

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