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The Effect of Photoperiod on Male Zebrafish Sex Hormones

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INTRODUCTION

The goal of this research was to explore how photoperiod affects the reproductive endocrine axis in zebrafish (*Danio rerio*). It is known that the reproductive endocrine axis controls zebrafish reproduction. Additionally, it is known that photoperiod determines both reproductive seasonality (long days promote reproduction) and daily timing of spawning (initiates at the start of the light phase). However, there is limited research examining the effects of photoperiod on those endocrine pathways controlling zebrafish reproduction. I hypothesized that in males, long-day photoperiods promote the synthesis of key reproductive hormones: the gonadotropins (FSH and LH). I tested this hypothesis by comparing gonadotropin transcript levels and gonadosomatic index (GSI) in male zebrafish that have been exposed to long photoperiods with those exposed to short photoperiods. Because zebrafish breed under long light conditions, I predicted that the zebrafish exposed to long light conditions will have higher levels of LH and FSH transcripts, and a higher GSI.

BACKGROUND

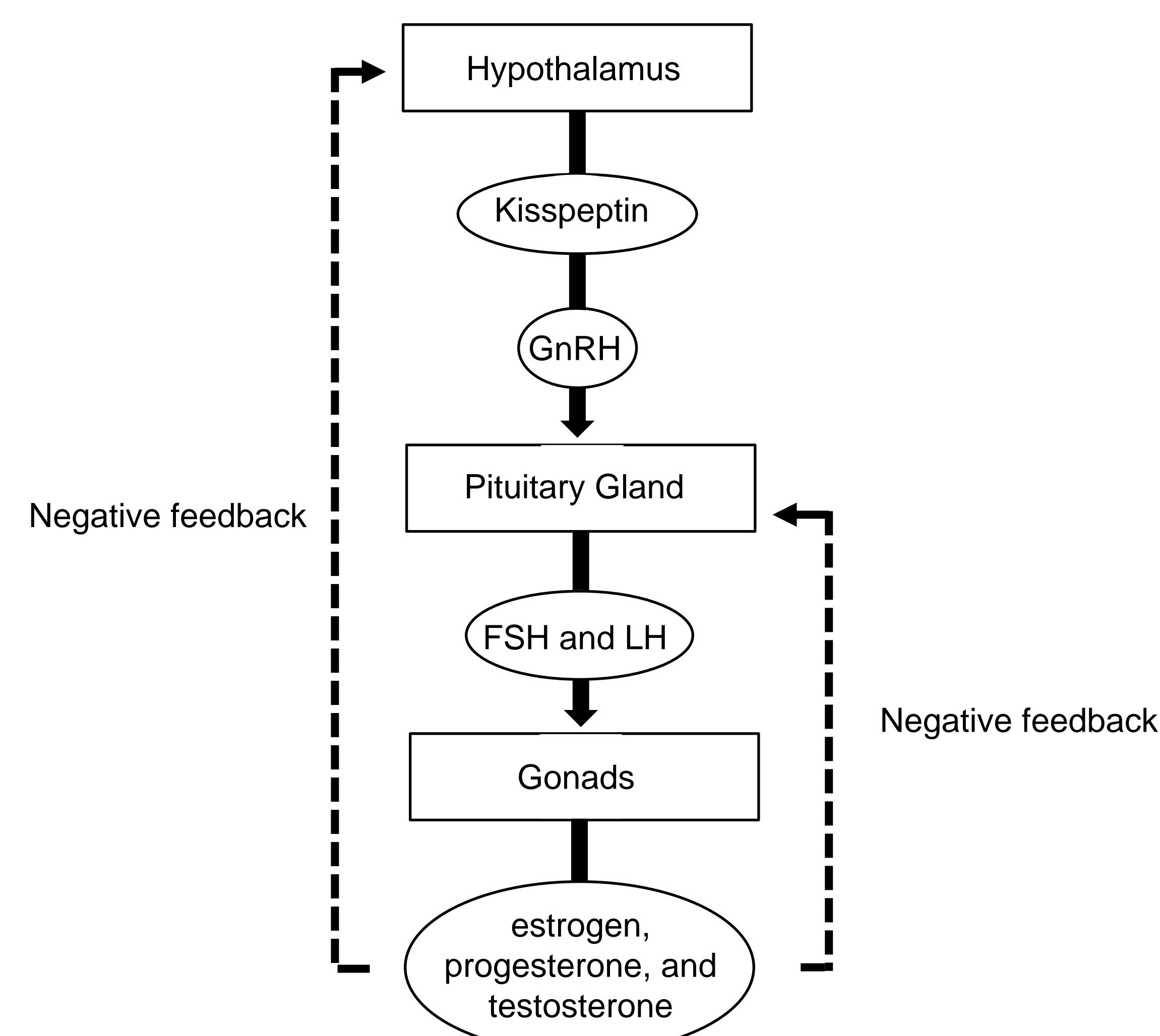


Figure 1. The Reproductive Endocrine Axis. In adult vertebrates, the gonadotropins include luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins are dimeric and consist of a common α -subunit and a hormone-specific β -subunit. In both sexes, FSH regulates gametogenesis. In males, FSH stimulates Sertoli cell proliferation. This allows for proper formation of sperm cells. LH regulates androgen production in Leydig cells (Zhang et al. 2015). Gonadotropin synthesis and release are primarily controlled by neurohormones from the brain and via negative feedback from the gonads. The hypothalamic neuropeptide gonadotropin-releasing hormone (GnRH) regulates the synthesis of LH and FSH in the pituitary. GnRH release, in turn, is regulated by another hypothalamic peptide, kisspeptin (Choi et al. 2010; Skorupskaite et al. 2014).

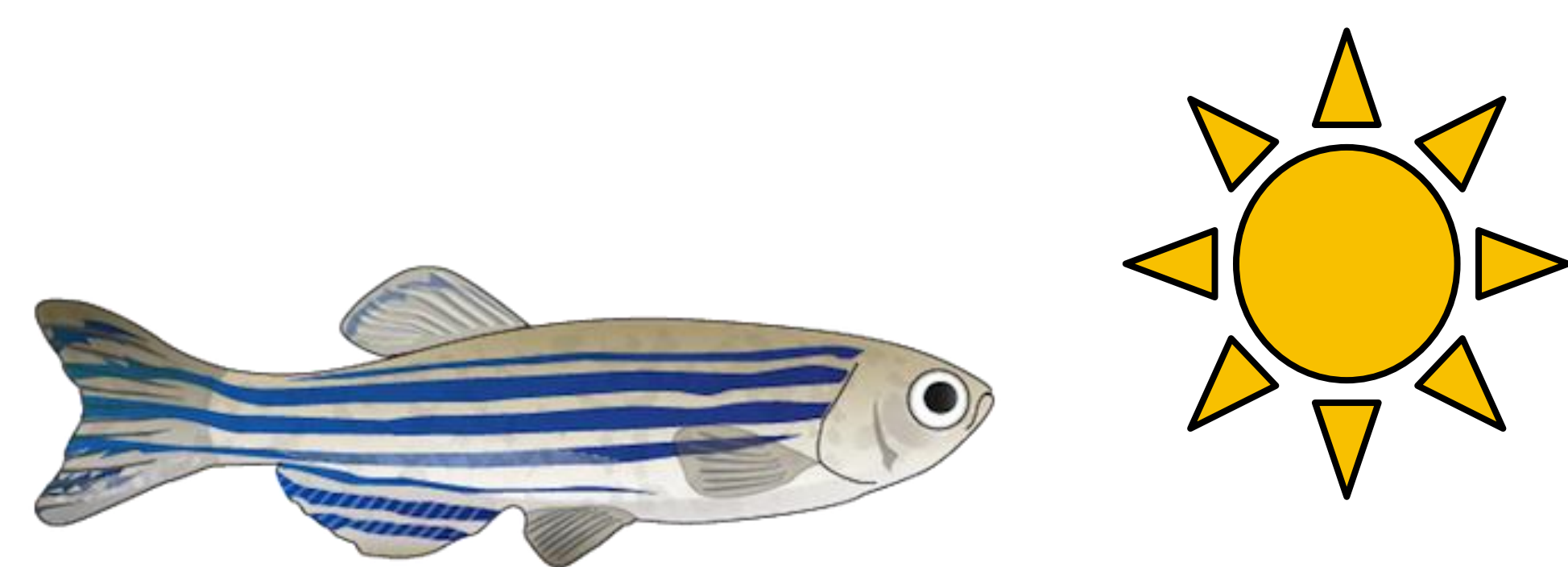


Figure 2. Photoperiodism in Zebrafish. Photoperiod can affect many aspects of reproduction in zebrafish. In a study conducted by Abdollahpour et al. (2020), zebrafish were kept in four different light-dark cycles for 42 days. The extended light group (16H light: 8H dark) produced the most larvae, and had the highest hatching rate, most embryos spawned, and the highest percent of ovulating females, compared to the shortened light groups (8H light: 16H dark and 12H light: 12H dark) and constant light group (24H light: 0H dark) (Abdollahpour et al. 2020).

METHODS

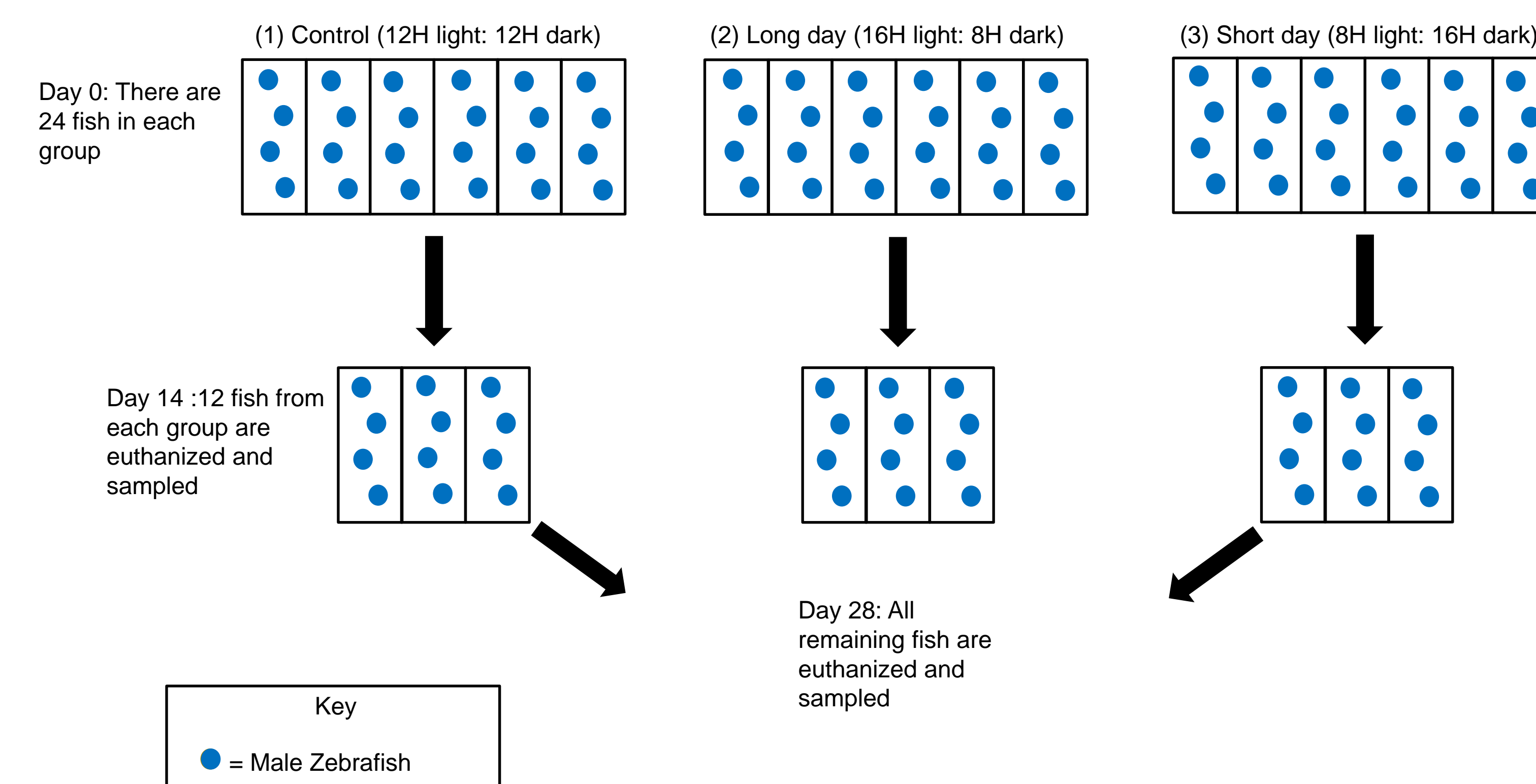


Figure 3. Experimental Groups and Design. Tanks were randomly assigned into three groups: (1) control group (12H light: 12H dark), (2) long-day (LD, 16H light: 8H dark), and (3) short day (SD, 8H light: 16H dark), based on treatments used by Abdollahpour et al. (2020). For each experimental group and the control, there were six tanks containing four fish each. The fishes were kept under their respective photoperiod for 28 days. On day 14, four fish from each of three replicate tanks per treatment were euthanized and sampled. This left three tanks remaining. On day 28, all remaining fish were euthanized and sampled.



Figure 4. Covering Tanks. Tanks were covered using electrical tape and trash bags. The electrical tape covered the backs of the tanks to prevent exposing fish to light. For the first 14 days, trash bags were removed from all tanks at 7:15 am. The trash bags were replaced at 3:15pm for the short-day group and 7:15pm for the control group. The lights in the lab automatically turned off at 11:15pm. For the last 14 days, time change had to be considered. For the last two weeks, trash bags were removed from the tanks at 6:15 am. The trash bags were replaced at 2:15pm for the short-day group and 6:15pm for the control group. The lights in the lab turned off at 10:15pm.

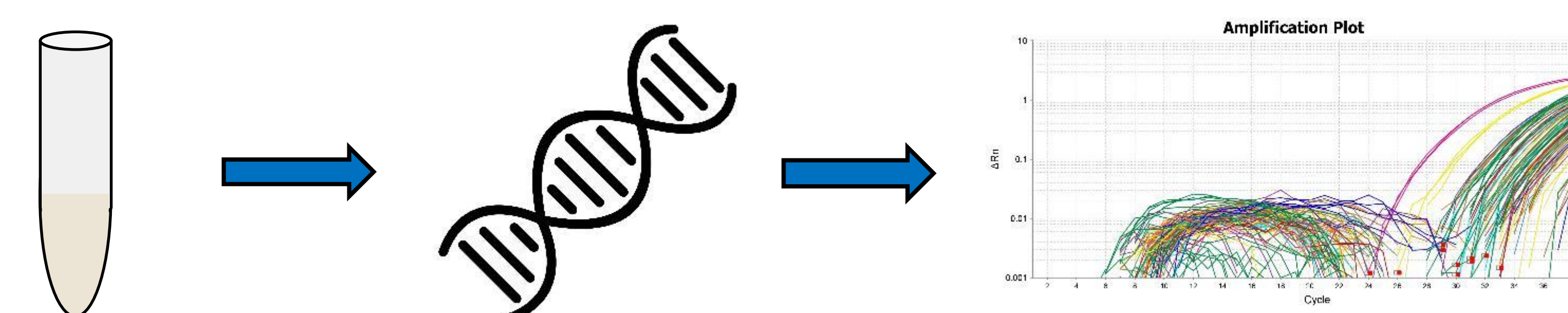


Figure 5. Methods. RNA was extracted using the Zymo Research Direct-zol RNA miniprep kit, following manufacturers instructions. RNA concentrations were measured using the Nanodrop and ranged from 11.4 ng/ μ L - 86ng/ μ L. The Maxima H Minus cDNA synthesis master mix with dsDNase was used to synthesize cDNA, following manufacturer instruction. Primers for FSH β , LH β , and the housekeeping gene EF1 α from Chen and Ge (2012) were tested. Then, qPCR was done on samples, and relative gene expression was analyzed using a two-way ANOVA and subsequent T-tests when $p \leq 0.05$. The Bonferroni correction was made for groups that required post-HOC T-tests ($p \leq 0.016$).

RESULTS AND CONCLUSIONS

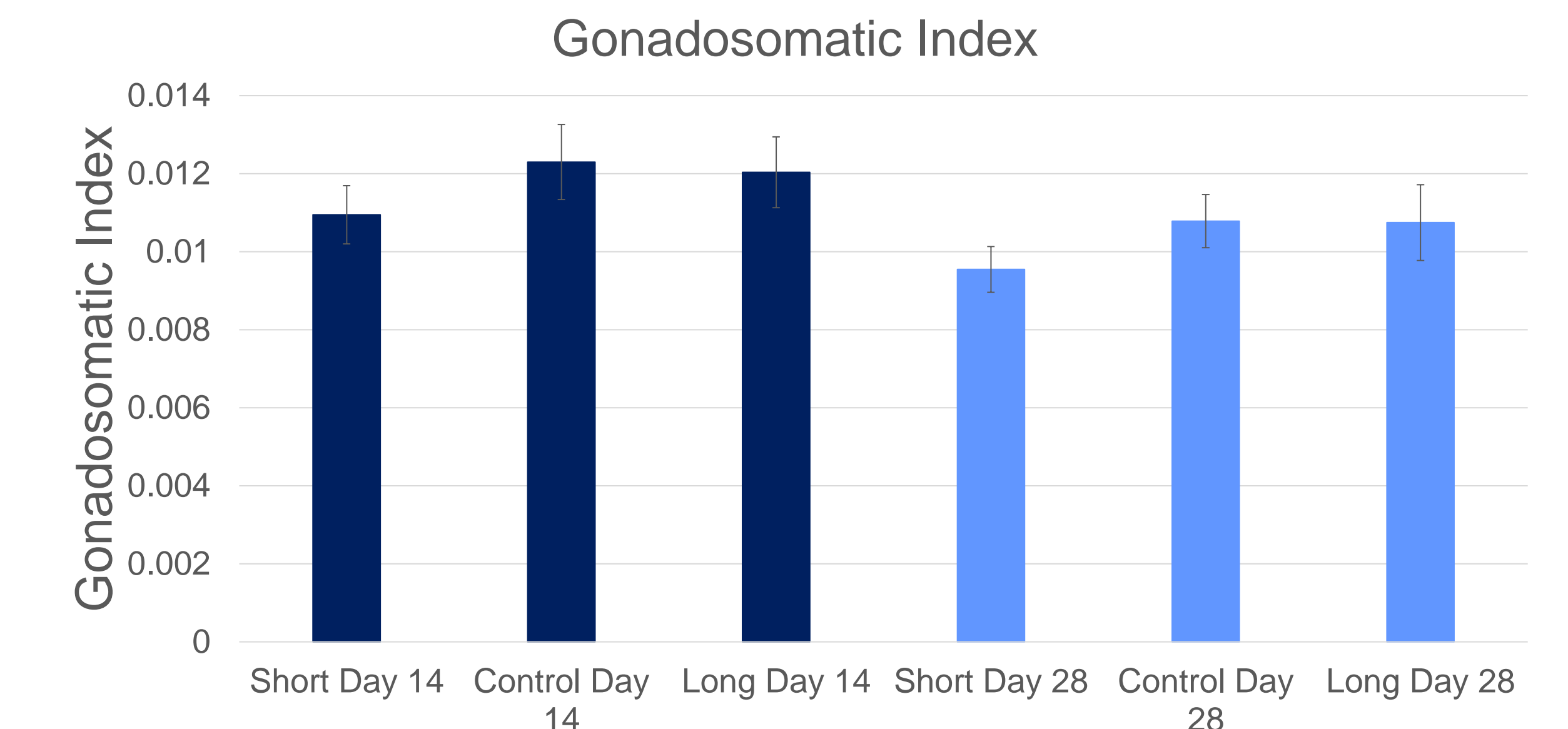


Figure 5. Gonadosomatic Index. Gonadosomatic index was calculated by dividing gonad weight by whole-body weight. There were no significant differences in gonadosomatic index between groups.

Figure 6. The Effect of Photoperiod on Relative Expression of FSH. Mean (\pm SE) fold change values were normalized to the housekeeping gene EF1 α . Mean transcript levels were significantly different between the 14-day control group and both the short-day and long-day groups at day 14. SD = short day, LD = long day. Asterisks signify $p \leq 0.0166$.

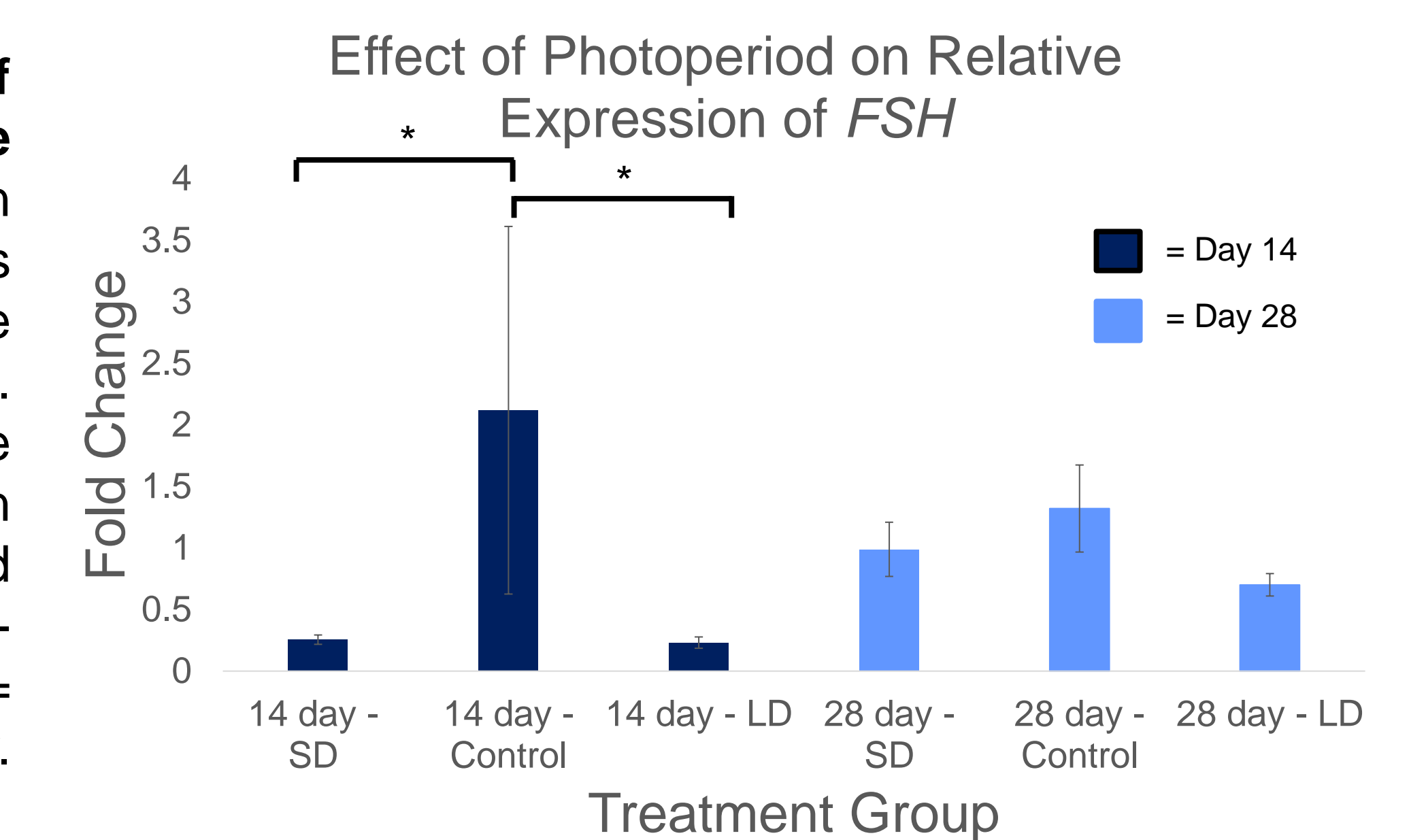
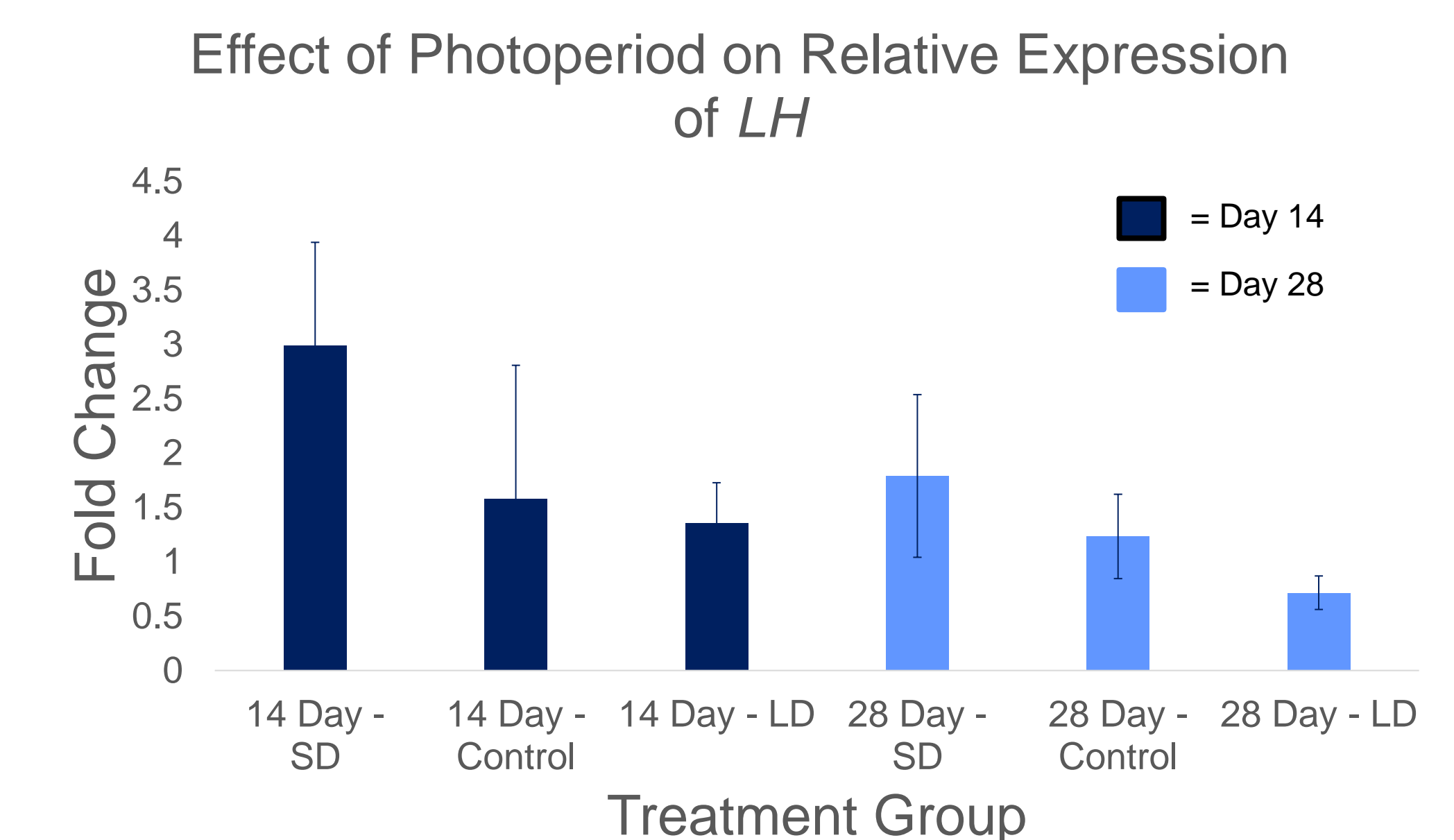


Figure 7. The Effect of Photoperiod on Relative Expression of LH. Mean (\pm SE) fold change values were normalized to the housekeeping gene EF1 α . Mean transcript levels were not significantly different. SD = short day, LD = long day.



RESULTS AND FUTURE DIRECTIONS

- There were no significant differences in gonadosomatic index between groups
- qPCR results show there were no significant differences in relative expression of LH between groups
- qPCR results showed a significant difference in relative expression of FSH between the control and short-day group, and the control and long-day group
- These results indicate photoperiod may affect FSH expression
- In the future, I hope to conduct more trials using more fish/tanks
- This would allow for a larger sample size

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