

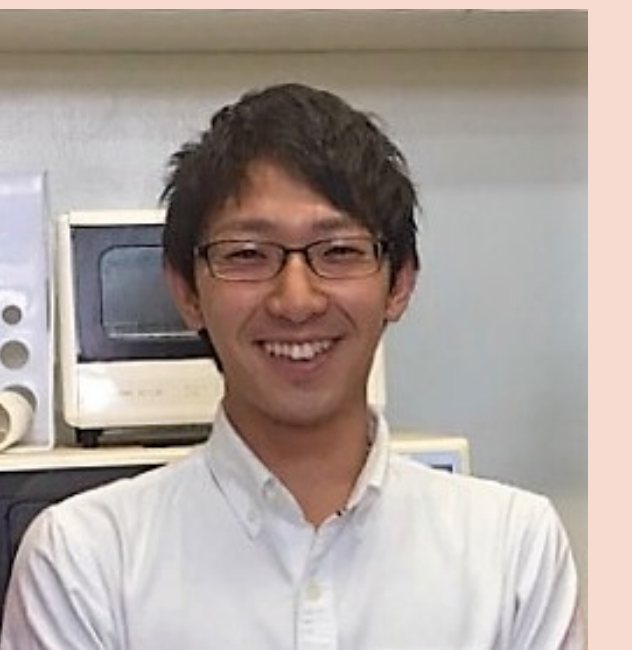


# The lunar phase-dependent expression change of clock genes and sex hormone genes in the honeycomb grouper

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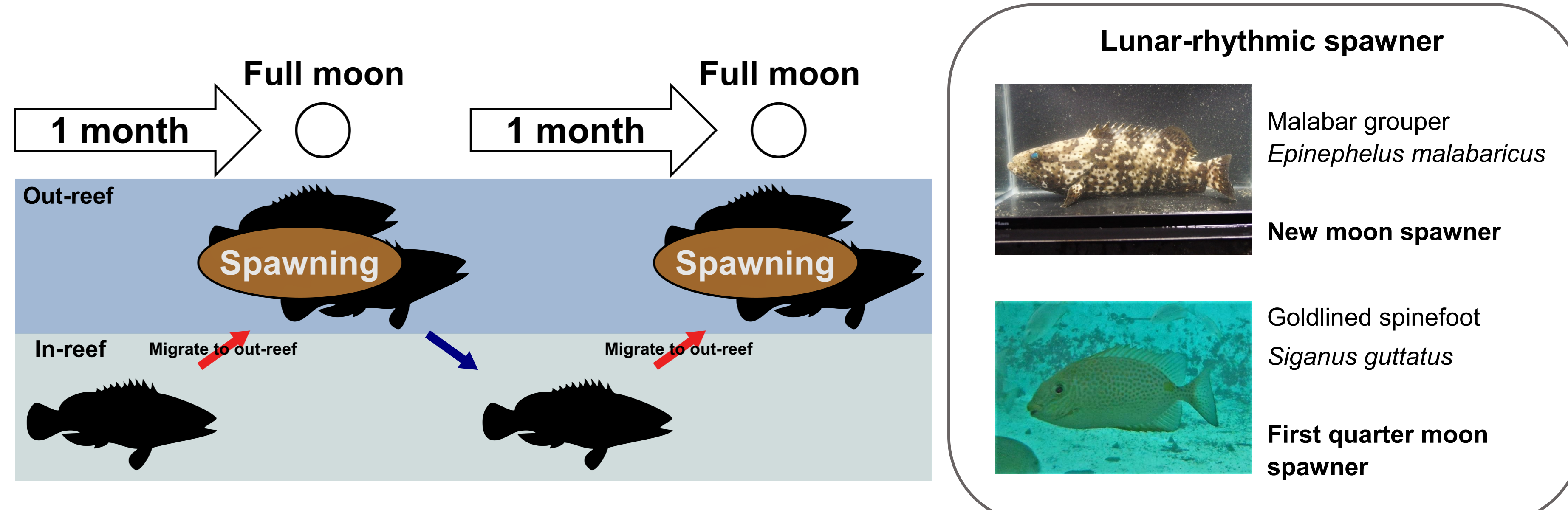


## INTRODUCTION

Groupers (Serranidae) display lunar periodic rhythms in reproductive events called **lunar-rhythmic spawning**.

### What is lunar-rhythmic spawning ?

It is a repetition of the synchronous gonadal development and spawning at a one-month interval around the specific lunar phase.



These organisms are supposed to perceive cues from the moon and convert them into endogenous signals. **However, studies on endogenous system generating lunar-rhythmicity are still lacking.**

## EXPERIMENT 1

◐; first quarter moon (FQM), ◯; full moon (FM), ◑; last quarter moon (LQM), ◐; new moon (NM)

- **Gonadosomatic index (GSI)** : GSI increased toward FM in June (Fig. 1).
- **Gene expression (*fshβ* and *lhβ*)** : Transcript levels of *fshβ* and *lhβ* in the pituitary increased around FQM in June. Abundance of *fshβ* decreased around LQM in June (Fig. 2).
- **Gene expression (*cry2*)**: Abundance of *cry2* in the diencephalon around LQM and NM was higher than that around last FM. Its abundance in the pituitary peaked around NM (Fig. 3).

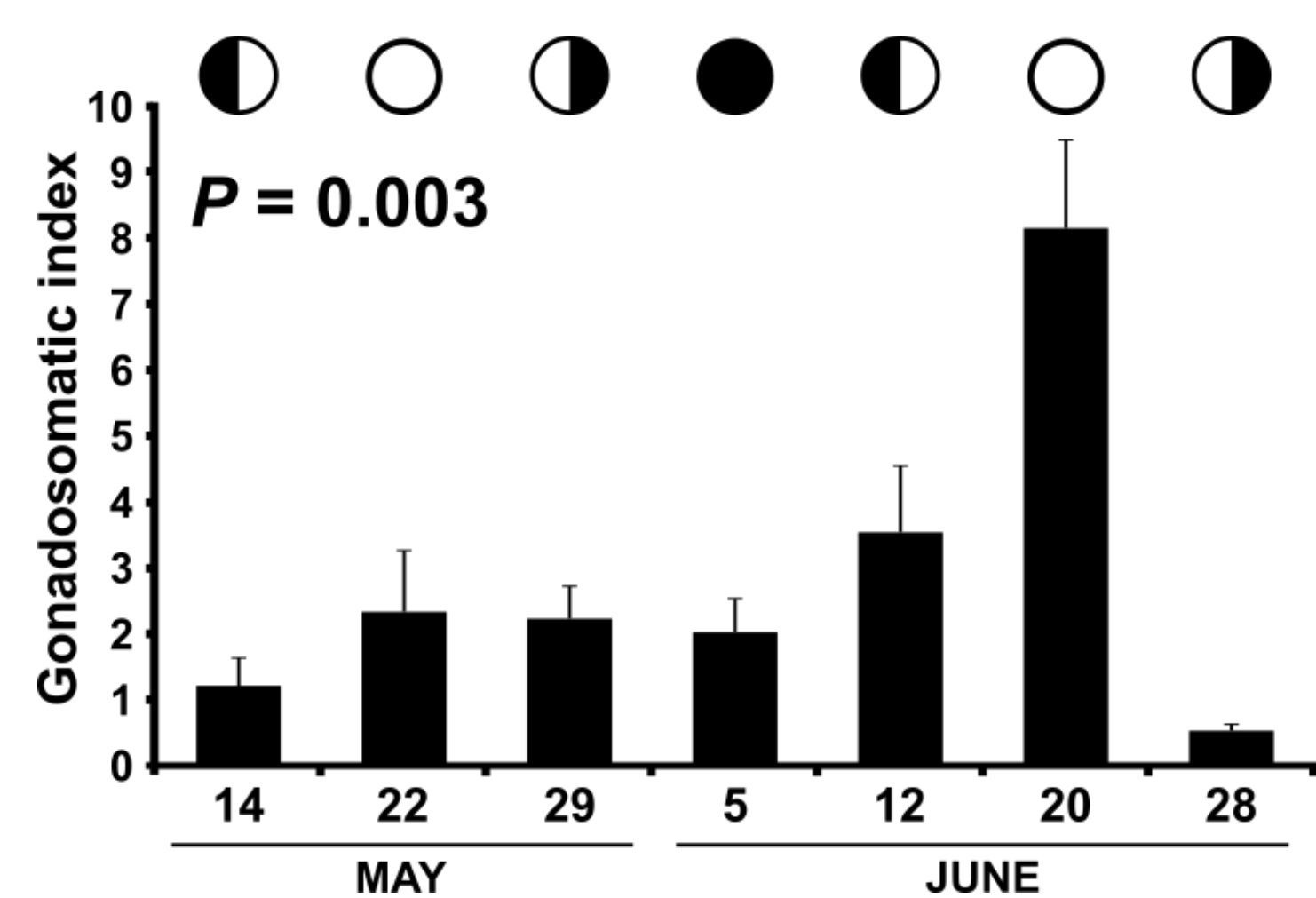


Fig. 1. Weekly changes in GSI in May to June, 2016.

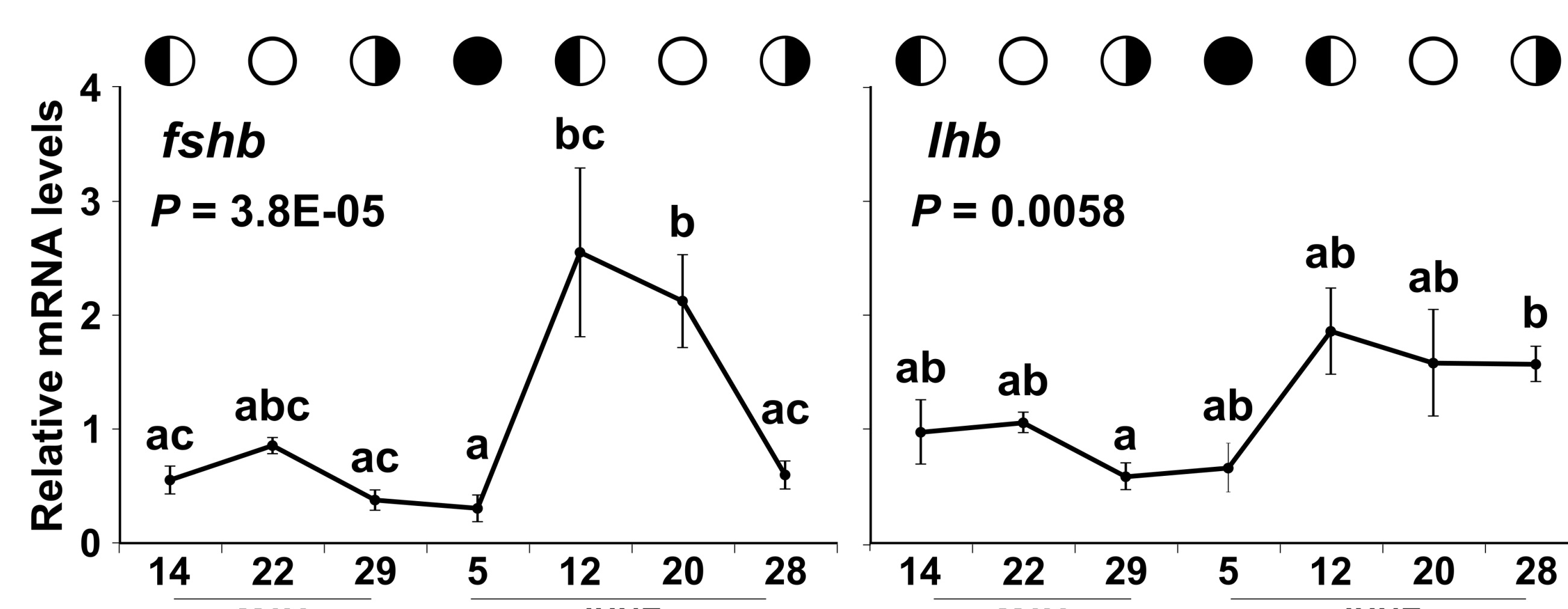


Fig. 2. Weekly changes in relative mRNA levels of *fshβ* and *lhβ* at 00:00.

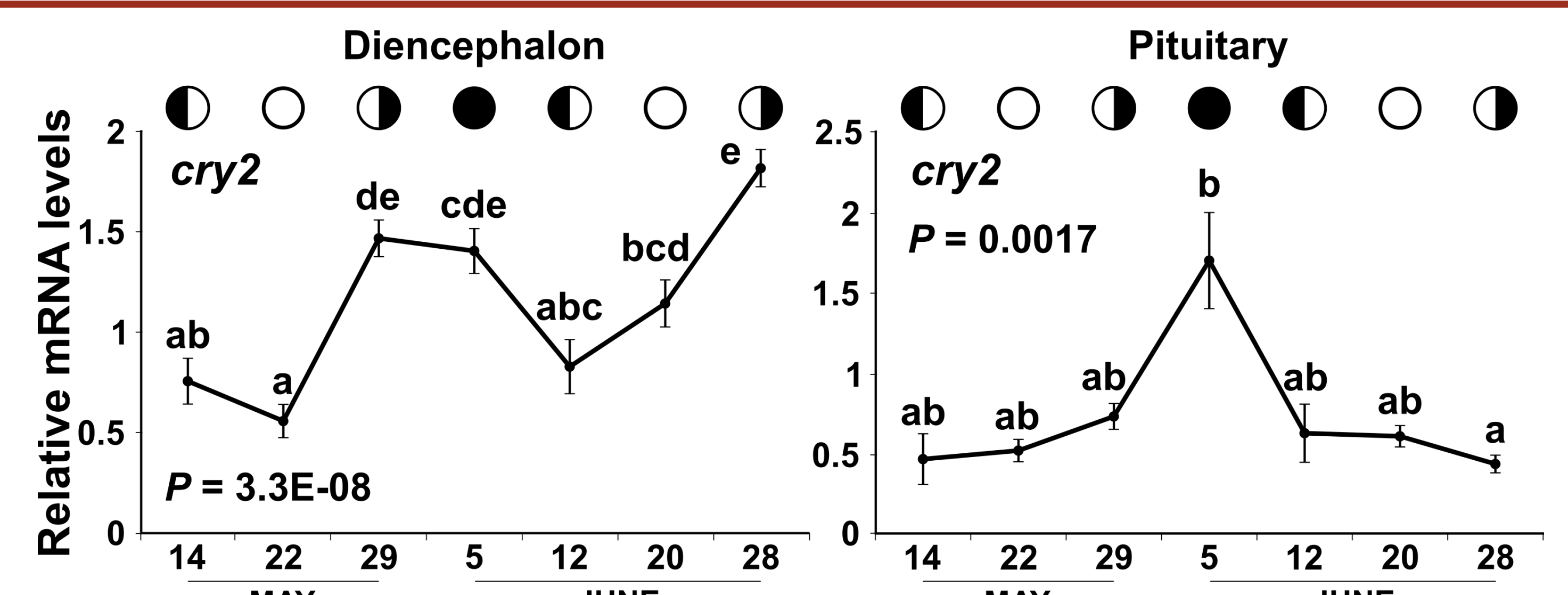


Fig. 3. Weekly changes in relative mRNA levels of *cry2* at 00:00.

## EXPERIMENT 2

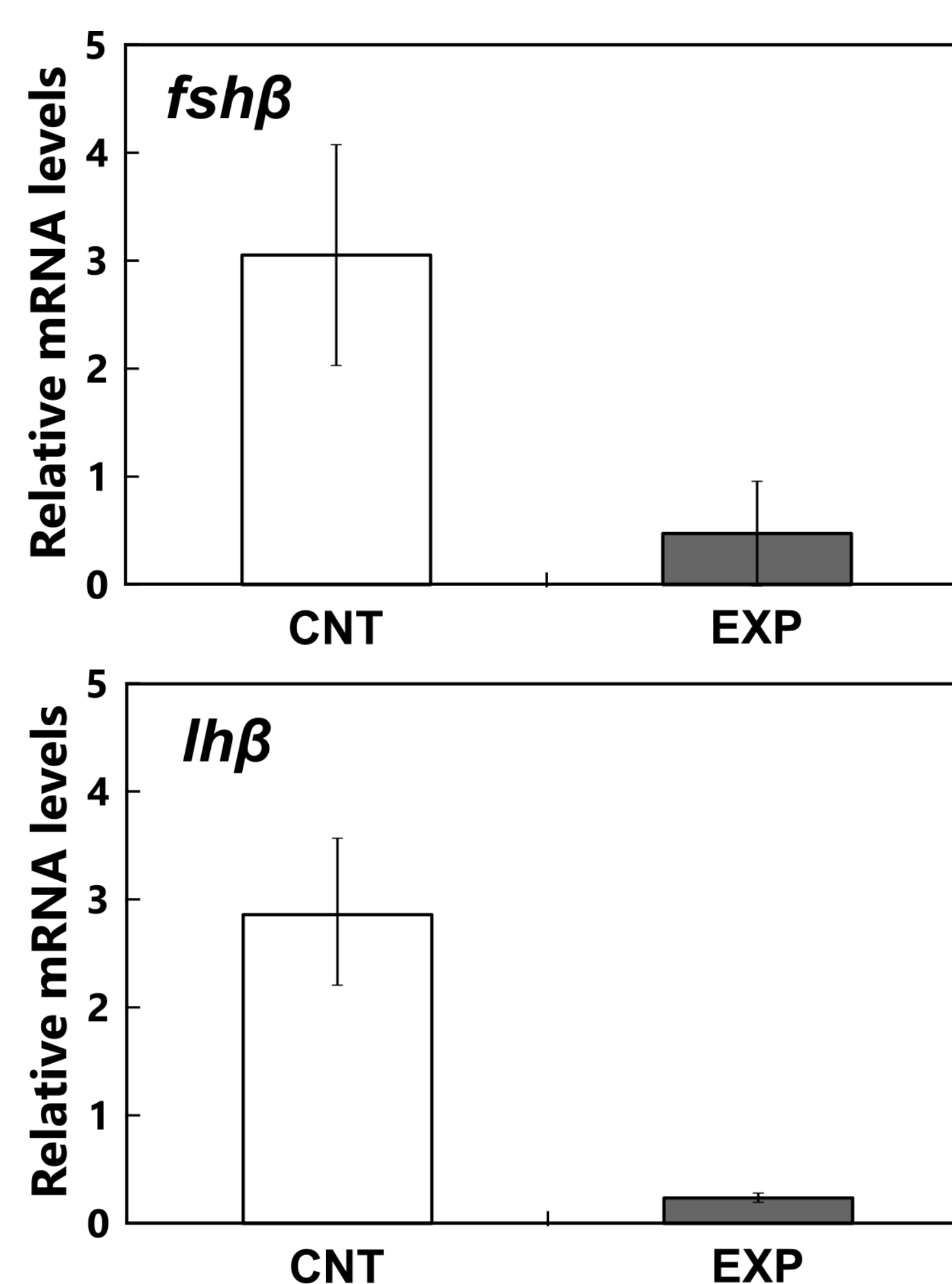


Fig. 4. The effect of melatonin implantation on the *fshβ* and *lhβ* expression at 12:00 in the FM in June, 2017.

Melatonin implantation lowered abundance of *fshβ* and *lhβ* in the pituitary (Fig. 4), but not that of *cry2* in the diencephalon (Fig.5).

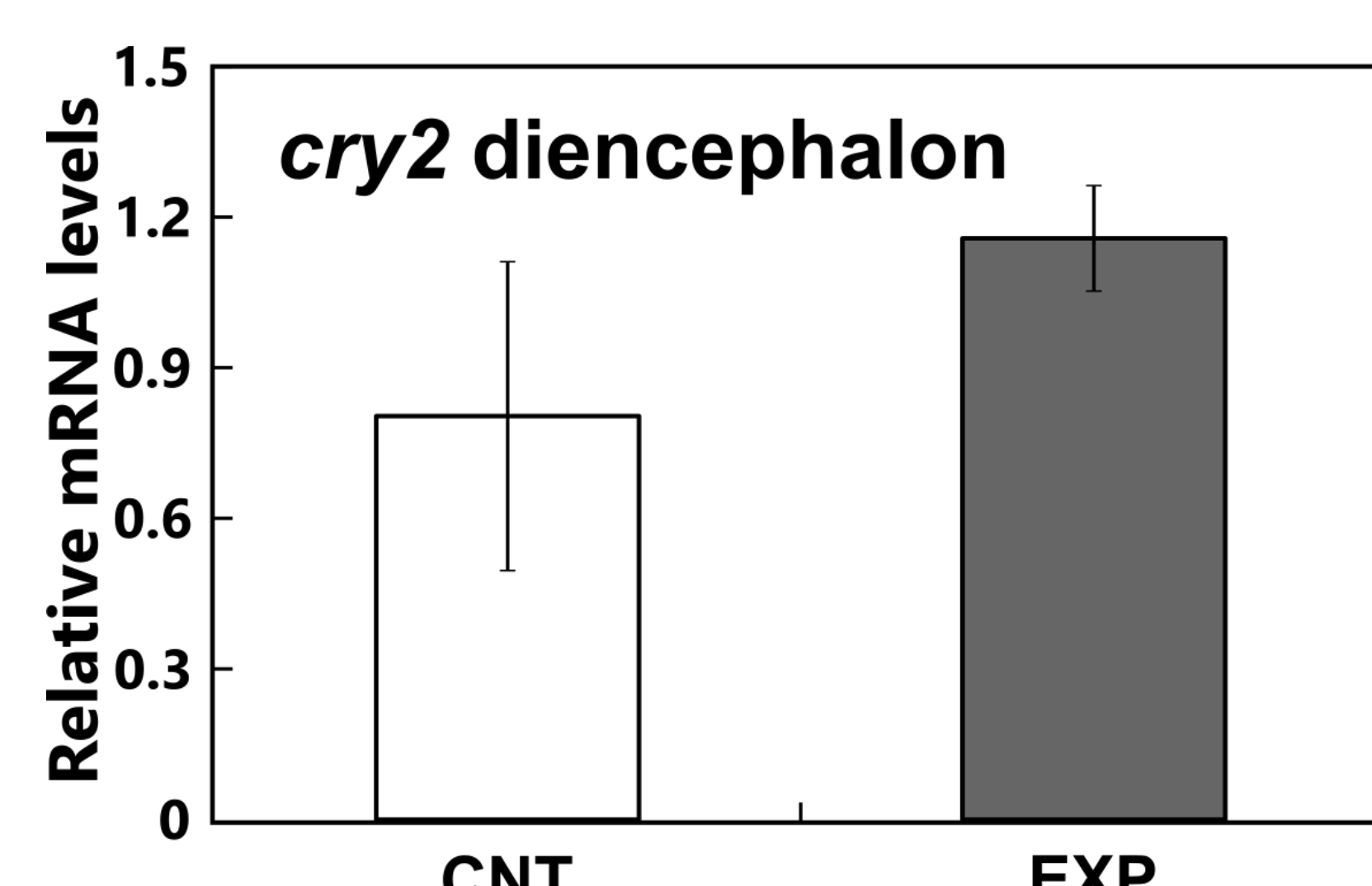


Fig. 5. The effect of melatonin implantation on the *cry2* expression in the diencephalon at 12:00 in the FM in June, 2017.

## AIM OF THIS STUDY

The aim of this study is to investigate **gene expression patterns of gonadotropin (*fshβ* and *lhβ*), and cryptochrome (*cry*) in the brain** of the honeycomb grouper *Epinephelus merra* during spawning season. We also checked **the effect of melatonin** on gonad development and these gene expressions.

## MATERIALS & METHODS

### Experiment 1



Honeycomb grouper *E. merra*

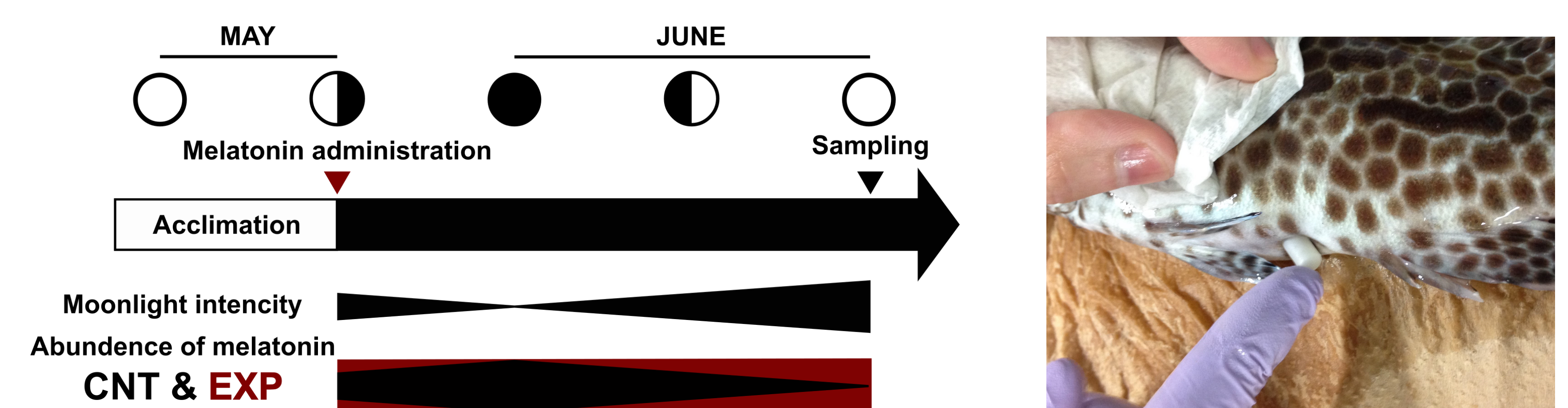
The collections were performed by hook and line. Captured fish were reared in outdoor tanks at Sesoko Station for 1-2 days.



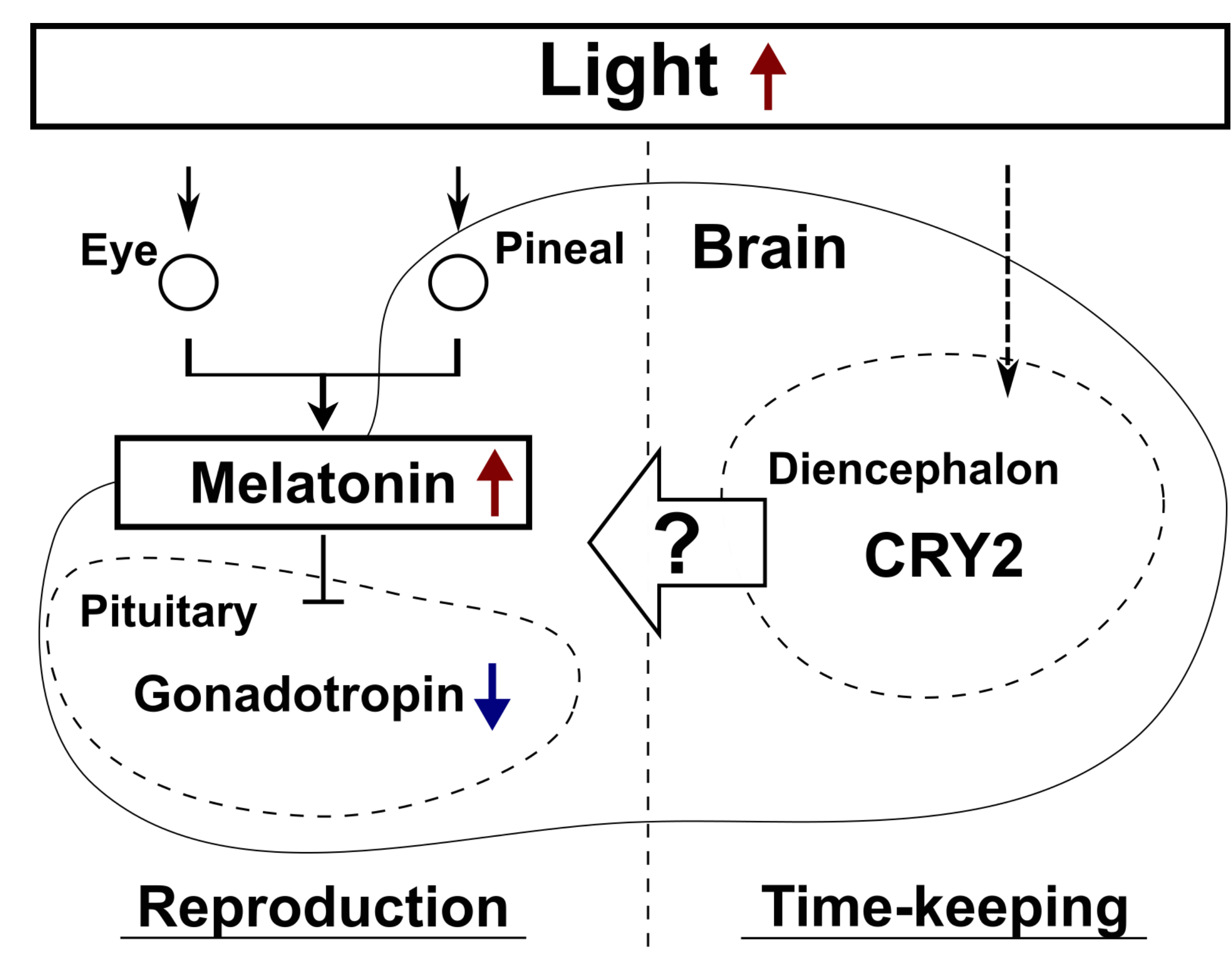
Fish were weekly collected from fringing reefs around the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan.

### Experiment 2

We investigated **the effect of melatonin administration on *fshβ*, *lhβ* and *cry2* genes**. Fish implanted osmotic pump (EXP) were kept for 3 weeks from LQM to FM. Sham operation was performed for CNT fish. They were sampled at 12:00.



## CONCLUSION & DISCUSSION



These results suggested that **“brightness of night”** suppresses *fshβ* and *lhβ* expressions through melatonin action. The transcriptional regulation of *cry2* might be involved in **time-keeping** at a one-month interval at the diencephalon upper melatonin.