

# Lunar-synchronized spawning in fish and moonlight

## ~Chronobiology in a moonlit night~

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### ◆ Background

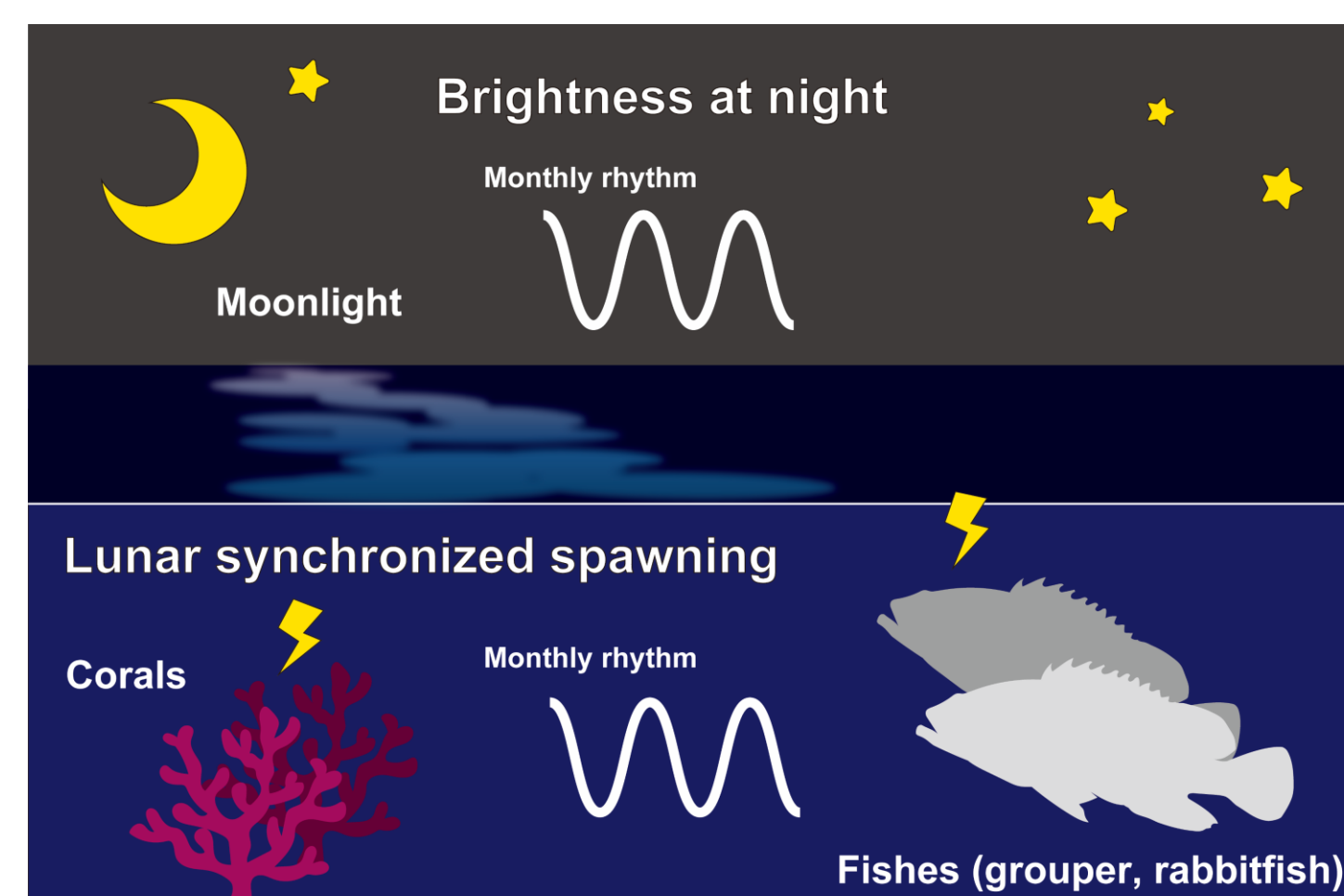


#### • *Cryptochrome*

In the fish, *cryptochrome* (*cry*), one of the clock genes, is possibly related to time-keeping for the spawning due to showing the monthly variation in the gene expression (Fukushiro et al., 2011; Fukunaga et al., 2020).

#### • Brightness at night

The transition of the moon phase makes the brightness at night variable. It has been demonstrated that lunar-synchronized spawning is known to be controlled by the variation in brightness at night (Takemura et al., 2009).



? The effect of brightness at night on the physiological process of spawning is unclear. ?

#### The aim of this study

- To reveal the effect of brightness at night on the spawning, investigated released egg amounts ... (a) and ovarian statement ... (b).
- To reveal the effect of brightness at night on the clock gene (*cry*), investigated these monthly patterns ... (c).

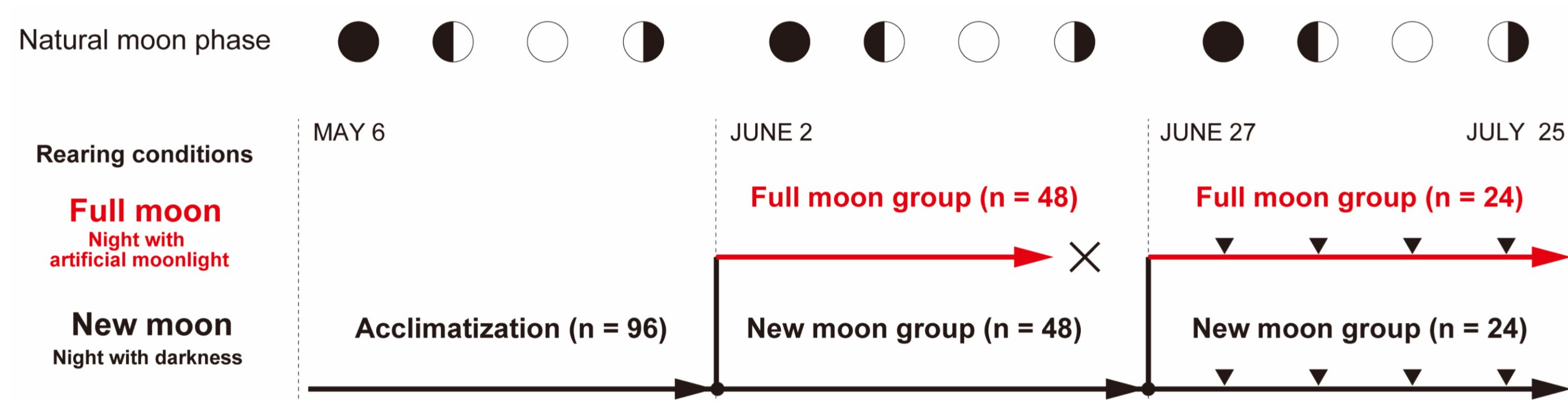
### ◆ Materials and methods

#### • Experimental species: Honeycomb grouper *Epinephelus merra*



- Small species (up to 30 cm) that inhabit the shallow reefs of tropical water
- A lunar synchronizer with a full-moon preference (Lee et al., 2002)
- The fish were collected around Sesoko Island, Okinawa, Japan, in April 2019.

#### • Rearing experiment (May 6 – July 25, 2019)



#### a. Monitoring released egg (3 months, May 6 – July 25)

Egg release was monitored weekly. Nylon filters (mesh size 500  $\mu$ m) were set at the drain of each tank to trap released eggs.

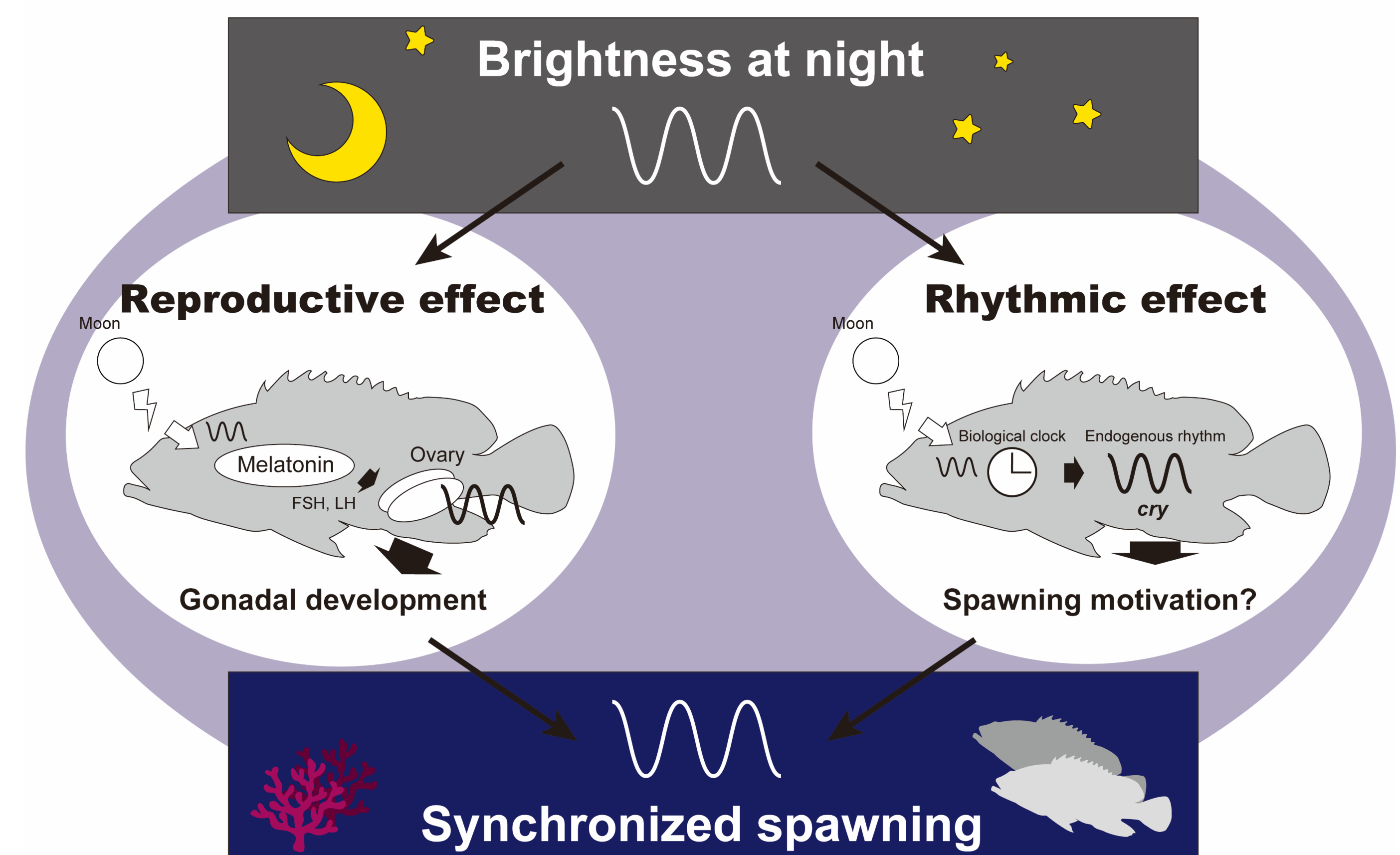
#### b. Ovarian observation (1 month, June 27 – July 25)

Small pieces of the ovary were fixed in Bouin's solution and histologically observed. The gonadosomatic index (GSI) was calculated as (ovarian mass/body mass)  $\times$  100.

#### c. Gene expression analysis (1 month, June 27 – July 25)

Total RNA extracted from the diencephalon and pituitary was applied to gene expression analysis for *cry1*, *cry2*, and *cry3* by qPCR.

### ◆ Discussion



### ◆ Results

#### • The effect of brightness at night on the spawning

Table 1. The presence of spawning through the experiment.

Periods	Rearing conditions	1	2	3	4
May 6 – June 1	Acclimatization	N.D.	-	-	+
June 2 – June 26	Full moon	+	+	+++	++
	New moon	+	+	+	+
June 27 – July 25	Full moon	-	-	+++	-
	New moon	-	-	+	-

- More eggs were laid in the full moon group.

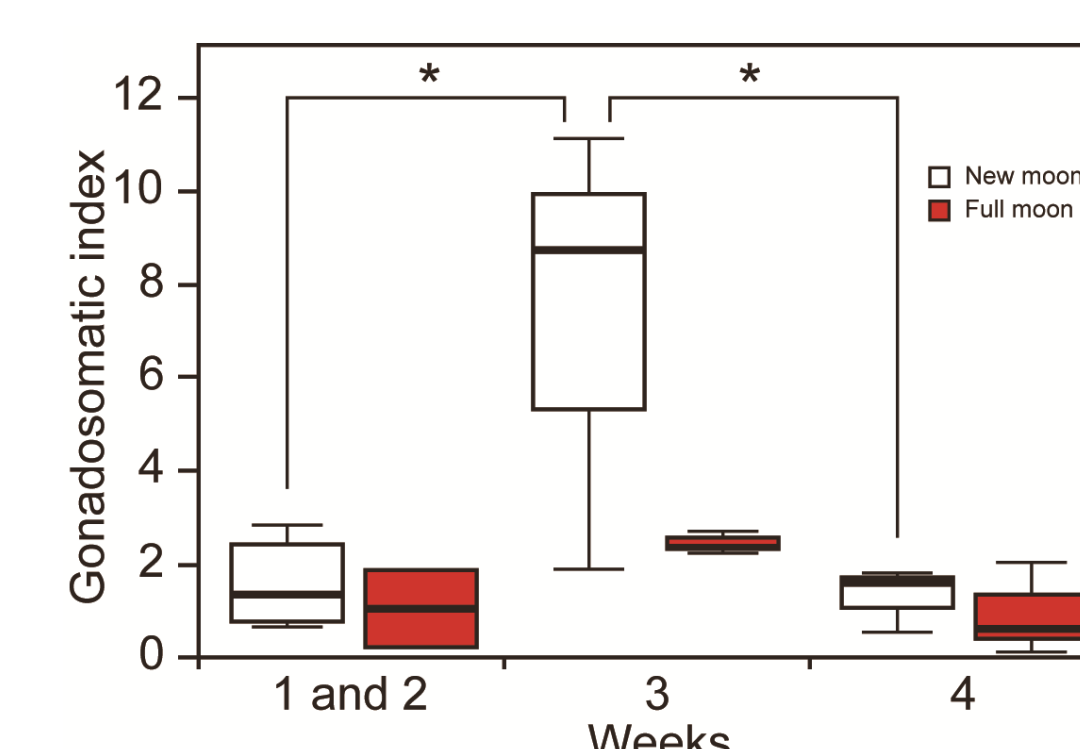


Fig. 1. The effect of artificial moonlight on the gonadal development.

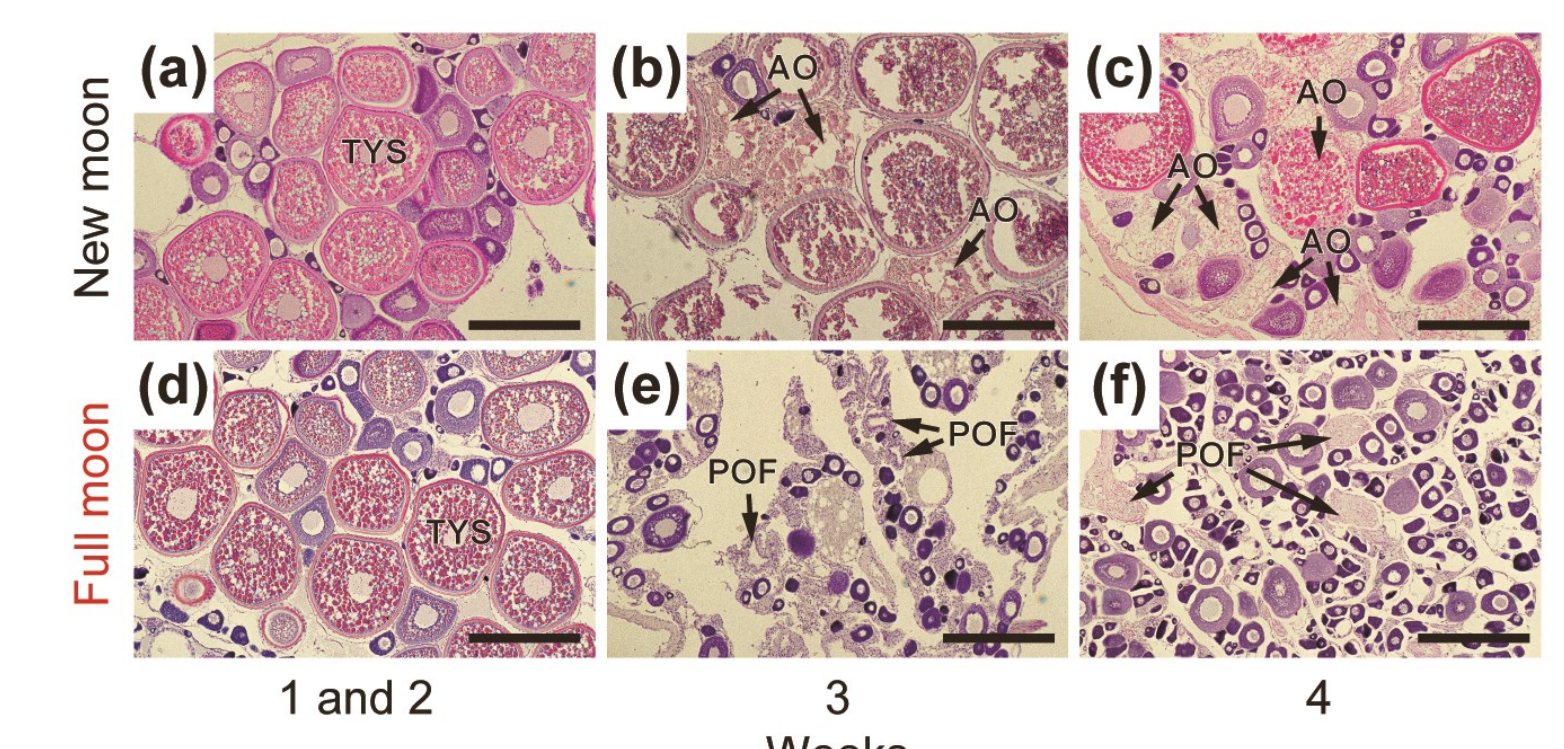


Fig. 2. The effect of artificial moonlight on the oocyte stage composition.

- Histological observation revealed the existence of atretic oocytes (AO) and post-ovulatory follicles (POF) in the ovary of the full and new moon groups, respectively.

→ The brightness at night could induce oocyte maturation.

#### • The effect of brightness at night on the clock genes (*cry*)

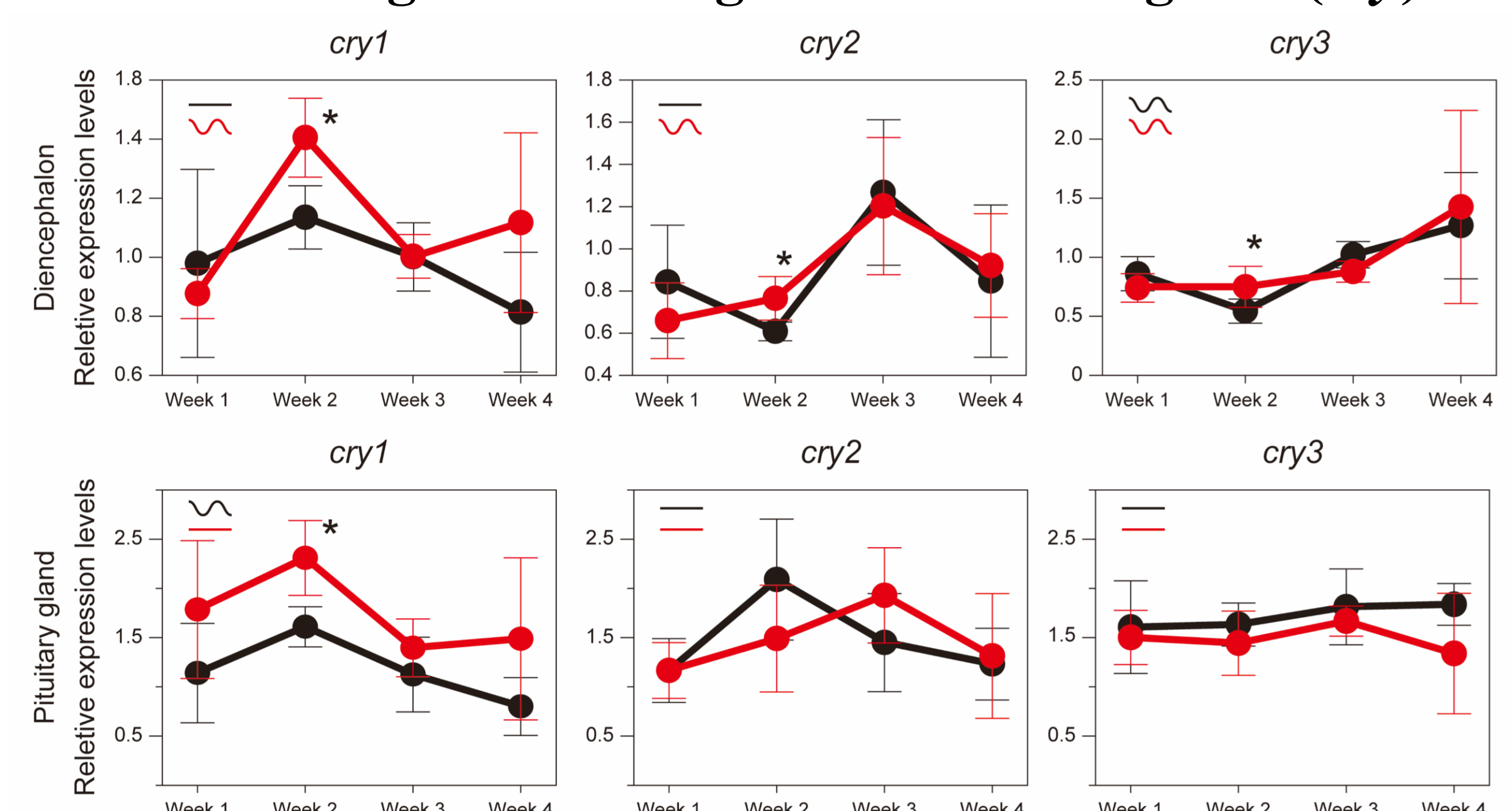


Fig. 3. The effect of artificial moonlight on the monthly patterns of *crys* expression in the diencephalon (upper) and pituitary gland (bottom).

- The monthly expression pattern of *crys* was altered by the artificial moonlight.
- In the diencephalon, the artificial moonlight seemed to help to generate the monthly rhythmicity of *cry* expression.

→ The brightness at night modified the monthly expression pattern of *crys* in the brain.