

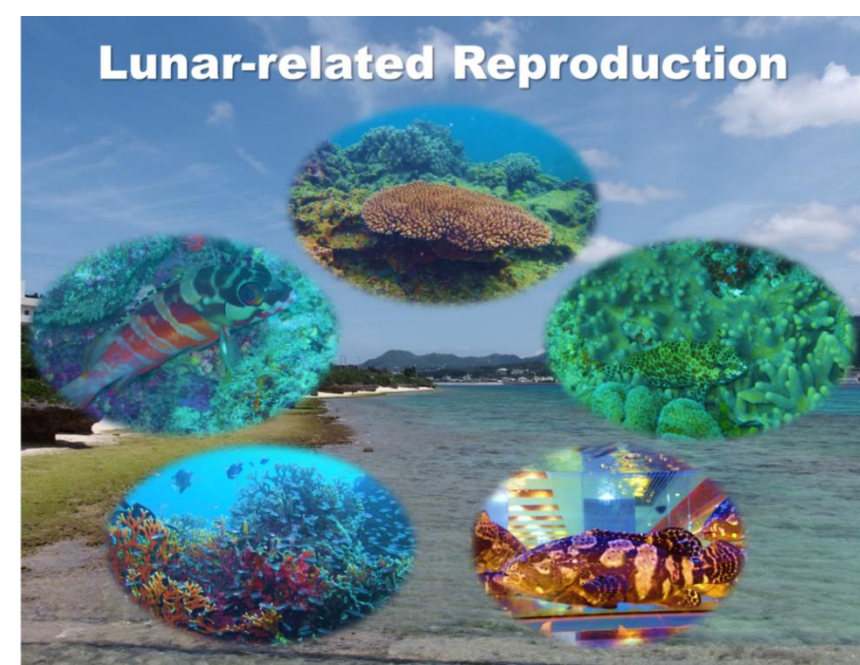


# Sequence and expression of clock genes in the brain of a full moon spawner, *Epinephelus merra*

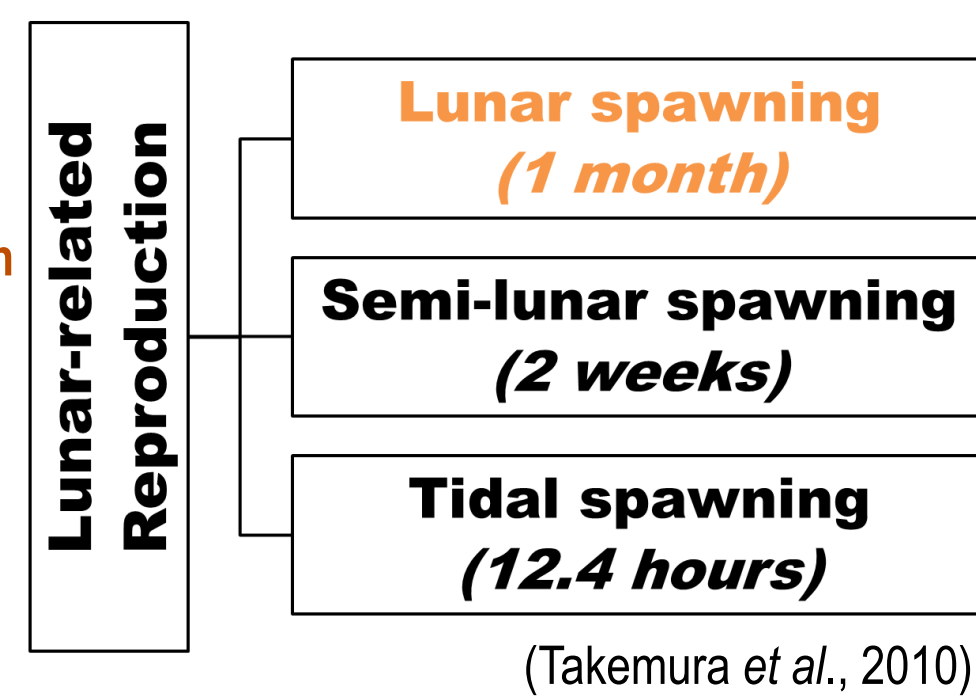
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## INTRODUCTION



Many tropic marine organisms show the lunar-related reproduction

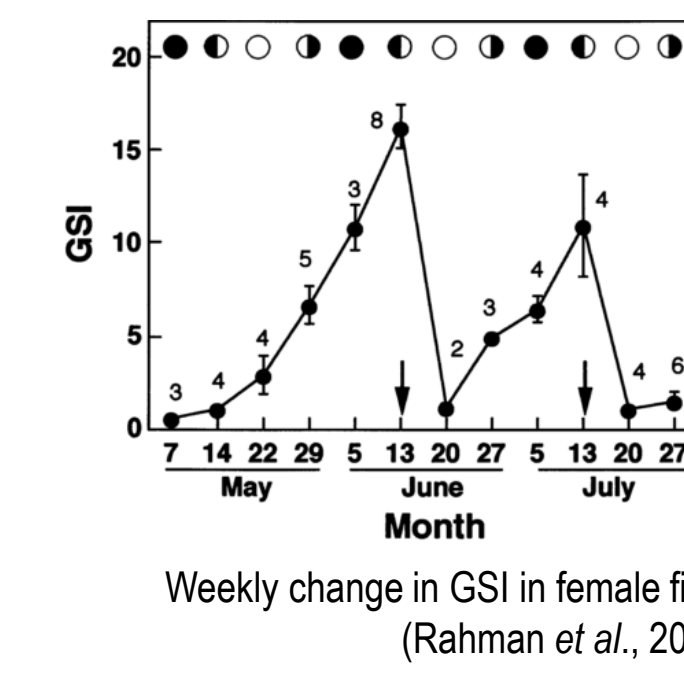


(Takemura et al., 2010)

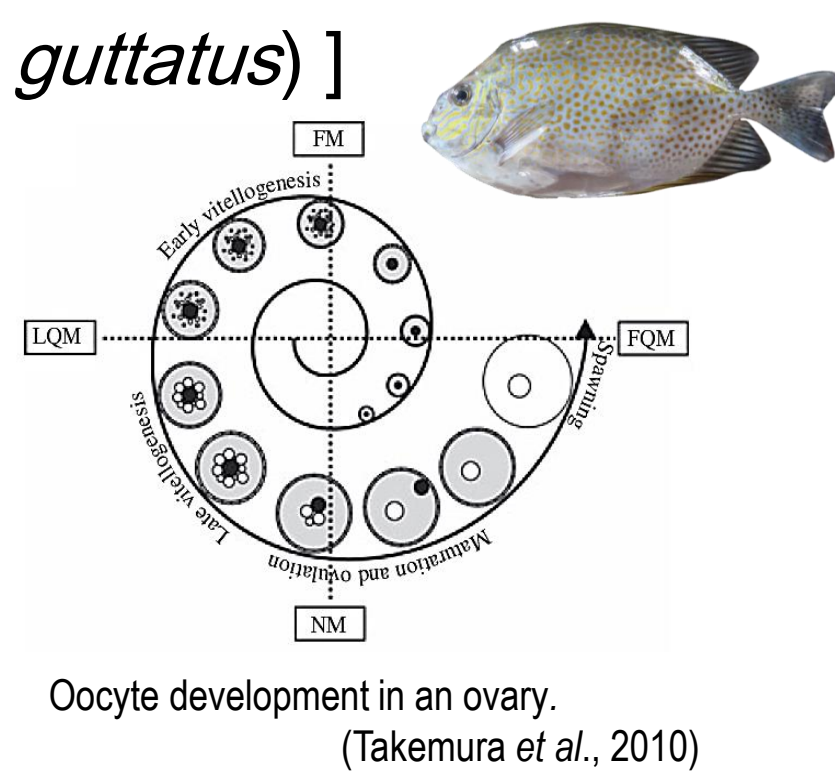
## Lunar spawning cycle

[ In goldlined spinefoot (*Siganus guttatus*) ]

"The lunar spawning cycle is a repetition of the synchronous gonadal development and spawning at 1 month intervals around a specific lunar phase."



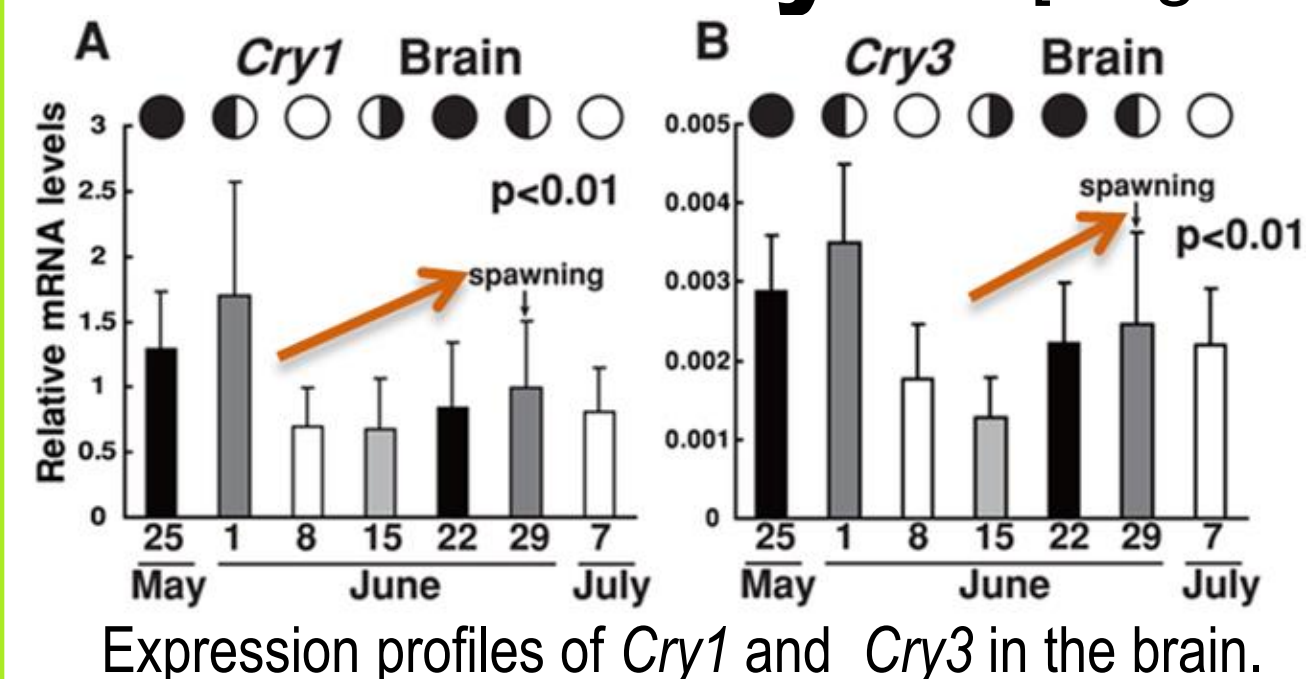
Weekly change in GSI in female fish. (Rahman et al., 2000)



Oocyte development in an ovary. (Takemura et al., 2010)

## Previous study

[ In goldlined spinefoot (*Siganus guttatus*) ]



Abundance of *Cry1* and *Cry3* raised toward the first quarter moon, which is the spawning lunar phase of this species.

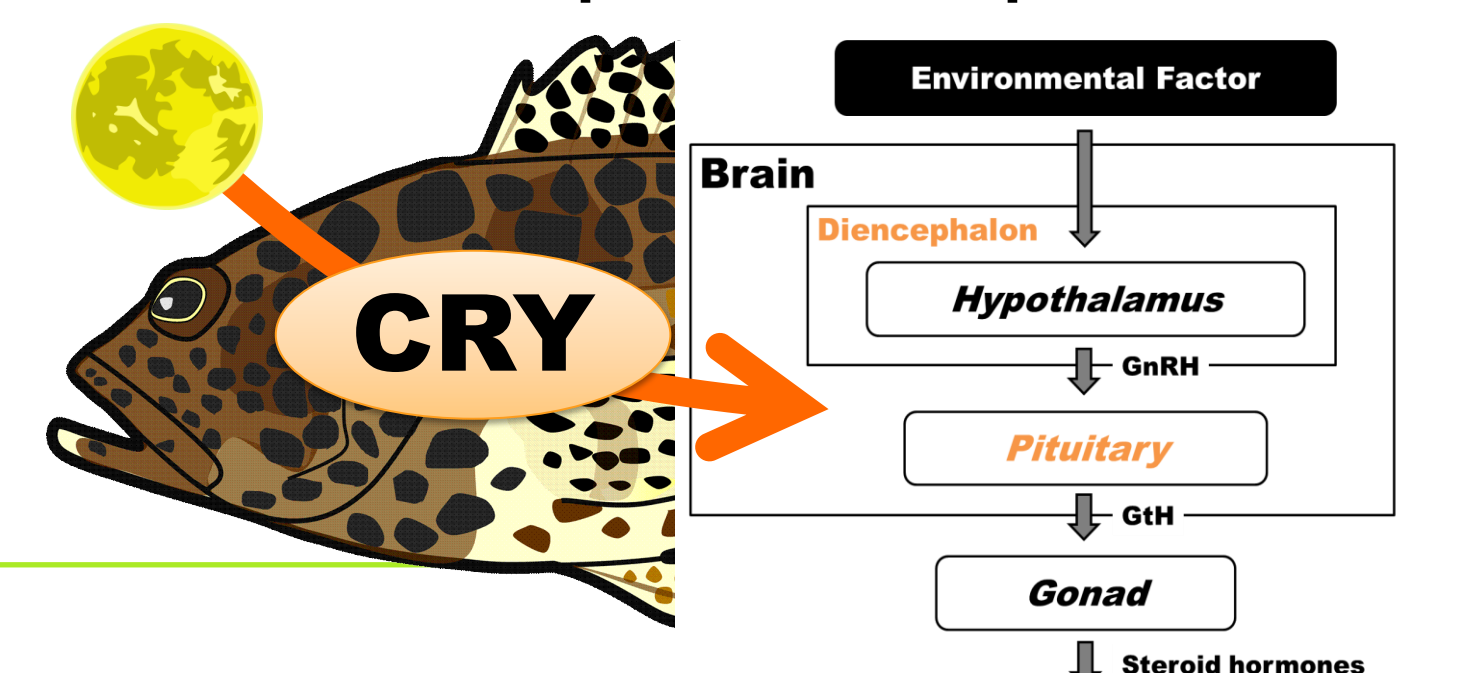
(Fukushiro et al., 2011)

## Hypothesis

CRY plays a role in entraining hormonal synthesis at the HPG axis to spawns lunar phase.

"It is considered that the higher parts of the HPG axis play important roles in the perception and regulation of lunar-related periodicity."

(Takemura et al., 2010)



## AIM

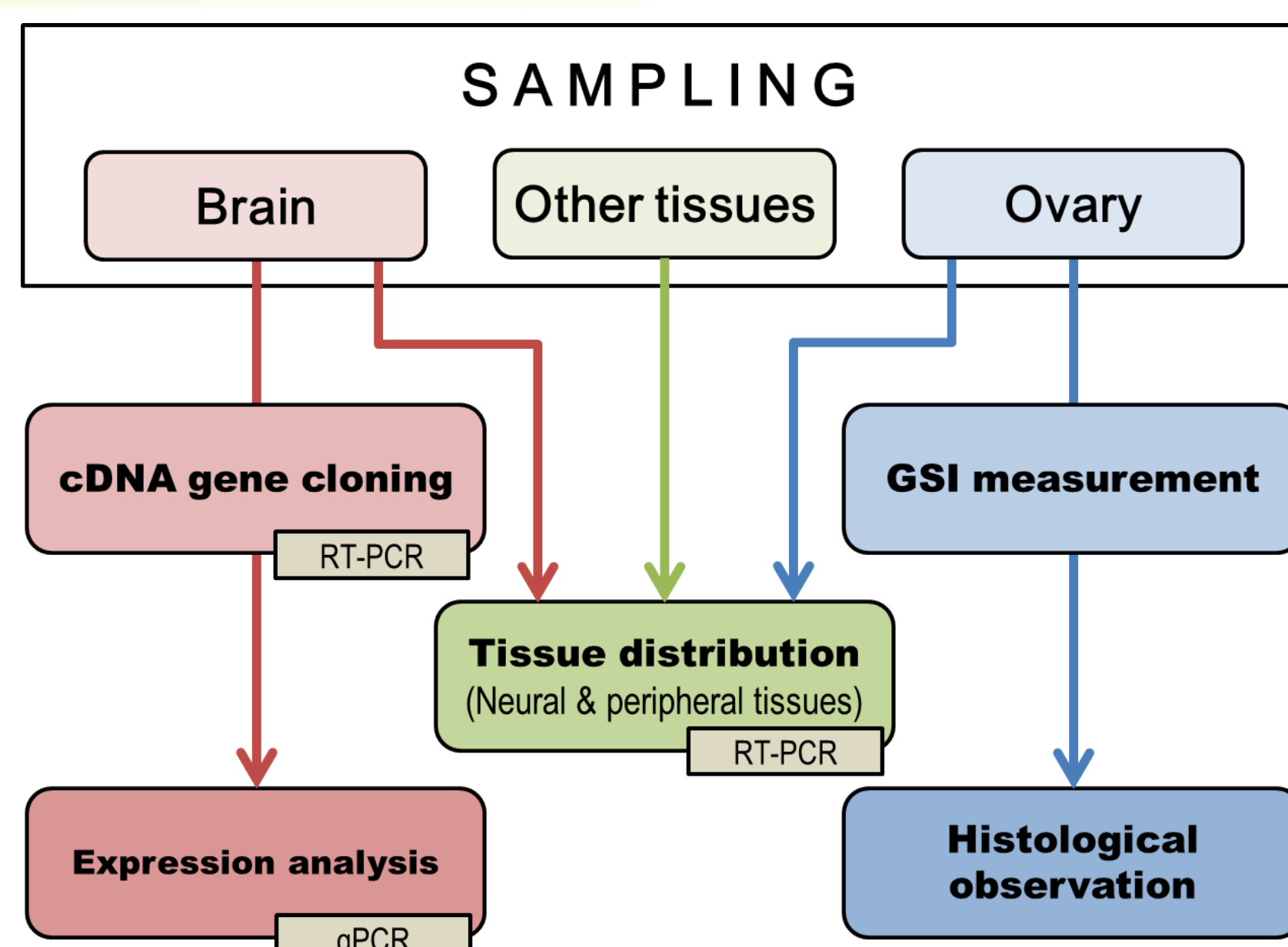
To clarify the expression pattern of *Cry* in the brain of honeycomb grouper (*Epinephelus merra*)

## MATERIALS & METHODS



### Honeycomb grouper (*E. merra*)

This species (upper 30 cm) inhabits shallow reefs in tropic and sub-tropic regions. It spawns around the **full moon** period in May and June in Okinawa, Japan.



- Gonadosomatic index (GSI):** GSI was measured around the periods of the new moon (NM), first quarter moon (FQM), full moon (FM) and last quarter moon (LQM) during the spawning season (Fig.1a). [GSI = (Gonad weight / Body weight) × 100]. Ovaries were sectioned at 5μm and stained with hematoxylin and eosin for histological observation (Fig. 1b).
- HgCry cloning:** Total RNA was extracted from the diencephalon (DI). *HgCry1*, *HgCry2*, and *HgCry3* were amplified using the primer sets that were designed based on some teleost fishes (Fig.2).
- Tissue distribution:** The abundance of *HgCrys* in neural and peripheral tissues was analyzed with reverse transcriptional PCR (RT-PCR) (Fig.3).
- Expression pattern:** Total RNA was extracted from DI and the pituitary (PT) during the spawning season in the periods of NM, FQM, FM, and LQM. Expression patterns of each gene were analyzed using quantitative real-time PCR (qPCR) (Fig.4).

## RESULT

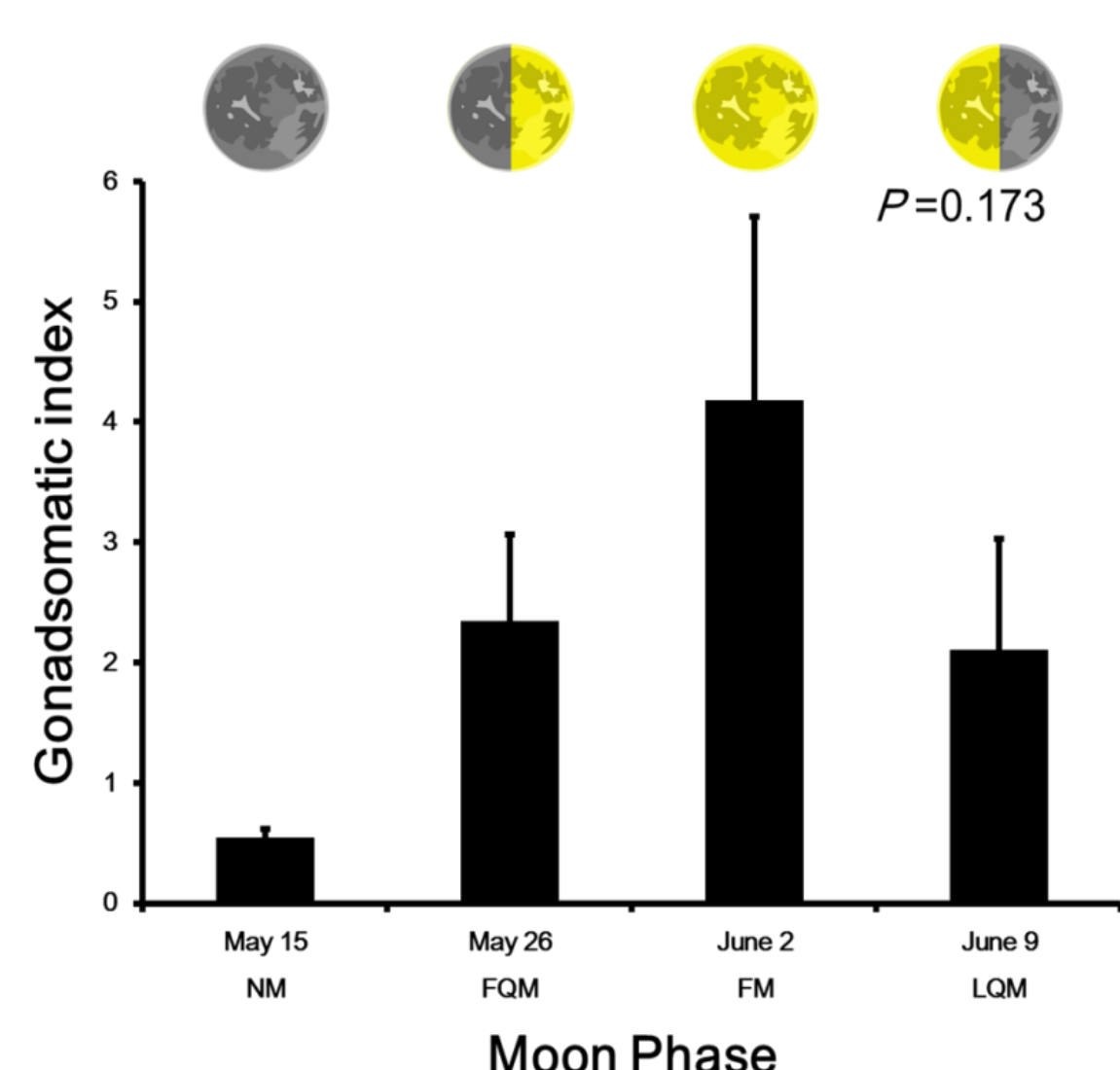


Fig. 1a. Weekly change in GSI.

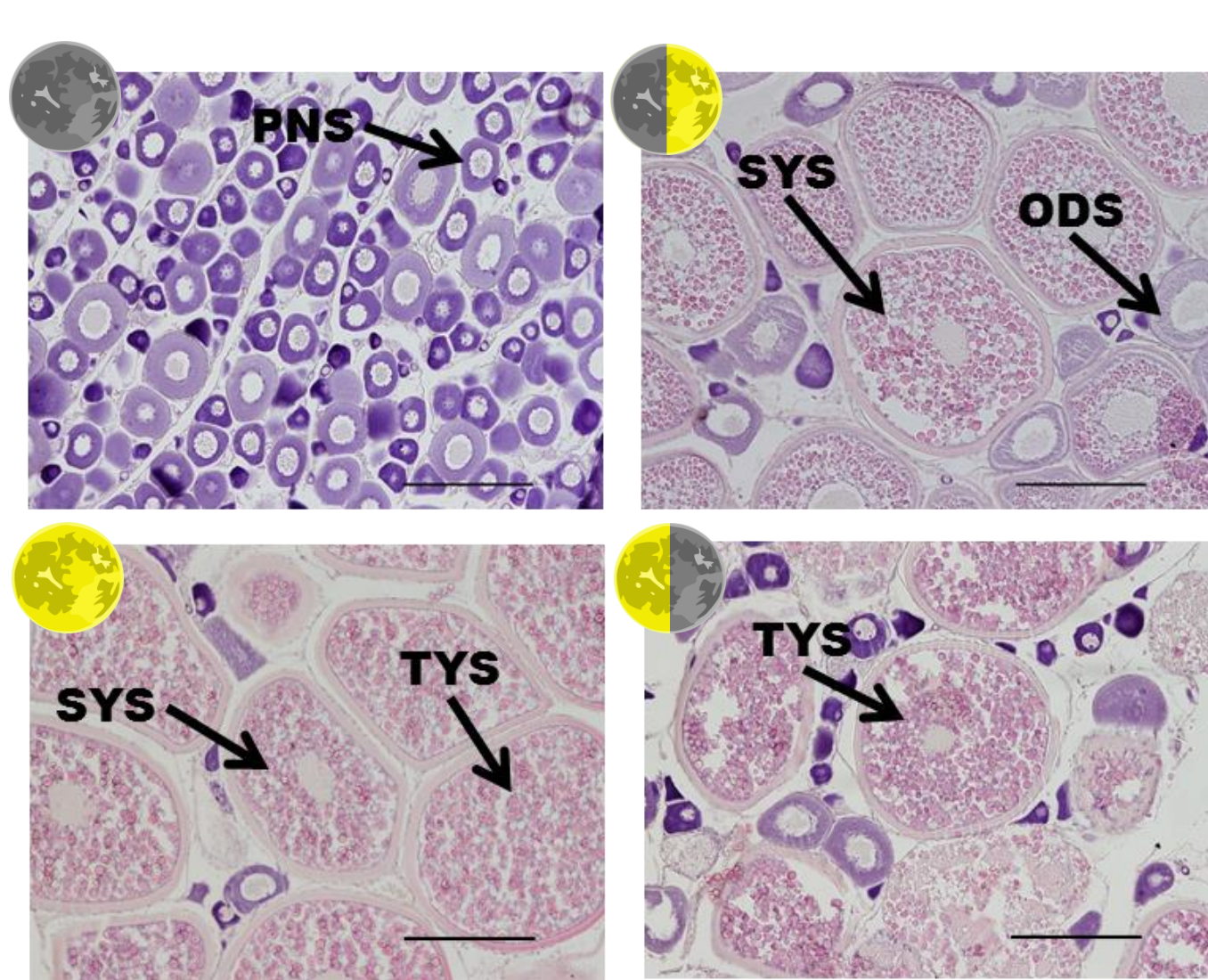


Fig. 1b. Weekly change in ovarian histology.

Ovarian activity increased around the full moon period.

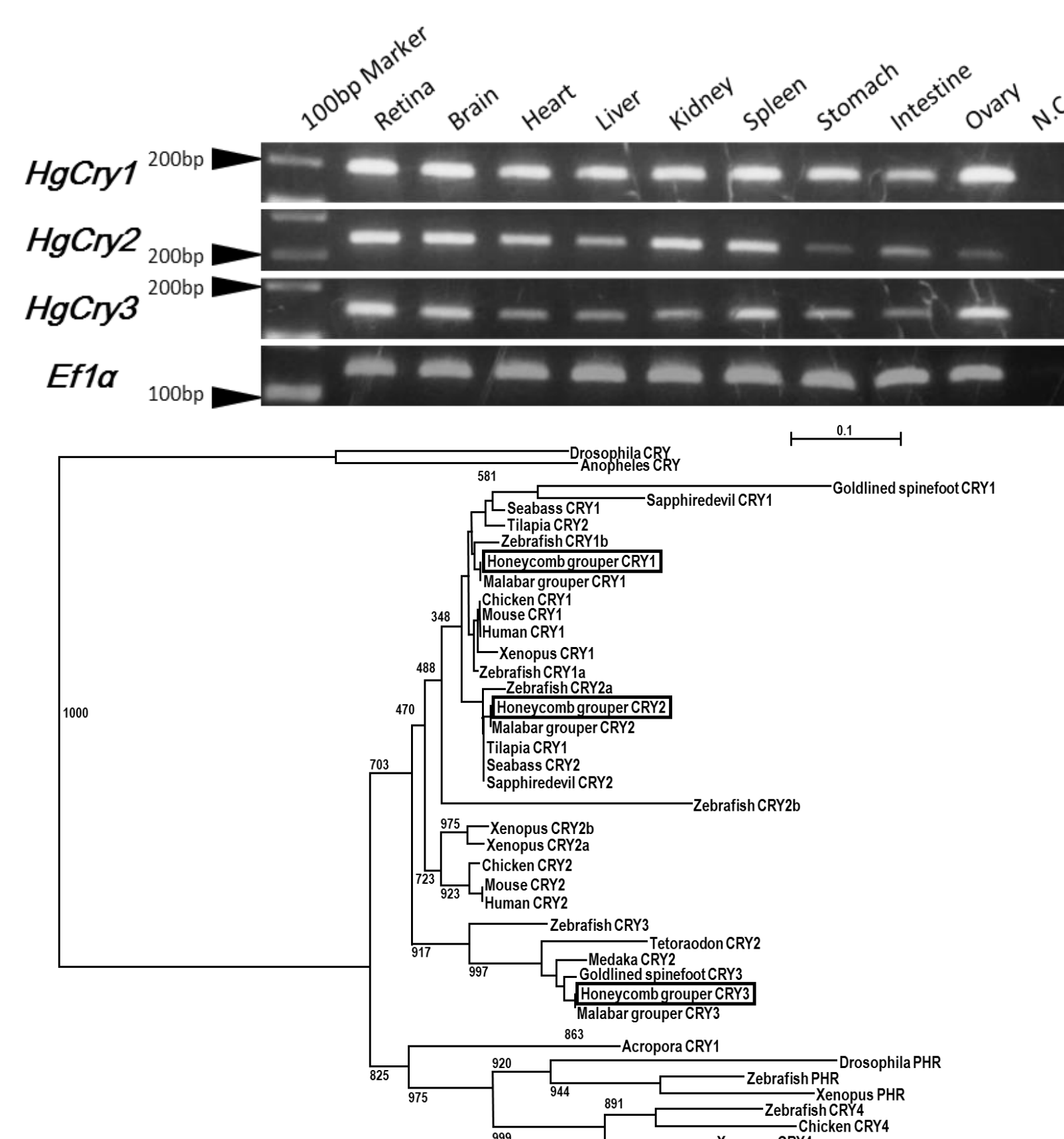


Fig. 2. Phylogenetic tree of CRY protein family.

CRYs of the honeycomb grouper were clustered into each CRY of teleosts.

Fig. 3. Tissue distribution of *HgCry*.

Three *HgCrys* were expressed in all the tissues tested in the present study.

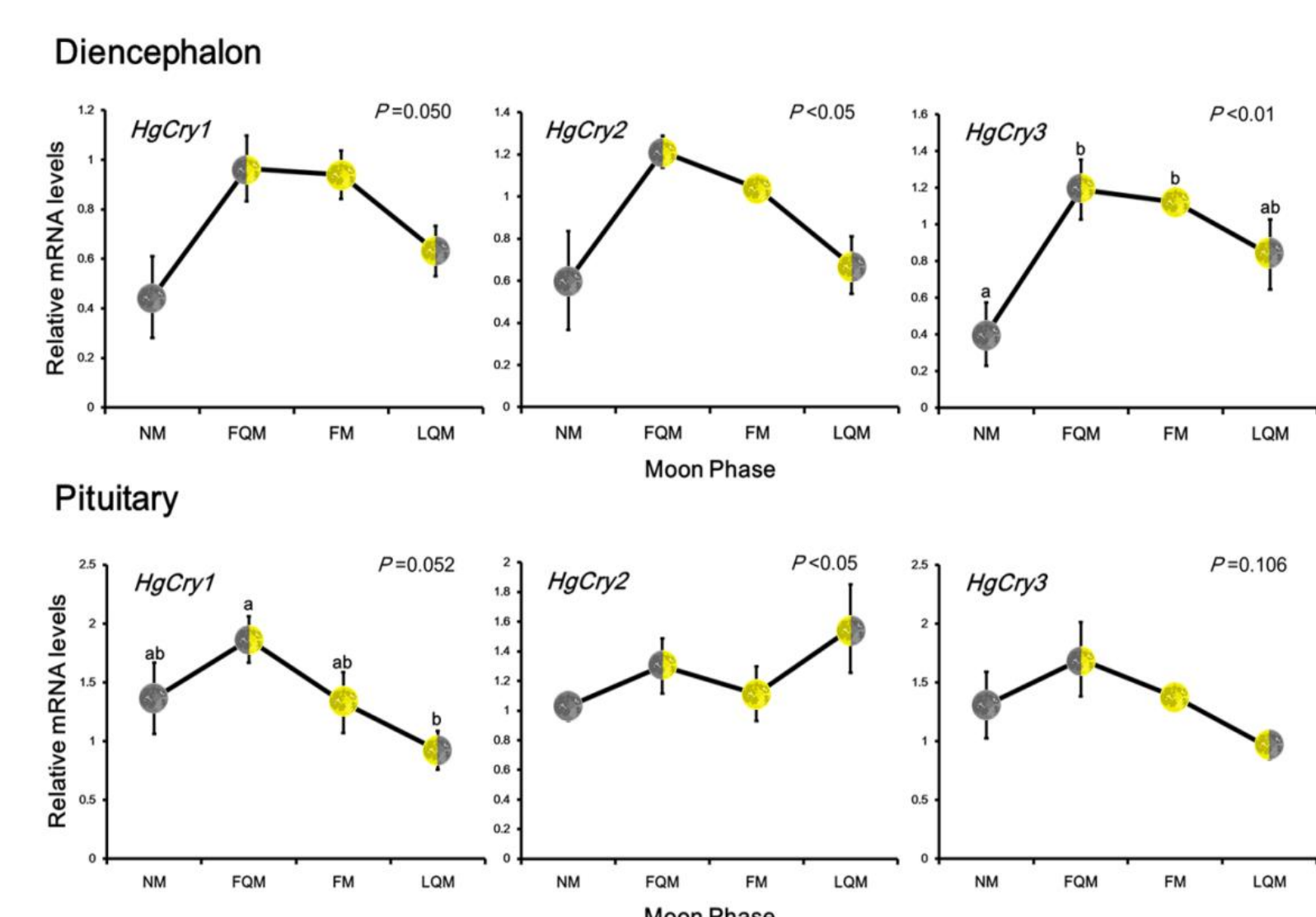


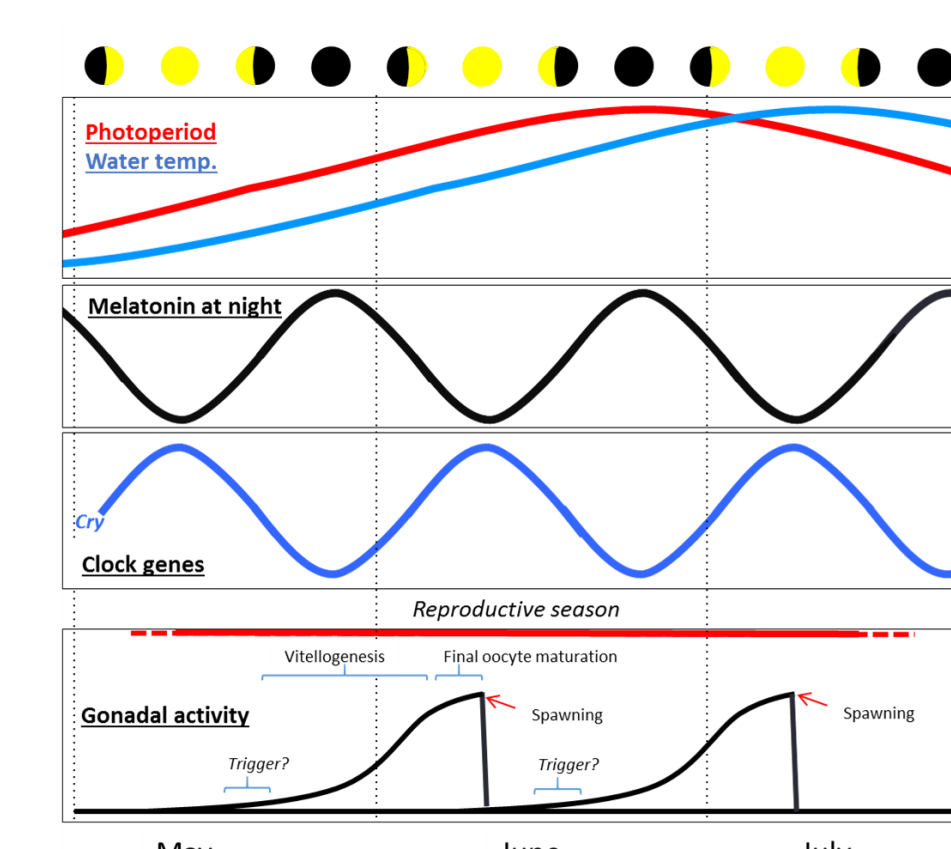
Fig. 4. Expression profiles of *HgCry*.

*HgCry* profiles showed lunar-related fluctuation in DI and PT.

## CONCLUSION

- Honeycomb grouper spawns around the full moon period. ← Ex. 1
- HgCry* abundance in DI and PT increases around the full moon period. ← Ex. 4

Major components of the circadian system are involved in oscillation of the lunar-related spawning cycle.



Possible profiles of night melatonin in the circulation and *Cry* in the brain during the spawning season.