Lunar and daily transcription of *Cryptochrome2* in the brain of a tropical grouper

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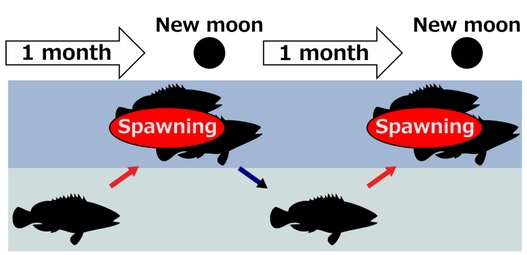
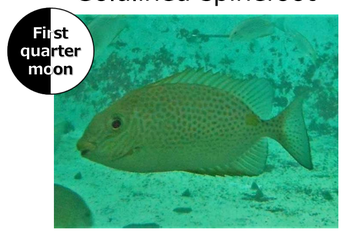
Background

- Lunar-synchronized spawning

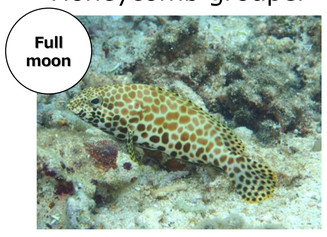
The tropic and sub-tropic areas have little seasonal changes in day length and temperature. The waxing and waning moon is an reliable cue to synchronize the timing of reproductive event for the dwellers. The lunar-synchronized spawning is often observed in tropical and sub-tropical fishes.



Goldlined spinefoot

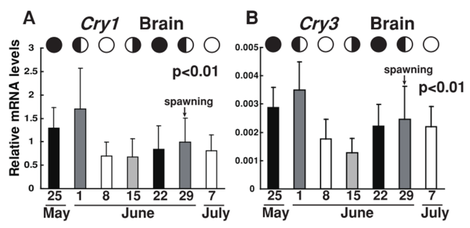


Honeycomb grouper

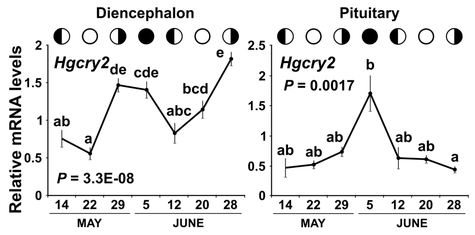


- Lunar change in circadian clock genes

In the brain of lunar-synchronized spawners, the expression of *Cryptochrome* (*Cry*) genes fluctuate with lunar cycles. It is suggested that *Cry* is involved in the lunar-synchronized spawning system.



Lunar change in the expression of *Cry* genes in the brain of goldlined spinefoot. Fukushiro *et al*. (2011)



Lunar change in the expression of *Cry2* gene in the brain of honeycomb grouper. Fukunaga (unpublished)

It is still unknown how the lunar-synchronized spawning is physiologically regulated in fish.

The aim of present study is to investigate the expression pattern of circadian clock gene (*Cry2*) and to accumulate knowledge about the mechanism of lunar-synchronized spawning using the orange-spotted grouper.

Materials and Methods

Orange-spotted grouper (*Epinephelus coioides*)

- Tropic and sub-tropic grouper fish
- Last quarter moon spawner (Toledo *et al*., 1993)
- Total length 148±2.0 mm, Body weight 64.5±1.6 g
- Obtained from Okinawa Prefectural Sea Farming Center, Motobu, Okinawa, Japan.
- January to February 2017.

Outdoor tank → EXP. 1, 3

- Fish were stored in outdoor tank (2 metric tons) at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus.
- **Natural lighting condition** with running seawater and aeration.

Indoor tanks → EXP. 2

- Fish (n=6) werw transferred to seven indoor tanks (60L) at Sesoko Station.
- **Controlled photoperiod (LD=12:12 or constant dark, DD**) with running seawater and aeration.

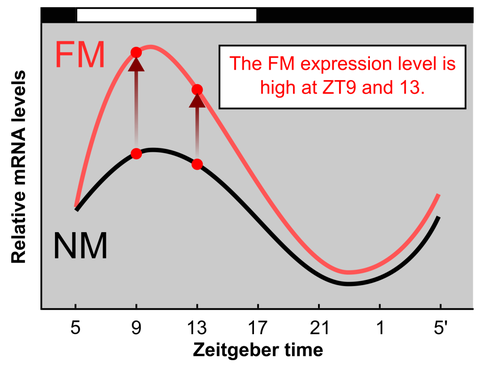
EXP. 1. Cloning of *OgCry2* gene

The partial amino acid fragment isolated from the brain of orange-spotted grouper was clustered into CRY2 clade of bony fish.

Phylogenetic tree analysis of CRY2 amino acid in the orange-spotted groper compare with other vertebrate.
The tree was generated using neighbor-jointing method with bootstrap confidence values based on 100 replicates.

Conclusion and Discussion

EXP. 2, 3. The expression profiles of *OgCry2*



The estimated expression profiles of *OgCry2*
The profiles fluctuates with circadian peak at day and monthly peak around the FM. As a result of that the width of amplitude of daily expression at day changes monthly.

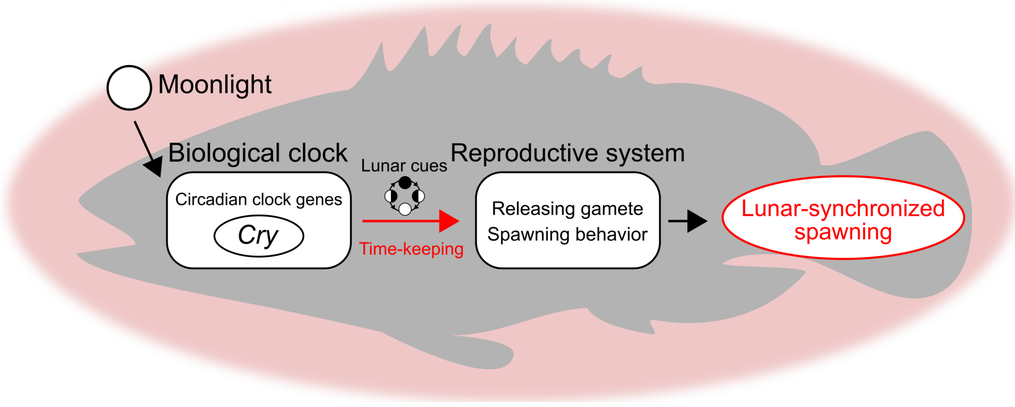
- *OgCry2* highly expressed in light phase and decreased in middle of dark (EXP. 2).
- The expression was higher around FM compare to other moon phase (EXP. 3).

These data suggested *OgCry2* fluctuates with daily and monthly cycles.

The role of circadian clock as a time-keeping for reproduction

Malabar grouper

In the Malabar grouper, the expression of *Cry2* in the brain exhibited lunar phase-dependent change and the transcript level was varied by **moonlight manipulation**. Yamashina (unpublished)



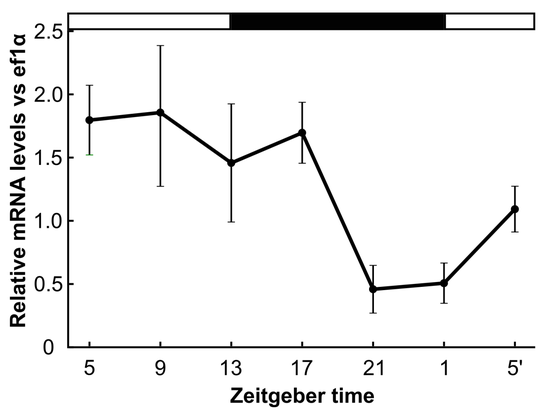
It is possible that the lunar phase-dependent change in circadian clock genes is regulated by moonlight and involves in time-keeping for reproduction.

Results

EXP. 2. Daily and circadian expressions of *OgCry2* by qPCR

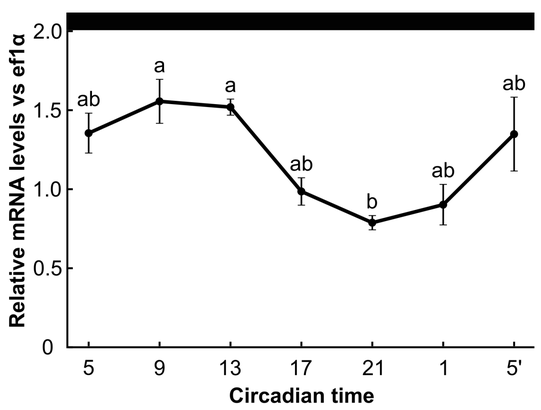
Daily fluctuation（LD=12:12）

The mRNA abundance of *OgCry2* was high from light to early dark phase and decreased in middle dark in LD condition.



Circadian fluctuation （DD）

The mRNA abundance of *OgCry2* was higher in subjective day than in subjective night in DD condition.

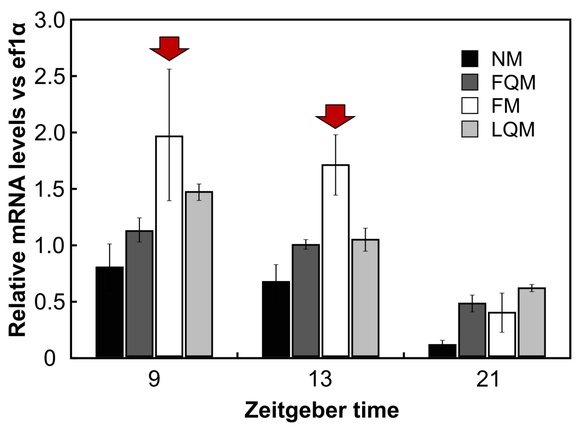


Daily and circadian variation of *OgCry2*
The expression level of *OgCry2* in the diencephalon was measured by real-time qPCR. Each data was represented as the mean ± SEM. Multiple comparison was analyzed using Kruskal-Wallis test together with Steel-Dwass test ($p<0.05$).

EXP. 3. Lunar phase-dependent expressions of *OgCry2* by qPCR

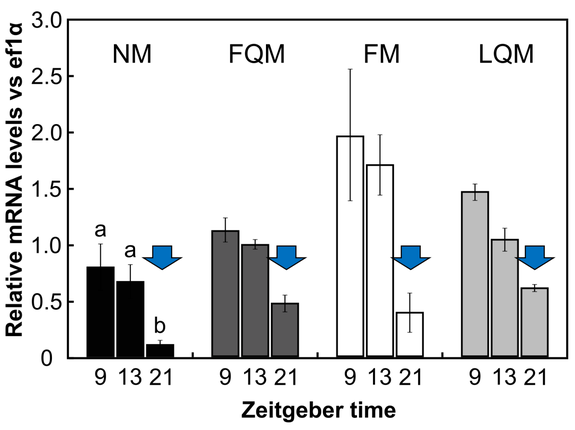
Moon phase comparison

The mRNA abundances of *OgCry2* around FM were higher than other moon phase in ZT9 and 13.



Time point comparison

The mRNA abundances of *OgCry2* in ZT21 were lower than other time point.



Lunar phase-dependent variations of *OgCry2*
The expression levels of *OgCry2* in the diencephalon were measured by real-time qPCR. The *OgCry2* gene in the diencephalon was compared among lunar phases. Each data was represented as the mean ± SEM. Multiple comparison was analyzed using Kruskal-Wallis test together with Steel-Dwass test ($p<0.05$).

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No conflict of interest declared with regard to the presentation.