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The Reproductive Cycle of the Humbug Damselfish *Dascyllus aruanus* According to the Changes in Lunar Phase in Micronesia

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Abstract – The moon is known to be an environmental factor that controls the reproductive cycle of fish, and fish have evolved a variety of reproduction patterns depending on the lunar phase. In this study, we examined the relationship between lunar phases and the reproductive cycle of the humbug damselfish *Dascyllus aruanus* inhabiting Weno Island, Chuuk Lagoon, Micronesia. We divided the one-month lunar cycle into eight phases, and measured the features of moonlight (peak wavelength and intensity) and indicators of fish maturity [gonadosomatic index (GSI) and sex hormones] in relation to the eight lunar phases. In addition, we investigated the daily rhythms of sex hormones in fish ovaries during the full moon phase. The results showed that the peak wavelength of moonlight was 430 nm (blue wavelength region) regardless of the lunar phase, and that moonlight intensity was highest during a full moon at 02:00. Furthermore, the GSI and sex hormones were both higher around the full moon phase. These findings support the hypothesis that humbug damselfish spawn once a month and that this event occurs at full moon, which is the phase of the moon with the strongest intensity. Based on these findings, we predict that blue wavelength, the dominant wavelength of moonlight, is one of the environmental factors influencing the monthly spawning of *D. aruanus*.

Keywords – blue wavelength, *Dascyllus aruanus*, lunar phase, Micronesia, reproductive cycle, sex hormone

1. Introduction

Fish typically have a wide range of reproductive strategies and reproductive cycles in order to survive and adapt to environmental changes, such as those of temperature, photoperiod, and salinity (Lowe-McConnell 1975; Takemura et al. 2004a). However, because the climate has less seasonal influence and maintains a stable environment in equatorial regions, the life cycles of fish that live near the equator are considered to be potentially influenced by alternative environmental factors such as phase changes of the moon, tidal amplitude, and electromagnetic fields, rather than by temperature and salinity changes (Robertson 1991; Takemura et al. 2004b).

One of the potential environmental factors affecting fish life cycles is the moon, which orbits the earth during a period of approximately 27.3 days and appears in various phases, as determined by the position of the sun. Differences in the phase of the moon result in differences in the intensity of moonlight reaching the Earth's surface and changes in tidal amplitude (highest at full moon, lowest at the new moon). The moon is accordingly known to be an important environmental factor affecting physiological changes in fish (Ikegami et al. 2014).

In particular, recent studies have reported changes in the reproductive cycle of fish in accordance with phase changes of the moon (Sugama et al. 2008; Takemura et al. 2010). Takemura et al. (2010) classified the spawning period of fish into three patterns in accordance with the changing phases of the moon: (1) a lunar spawning cycle period of 1 month, (2) a semi-lunar spawning cycle period of 2 weeks, and (3) a tidal spawning cycle period of 1 day at the highest spring tide. However, the relationship between moon phase changes and the ovary development cycle has to date only been reported for a few fish species (Colin et al. 1987; Takemura et al. 2004b). Previous studies have shown that fish start to spawn during the full moon phase, which coincides with the highest difference in water flux during a particular month, in order to improve their survival and achieve widespread dispersal

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of their offspring using geophysical phenomena related to moon phase changes (Leatherland et al. 1992; Takemura et al. 2010). In addition, at full moon when the intensity of moonlight is strong, fish appear to undergo spawning migration and develop aggressive behavior before spawning to protect their offspring (Johannes 1978; De Vries et al. 2004). However, there has been insufficient research on the relevance of phases of the moon with respect to fish spawning, and on the physiological aspects associated with spawning, including the development of ovaries in response to maturity-related hormone and histological changes, and their correlations.

Maturity and sexual development in fish is known to be regulated by various sex hormones, such as the gonadotropin-releasing hormone (GnRH), gonadotropic hormones (GTH), and sex steroid hormones (e.g., estrogen and testosterone), as centered on the hypothalamic-pituitary-gonadal (HPG) axis (Lee et al. 2001; Moussavi et al. 2012). GnRH regulates the secretion of GTH, which include the pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH consist of two subunits, the α-subunit and the β-subunit (Jameson et al. 1983; Maurer 1987). The β-subunits are different and confer biological specificity (Pierce and Parsons 1981). These hormones are involved in the development and maturation of the ovaries via activation of GnRH receptors in the pituitary gland (Andrews et al. 1988). 17β-Estradiol (E2), which play an important role in sexual development and sex differentiation, such as oogenesis in the ovary and regulation of gonadotropin expression via binding of estrogen receptors and the gonadal hormone secreted by the GTH (Ishibashi and Kawashima 2001; Hess 2003). In addition, melatonin, which is mainly secreted from the pineal gland and retina, acts as a circadian neuroendocrine signal that is closely related to biological rhythms (Falcón et al. 2007). Melatonin also serves to promote the growth of organs in combination with the melatonin receptor; however, this receptor has been reported to suppress sexual maturation by reducing the secretion of FSH and LH (Zeman 1993; Sébert et al. 2008).

In this study, we used the humbug damselfish, *Dascyllus aruanus* (Perciformes: Pomacentridae), which is widely distributed in the Chuuk Lagoon in the South Pacific islands of Micronesia, in order to determine changes in reproductive patterns in relation to changes in moon phase. Chuuk Lagoon is a Pacific coral reef area that lies close to the equator, and has a stable environment in which there is little seasonal change in temperature and salinity. Notably, because waters of the coral reef areas of Micronesia have very shallow average depth, and thus external light is transmitted to the sea bed, it is a good environment for marine organisms to inhabit, as well as providing excellent conditions in which to conduct light-related research (KIOST 2009). Furthermore, because, unlike urban areas, this area is not exposed to external artificial lighting at night, we consider that Micronesia is an ideal location to conduct research on the effects of moon phase and moon light on marine organisms.

In this study, we measured changes in the wavelength and intensity of moonlight depending on the phase of the moon. In addition, we analyzed the gonadosomatic index (GSI), the plasma concentration of maturity-related hormones (GnRH, FSH, LH, and E_2), and developmental changes in the ovary, and plasma concentration of melatonin, and examined their correlation with the reproductive cycle.

2. Materials and Methods

Experimental fish and lunar phases

This study was conducted from June 24, 2015 until 20 July 20, 2015 at Weno Island in Chuuk Lagoon, Micronesia (7°27'N; 151°53'E). During each lunar phase, we collected 10 female humbug damselfish (*D. aruanus*) (mean length, 4.7 ± 1.2 cm; mean mass, 3.7 ± 0.8 g) off the coast of the Korea South Pacific Ocean Research Center (KSORC) located on the western side of Weno Island, using a fence and hand nets 1 day before experimental sampling, and maintained these fish in small sea cages in front of the KSORC for 1 day until the experiment. We performed experiments in eight equal parts by dividing one cycle of the moon into eight lunar phases [first quarter (June 24); waxing gibbous (June 28); full moon (July 2); waning gibbous (July 6); last quarter (July 9); waning crescent (July 13); new moon (July 16); waxing crescent (July 20)]. We did not feed the experimental fish, which fed on natural food in the sea.

The fish were killed by spinal transection in order to collect the ovary and pituitary under dim light provided by an attenuated white fluorescent bulb at 02:00. Before being killed, the fish were placed in 2-phenoxyethanol (Sigma, St. Louis, MO, USA) to minimize stress prior to blood collection. Blood was collected rapidly from the caudal vein using a 1-mL syringe coated with heparin. Plasma samples were separated by centrifugation (4°C, 1,000 × g for 15 min) and stored at −80°C until analysis. The tissues were removed from the fish, immediately frozen in liquid nitrogen, and stored at

−80°C until analysis.

Characteristic analysis of moonlight

We measured the peak wavelength (nm) and intensity (lux) of moonlight on the water surface according to the lunar phase changes, using a portable spectrometer (MR-16; Rainbow Light Technology Co. Ltd., Taoyuan, Taiwan) and photo-radiometer (HD 2102.1; Delta OMH Co., Caselle di Selvazzano, Italy), respectively.

Gonadosomatic index (GSI)

The female fish were killed by spinal transection under dim light provided by an attenuated white fluorescent bulb. The fish were placed in 2-phenoxyethanol (Sigma, St. Louis, MO, USA) for ovary collection. Initially, we measured the weight of the whole body (BW) and ovary (GW) to calculate the GSI, using the formula: GSI = GW × 100/BW (Crim and Glebe 1990).

Plasma parameter analysis

Plasma GnRH, FSH, LH, E_2, and melatonin levels were analyzed by immunoassay using ELISA kits [gonadotropin-releasing hormone (catalog no. MBS701579; Mybiosource, USA), Follicle Stimulating Hormone (MBS035576; Mybiosource), Luteinizing Hormone (MBS9358744; Mybiosource), Estradiol (E_2) (MBS700179; Mybiosource) and Fish Melatonin ELISA kit (MBS013211; Mybiosource)].

An anti-antibody that was specific to the antibody of hormones (GnRH, FSH, LH, E_2, and melatonin) was pre-coated onto a microplate. Fifty microliters of plasma were added per well, followed by 100 μL of HRP-conjugate, and then 50 μL antibody. Plate contents were mixed well and then incubated for 1 hour at 37°C. After the final wash, any remaining wash buffer was removed by aspirating or decanting. Fifty microliters of Substrate A and Substrate B were then added to each well, followed by incubation for 15 minutes at 37°C in the dark. Following incubation, 50 μL of stop solution was added to each well. The optical density of each well was determined within 10 minutes, using a microplate reader set to 450 nm.

Histological analysis

The ovaries of each experimental group [1. Lunar cycle experiment: sampled at 4 lunar phases (first quarter, full moon, last quarter, and new moon) at 02:00 h; 2. Daily experiment: sampled at 4-hour intervals from 18:00 h during full moon (July 2) to 06:00 h (July 3)] were fixed in Bouin's solution to analyze the ovaries during sexual maturation. The samples were dehydrated in increasing ethanol concentrations, clarified in xylene, and embedded in paraffin. Sections (5 μm thick) were selected and stained with hematoxylin–eosin for observation under a fluorescence microscope (Eclipse Ci; Nikon, Tokyo, Japan). Images were captured using a digital camera (DS-Fi1c; Nikon).

Statistical analysis

All data were analyzed using the SPSS statistical package (version 10.0; SPSS Inc., USA). A one-way ANOVA followed by Tukey's post hoc test was used to compare differences in the data ($P < 0.05$). The values are expressed as the means ± standard error (SE).

3. Results

Changes in moonlight

The average peak wavelength (nm) of moonlight according to the lunar phase changes on the water surface was 420 nm, and the values were similar regardless of lunar phase. The intensity of moonlight was highest during a full moon (10 lux) and lowest during the waning crescent phase (1.9 lux). However, we were unable to measure the wavelength value during the waning crescent phase due to increasing sunlight (Table 1).

Changes in gonadosomatic index (GSI)

The GSI of female humbug damselfish was significantly higher during the waxing gibbous phase than during the other lunar phases. After the waxing gibbous phase, the value of GSI tended to decrease gradually until the waning crescent phase (Fig. 1).

Table 1. Values of the peak wavelength (nm) and intensity (lux) of moonlight at the water surface according to lunar phase. The asterisk indicate lowest (*) value of moonlight intensity. N/A mean not applicable

Lunar phase	Peak wavelength (nm)	Intensity (lux)
First quarter moon	424	5.2
Waxing gibbous	425	7.2
Full moon	412	10.1
Waning gibbous	415	6.9
Last quarter moon	420	4.8
Waning crescent	422	2.9*
New moon	N/A	N/A
Waxing crescent	425	3.2

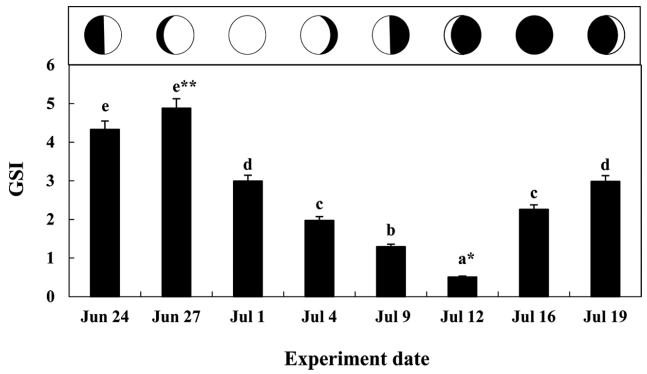

Fig. 1. Change in gonad somatic index (GSI) in the humbug damselfish during the lunar phase cycle. Ovaries were taken from the fish at night time (02:00 a.m.). Lunar phases are indicated by schematic moon images as ◐, the first quarter moon; ☾, waxing gibbous; ○, the full moon; ☽, waning gibbous; ◑, the last quarter moon; ◔, waning crescent; ●, the new moon; and ◕, waxing crescent. The asterisks indicate lowest (*) and highest (**) values. Values with different letters are significantly different ($P < 0.05$), and all values are means ± SE ($n = 10$)

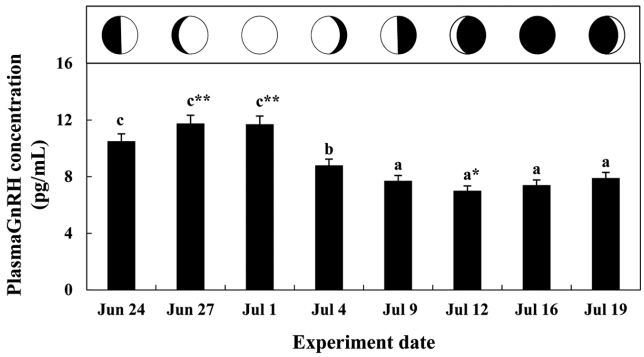

Fig. 2. Change in GnRH plasma concentration in the humbug damselfish during the lunar phase cycle. The plasma was taken from the fish at night time (02:00 a.m.). Lunar phases are indicated by schematic moon images as ◐, the first quarter moon; ☾, waxing gibbous; ○, the full moon; ☽, waning gibbous; ◑, the last quarter moon; ◔, waning crescent; ●, the new moon; and ◕, waxing crescent. The asterisks indicate lowest (*) and highest (**) values. Values with different letters are significantly different ($P < 0.05$), and all values are means ± SE ($n = 10$)

Changes in plasma GnRH concentrations

The plasma GnRH concentrations during the waxing gibbous and full moon phases were significantly higher than those during the other lunar phases, whereas the concentrations during the waning crescent phase were significantly lower. The plasma concentration of GnRH during the waxing gibbous phase (approximately 1.7-fold) was significantly higher than that during the waning crescent phase. Notably, plasma concentrations of GnRH showed a tendency to decrease and increase between the full moon and the new moon (Fig. 2).

Changes in FSH and LH concentrations

Similar to the pattern of GnRH plasma concentration, the plasma concentration of FSH was higher during the waxing gibbous and the full moon phases than during the other lunar phases, whereas concentrations during the waning crescent phase were significantly lower (Fig. 3A).

Similarly, the plasma concentration of LH was also higher during the waxing gibbous and full moon phases than during the other lunar phases, whereas the concentrations during the last quarter and waxing crescent phases were significantly lower (Fig. 3B).

Changes in plasma E_2 concentrations

The plasma E_2 concentrations during the waxing gibbous and full moon phases were significantly higher than during the other lunar phases, whereas the concentration during the

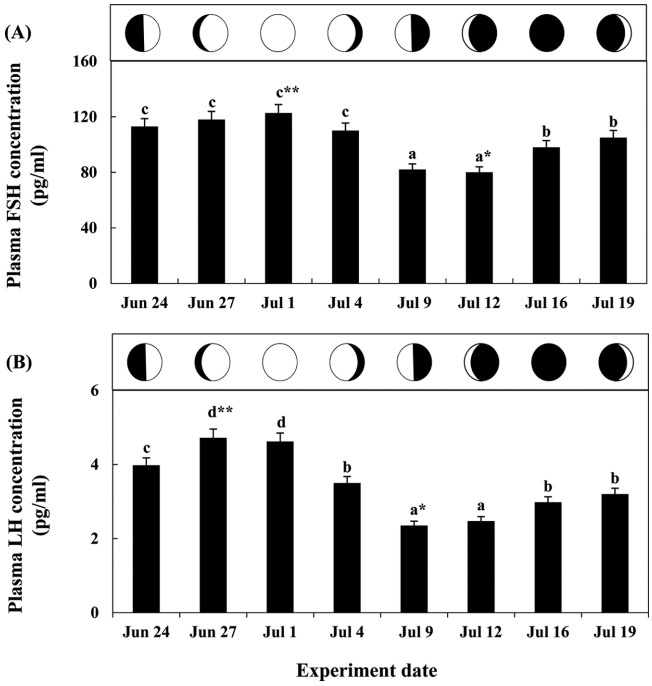

Fig. 3. Change in plasma FSH concentration (A) and LH concentration (B) in the humbug damselfish during the lunar phase cycle. The pituitary and plasma were taken from the fish at night time (02:00 a.m.). Lunar phases are indicated by schematic moon images as ◐, the first quarter moon; ☾, waxing gibbous; ○, the full moon; ☽, waning gibbous; ◑, the last quarter moon; ◔, waning crescent; ●, the new moon; and ◕, waxing crescent. The asterisks indicate lowest (*) and highest (**) values. Values with different letters are significantly different ($P < 0.05$), and all values are means ± SE ($n = 10$)

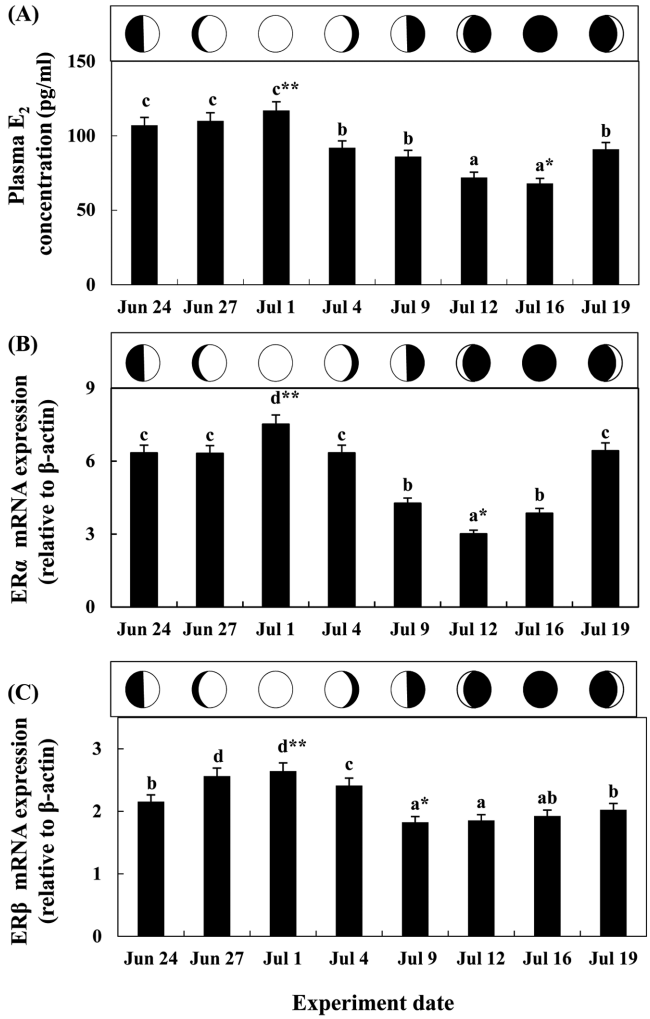

Fig. 4. Change in plasma E_2 concentration in the ovary, and in the humbug damselfish during the lunar phase cycle. The tissues and plasma were taken from the fish at night time (02:00 a.m.). Lunar phases are indicated by schematic moon images as ◐, the first quarter moon; ○, waxing gibbous; ○, the full moon; ○, waning gibbous; ◑, the last quarter moon; ●, waning crescent; ●, the new moon; and ●, waxing crescent. The asterisks indicate lowest (*) and highest (**) values. Values with different letters are significantly different ($P < 0.05$), and all values are means ± SE ($n = 10$)

new moon phase was significantly lower. The plasma concentration of E_2 during the full moon phase (approximately 1.72-fold) was significantly higher than that during the new moon phase. Notably, the plasma concentrations of E_2 showed a tendency to decrease and increase between the full moon and the new moon (Fig. 4).

Changes in plasma melatonin concentrations

The plasma concentration of melatonin was lower during the full moon phase than during the other lunar phases,

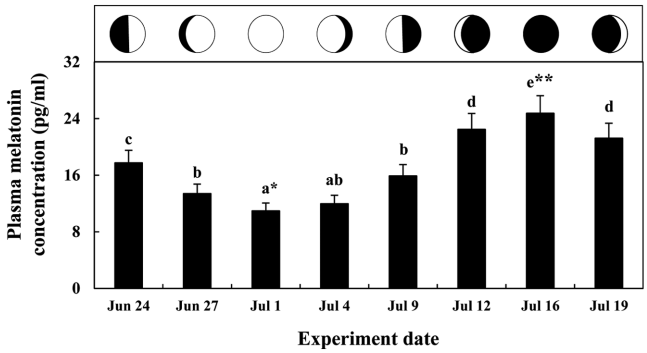

Fig. 5. Change in melatonin concentration in the tissues of the humbug damselfish during the lunar phase cycle. The plasma was taken from the fish at night time (02:00 a.m.). Lunar phases are indicated by schematic moon images as ◐, the first quarter moon; ○, waxing gibbous; ○, the full moon; ○, waning gibbous; ◑, the last quarter moon; ●, waning crescent; ●, the new moon; and ●, waxing crescent. The asterisks indicate lowest (*) and highest (**) values. Values with different letters are significantly different ($P < 0.05$), and all values are means ± SE ($n = 10$)

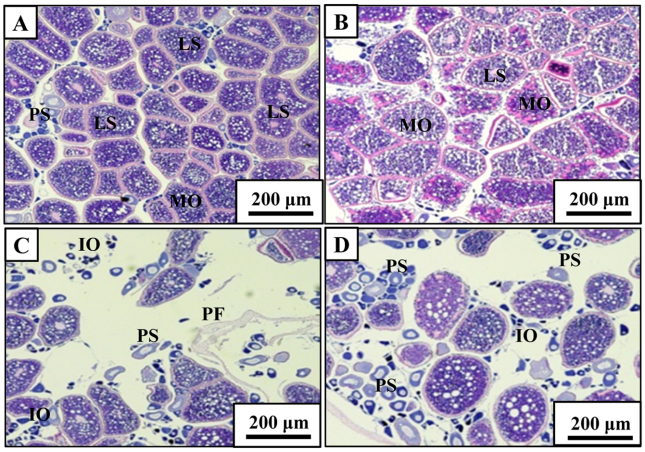

Fig. 6. Change in daily histological observations of ovaries in the humbug damselfish ovary during the lunar phase cycle [the first quarter moon (A), the full moon (B), the last quarter moon (C), the new moon (D)]. The ovaries were taken from the fish at night time (02:00 a.m.). Immature oocyte (IO); Pre-vitellogenic stage (PS); Last vitellogenic stage (LS); Mature oocyte (MO); Postovulatory follicle (PF). Scale bar = 200 μm

whereas the concentration during the new moon phase was significantly higher (Fig. 5A).

Histological observations

To investigate ovary morphology, we performed histological studies of ovary samples, as shown in Figs. 6 and 8.

In the lunar cycle experimental groups, the ovaries of fish were mainly found to contain final vitellogenic oocytes

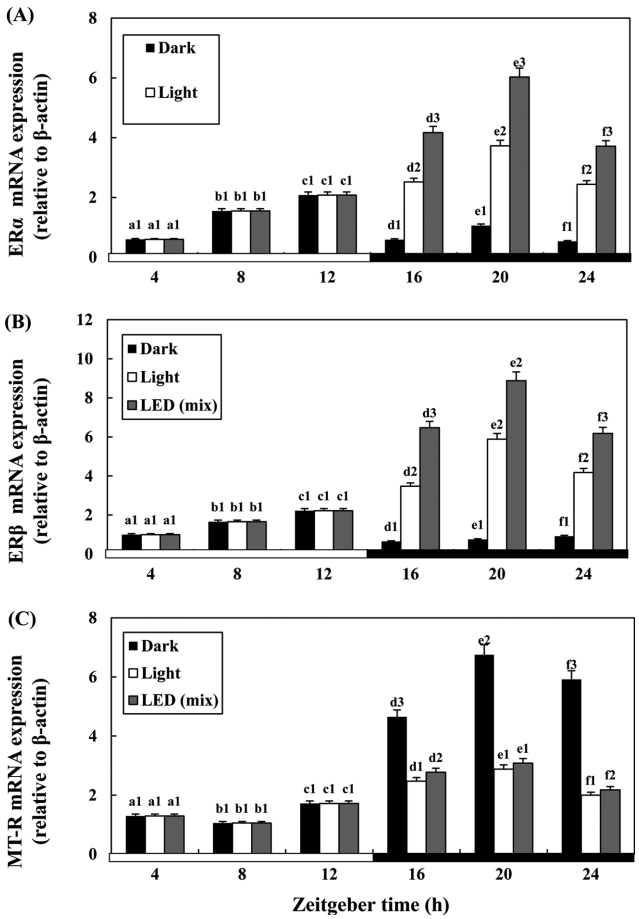

Fig. 7. Change in ERα (A), ERβ (B), and MT-R (C) mRNA expression in the oocytes at ZT 4, 8, 12, 16, 20, and 24. The in vitro-cultured oocytes were exposed to mixed [similar to the intensity and wavelength of the full moon; mixed LEDs of blue (455 nm, 50%), green (520 nm, 20%) and red (640 nm, 30%)] for the experimental group, and exposed to no-light (Dark) for the control group and to fluorescent bulb (Light) for the negative control at night time. In addition, both experimental groups were exposed to white fluorescent bulbs for the control group during the day time. The numbers indicate the same time points, and the lower-case letters indicate significantly different values at the same time point ($P < 0.05$). All values are means ± SE ($n = 5$)

during the first quarter moon (Fig. 6A) and matured oocytes during full moon (Fig. 6B). However, during the last quarter phase, the ovaries contained mainly immature oocytes, and had a postovulatory follicle (Fig. 6C). During the new moon phase, the ovaries contained mainly oocytes at the pre-vitellogenic stage (Fig. 6D).

In the daily experimental groups, the ovaries of fish were mainly found to contain matured oocytes at 18:00 (ZT 12), 22:00 (ZT 16), and 02:00 (ZT 20) (Fig. 8A, B, and C). However, after 06:00 (ZT 24), ovulation appeared to occur, and a postovulatory follicle was observed (Fig. 8D).

Table 2. The frequencies of ovary stages in the histological observations according to moon phase. Immature oocyte (IO); Pre-vitellogenic stage (PS); Last vitellogenic stage (LS); Mature oocyte (MO); Postovulatory follicle (PF)

Moon phase	IO	PS	LS	MO	PF
First quarter	−	+	++	+	−
Full moon	−	−	++	++	−
Last quarter	++	+	−	+	+
New moon	+	++	+	+	−

−, not present; +, minor presence; ++, major presence

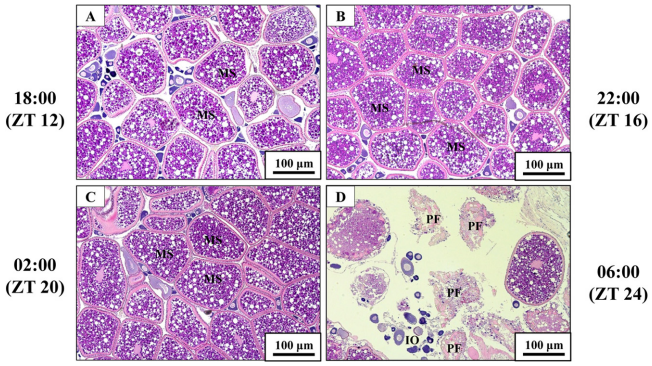

Fig. 8. Changes in daily histological observations of ovaries, 18:00 (ZT 12) (A), 22:00 (ZT 16) (B), 02:00 (ZT 20) (C), and 06:00 (ZT 24) (D) during the full moon (July 2). Immature oocyte (IO); Pre-vitellogenic stage (PS); Last vitellogenic stage (LS); Mature oocyte (MO); Postovulatory follicle (PF). Scale bar = 100 μm

4. Discussion

In this study, to understand the effects of changes in moon phases on fish reproductive cycle, maturation, and spawning, and their correlation, we measured the wavelength and intensity of moonlight, which are expected to vary depending on the phase shift, and variation in the concentration of genes associated with sex hormone that are controlled by the HPG central axis.

Investigation of the changes in the GSI in response to changes in moon phases showed that the GSI increased significantly until the waxing gibbous phase (immediately before full moon) after showing the lowest value during the new moon phase. However, the GSI decreased significantly after the full moon.

Consistent with these results, Takemura et al. (2004b) reported that the GSI value was significantly higher during the first quarter moon in rabbitfishes that inhabit coral reefs, suggesting that the moon phase influences the maturation and spawning of fish. Hsiao et al. (1994) also reported that GSI values started to increase from the new moon and were

significantly higher at full moon in female killifish (*Fundulus heteroclitus*).

By measuring the wavelength of each moon phase in Chuuk Lagoon, we confirmed that it was emitted near the blue wavelength (430 nm), regardless of the moon phase. The intensity of the light was strongest during the full moon (10.1 lux); however, light intensity of the new moon could not be measured using an illumination photometer because the intensity was too weak. Blue wavelengths of light have previously been reported to promote maturation and increase the secretion of sex hormones in fish (Park et al. 2013; Kim et al. 2014; Choi et al. 2015a). Furthermore, Shin et al. (2013) reported that GSI value, plasma estrogen concentration, and expression of ER mRNA were significantly increased in female yellowtail damselfish (*Chrysiptera parasema*) exposed to blue wavelength compared with fluorescent light.

In the present study, we also analyzed the changes in sex hormones secreted by the HPG axis and plasma melatonin concentration to confirm the relevance of moon phase shift with respect to maturity and spawning in the humbug damselfish. We found that the concentrations of sex maturation hormones were significantly higher during the full moon phase, coinciding with the strongest intensity of moonlight, and was lowest during the new moon phase when moonlight intensity is at its weakest and irradiation time is shortest. Similar to previous results, our histological observations of the ovaries according to moon phase shift indicated that oocytes were most developed at full moon. However, in contrast to the aforementioned observations, we confirmed that the plasma melatonin concentrations were lowest at full moon when moonlight intensity is at its strongest and irradiation time is longest, and the highest concentrations coincided with the new moon. Consistent with these findings, Choi et al. (2015b) reported the effect of melatonin on regulating maturation, and showed that GSI values, and plasma E_2 concentrations of yellowtail damselfish (*C. parasema*) exposed to a long-day photoperiod environment (14-Light:10-Dark) were significantly increased compared with fish exposed to 12-L:12-D (control light conditions). In contrast, the plasma melatonin concentrations were significantly decreased. These authors accordingly suggested that secretion of maturity-related hormones appears to be dependent on the amount of melatonin, and the concentrations of melatonin acts as a main factor in the regulation (inhibition) of ovary development. Furthermore, because MT-R is present in the neurons that synthesize the gonadotropin-inhibitory hormone (GnIH), which suppresses the secretion of GnRH, Ubuka et al. (2005) reported that melatonin, which is sensitively regulated by light, inhibits maturation by stimulating the secretion of GnIH.

A full moon, which produces the strongest intensity and the longest irradiation time of moonlight to the water surface, appears to have an effect on humbug damselfish maturation due to the longest exposure time to blue wavelength light, and we suggest that this species begins spawning in response to the full moon and has a reproductive cycle period of 1 month (lunar spawning cycle).

In this study, as the experimental fish is small, only a limited amount of blood could be obtained from each animal. These samples were used to measure various hormones in this study, but there was simply insufficient blood per fish to extend the measurements to other hormones. However, since the changes in E_2 concentration measured in this study were similar to those of other sex-related hormones (GnRH, FSH, and LH), as well as changes in GSI, we consider that the purpose of this study, to investigate the relationship between the lunar cycle and maturity in humbug damselfish.

In summary, on the basis of the results of the present study, we suggest that (1) female humbug damselfish inhabiting Chuuk Lagoon spawn once a month, and (2) that the spawning time of this species appears to commence during the full moon phase, which coincides with high intensities of blue wavelength light and irradiation dosage. Furthermore, (3) the fluctuation in hormones according to phase shift at full moon, such as a decrement in melatonin, which inhibits sex hormone secretion, and an increment in maturity-related hormones, has an effect on the spawning of humbug damselfish. In particular, (4) on the basis of observation in the histological analysis of ovaries, we estimated that the spawning time of this species occurs after 02:00.

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References

Andersson E, Fjelldal PG, Klenke U, Vikingstad E, Taranger GL, Zohar Y, Stefansson SO (2001) Three forms of GnRH in the brain and pituitary of the turbot, *Scophthalmus maximus*: immunological

characterization and seasonal variation. Comp Biochem Phys B **129**:551–558

Andrews WV, Maurer RA, Conn PM (1988) Stimulation of rat luteinizing hormone-beta messenger RNA levels by gonadotropin releasing hormone. Apparent role for protein kinase C. J Biol Chem **263**:13755–13761

Choi CY, Shin HS, Kim NN, Yang S-G, Kim B-S, Yu YM (2015a) Time-related effects of various LED light spectra on reproductive hormones in the brain of the goldfish *Carassius auratus*. Biol Rhythm Res **46**:671–682

Choi YJ, Kim B-S, Choi CY (2015b) Effect of long-afterglow phosphorescent pigment on reproductive parameters and ovarian maturation in the yellowtail damselfish, *Chrysiptera parasema*. Comp Biochem Phys A **182**:113–120

Colin PL, Shapiro DY, Weiler D (1987) Aspects of the reproduction of two groupers, *Epinephelus guttatus* and *E. striatus* in the West Indies. B Mar Sci **40**:220–230

Crim LW, Glebe BD (1990) Reproduction. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 529–547

DeVries P, Goetz F, Fresh K, Seiler D (2004) Evidence of a lunar gravitation cue on timing of estuarine entry by Pacific salmon smolts. T Am Fish Soc **133**:1379–1395

Falcón J, Besseau L, Sauzet S, Boeuf G (2007) Melatonin and neuroendocrine regulations in fish. J Soc Biol **201**:21–29

Hasio S-M, Greeley MS, Wallance RA (1994) Reproductive cycling in female *Fundulus heteroclitus*. Biol Bull **186**:27l–284

Hess RA (2003) Estrogen in the adult male reproductive tract: a review. Reprod Biol Endocrin **1**:52

Ikegami T, Takeuchi Y, Hur S-P, Takemura A (2014) Impacts of moonlight on fish reproduction. Mar Genomics **14**:59–66

Ishibashi O, Kawashima H (2001) Cloning and characterization of the functional promoter of mouse estrogen receptor β gene. Biochim Biophys Acta **1519**:223–229

Jameson L, Chin WW, Hollenberg AN, Chang AS, Habener JF (1983) The gene encoding the beta subunit of rat LH. J Biol Chem **259**:15474–15478

Johannes RE (1978) Reproductive strategies of coastal marine fishes in the tropics. Environ Biol Fish **3**:65–84

Kim NN, Choi YJ, Shin HS, Lim JR, Han JM, Cho JH, Choi CY (2014) The effect of LED light spectra on antioxidant system by thermal stress in goldfish, *Carassius auratus*. Mol Cell Toxicol **10**:47–58

KIOST (2009) Development of overseas marine bioresources and a system for their utilization. Korea Institute of Ocean Science and Technology (KIOST), Ansan, 341 p

Leatherland JF, Farbridge KJ, Boujard T (1992) Lunar and semi-lunar rhythms in fishes. In: Ali MA (ed) Rhythms in fishers. Plenum Press, New York, pp 83–107

Lee YH, Du JL, Yen FP, Lee CY, Dufour S, Huang JD, Sun LT, Chang CF (2001) Regulation of plasma gonadotropin II secretion by sex steroids, aromatase inhibitors, and antiestrogens in the protandrous black porgy. *Acanthopagrus schlegeli* Bleeker. Comp Biochem Phys B **129**:399–406

Lowe-McConnell RH (1975) Fish communities in tropical freshwaters. Longman Inc., New York, 337 p

Maurer RA (1987) Molecular cloning and nucleotide sequence analysis of complementary DNA for the beta subunit of rat FSH. Mol Endocrinol **1**:717–723

Moussavi M, Wlasichuk M, Chang JP, Habibi HR (2012) Seasonal effect of GnIH on gonadotrope functions in the pituitary of goldfish. Mol Cell Endocrinol **350**:53–60

Pierce JG, Parsons TF (1981) Glycoprotein hormones: structure and function. Annu Rev Biochem **50**:465–495

Park MS, Shin HS, Kim NN, Lee J, Kil G-S, Choi CY (2013) Effects of LED spectral sensitivity on circadian rhythm-related genes in the yellowtail clownfish, *Amphiprion clarkii*. Anim Cells Syst **17**:99–105

Robertson DR (1991) The role of adult biology in the timing of spawning of tropical reef fishes. In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press, San Diego, pp 356–386

Sébert ME, Legros C, Weltzien FA, Malpaux B, Chemineau P, Dufour S (2008) Melatonin activates brain dopaminergic systems in the eel with an inhibitory impact on reproductive function. J Neuroendocrinol **20**:917–929

Shin HS, Kim NN, Choi YJ, Habibi HR, Kim JW, Choi CY (2013) Light-emitting diode spectral sensitivity relationship with reproductive parameters and ovarian maturation in yellowtail damselfish, *Chrysiptera parasema*. J Photoch Photobio B **127**:108–113

Sugama N, Park JG, Park YJ. Takeuchi Y, Kim SJ, Takemura A (2008) Moonlight affects nocturnal Period2 transcript levels in the pineal gland of the reef fish *Siganus guttatus*. J Pineal Res **45**:133–141

Takemura A, Sri Susilo E, Rahman MS, Morita M (2004a) Perception and possible utilization of moonlight intensity for reproductive activities in a lunar-synchronized spawner, the golden rabbitfish. J Exp Zool Part A **301**:844–851

Takemura A, Rahman MD, Nakamura S, Park YJ, Takano K (2004b) Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes. Fish Fish **5**:317–328

Takemura A, Rahman MS, Park YJ (2010) External and internal controls of lunar-related reproductive rhythms in fishes. J Fish Biol **76**:7–26

Ubuka T, Bentley GE, Ukena K, Wingfield JC, Tsutsui K (2005) Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain. P Natl Acad Sci USA **102**:3052–3057

Zeman M, Výboh P, Juráni M, Lamosová D, Kostal L, Bilcík B, Blazícek P, Jurániová E (1993) Effects of exogenous melatonin on some endocrine, behavioural and metabolic parameters in Japanese quail *Coturnix coturnix japonica*. Comp Biochem Phys A **105**:323–328