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Research Paper

Dark environment affects circadian rhythms of gut bacterial community in the mud crab *Scylla paramamosain**

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Abstract The circadian rhythms of gut bacterial community are essential for the health of the host. Although previous studies have shown that environmental light cycles synchronize circadian feeding patterns in animals and regulate microbial communities through environmental fluctuations, the role of light in regulating the circadian rhythms of gut bacterial community in crustaceans and the impact of dark environments on these rhythms remain unclear. To address this issue, we used the mud crab (*Scylla paramamosain*) as a model to evaluate the circadian rhythms of gut bacterial community in different photoperiods (12-h L∶12-h D and 0-h L∶24-h D), and identified the microbes exhibiting circadian rhythms influenced by light exposure and microbial circadian clocks. The study found that 12-h L∶12-h D photoperiod optimally supports mud crab growth performance, animal welfare, and circadian microbial balance. While gut microbiota exhibited rhythmic fluctuations in different photoperiods, the community composition analysis revealed significant differences between light/dark and continuous dark conditions. The circadian rhythmic microbes regulated by light exposure were primarily dominated by Spirochaetes, while the dark environment significantly reduced the relative abundance of Alpha-proteobacteria and Bacteroidetes (P<0.05), with Fusobacteria serving as a representative microorganism. Additionally, the dark environment caused significant fluctuations in functional pathways related to environmental stress response (P<0.05), such as the FoxO signaling pathway, lysosome, and RNA transport, suggesting that a complete darkness environment disrupts the biological clock. These findings elucidated the regulatory mechanisms of circadian rhythms in the gut bacterial community of mud crabs and highlighted the significant role of light in shaping gut bacterial community.

Keyword: *Scylla paramamosain*; photoperiod; circadian rhythm; gut bacterial community

1 INTRODUCTION

The Earth's rotation causes the light-dark cycles, which has influenced living organisms throughout evolution. Therefore, most organisms on the Earth have a natural clock (Sharma, 2003; Gerhart-Hines and Lazar, 2015). This natural clock encodes the rhythmicity of biological activities in the daily 12-h light/12-h dark cycle in the most areas of the world, and this endogenous biological rhythm is known as the circadian rhythm (Hussain and Pan, 2015; Schilperoort et al., 2020; Zhang and Liu, 2023). These rhythms facilitate temporal mutualism, reducing competition among sympatric species, and ultimately promote the survival of organisms in

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rhythmic environments (Sartor et al., 2019; Zhao et al., 2021). Thus, investigating the rhythms of bacterial populations can enhance aquaculture management to promote better health and well-being of cultured species.

Light is a composite environmental factor that mainly includes three elements: photoperiod, light spectrum, and light intensity. Among these, photoperiod is the most regular and provides organisms with a seasonally stable rhythmic environment, profoundly affecting their physiological and behavioral processes (Shi et al., 2022). Previous studies have shown that in a 12-h L∶12-h D photoperiod, the clock genes, such as *cry1*/*2*, *per2*, *cry1a*, and *timeless*, in the pituitary of the spotted sea perch (*Lateolabrax maculatus*) and eyestalks of the mud crab (*Scylla paramamosain*) exhibit circadian rhythms (Yuan et al., 2020; Chen et al., 2025); moreover, under continuous light and dark conditions, the Chinese mitten crab (*Eriocheir sinensis*) showed the greatest sensitivity to light on day 2. The expression of genes related to immune response also varied in different photoperiods (Zhang et al., 2023).

Light exposure acts as an upstream factor that regulates the circadian rhythm of the gut microbiota (Wu et al., 2018). Under normal light conditions, the gut bacterial communities exhibit circadian rhythms (Liang et al., 2015; Kaczmarek et al., 2017). When the endogenous biological rhythm aligns with external light signals, it supports the organism's behavior, immune function, and overall health. However, prolonged exposure to darkness can disrupt the circadian rhythm, reduce the diversity of the gut microbiota, and consequently influence its composition, structure, and function (Guo et al., 2019). So far, most research on the relationship between light/dark cycles and gut microbiota are focused on fish (Peyric et al., 2013; Fortes-Silva et al., 2016). Dark environment treatment could reduce the abundance of Bacteroidetes and Firmicutes in the gut of zebrafish (*Danio rerio*) (Feng et al., 2021). Furthermore, the relative abundances of bacteria from *Ruegeria*, *Vibrio*, and *Actibacter* in the gut of the Pacific white shrimp (*Litopenaeus vannamei*) exhibited significant diel oscillations (Jiao et al., 2021). Hence, the gut, as the primary site for the digestion, absorption, and metabolism of nutrients, presents the potential targets for studying the response of crabs to different light/dark photoperiods.

The mud crab (*S. paramamosain*), represents a significant marine economic species in China, primarily distributed along the coastal waters of Zhejiang, Fujian, Taiwan, Guangdong, Guangxi, and Hainan (Lin et al., 2007). Recent studies have examined the effects of different light/dark cycles on survival, growth performance, and lipid metabolism in juvenile *S. paramamosain*. Based on these findings, a 12-h L∶12-h D photoperiod has been recommended for mud crabs during the juvenile grow-out phase in commercial farming operations (Chen et al., 2021, 2023). However, there is limited understanding of how a dark environment affects the circadian rhythm of the gut microbiome in juvenile mud crabs. In this study, juvenile crabs were placed in 12-h L∶12-h D light dark (LD) and constant dark 0-h L∶24-h D (DD) environment for 2 weeks. Subsequently, the culture was continued for an additional 2 d, during which gut samples from the crabs were collected every 6 h to identify microorganisms exhibiting circadian rhythms in LD and DD groups. Our results reveal significant differences in gut microbial communities between LD and DD groups, demonstrating that circadian microorganisms respond differently under altered light conditions. These findings enhanced our understanding of the adaptive capacity of circadian rhythms in mud crabs and offered a reference for manipulating the composition and function of their gut microbial community via controlled light exposure.

2 MATERIAL AND METHOD

2.1 Experimental design and sample collection

All experimental procedures and animal care were conducted in accordance with the Animal Research Institute Committee Guidelines of Ningbo University, China, and were approved by the Institutional Animal Care and Use Committee (IACUC) of Ningbo University.

A total of 300 wild juvenile mud crabs (19.54± 3.26 g) were captured from the intertidal zone of Sanmen Bay, Taizhou, China and then immediately transferred to the laboratory under dark, oxygenated conditions within 2 h. Each crab was placed individually in a plastic basket (320 mm×190 mm× 170 mm) to avoid cannibalism. These baskets were evenly transferred into 20 canvas pools (1.5 m×1.0 m× 0.6 m), each containing 300 L of natural seawater with the following conditions: salinity 23–24, temperature 27–28 °C, pH 8.0–8.2, ammonia nitrogen <0.5 mg/L, and dissolved oxygen 6–6.5 mg/L.

The pools were evenly divided into two groups: the LD group, exposed to a light intensity of 12 W/m^2 and a 12-h L∶12-h D natural photoperiod (with the light period from 6:00 to 18:00), and the DD group, which was maintained under a 0-h L∶24-h D regime (constant darkness). Here, full-spectrum LED lamps were used as light source and suspended above the rearing pools. Meanwhile, black-out cloth was used as a cover to prevent any light interference. From 16:00 to 18:00 daily, each crab was provided with the chilled meat of a clam (*Ruditapes philippinarum*) as their dietary intake. The dead crabs and food debris were removed at 20:00, and then 25% of seawater was replaced by fresh natural seawater every 2 d.

Two weeks later, eight crabs of each group were randomly collected from 10 pools every 6 h at Zeitgeber Times (ZT) 0, 6, 12, 18, 24, 30, 36, 42, and 48 (ZT0, ZT24=light off, ZT12, ZT36=light on) under 12-h L∶12-h D conditions, with corresponding Circadian Time (CT) points (CT0, 6, 12, 18, 24, 30, 36, 42, and 48, where CT0=subjective light off) collected under constant darkness (Fig.1a). A total of 144 crabs were collected and anesthetized on ice. The entire gut of each crab was obtained and stored at -80 ℃ for DNA extraction.

2.2 DNA extraction and 16S rRNA gene sequencing analysis

Total DNA was extracted from each gut sample using the PowerFecal™ DNA extraction kit (QIAGEN, Germany) according to the manufacturer's protocol. After purification by DNA purification kit (TaKaRa, Japan), the concentration and purity of the extracted DNA were measured using a NanoDrop Spectrophotometer (ND-2000, Thermo, USA) and the qualified DNA was used for subsequent analysis. The V3–V4 hypervariable region of the 16S rRNA gene was amplified using polymerase chain reaction (PCR) with a dual-indexed primer pair (338F: 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806R: 5′-GGACTACHVGTWTCTAAT-3′) (Castrillo et al., 2017). To reduce the bias caused by PCR amplification, PCR were conducted in triplicate for each sample. The target PCR product was verified by 1% agarose gel electrophoresis and further purified after passing quality control. DNA library sequencing was then carried out using the Nova 6000 platform (Illumina, USA). The amplicon sequencing data were processed according to the USEARCH pipeline (http://www.drive5.com/usearch/). In brief, following primer trimming and quality score-based filtering, the remaining sequences were binned into amplicon sequence variants (ASVs) with 100% nucleotide similarity using UCLUST (Edgar et al., 2011). Taxonomic classification of each ASV was carried out through the Silva (v.123) (http://www.arb-silva.de/). All sample data were normalized to the minimum depth to eliminate biases caused by inconsistent sequencing depths among samples. The 16S rRNA gene sequence data obtained in this study have been deposited in the National Center for Biotechnology Information (NCBI) with the accession number PRJNA1207210.

2.3 Cosinor analysis

To explore the changes in the circadian rhythms of gut bacteria in the mud crab under dark environments, we employed the CircaCompare and Cosinor2 packages in R software (v4.3.2) to identify significantly rhythmic ASVs in the gut bacterial communities of LD and DD groups over 24 h ($P<0.05$). These ASVs were then fitted to a 24-h cosine model using nonlinear least squares. Based on rhythmicity parameters, the possible rhythmic changes in abundance due to light exposure under circadian clock control could be categorized as follows: no change, loss or gain of rhythmicity, dampening or increase in the amplitude, phase advancement or delay and base shift (Lopez et al., 2021). The cosinor model used in CircaCompare can be described by the following equation (Parsons et al., 2020):

$$\hat{Y}=k+k_1\times X+(\alpha+\alpha_1\times X)\times\cos[r-(\varphi+\varphi_1\times X)],$$

where $\hat{Y}$ represents the response or outcome variable, which is the normalized abundance. r is the time in radians. Using the summary function from the CircaCompare package, the model will output the estimated parameters (k, k_1, α, α_1, φ, and φ_1), their standard errors, P-values, and 95% confidence intervals. The estimated parameters, k, α and φ represent the mean, amplitude, and phase of the control group, respectively, while k_1, α_1, and φ_1 represent the differences between the treatment and control groups in terms of mean, amplitude, and phase. The "ggvenn" and "treemapify" packages were used to classify the rhythms in each group.

2.4 Statistical analysis

Statistical analyses were preformed using R software (v4.3.2). The vegan package was employed to calculate the α-diversity indices for each sample,

including the Shannon index and ASV richness. Statistical differences in these indices between groups were assessed using the Benjamini-Hochberg (BH) *P*-value correction and the Kruskal-Wallis test. Principal Coordinate Analysis (PCoA) based on the Bray-Curtis dissimilarity matrix was used to explore the dissimilarity of gut bacterial communities in the mud crab. PICRUSt2 was utilized to predict the functional potentials of gut bacteria (Douglas, 2018). All data visualizations were created using the "ggplot2" package (http://www.r-project.org).

3 RESULT

3.1 Change of gut bacterial community under dark environment

After quality control of 16S rRNA gene amplicon sequencing data, a total of 13 123 396 high-quality sequences were generated from 144 gut samples of mud crabs, with an average of 91 135±14 666 reads for each. After normalization, a minimum 52 398 reads per sample was used for subsequent analysis. Measurement of α-diversity showed no significant differences in the Shannon index between two groups during 48 h (P>0.05) (Fig.1b). By contrast, the bacterial community in DD group presented a significantly increased ASV richness relative to that in LD group at ZT6/CT6 (P<0.05) (Fig.1c). We found that the overall bacterial community composition did not differ at Zeitgeber time intervals (Supplementary Fig.S1). However, the significant and detectable differences at the phylum/Proteobacterial class level were observed (Fig.1d). In detail, in both the LD and DD groups, the gut bacterial communities were dominated by Proteobacteria classes, Fusobacteria and Bacteroidetes, with Epsilon-proteobacteria, Fusobacteria and Bacteroidetes accounting for over 60% of the communities across all groups and time points. Compared to the LD group, the DD group showed an increase in Epsilon-proteobacteria and a decrease in Fusobacteria and Bacteroidetes. In addition, dark environment can also increase the abundance of Spirochaetes. For example, at ZT48/CT48, the relative abundance of Spirochaetes in the DD group (5.3%) was significantly higher than that in the LD group (1.9%) (P<0.05).

3.2 Change in circadian rhythms of gut bacterial community in dark environment

We found that 141 ASVs of the total of 1 565 ASVs presented circadian rhythms in the gut bacterial community of the mud crabs in LD group. In comparison, 148 ASVs of the total of 1 662 ASVs in the DD group displayed circadian rhythms. Dark environment induced six categories of rhythmic changes of ASVs, including no effect (nine ASVs), loss of rhythmicity (141 ASVs), gain of rhythmicity (130 ASVs), amplitude change (one ASV), phase shift (six ASVs) and base shift (two ASVs) (Fig.2a & b). Obviously, most of ASVs lost or gained rhythmicity under dark environment with only 18 ASVs maintaining a significant oscillation (P<0.05) (Supplementary Fig.S2 & Table S1). From the perspective of relative abundance, ASVs belonging to both the rhythmic and arrhythmic categories dominated the rhythmic changes. Notably, the ASVs in both categories fluctuated significantly during the dark environment period in the DD group. At CT6, the relative abundances of ASVs belonging to the rhythmic and arrhythmic categories were 2.0% and 3.7%, respectively. Overall, the relative abundance of rhythmic ASVs in the DD group (3%) was lower than in the LD group (7.6%), while the relative abundance of arrhythmic ASVs in the DD group (3.9%) was slightly higher than in the LD group (3.6%) (Fig.2c).

For the ASVs within loss of rhythmicity, the Shannon index in the DD group was significantly higher at ZT24/CT24 compared to that in the LD group (P<0.05) (Fig.3a) with significantly higher ASV richness at ZT12/CT12 (P<0.05) (Fig.3b). The dark environment reduced the total relative abundances of ASVs within loss of rhythmicity at ZT6/CT6. However, the average relative abundance of Spirochaetes ASVs belonged to (mainly ASV_7 belonging to Spirochaetia) in the DD group (3.58%) was higher than in the LD group (3.50%) (Fig.3c & d). Although the ASVs within loss of rhythmicity were mainly from Bacteroidetes (33 ASVs), Alpha-proteobacteria (36 ASVs), Gamma-proteobacteria (13 ASVs), and Epsilon-proteobacteria (nine ASVs), their total relative abundance was lower than 0.2% (Fig.3c).

We next focused on the fluctuations of these ASVs lost rhythmicity under light/dark cycles and classified each ASV based on the significance of their phase. We found two fluctuation patterns including phase 1 and phase 2 for these ASVs (Supplementary Fig.S3a). Overall, the rhythm characterized by phase 1 displayed a cyclical pattern of rising, followed by falling, and then rising again over a 24-h period, with the peak typically occurring at ZT6. The ASVs belonging to Bacteroidetes (27)

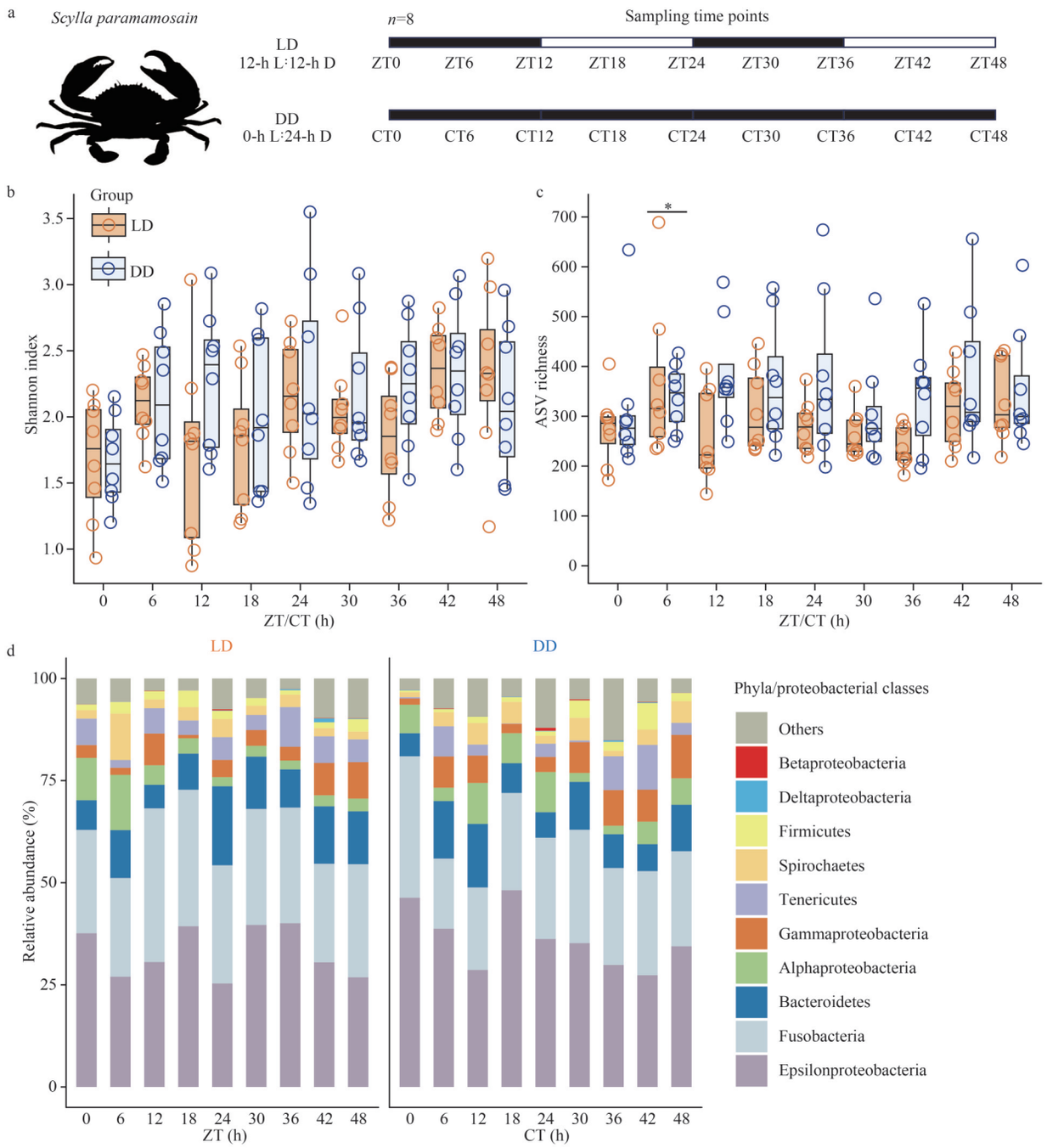


Fig.1 Experimental design and the changes in the α-diversity and composition of gut bacterial communities of the mud crab caused by dark environment

a. sampling strategy. The black part of the bar represents darkness whereas the white part represents light; b. Shannon index; c. ASV richness. * means $P<0.05$; d. average relative abundances of phyla/proteobacteria classes. ZT: the diurnal rhythm under 12-h L:12-h D light/dark cycles; CT: the free-running rhythm under 0-h L:24-h D, the same hereinafter.

and Alpha-proteobacteria (21) played a dominant role in contributing to this rhythm (Supplementary Fig.S3b). It is worth noting that both Spirochaetes and SR1 only exhibited the rhythm represented by phase 1. By contrast, the rhythm represented by phase 2 exhibited the opposite trend to phase 1. The ASVs belonging to Verrucomicrobia only exhibited the rhythm represented by phase 2, peaking at ZT42.

For the ASVs within gain of rhythmicity, dark environment resulted in the significantly increased Shannon index at CT24 and CT42 ($P<0.05$) (Fig.4a), but the highly decreased ASV richness at

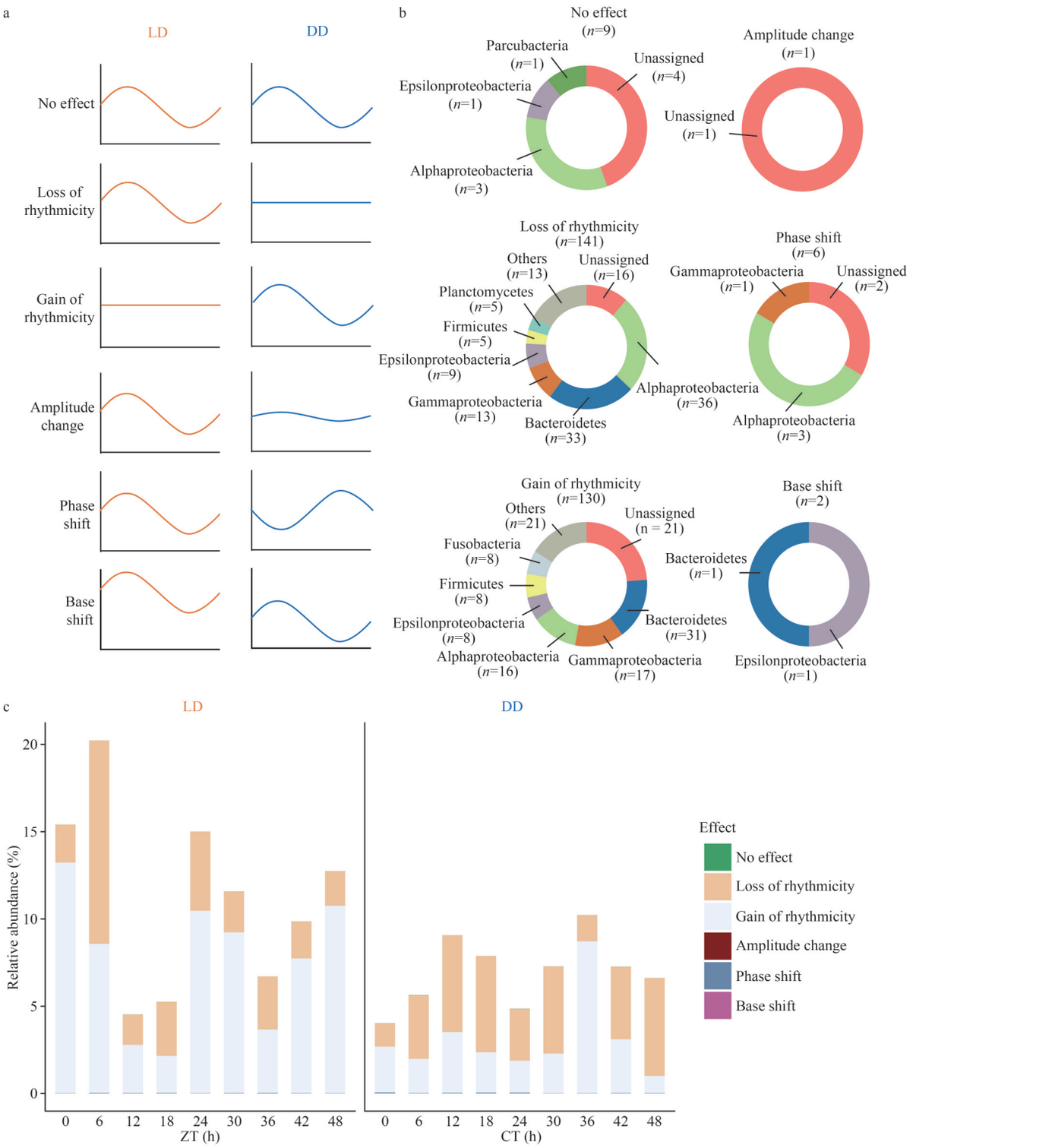


Fig.2 Effect of dark environment on the circadian rhythms of gut bacterial community in the mud crab

a. possible changes to rhythmicity include no effect, loss or gain of rhythmicity, dampening or increase in the amplitude, phase advancement or delay, and base shift; b & c. numbers and relative abundances of ASVs within six rhythm categories, respectively.

CT6, CT24, and CT42 (Fig.4b). From the perspective of community composition, dark environment decreased the total relative abundances of ASVs within gain of rhythmicity during 48 h, except at CT12, CT18, and CT36. Among them, the relative abundance of Alpha-proteobacteria ASVs

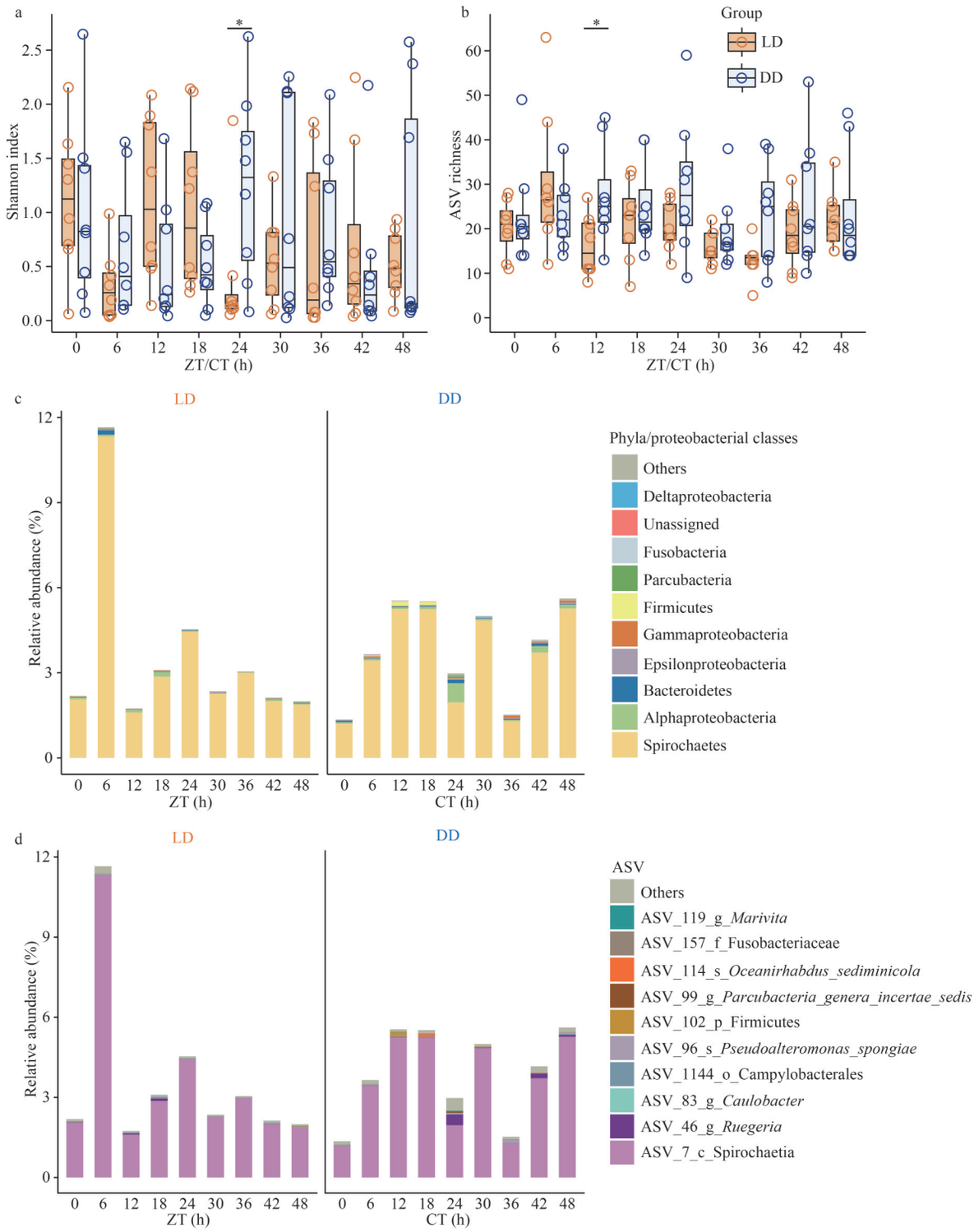


Fig.3 Alpha-diversity and composition of the ASVs that lost rhythmicity during 48 h

a. Shannon index; b. ASV richness. * $P<0.05$; c. top 10 phyla/proteobacterial classes; d. top 10 ASVs.

belonged to (mainly ASV_14 belonging to *Maritalea*) had the most decrease at CT0 and CT6. And compared to the LD group, the relative abundances of Bacteroidetes ASVs belonged to (mainly ASV_11 belonging to Bacteroidales) in the DD group remained at a lower abundance across all time points (Fig.4c & d). Furthermore, the ASVs gained rhythmicity under the constant dark

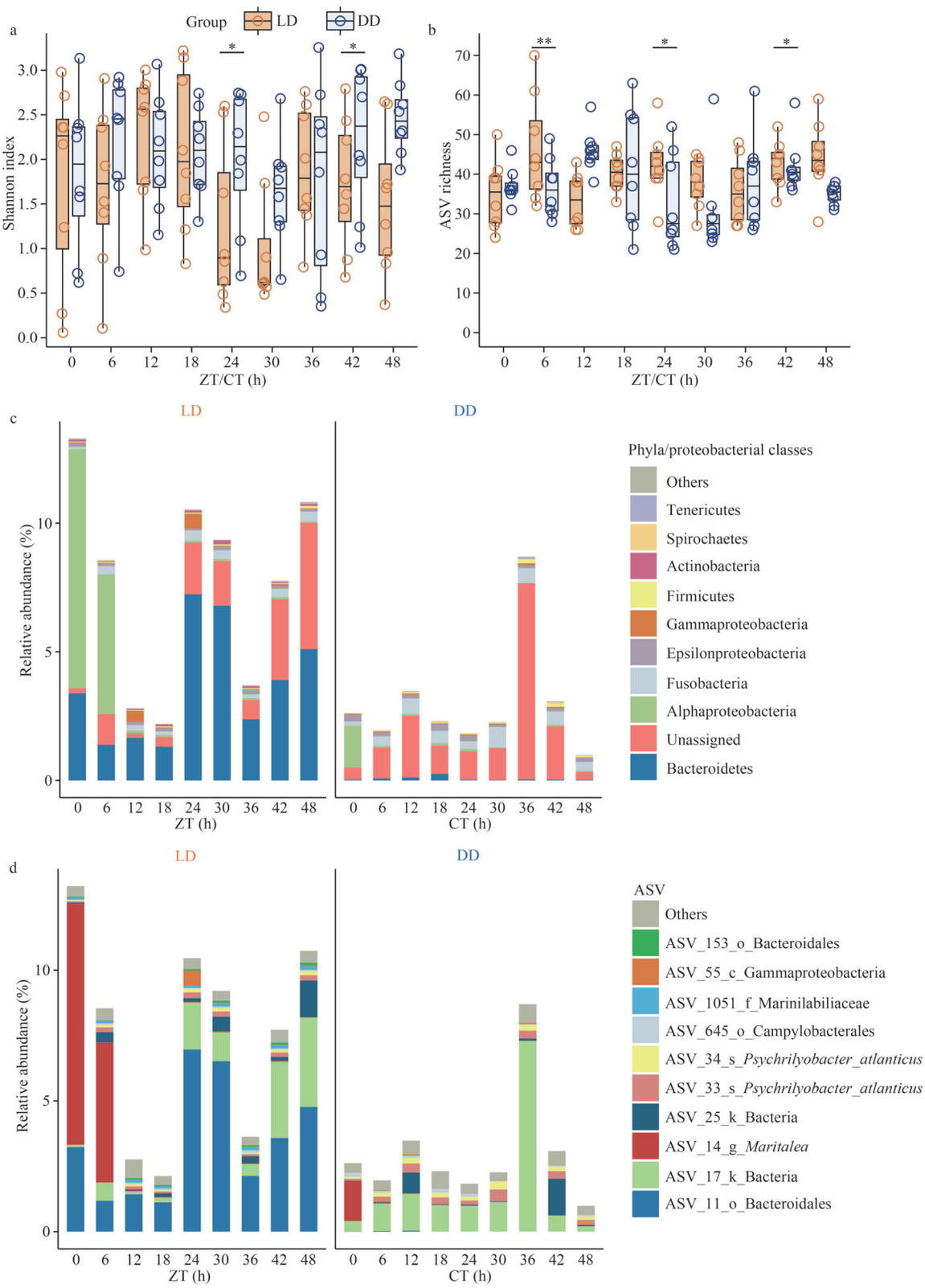


Fig.4 Alpha-diversity and composition of the ASVs that gained rhythmicity during 48 h

a. Shannon index; b. ASV richness. *: $P<0.05$, **: $P<0.01$. c. top 10 phyla/proteobacterial classes; d. top 10 ASVs.

environment also exhibited two fluctuation patterns, including phase 1 and phase 2 (Fig.5a). The rhythm represented by phase 1 exhibited a 24-h periodic fluctuation, with activity peaks occurring at CT12 and CT36 in most cases. ASVs belonging to Bacteroidetes (18), Gamma-proteobacteria (6), Firmicutes (6), and Fusobacteria (6) were the dominant contributors to this rhythm (Fig.5b). Additionally, eight phyla/Proteobacteria classes, including Delta-proteobacteria, Parcubacteria, Spirochaetes, and Verrucomicrobia only exhibited the rhythm associated with phase 1, while Actinobacteria and Planctomycetes exclusively displayed fluctuations corresponding to phase 2. Actinobacteria reached its peak at CT6 and CT24, while Planctomycetes exhibited the highest activity at CT24.

3.3 Circadian rhythm influences functional pathways related to gut bacterial community structure in the mud crab

To investigate the functions of the gut bacteria in mud crabs under LD and DD conditions, we performed functional predictions for all the gut bacteria. Results show that a total of 283 functions were involved in the gut bacteria of the LD group, while 286 functions were involved in the gut bacteria of the DD group. We presented the top 30 functions with the highest relative abundance in the LD and DD communities, most of which were related to functions and metabolism (Fig.6). We found that most functions in the DD group had higher abundances at CT12, CT42, and CT48. Although the gut bacterial functions in the DD group generally exhibited a similar trend to those in the LD group, the fluctuations are relatively stable. At CT6, the abundance of most functions, except for methane metabolism and bacterial secretion system, was significantly lower in the DD group compared to the LD group (P<0.05). In addition, at CT24, most of the functional abundances in the gut bacteria of the DD group were opposite to those in the LD group.

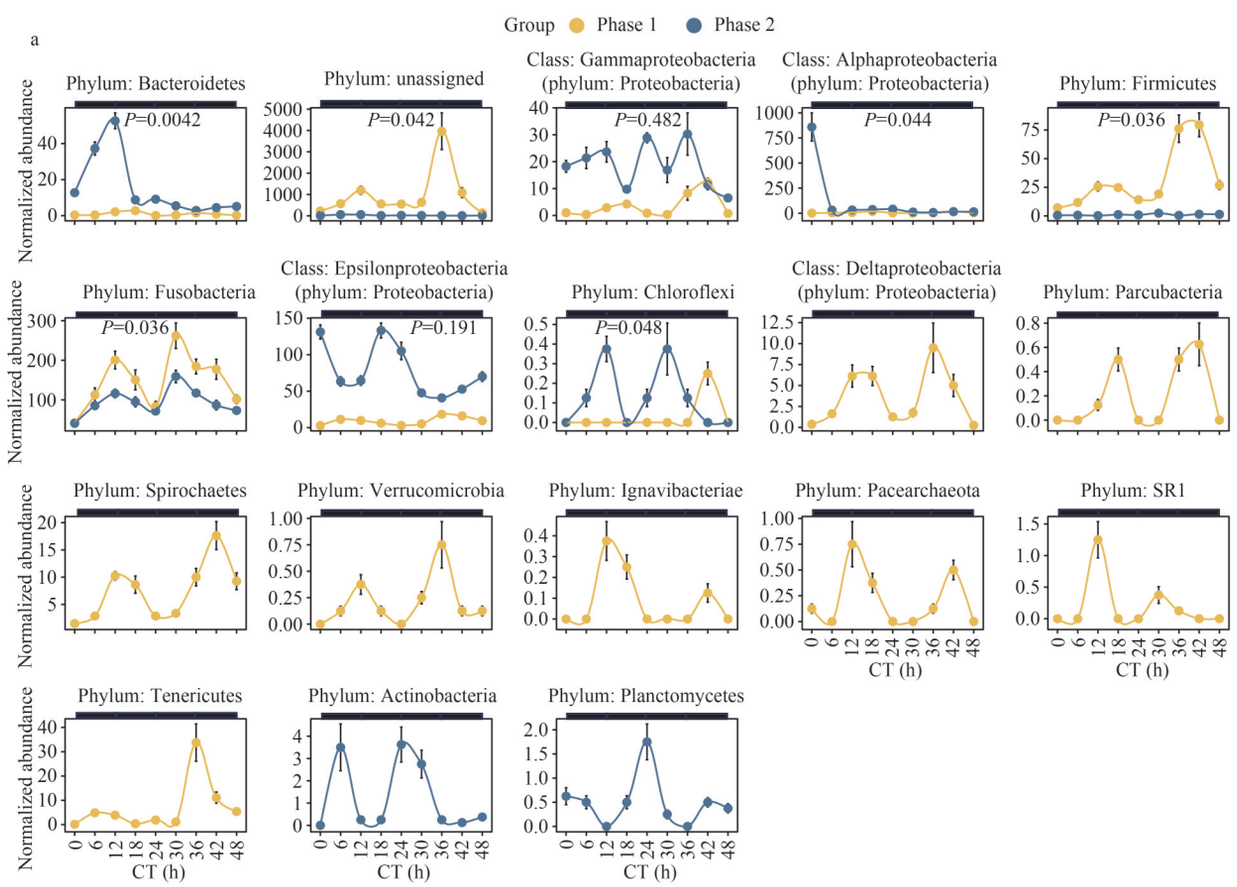
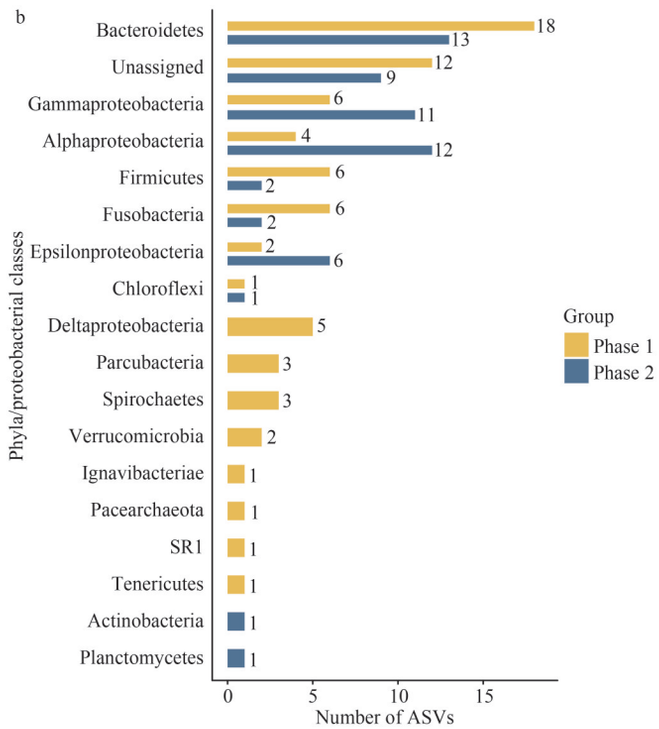


Fig.5 Diurnal oscillation (a) of the ASVs that gained rhythmicity and their numbers (b) at the phyla/Proteobacteria classes levels

P value: significance between different phases derived from cosinor analysis. The black part of the bar represents darkness.

To be continued

Fig.5 Continued



To further investigate the effect of dark environment on the functional potentials of the gut bacterial community in the mud crab. The PICRUSt2 analysis based on a cosine model was performed on the two categories of ASVs within loss of rhythmicity and gain of rhythmicity. We found that dark environment caused the loss of rhythmicity of four functional pathways, including basal transcription factors, furfural degradation, type I polyketide structures, and zeatin biosynthesis ($P<0.05$) (Supplementary Fig.S4). Meanwhile, dark environment caused the gain of rhythmicity of 12 functional pathways, including the pathways of FoxO signaling pathway, lysosome, thyroid hormone signaling pathway, non-homologous end-joining, RNA transport, betalain biosynthesis, caffeine metabolism, dioxin degradation, monoterpenoid biosynthesis, pentose and glucuronate interconversions, polycyclic aromatic hydrocarbon degradation, and sphingolipid metabolism ($P<0.05$). Among them, the abundances of the functional pathways of FoxO signaling pathway, lysosome, and thyroid hormone signaling pathway increased at both CT12 and CT30, while the abundance of polycyclic aromatic hydrocarbon degradation was lowest at CT30.

4 DISCUSSION

The regulation of food intake, nutrient absorption, lipid metabolism, and oxidative stress by photoperiod is crucial for the growth and immune response of marine organisms (Ma et al., 2021; Di et al., 2023; Goldstein et al., 2023). Early studies on the circadian rhythms of marine organisms focused on mainly fish (Ellison et al., 2021; Vera et al., 2023), and many studies have shown that the gut microbiota also exhibited circadian rhythms (Lutfi et al., 2021; Wang et al., 2024). In this work, we for the first time demonstrated the daily variations in the gut microbiota composition and structure of mud crabs under LD and DD conditions, showing that the gut microbiota exhibited circadian rhythm under both conditions, which contradicts to previous findings that gut microbiota lost its circadian rhythm under dark conditions and may be attributed to the relatively fixed feeding time in our study. Timed feeding could influence the circadian rhythm of the gut microbiota, contributing to the maintenance of a stable rhythm under dark conditions, which has been confirmed recently (Niu et al., 2024). Additionally, the short duration of the experiment may have allowed the gut, due to its intrinsic peripheral rhythm, to sustain endogenous oscillations for a period of time. We found that all rhythmic changes could be categorized into six types. Although 130 ASVs gained their rhythmicity under DD conditions, 141 ASVs lost rhythmicity, which indicates that circadian rhythms of gut microbiota

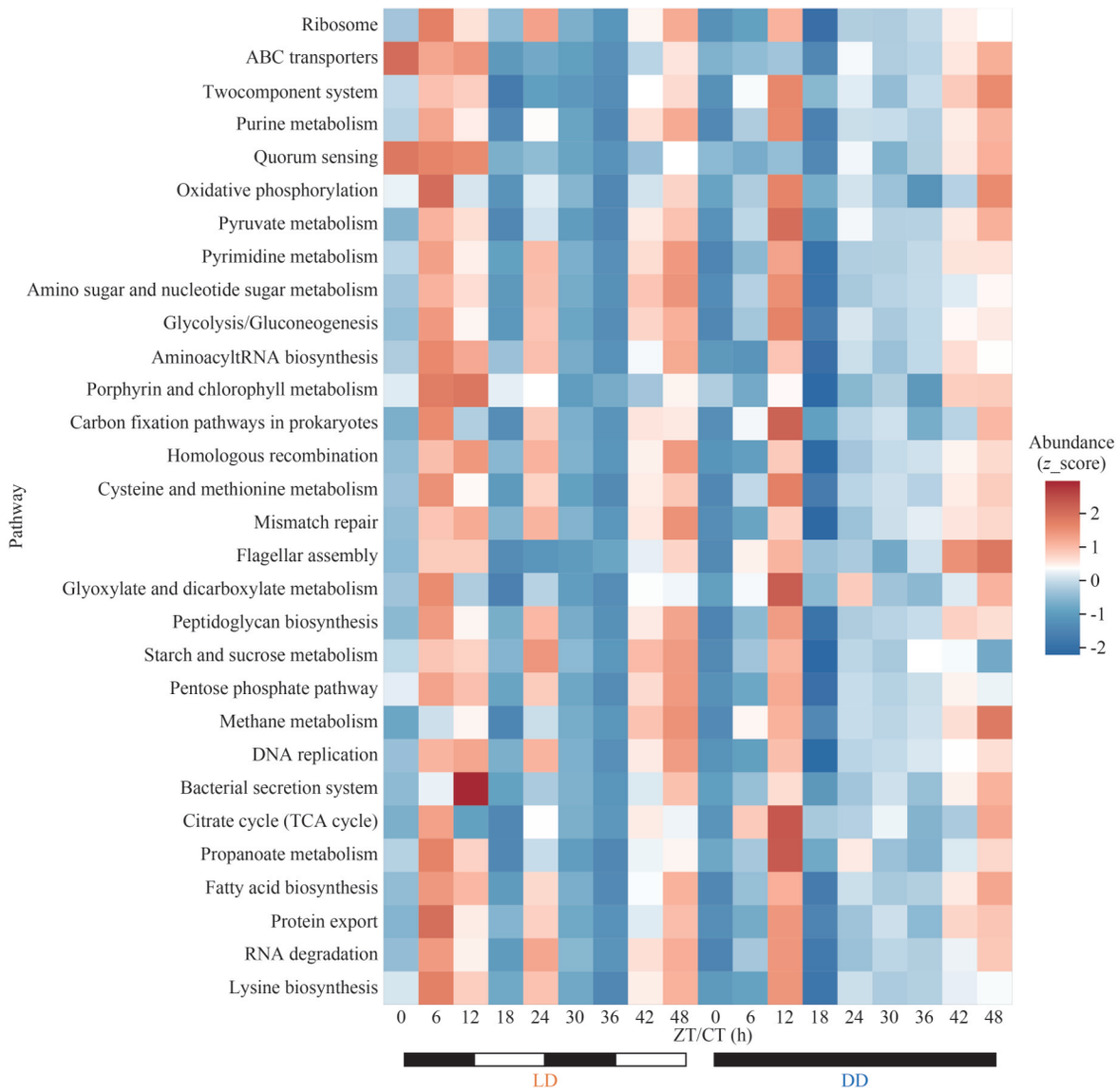


Fig.6 The dynamic changes in the top 30 functional pathways of the community over time under LD and DD conditions

Dates are expressed as mean±SEM.

composition are guided by environmental photoperiod signals. However, under DD treatment, the microbial behavior controlled by the endogenous circadian rhythm mechanism leads to the loss of rhythmicity in 141 ASVs, which may result in dysfunctions in metabolic regulation, immune response, and microbiota balance, ultimately affecting the host's health, adaptability, and growth development.

During our experiment, we found significant differences in the gut microbiota composition adapted to different light conditions under the same farming conditions. Species relative abundance analysis revealed that for the ASVs that lost their rhythmicity, the ASV_7 belonging to *Spirochaetes* exhibited significant daily fluctuations in the LD group, with its abundance dominating among all the ASVs that lost rhythmicity. Although ASV_7 significantly increased at CT36 under constant darkness conditions, its relative abundance was generally higher than in the LD group. *Spirochaetes* are among the dominant microbial taxa in the intestines of dark-spotted pufferfish (*Takifugu obscurus*) and Atlantic salmon (*Salmo salar*), often associated with parasitic infections that lead to diseases (Llewellyn et al., 2016; Egerton et al., 2020; Wei et al., 2024). Studies have shown that when organisms are exposed to infection or injury

risks, immune factors and infections can influence the expression of the molecular clock (Cavadini et al., 2007; Okada et al., 2008; De Leone et al., 2020) and the subsequent rhythmic phenotypes (Marpegán et al., 2005; Shirasu-Hiza et al., 2007). Disruption of circadian rhythms may increase the incidence of gastrointestinal diseases (Swanson and Burgess, 2017), suggesting that DD treatment may induce the proliferation of pathogenic bacteria. Bacteroidetes is one of the important phyla in the gut, playing a critical role in energy absorption, obesity and maintaining microbiota balance (Komaroff et al., 2017). In this study, we observed significant differences in the gut microbiota composition under LD and DD conditions for ASVs exhibiting rhythmicity. Under DD conditions, the relative abundance of ASV_11 from the Bacteroidales was significantly reduced. This finding is consistent with the results observed in zebrafish gut microbiota changes (Feng et al., 2021), suggesting that dark environments may negatively affect the metabolism of the mud crab.

Phase is an important parameter in the variation of circadian rhythms in microorganisms, describing the specific time points or states within the microbial biological clock cycle (Liu et al., 2024). By assessing phase significance, we refined the activity of the gut microbiota and found that certain microorganisms, including Bacteroidetes, Firmicutes, Planctomycetes, Verrucomicrobia, Actinobacteria, Parcubacteria, Alpha-proteobacteria, Gamma-proteobacteria, Delta-proteobacteria, Epsilon-proteobacteria, and Spirochaetes exhibit circadian oscillations under both LD and DD conditions, which is consistent with findings in shrimp (Jiao et al., 2021). Moreover, these microorganisms show inconsistent fluctuation patterns under LD and DD conditions, highlighting the importance of light in regulating circadian rhythms. Therefore, this study suggests that the circadian rhythms of gut microbiota in the blue crab are not synchronized with the host's biological clock under light exposure.

Our study revealed significant daily dynamics in the gut microbiota of mud crabs and provided new insights into the temporal dynamics of the gut microbiome in this species. Although research on this topic remains relatively limited, our data show that a substantial proportion of bacteria in the gut microbiome exhibited rhythmic changes in relative abundance. These oscillations reflect the microbiota's adaptive response to the light/dark cycle, indicating that microbiome functional changes follow a distinct temporal structure. Specifically, under both LD and DD conditions, the microbiota functions exhibit notable fluctuations. The light/dark cycle regulates microbial metabolism and physiological activities, helping the community optimize its growth and functional performance. This finding is consistent with previous studies, which suggest that environmental light serves as an upstream factor for the circadian rhythm, influencing microbial gene expression and metabolic pathways to ensure that the microbiota exhibits distinct functions at different times (Zhao et al., 2022a). Under the light/dark cycle, we observed oscillations in functions such as Basal transcription factors, Furfural degradation, Type I polyketide structures and Zeatin biosynthesis, all of which are closely linked to microbial adaptation, energy metabolism and secondary metabolite production (Einset, 1986; Wang et al., 2020; Osorio-González et al., 2022). In contrast, the oscillations of microbial functions under dark conditions display a more complex pattern, which may be due to circadian rhythm disruption caused by the dark environment, prompting the microbiota to adapt through more complex metabolic and stress responses in the absence of light (Lopez et al., 2021; Liu et al., 2022). Specifically, under constant dark conditions, the microbiota may rely on endogenous regulatory mechanisms, such as the FoxO signaling pathway, lysosome and non-homologous end-joining, to cope with environmental changes (Zhao et al., 2022b). These oscillations in pathways suggest that the microbiota may adopt stress responses under dark conditions to maintain metabolic homeostasis and cope with environmental stress.

Collectively, these findings highlight light as the upstream environmental factor driving circadian oscillations in the gut microbiota of mud crabs. The disruptions of circadian rhythms caused by the dark environment may negatively affect critical functional pathways involved in metabolism and immune regulation. However, all of our analyses are based on the relative abundance of microbial communities and did not involve changes at the gene expression level. Therefore, future research should further explore gene expression and metabolic network changes in the gut microbiota of mud crabs under different light conditions to better understand its role and adaptive mechanisms within ecosystems.

5 CONCLUSION

Overall, our study confirmed that the dark environment influenced the circadian rhythm of gut bacterial community in the mud crab, revealing six distinct types of microbial variations that exhibited significant day/night fluctuations. Results show that different light conditions caused notable shifts in the composition and structure of the gut microbiota, and some strains lost rhythmicity while others gained it. Importantly, as changes in microbiota structure can affect its function, we emphasized the potential role of light in regulating both the composition and functional dynamics of the gut microbiota. These functional implications strongly suggest avoiding constant dark conditions in aquaculture practice, with our experimental data specifically supporting the implementation of 12-h L∶12-h D photoperiod during the grow-out phase for optimal commercial operations. This work stimulated further exploration of the interplay between the circadian clock of mud crabs and microbial circadian rhythmicity, with significant implications for aquaculture management and gut microbiome research.

6 DATA AVAILABILITY STATEMENT

All data generated and analyzed for this study are included in this published article and additional online supplementary materials.

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Electronic supplementary material

Supplementary material (Supplementary Table S1 and Figs.S1–S4) is available in the online version of this article at https://doi.org/10.1007/s00343-025-5068-2.