



OPEN Temporal dynamics of courtship and spawning in medaka under laboratory conditions revealed by 24 h video monitoring

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Understanding the biological phenomena in model organisms typically relies on laboratory studies. However, the ecological validity of these findings is often uncertain when natural behaviors remain understudied. The medaka (*Oryzias latipes*) is a widely used model in reproductive and behavioral research; however, the timing of spawning in natural settings has rarely been directly observed. Recent fieldwork has suggested that medaka spawn several hours before sunrise, in contrast to the common assumption in laboratory-based studies that spawning occurs within an hour before or after exposure to light. In this study, we conducted continuous 24 h video recordings of medaka pairs under controlled laboratory conditions (14 L:10D photoperiod) to quantify diel variations in courtship and spawning behavior. Spawning occurred mostly between 08:00 and 11:00, peaking immediately after lights were turned on (08:00). Courtship behavior began during the dark period, increased before lights-on, and peaked between 07:00 and 09:00. These results exhibited a consistent delay of 3–4 h compared to (semi-)natural conditions. Our results highlight the influence of environmental conditions on reproductive timing in medaka. The temporal shifts observed in this study underscore the importance of incorporating natural temporal dynamics into experimental protocols to ensure accurate behavioral studies with model organisms.

Keywords Spawning, Courtship, Model organism, Medaka, *Oryzias latipes*, Video observation

Model organisms play a central role in elucidating biological mechanisms, often through studies conducted in highly controlled laboratories, which enable the precise control of environmental variables and facilitate fundamental discoveries in biology. However, the extent to which such findings generalize to natural environments remains uncertain, as these controlled conditions often omit key ecological factors, resulting in gaps in our understanding of behavior^{1,2}. This disconnection raises critical concerns regarding the ecological validity and scientific rigor of laboratory-based research. Environmental differences between laboratory and field conditions can induce substantial shifts in behavioral and physiological responses. The relevance of observed patterns to natural systems becomes questionable when experimental settings fail to replicate key ecological variables². In response, there is a growing recognition of the need for comparative studies in which behavioral and physiological traits are evaluated in both laboratory and field conditions^{3–7}.

These concerns are particularly relevant for chronobiological research using model organisms, where laboratory-imposed light/dark cycles may distort natural timing mechanisms. Circadian rhythms and diel activity patterns are approximately 24 h biological rhythms driven by endogenous clock mechanisms, serving as fundamental regulatory mechanisms for physiological functions and behaviors across animal taxa^{8–10}. In many fish, birds, and mammals, various behaviors including foraging, locomotion, predator avoidance, and reproductive activities are synchronized with environmental rhythms such as photoperiod and temperature. These behaviors are controlled by the interactions between internal clocks and external stimuli. Activity patterns such as nocturnal or diurnal behaviors are understood as adaptations to reproduction, predation pressure, and the availability of food resources^{8–10}. Therefore, evaluating behavioral rhythms recorded under laboratory conditions within ecological contexts that consider natural conditions and adaptive backgrounds is essential for understanding animal behavior and its underlying mechanisms.

Despite its long history as a model organism, the ecological biology of medaka (*Oryzias latipes*), particularly its reproductive ecology, remains poorly understood in natural settings⁴. Medaka are widely used in physiology,

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genetics, developmental biology, behavioral science, and biomedical research due to their small body size, ease of rearing, pronounced sexual dimorphism, short generation time, transparent eggs, and a compact genome^{11,12}. Due to the ease of observation, numerous studies on mate choice^{13–16}, mate guarding behavior^{17–19}, and alternative reproductive tactics^{20–22} have been conducted in laboratory settings. Importantly, medaka exhibit robust circadian rhythms that regulate physiological and behavioral processes, including reproduction, which are entrained by environmental light-dark cycles^{23–28}. However, these features are the products of natural and sexual selection, and their functional significance cannot be fully understood without observations under ecologically relevant conditions.

Conventional laboratory studies have suggested that medaka typically initiate mating within 1 h before or after the onset of light^{29–32}. However, these conclusions were largely drawn from indirect methods, such as the developmental staging of fertilized eggs, or visual observations restricted to daylight hours, leaving the exact timing of spawning initiation unresolved. Although laboratory studies have shown that ovulation in females is completed at night³², recent studies have demonstrated that general activity levels begin to increase several hours before the onset of light^{26,27}. The results of our recent video-based observations conducted in the field and semi-natural settings have challenged this conventional understanding. In the field, post-spawning females were recorded as early as midnight, before sunrise at approximately 05:00²⁸. Similarly, under semi-outdoor conditions, peak spawning occurred between 02:00 and 04:00, before sunrise at approximately 04:45³³. These findings strongly suggest that spawning in natural environments begins several hours before sunrise, in contrary to the assumptions made in laboratory-based studies.

These findings prompt a critical reassessment of the timing and context of reproductive behavior in medaka and underscore the importance of integrating laboratory and field perspectives. Quantifying the behavioral differences between these settings is essential for optimizing experimental protocols that better reflect natural conditions and for designing studies that elicit ecologically meaningful behaviors. To date, there have been no published reports on the precise timing of spawning initiation in medaka in laboratory aquariums, and systematic comparisons with field data are limited. Bridging this gap is particularly crucial in model organism research, in which both scientific rigor and ecological validity must be maintained to ensure meaningful inferences in both the basic and applied sciences.

This study was designed to investigate the temporal dynamics of courtship and spawning in laboratory-reared medaka. To minimize confounding effects caused by genetic divergence, we used a uniform commercial strain (“himedaka”) commonly used in laboratory research. This allowed us to assess the environmental modulation of reproductive behavior by comparing 24 h behavioral observations conducted under controlled indoor and semi-outdoor conditions^{28,33}. Specifically, 24 h behavioral observations were carried out to (1) identify the female spawning initiation time and (2) elucidate the temporal changes in the intensity of male courtship behavior. By comparing the results obtained in the present study with those of our previous field and semi-field observational studies^{28,33}, we aimed to clarify the differences in courtship and spawning behaviors between laboratory and (semi-)natural environments and to examine the factors that affect these differences. Ultimately, the results of this study contribute to a more ecologically grounded interpretation of laboratory findings and inform the development of experimental protocols that incorporate natural temporal patterns.

Methods

Study fish and rearing

To ensure a direct comparison with our previous semi-outdoor study, the same Himedaka strain was selected from the same local pet shop in Kondo and Awata (2025)³³. This commercially available strain is widely used in laboratory studies^{11,12} to enhance the reproducibility and comparability of our findings with those of other studies. These individuals were maintained under the following conditions for one month before being used in experiments: Eight breeding tanks (90.5 × 60.5 × 21.0 cm, length × width × height) were set up in the laboratory, with approximately 100 individuals housed in each tank. Water temperature was maintained at 26 ± 1 °C, and a 14 L:10D photoperiod was used (lights on at 08:00 and off at 22:00). Fish were fed Tetramin (Tetra, Melle, Germany) three times per day. Spawning activity was monitored daily during the acclimation period.

Experimental settings and video recording

Experiments were conducted from February 5 to 15, 2025, under the same temperature and photoperiod conditions as those described above, following the protocol described by Kondo and Awata (2025)³³. Experimental tanks (22.5 × 15.8 × 5.5 cm; L × W × H) were filled with 950 mL of water, resulting in a depth of approximately 2.7 cm.

Each night, between 20:00 and 21:00, one male and one female from each breeding tank were selected and introduced into the experimental tank at 21:00. A total of 35 male–female pairs (2–4 pairs per day) were tested (Supplementary Table S1).

Continuous 24 h video recordings were performed using an AURORA PRO C011300 camera (SiOnyx, Beverly, MA, USA) equipped with a 512 GB SD card (SanDisk, Milpitas, CA, USA). Nighttime recordings (22:00–08:00) were conducted in infrared mode using 940 nm illumination (EnergyPower, Hong Kong, China), which falls outside the visual sensitivity range of medaka and does not affect their behavior^{34–37}. Daytime recordings (08:00–22:00) were performed in standard mode. No food was provided during the experiments.

At the end of the 24 h period (21:00 the following day), the fish were anesthetized by immersion in a solution of FA100 (DS Pharma Animal Health, Osaka, Japan) diluted 1:2,000 (0.25 mL per 500 mL of water), and body mass was measured with an electronic balance (HT-120, A&D, Tokyo). After the measurements were taken, the individuals were returned to their original breeding tanks. Each animal was examined at least once.

Behavioral analysis

Video data were analyzed using ELAN version 6.8 annotation software. To assess diel behavioral variation, 10 min video clips were extracted from the 20–30 min segment of each hour, yielding 24 clips per pair of medaka. Previous studies have shown that spawning behavior in medaka typically follows a stereotypical sequence³⁸: (1) following, in which the male follows the female; (2) quick circle, in which the male swims rapidly around the female; (3) wrapping, in which the male encircles the female with its dorsal and anal fins; (4) egg and sperm release; and (5) leaving the female. After spawning, females carry eggs attached to their abdomen and deposit them on the aquatic vegetation. Behavioral sequences during spawning, including egg and sperm release, were classified according to established criteria, and spawning time was defined as the moment of initiation of the spawning act, which was visually identified from the recordings^{33,39}. Furthermore, based on previous work^{28,33}, we quantified two courtship behaviors from each 10 min clip: (1) the total duration of the following and (2) the frequency of quick circles.

Statistical analysis

The mean body mass of males was 0.29 g (SD = 0.01; range: 0.21–0.39; $n = 35$), and that of females was 0.31 g (SD = 0.01; range: 0.23–0.50; $n = 35$; see Supplementary Table S1).

All statistical analyses were conducted using R version 4.4.1 (R Core Team 2024)⁴⁰. To estimate peak spawning timing, we fitted a gamma distribution to the observed spawning events. Temporal changes in courtship behavior (following duration and quick-circle frequency) were analyzed using the generalized additive mixed models (GAMMs) implemented in the *mgcv* package. Behavioral data were quantified from 10 min video segments extracted hourly. For duration analysis, a Gaussian distribution was used. For the quick-circle frequency analysis, a negative binomial distribution was used to account for overdispersion. In both models, the time of day (hours) was included as a smooth function, and male ID was specified as a random intercept effect. Model significance was assessed using likelihood ratio tests, with statistical significance defined as $p < 0.05$.

Results

Spawning behavior

A total of 35 spawning events were recorded between 07:23 and 13:47 (Supplementary Movie S1). The majority (25 of 35 events, 71%) occurred between 08:00 and 11:00, with a distinct peak shortly after the lights were turned on at 08:00 (Fig. 1a; Supplementary Table S1). Twelve events (34%) occurred within 1 h of or after lights were turned on. In total, 31 spawning events (89%) occurred after light onset, whereas only four events (11%) occurred before light onset.

Courtship behavior

To examine diel variations in courtship behavior, we analyzed 24 h recordings of all 35 spawning pairs. “Following” was observed consistently throughout the day, with an average duration of 181.8 ± 6.0 s per 10 min video segment ($n = 840$ observations from 35 males; mean $\pm$ SE; Supplementary Movie S2). The following duration increased during the dark period, peaked between 07:00 and 09:00, and then decreased (GAMM: deviance = 4075648; $df = 8.75$; $p < 0.0001$; Fig. 1b).

“Quick circle” occurred 476 times in total, averaging 0.57 ± 0.07 occurrences per video segment ($n = 840$; Supplementary Movie S3). The temporal pattern of the quick circle mirrored the following: it increased before light onset, peaked between 07:00 and 09:00, and declined substantially after 12:00 (GAMM: deviance = 102.56; $df = 7.71$; $p < 0.0001$; Fig. 1c). Most post-mating males did not perform a quick circle after 12:00.

Discussion

Reproductive time is a key ecological trait that significantly influences fitness. In medaka, spawning has been reported to occur within 1 h before or after light exposure^{29–32}; however, these conclusions were based on indirect observations rather than direct monitoring under dark conditions. In the current study, we provided the first direct evidence of spawning initiation and courtship rhythms under controlled laboratory conditions, using continuous video surveillance. Our findings are consistent with previous reports that spawning primarily occurs shortly after lights-on; however, we observed that courtship behavior begins during the dark period. This previously undocumented nocturnal initiation of courtship behavior represents a significant revision to the conventional understanding of medaka reproductive behaviors in laboratory settings.

Our 24 h continuous video monitoring methodology revealed that medaka initiate courtship activities during the dark period, a phenomenon that has not been previously reported in laboratory settings. Although it has been reported previously that the general activity level of medaka increases several hours before lights-on^{26,27}, our detailed behavioral analysis has, for the first time, identified specific reproductive behaviors that drive the pre-dawn period. This methodological advancement allowed us to document the complete chronological sequence of reproductive behaviors, providing a behavioral foundation for previously unexplained activity patterns. Moreover, prior research has shown that medaka can spawn in the dark⁴¹ and that olfactory cues play an integral role in reproductive process⁴². The occurrence of nocturnal courtship suggests that medaka can detect and respond to reproductive cues via non-visual modalities, most likely by relying on olfaction.

A key finding of this study was the consistent 3–4 h delay in the timing of courtship and spawning behaviors in laboratory settings compared to field and semi-natural environments^{28,33}, despite similarities in overall behavioral patterns. These results strongly indicate that environmental conditions play a primary role in modulating reproductive timing. Previous studies have shown that medaka populations from different geographic regions exhibit variation in the environmental cues that trigger reproduction, such as temperature and photoperiod⁴³. Genetic analyses have identified candidate genomic regions associated with these circadian

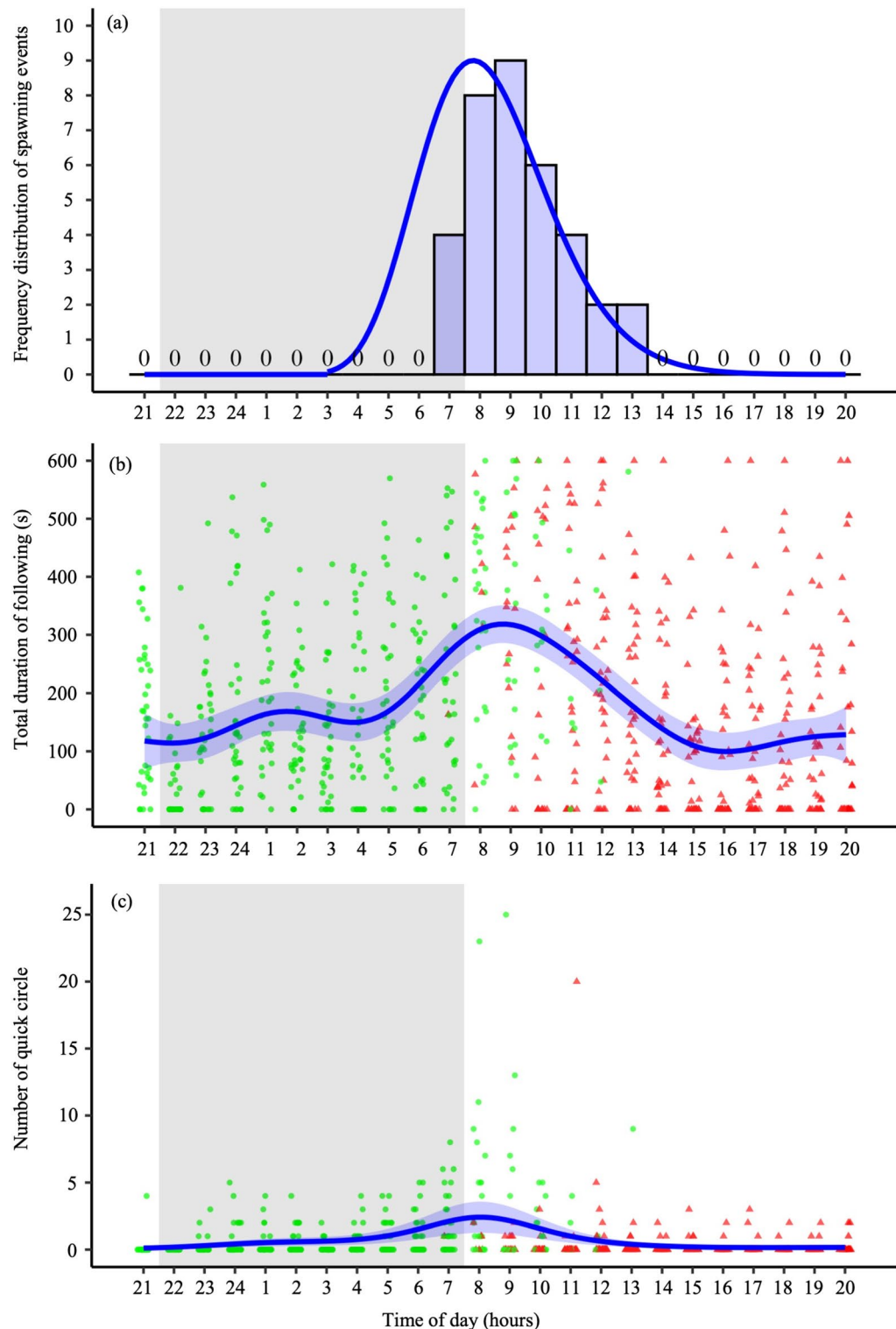


Fig. 1. (a) Frequency distribution of spawning events observed in each hour over 24 h in medaka, *Oryzias latipes* ($n = 35$). The peak of spawning events was determined using gamma distribution fitting. (b) and (c) Changes in courtship behaviors from 21:00 to 20:00 over 24 h. (b) The total following duration (sec/10 min) and (c) the number of quick-circle behaviors (number/10 min). Each plot (green circle: pre-mating male; red triangle: post-mating male) signifies the observed values from the analyzed videos. The regression curves were based on the generalized additive mixed models (GAMMs) using all data ($n = 840$ observations from 35 males), and the shading indicates the 95% confidence intervals.

rhythms⁴⁴, and additional studies have reported differences in fecundity among populations along with their genetic underpinnings⁴⁵. These findings highlight the potential contribution of genetic background to the reproductive traits of medaka. However, in the current study, identical commercial medaka strains (himedaka) from the same source were used under both laboratory and semi-natural conditions³³. Similar experimental procedures were applied, including 24 h video monitoring, comparable tank dimensions, and uniform behavioral analysis protocols. The main difference lies in the environmental conditions: artificial lighting with abrupt on/off cycles and constant temperature in the laboratory, versus natural photoperiod with gradual light transitions and fluctuating ambient temperatures in semi-natural conditions. The consistent behavioral delay observed under laboratory conditions, despite genetic and methodological uniformity, supports the conclusion that environmental factors, rather than genetic variation, are responsible for the observed temporal shift in reproductive behavior.

Several factors may explain these observed temporal shifts. The most immediate explanation involves a discrepancy between the artificial light cycle in the laboratory (lights on at 08:00 and lights off at 22:00) and the natural timing of sunrise and sunset. As medaka behavioral rhythms are highly sensitive to photoperiod, entrainment into this artificial schedule likely influences the timing of reproductive behaviors^{41,43,44}. Given the known role of thyroid-stimulating hormones in the photoperiodic regulation of seasonal reproduction in birds, mammals^{46–48}, and fish⁴⁹, such shifts in photoperiodic signaling could also modulate reproductive timing in medaka.

However, photoperiod differences alone may not fully account for temporal shifts. In natural environments, ambient light intensity gradually increases during the pre-dawn period, whereas laboratory lighting typically operates on an abrupt on/off cycle. This qualitative difference may have affected the onset of behavioral expression. Diurnal temperature fluctuations may have also contributed to this phenomenon. Although natural habitats exhibit temperature minima during the night and early morning, our laboratory setup maintained water temperature at a constant 26 ± 1 °C. As temperature influences both physiological processes and behavioral rhythms across taxa, it is plausible that thermal stability affects spawning timing. Given that temperature has been identified as a critical cue in the seasonal reproduction of bony fish^{24,50}, even endothermic species^{51–53}, the absence of naturalistic thermal variability in laboratory settings may further distort reproductive timing.

This study has certain limitations. First, our findings were based solely on a single laboratory strain (himedaka), and caution should be exercised when extrapolating these results to wild populations. Second, although our results demonstrated temporal patterns in reproductive behavior, we did not assess the day-to-day repeatability of these behaviors in individuals. Repeated observations of the same individual over multiple days are valuable to evaluate the consistency and robustness of individual reproductive rhythms. Third, although we determined the timing of spawning, the exact timing of ovulation was not directly measured. Although previous studies have suggested that ovulation is completed at night³², the precise timing under laboratory conditions remains unknown. Further studies employing fine-scale hormonal or physiological monitoring are needed to elucidate the relationship between ovulation and courtship. Finally, the adaptive value of nocturnal reproduction, such as the avoidance of visual predators, warrants further investigation. Assessing the risk of predation on medaka and their eggs across diel cycles in natural environments would provide insights into the potential selective pressures shaping reproductive timing.

By showing that courtship initiation during the dark period occurs in both laboratory and field environments, albeit with a consistent time lag, this study highlights the need to bridge experimental and ecological contexts^{1,2}. The 3–4 h delay in reproductive timing observed in this study exemplifies such a gap between laboratory and field conditions. These temporal mismatches may have far-reaching implications for experimental design, particularly for the investigation of diel variations in hormone secretion, gene expression, and reproductive behavior in medaka. Our findings suggest that sampling schedules and analytical frameworks should account for field-based temporal dynamics to avoid systematic biases in model organisms. In conclusion, our study demonstrates that medaka initiate reproductive behaviors earlier in (semi-)natural environments than in laboratory conditions, primarily because of differences in environmental cues such as light transitions and temperature. These findings underscore the need to integrate ecologically relevant parameters into experimental protocols involving model organisms.

Data availability

Data are included as Supplementary Information.

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Author contributions

Y. Kondo, R. K., Y. Kobayashi and S. A. designed the study. Y. Kondo conducted aquarium experiments and collected data. Y. Kondo and S. A. performed the analyses. Y. Kondo, R. K., Y. Kobayashi and S. A. wrote and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical standard

All experimental procedures were conducted in accordance with the ARRIVE guidelines⁵⁴ and with the approval of Osaka Metropolitan University (Approval Number: S0092). All procedures involving animals adhered to the most recent ASAB/ABS ethical guidelines and were approved by the Animal Care and Use Committee of the Osaka Metropolitan University, Japan. Housing and experimental methodologies were developed to minimize stress in medaka. After the experiment, the fish were returned to their original tanks. We did not observe any behaviors indicative of stress.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-11082-y>.

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