A Staggered L1 Plating Strategy Enables Daily Availability of Synchronized *Caenorhabditis elegans* L4 Worms

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Article

A Staggered L1 Plating Strategy Enables Daily Availability of Synchronized *Caenorhabditis elegans* L4 Worms

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Abstract

Caenorhabditis elegans (*C. elegans*) is a non-parasitic roundworm, widely used as an animal model in biological research due to its genetic tractability, short lifecycle, and suitability for high-throughput experimental studies. Synchronization is commonly employed strategy to obtain age-matched *C. elegans* populations, particularly at the L4 stage for experimental studies. However, the developmental interval required for L1 larvae to reach the L4 stage limits flexibility for spontaneous experiments. Furthermore, conventional synchronization protocols become time-consuming, labor-intensive, and repetitive when multiple experimental replicates are required over consecutive days. Here, we present a staggered L1 plating strategy that enables the daily availability of synchronized L4 worms. In this approach, a large worm population is bleached to obtain growth-arrested L1 larvae, which are then plated in fractions onto NGM plates seeded with paraformaldehyde-killed OP50 at 0, 1, 2, 3, 4, 5, and 6 days post-hatching. Our results show no significant differences in body length, body volume, brood size, embryonic viability, or locomotion (body length per second [BLPS] and body bends per second [BBPS]) among worms plated at different time points. Together, these findings demonstrate that this method provides a simple, efficient, and reproducible strategy for ensuring daily access to synchronized L4 populations, thereby facilitating both routine and spontaneous experiments.

Keywords: *Caenorhabditis elegans*; synchronization; L4 stage; growth-arrested L1 larvae; bleaching; daily availability; brood size; locomotion

1. Introduction

Caenorhabditis elegans (*C. elegans*) is a free-living, non-parasitic nematode widely recognized as a powerful model organism in biological and biomedical research [1,2]. Its wide usage is attributed to several advantageous features, including a fully mapped cell lineage [3,4], well-annotated genome [5,6], short life cycle (~3 days at 20 °C) [7], optical transparency [4,8], and ease of genetic manipulation [9,10]. These characteristics have enabled their extensive use in diverse areas such as developmental biology [3,4,11], neurobiology [12,13], aging [14,15], metabolism [16,17], toxicology [18,19], and disease modeling [20,21]. Furthermore, the availability of numerous mutant and transgenic strains, along with standardized culture techniques, has established *C. elegans* as a robust and versatile experimental system [6,7].

A fundamental requirement for most *C. elegans* experiments is the use of a **synchronous population**, as developmental stage-specific differences can significantly influence physiological, molecular, and behavioral readouts. Heterogeneous populations often introduce variability and

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reduce reproducibility, particularly in studies involving gene expression profiling, lifespan analysis, stress responses, and behavioral assays [7,22]. Therefore, synchronization of worm populations has become a critical step in experimental workflows. Historically, several methods have been employed to achieve synchronization. Early approaches relied on **manual selection of worms at specific developmental stages using pipetting or worm picks**, which, although effective, are labor-intensive and prone to operator bias [23,24]. To improve throughput, **filtration-based methods** were introduced to separate worms based on size using mesh filters, enabling enrichment of specific larval stages [25,26]. Another commonly used strategy involves the use of **5-fluoro-2′-deoxyuridine (FUdR)**, which inhibits DNA synthesis and prevents progeny production, thereby maintaining age-synchronized adult populations [27]; however, FUdR has been reported to influence lifespan and stress responses, potentially confounding experimental outcomes [23,28]. More recently, microfluidics-based approaches and automated sorting systems have also been explored to achieve high-precision synchronization [29]. Among all approaches, **alkaline hypochlorite treatment (bleaching)** followed by starvation-induced L1 arrest remains the most widely adopted method due to its simplicity, scalability, and ability to generate highly synchronized populations [7,22,30,31]. A defining feature of this method is the induction of **L1 developmental arrest under starvation**, also known as L1 diapause. In the absence of food, newly hatched larvae halt development and enter a metabolically regulated quiescent state that enables survival for extended periods [32,33]. This state is actively controlled by nutrient-sensing and energy homeostasis pathways, including **AMPK, insulin/IGF-1 signaling (DAF-2/DAF-16), and TOR signaling**, which collectively shift cellular processes from anabolic growth to energy conservation [17,32,33]. Reduced metabolic rates during this stage are strongly associated with enhanced survival, highlighting the importance of metabolic regulation in L1 arrest [32]. L1 starvation is accompanied by several **physiological and cellular adaptations**, including global cell cycle arrest, suppression of germline proliferation [34], activation of stress response pathways [35], and utilization of internal energy reserves such as lipids [32]. Short-term starvation is generally reversible, allowing worms to resume normal development upon refeeding with minimal impact on morphology, behavior, or reproduction [35,36]. In contrast, **prolonged starvation can lead to cumulative physiological stress**, including delayed recovery, increased reactive oxygen species (ROS), protein aggregation, and reduced reproductive fitness [32,35]. In more extreme or repeated starvation paradigms, long-term metabolic and transgenerational effects have also been reported, mediated through pathways such as insulin signaling [37].

Despite the widespread use of L1 arrest for synchronization, an important yet underexplored question is whether **short-term starvation (≤ 7 days)** impacts the developmental and physiological integrity of *C. elegans* larvae. In routine laboratory practice, L1 larvae are often maintained under starvation for varying durations prior to use, raising the possibility that such differences may influence downstream outcomes [7,22,32,35]. While prolonged starvation is known to induce metabolic stress and impair recovery, it remains unclear whether **short-term L1 arrest within a practical experimental window** leads to measurable alterations in growth, reproduction, or behavior. Addressing this question is particularly important for determining whether starved L1 larvae can be **reliably used as equivalent to freshly hatched, non-starved synchronized L1 populations** across multiple days. Establishing this would enable a **continuous, week-long availability of synchronous L1 worms** from a single synchronization event, thereby reducing repetitive labor and improving experimental flexibility and reproducibility.

In the present study, we investigated the impact of **staggered L1 plating on developmental, physiological, and behavioral outcomes in** *C. elegans*. Synchronized L1 larvae were generated via bleaching and maintained under starvation, followed by plating at defined intervals (0-6 days post-hatching). We then assessed key phenotypic parameters, including body length, body volume, brood size, embryonic viability, and locomotion (BLPS and BBPS), to determine whether variations in short-term starvation duration influence organismal outcomes. Our findings provide a practical and

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reproducible strategy for achieving **daily availability of synchronized L4 worms**, while maintaining physiological consistency across experimental time points.

2. Materials and Methods

2.1. Reagents

2.1.1. Bleaching Solution (2X)

The bleaching solution was prepared as previously described [38]. For the preparation of 2X bleaching solution, 0.3 mL of 4% sodium hypochlorite was mixed with 0.625 mL of 1 M NaOH and 0.125 mL of double-distilled water to obtain a final volume of 1 mL.

2.1.2. M9 Buffer

M9 buffer was prepared as previously described [38]. For M9 buffer preparation, Na_2HPO_4 (0.6 g), KH_2PO_4 (0.3 g), and NaCl (0.5 g) were dissolved in double-distilled water, after which 0.1 mL of 1 M $MgSO_4$ was added. The mixture was brought to a final volume of 100 mL, sterilized by autoclaving, and allowed to cool to room temperature prior to use.

2.1.3. Nematode Growth Medium (NGM)

Nematode growth medium (NGM) was prepared as previously described [38]. For preparation of NGM (1 L), 3 g NaCl, 2.5 g peptone, and 17 g agar were dissolved in 975 mL double-distilled water and autoclaved at 121 °C for 20 min. Once cooled to ~55 °C, the medium was supplemented with 1 mL each of 1 M $CaCl_2$ and $MgSO_4$, 25 mL of 1 M potassium phosphate buffer (pH 6.0), 1 mL of cholesterol solution (5 mg/mL in ethanol), and 1.25 mL of nystatin (10 mg/mL in 70% ethanol). The medium was poured into sterile Petri dishes, allowed to solidify, and stored at 4 °C.

2.2. C. elegans Strain, PFA-Killed OP50 and Culture Conditions

The culture conditions were followed as previously described [38]. Wild-type *C. elegans* (N2 Bristol) were cultured on 60 mm NGM plates seeded with 30 µL of PFA-killed *E. coli* OP50. OP50 cultures (500 mL) were grown overnight at 37 °C with shaking (200 rpm) and then treated with 1.25% PFA for 2 h. Cells were washed four times with sterile double-distilled water and resuspended in S-complete buffer at ~5 × 10^{10} cells/mL (250 mg/mL). Worms were maintained at 20 °C. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

2.3. Synchronization of C. elegans Population

Synchronization was performed as previously described [38]. Gravid adults from confluent 60 mm NGM plates were collected using 1 mL of M9 buffer and transferred to a 15 mL tube. The volume was adjusted with 13 mL M9 buffer and centrifuged at 1500 rpm for 2 min. After removing the supernatant, the pellet was washed repeatedly with 14 mL M9 buffer until free of bacteria. The pellet was resuspended in 1 mL M9, mixed with 1 mL of 2X bleaching solution, and vortexed at 2500 rpm for 6 min. The reaction was stopped by adding 12 mL M9 buffer, followed by centrifugation at 2000 rpm for 1 min. The pellet was washed three times with M9 buffer, resuspended in 1 mL M9, and incubated at 20 °C with shaking (30 rpm) for 15 h to allow hatching of synchronized L1 larvae.

2.4. Staggered Plating of Synchronous Starved L1 Larvae

The synchronized L1 suspension was aliquoted and plated onto NGM agar plates seeded with PFA-killed *E. coli* OP50 at defined time intervals of 0, 1, 2, 3, 4, 5, and 6 days post-hatching. Prior to plating, L1 larvae were gently resuspended to ensure a homogeneous distribution. A schematic representation of the staggered plating strategy is provided in Figure 1. Following plating, worms

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were incubated at 20 °C and allowed to develop to the L4 stage. The timing of development was monitored, and worms were collected at the L4 stage for downstream assays. All plates were prepared under identical environmental and culture conditions to minimize variability.

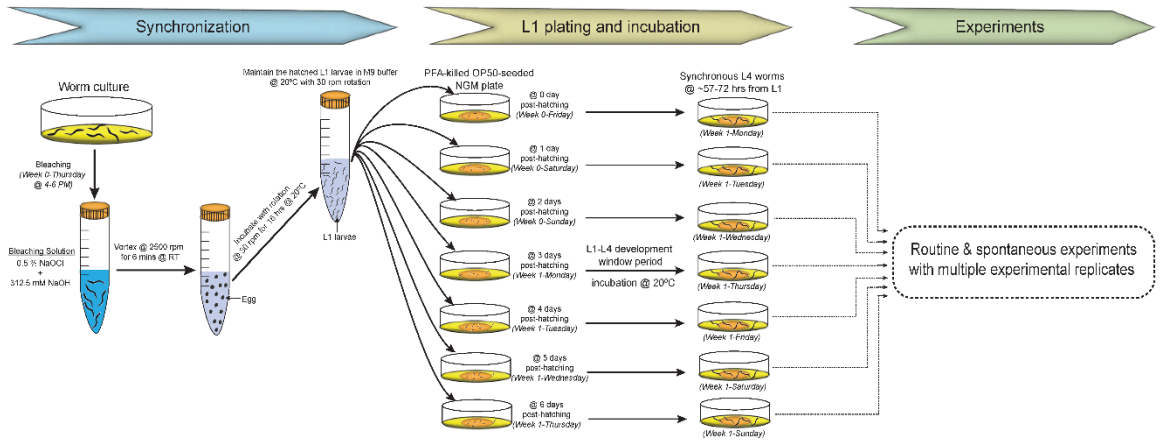

Figure 1. Staggered L1 plating strategy for continuous daily availability of synchronized *C. elegans* L4 worms. Schematic representation of the synchronization workflow. Gravid adult worms were subjected to bleaching (0.5% NaOCl and 312.5 mM NaOH) to isolate embryos, which were incubated in M9 buffer at 20 °C with gentle rotation (30 rpm) for ~16 h to obtain synchronized, growth-arrested L1 larvae. The L1 population was maintained under starvation and aliquots were plated onto PFA-killed OP50-seeded NGM plates at defined time intervals of 0, 1, 2, 3, 4, 5, and 6 days post-hatching. Following plating, worms were incubated at 20 °C and developed through larval stages (L1-L4) within a ~57-72 h window. This staggered plating strategy generates a continuous pipeline of synchronized L4 populations across consecutive days, enabling routine and spontaneous experiments with multiple replicates from a single synchronization event.

2.5. Body Length and Body Volume Measurement

Body length and body volume of *C. elegans* were quantified using the WorMachine image analysis platform [39]. Worms from different developmental stages (L1 to gravid adult), derived from staggered plating at defined time intervals, were imaged and analyzed. Bright-field images were acquired using a Nikon SMZ25 stereomicroscope equipped with a high-resolution monochrome camera (OPTO-EDU 20MPA). All images were captured at 1× zoom under consistent illumination settings to ensure uniform image quality across experimental groups. Captured images were processed using WorMachine implemented in MATLAB (version R2024a), following the standard protocol described in the WorMachine manual (https://github.com/adamhak/WorMachineClient) [39]. The software automatically identifies individual worms, segments them from the background, and extracts morphological parameters using skeletonization and feature-extraction algorithms. Body length was calculated based on the worm's skeleton, while additional parameters, including midwidth and thickness, were obtained from the software output. Worm body volume was calculated in Microsoft Excel using an ellipsoidal approximation according to the formula: $Volume = \pi \times \left[\frac{midwidth}{2}\right] \times \left[\frac{thickness}{2}\right] \times length$**.** A minimum of 150 worms per condition were analyzed in each experiment, with a total of 500 worms per condition across three independent experiments. All images were analyzed using identical processing parameters to ensure consistency across samples. Worms that were overlapping, touching, or improperly segmented were excluded from analysis to maintain data accuracy.

2.6. Brood Size and Embryonic Viability Scoring

Brood size and embryonic viability were assessed as previously described [40]. Synchronized L4-stage hermaphrodites obtained from staggered plating at defined time intervals were individually transferred onto **35 mm NGM plates** seeded with PFA-killed *E. coli* OP50 and maintained at 20 °C.

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Each worm was allowed to lay progeny for a 24 h period, after which the adult worm was transferred daily to a fresh plate for a total of 3 consecutive days. Plates containing laid embryos were incubated at 20 °C for an additional 24 h to allow viable embryos to hatch. Following incubation, the number of live larvae and unhatched embryos on each plate was counted under a stereomicroscope (Nikon SMZ745T). Unhatched embryos after the incubation period were considered non-viable, while hatched larvae were considered viable progeny, consistent with established methods [40]. Brood size was calculated as the total number of progeny (live larvae plus unhatched embryos) produced per worm across all days. Embryonic viability (%) was calculated using the formula: $Embryonic\ viablity\ (\%) = \left[\frac{number\ of\ live\ larvae}{number\ of\ live\ larvae + number\ of\ unhatched\ embryo}\right] \times 100$. For each condition, **three individual worms were analyzed per experiment,** with a total of **nine worms per condition across three independent experiments.** All assays were performed under identical experimental conditions to ensure reproducibility.

2.7. Locomotion Analysis

Locomotion of *C. elegans* was analyzed at the L4 stage of worms derived from staggered plating at defined time intervals using video-based tracking [41–43]. Worms were maintained on **35 mm nematode growth medium (NGM) plates from L1 plating until they reached the L4 stage,** after which locomotion recordings were performed. Videos were captured using a Nikon SMZ25 stereomicroscope equipped with a high-resolution monochrome camera (OPTO-EDU 20MPA). Recordings were acquired at **1× zoom**, with a frame rate of **28 frames per second (FPS)** and a total duration of **60 seconds** under consistent illumination conditions. For locomotion analysis, two parameters were quantified: **body length per second (BLPS)** and **body bends per second (BBPS).** For BLPS measurements, videos of worms crawling on NGM agar plates were recorded. For BBPS measurements, **200 µL of M9 buffer** was added onto the NGM plate to induce swimming behavior before video acquisition. Videos were analyzed using **ImageJ (version 1.54f)** with the **wrMTrck plugin (version 1.04),** following the standard protocol recommended in the wrMTrck manual (https://www.phage.dk/plugins/download/wrMTrck.pdf). The plugin performs automated tracking of individual worms and extracts locomotion parameters, including speed normalized to body length (BLPS) and frequency of body bends (BBPS), based on frame-by-frame movement and posture analysis. A minimum of 150 worms per condition were analyzed in each experiment, with a total of 500 worms per condition across three independent experiments. All recordings and analyses were performed under identical experimental conditions to ensure consistency across samples.

2.8. Statistical Analysis

Data are presented as the mean ± standard error of the mean (SEM) from at least three independent experimental replicates. Statistical analyses were performed using GraphPad Prism v.10. A two-way analysis of variance (ANOVA) was used to assess differences in body length and body volume. A one-way ANOVA test was used to evaluate differences in brood size, embryonic viability and locomotion. The value of $P < 0.05$ was considered significant. Statistical significance indicators are shown in the respective figures.

3. Results

3.1. Short-Term L1 Starvation Does not Affect Developmental Progression in C. elegans

To investigate whether short-term L1 starvation affects the *C. elegans* development, synchronized L1 larvae obtained from bleaching were plated in a staggered manner onto NGM agar plates seeded with PFA-killed OP50 at defined intervals of 0-6 days post-hatching (Figure 1). Brightfield images of worms were captured at different development stages, including L1, L2, L3, L4, young adult (YA), and gravid adult (GA). The captured images were analyzed to quantify body length and body volume. We observed that there was no significant difference in body length *(F(6,*

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20958) = 1.311; P = 0.2481) (Figure 2A) or body volume (Figure 2B) *(F(6, 20958) = 1.141; P = 0.3352)* among worms plated the different time intervals (0-6 days post-hatching). These results suggest that short-term L1 starvation (< 7 days) does not significantly affect developmental progression in *C. elegans.*

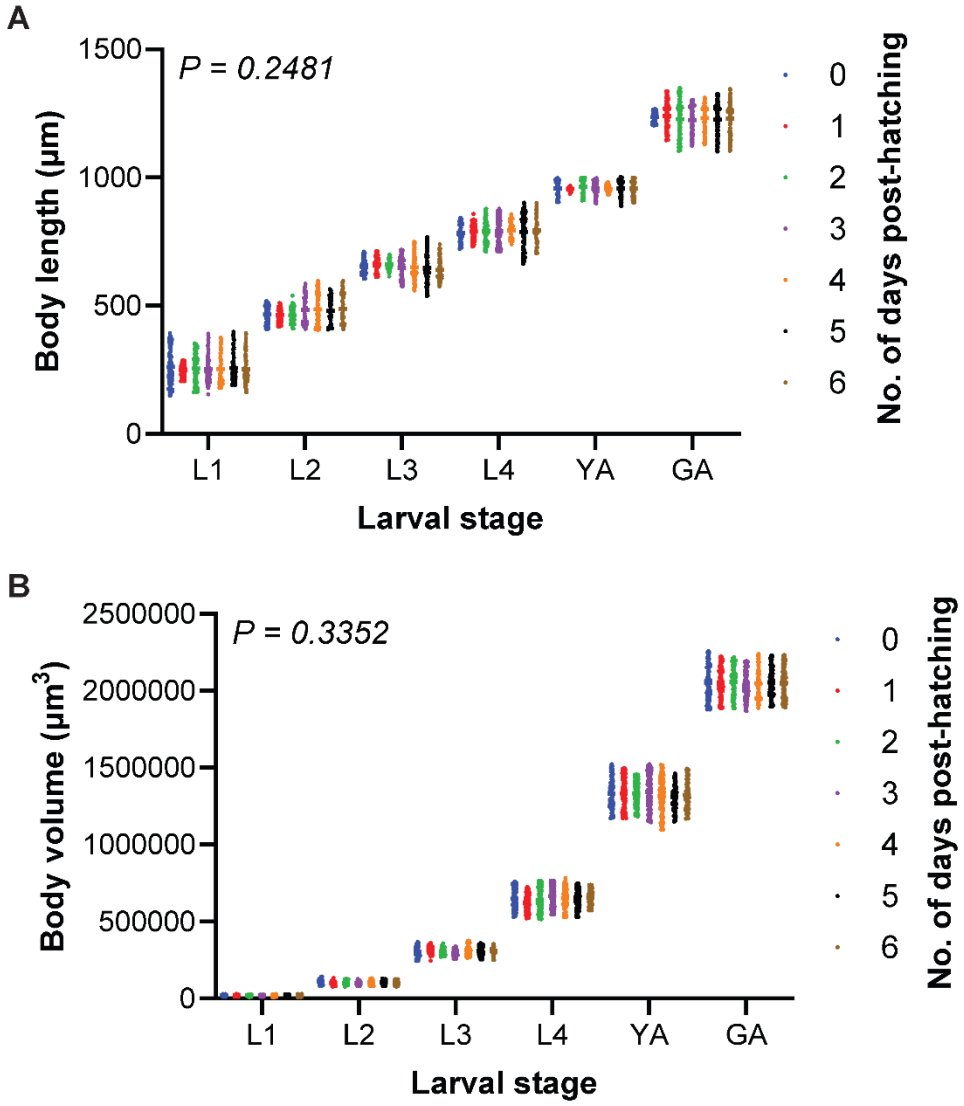

Figure 2. Effect of short-term L1 starvation on developmental progression and growth parameters. (A) Body length (µm) and **(B)** body volume (µm³) of *C. elegans* measured across developmental stages (L1, L2, L3, L4, young adult (YA), and gravid adult (GA)) for worms plated at different time intervals (0-6 days post-hatching). No significant differences were observed in body length (*P = 0.2481*) or body volume (*P = 0.3352*) across the different starvation durations, indicating that short-term L1 arrest does not affect developmental growth trajectories. Data represented as mean ± SEM from three independent experiments, and two-way ANOVA was applied to calculate statistical significance.

3.2. Short-Term L1 Starvation Does not Affect Fecundity and Fertility in C. elegans

To investigate whether short-term L1 starvation affects reproductive capacity, synchronized L1 larvae were plated at defined intervals (0-6 days post-hatching) and allowed to develop to the L4 stage (Figure 1). Individual L4 hermaphrodites were transferred onto fresh NGM plates seeded with PFA-killed OP50 and monitored for reproductive output. Brood size was determined by counting the total number of progenies produced over the reproductive period, while embryonic viability was assessed as the percentage of eggs that successfully hatched. We observed that there was no significant difference in brood size (*F(6, 56) = 0.2137; P = 0.971*) (Figure 3A) or embryonic viability

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(*F(6, 56) = 0.6154; P = 0.717*) (Figure 3B) among worms derived from different starvation durations (0-6 days post-hatching). These results suggest that short-term L1 starvation does not significantly affect fecundity or fertility in *C. elegans*.

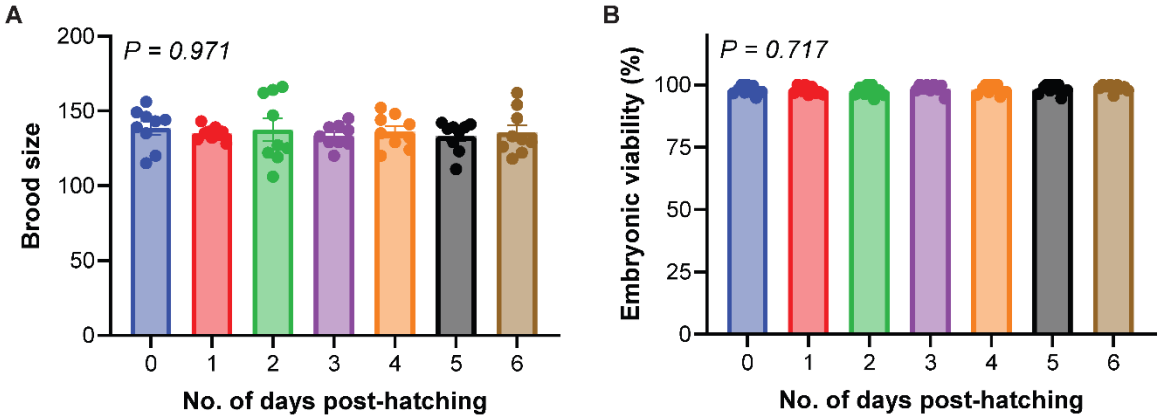

Figure 3. Effect of short-term L1 starvation on reproductive capacity. (A) Brood size and **(B)** embryonic viability (%) of *C. elegans* worms derived from L1 larvae plated at different time intervals (0-6 days post-hatching). No significant differences were observed in brood size (*P = 0.971*) or embryonic viability (*P = 0.717*) across the different conditions, demonstrating that short-term L1 starvation does not impair reproductive output or embryo viability. Data represented as mean ± SEM from three independent experiments, and one-way ANOVA was applied to calculate statistical significance.

3.3. Short-Term L1 Starvation Does not Affect Locomotion in C. elegans

To investigate whether short-term L1 starvation influences locomotor activity, synchronized L1 larvae were plated at defined intervals (0-6 days post-hatching) and allowed to develop to the L4 stage (Figure 1). Locomotion was assessed using video-based tracking, and two parameters were quantified: body length per second (BLPS; μm/s) and body bends per second (BBPS). We observed that there was no significant difference in BLPS (*F(6, 3493) = 1.456; P = 0.1894*) (Figure 4A) or BBPS (*F(6, 3493) = 1.553; P = 0.1569*) (Figure 4B) across worms derived from different starvation durations (0-6 days post-hatching). These findings indicate that short-term L1 starvation does not significantly affect locomotor behavior in *C. elegans*.

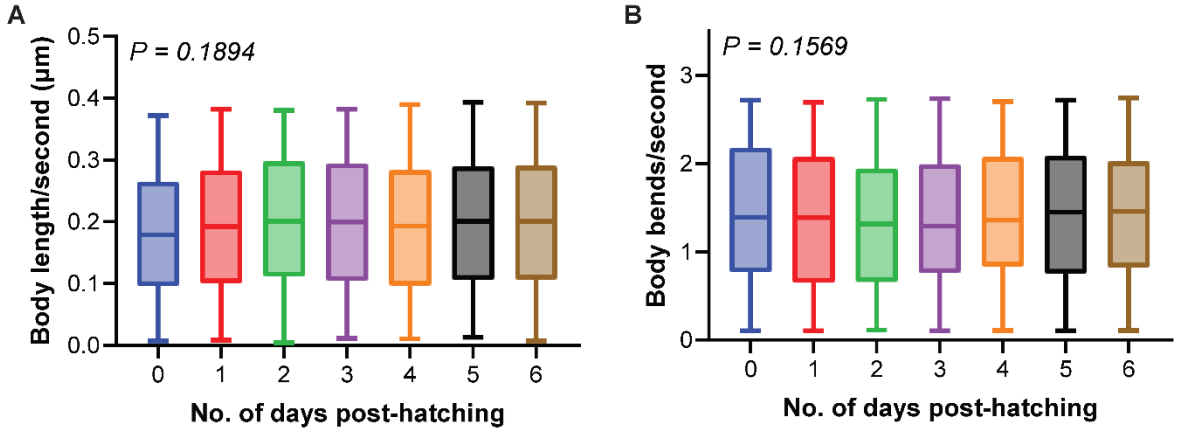

Figure 4. Effect of short-term L1 starvation on locomotion behavior. (A) Body length per second (BLPS; μm/s) and **(B)** body bends per second (BBPS) of *C. elegans* at the L4 stage derived from L1 larvae plated at different time intervals (0-6 days post-hatching). No significant differences were observed in BLPS (*P = 0.1894*) or BBPS (*P = 0.1569*) across the conditions, indicating that short-term L1 starvation does not affect locomotor activity. Data are presented as box-and-whisker plots derived from three independent experiments, and one-way ANOVA was applied to calculate statistical significance.

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4. Discussion

In the present study, we investigated whether short-term L1 starvation (< 7 days) influences developmental, reproductive, and behavioral outcomes in *C. elegans*, and whether such a paradigm can be practically leveraged to generate a continuous supply of synchronized L4 populations. Our results demonstrate that staggered plating of starved L1 larvae across a 7-day window (Figure 1) does not significantly affect developmental progression, body size (Figure 2), reproductive capacity (Figure 3), or locomotor behavior (Figure 4). Collectively, these findings establish that short-term L1 arrest is physiologically well tolerated and can be exploited as a robust synchronization strategy for routine experimental workflows.

A key observation of this study is that developmental progression remains unaffected across worms derived from L1 larvae starved for up to 6 days (Figure 2). This finding aligns with previous studies demonstrating that *C. elegans* can maintain developmental competence during L1 arrest and resume normal growth upon refeeding. L1 diapause is a metabolically regulated quiescent state that preserves organismal integrity during nutrient deprivation [44,45]. Earlier work has also shown that developmental arrest during starvation is reversible and does not inherently compromise organismal viability [36]. Our findings extend these observations by demonstrating that, within a practical experimental window (< 7 days), developmental outputs such as body length and volume remain quantitatively unchanged. These results are further supported by studies showing that L1 arrest involves adaptive metabolic reprogramming rather than pathological stress. Starved L1 larvae exhibits reduced metabolic rates and activation of conserved nutrient-sensing pathways, including AMPK and insulin/IGF-1 signaling, which promote survival during nutrient deprivation [32,45]. In contrast, prolonged starvation has been associated with delayed recovery, increased physiological stress, and altered developmental timing [35]. The absence of such effects in our study suggests that short-term starvation does not reach the threshold required to induce detrimental physiological changes.

In addition to developmental parameters, we observed no significant differences in brood size or embryonic viability across starvation durations (Figure 3). These findings are consistent with previous reports indicating that short-term L1 arrest does not impair reproductive capacity. During starvation, germline proliferation is temporarily halted but resumes upon refeeding, allowing normal reproductive output [44]. However, prolonged or repeated starvation has been shown to reduce fertility and induce transgenerational effects mediated through insulin signaling pathways [46,47], suggesting that the absence of reproductive defects in our study is likely due to the limited duration of starvation. Locomotion analysis further revealed no significant differences in crawling (BLPS) or thrashing (BBPS), indicating preserved neuromuscular function (Figure 4). Previous studies have shown that severe metabolic or oxidative stress can impair locomotion in *C. elegans [48–50]*. However, the absence of locomotor defects in our study suggests that short-term L1 starvation does not induce sufficient physiological stress to disrupt motor function, further supporting the resilience of the L1 arrest state.

Despite these consistencies, some studies have reported that starvation duration can influence recovery dynamics without necessarily altering final developmental outcomes. For example, extended L1 arrest has been shown to significantly prolong recovery time following refeeding, while developmental progression remains largely preserved, even after prolonged starvation (> 8 days) [35]. This suggests that while endpoint measurements such as growth and reproduction remain stable, underlying temporal and metabolic processes may still be affected. Another important consideration is the impact of starvation on lifespan. Previous studies have demonstrated that even short-term starvation or alterations in early-life nutritional status can influence longevity in *C. elegans* through modulation of insulin/IGF-1 signaling, AMPK activity, and metabolic rate [32,36,45]. Therefore, although our results indicate that short-term L1 starvation does not affect development, reproduction, or locomotion, it may still confound lifespan outcomes. Consequently, the staggered L1 plating strategy described in this study should not be applied to experiments focused on aging or lifespan, where even subtle metabolic alterations may significantly influence results.

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From a methodological perspective, the staggered L1 plating strategy addresses a major limitation in *C. elegans* research, particularly the need for repeated synchronization to obtain stage-matched populations. Conventional synchronization methods, including bleaching and timed egg laying, are labor-intensive and introduce variability due to handling and timing differences [7,22]. By demonstrating that L1 larvae can be maintained under starvation for up to 7 days without compromising key physiological outputs, this study provides a simple and scalable approach for generating daily synchronized L4 populations from a single synchronization event. This approach reduces experimental variability, minimizes labor, and enhances flexibility for both routine, spontaneous, and time-sensitive experiments. Nevertheless, certain limitations should be acknowledged. This study focused on phenotypic endpoints and did not assess molecular changes such as gene expression, metabolic flux, or stress signaling pathways. Subtle molecular alterations may occur without manifesting as overt phenotypic differences. Additionally, experiments were conducted under controlled laboratory conditions using PFA-killed OP50, and variations in microbial metabolism or environmental conditions may influence outcomes in other experimental settings. Finally, starvation durations beyond 7 days were not evaluated and may lead to different physiological consequences.

5. Conclusion

In conclusion, our findings demonstrate that short-term L1 starvation does not adversely affect developmental progression, reproductive capacity, or locomotor behavior in *C. elegans*. These results not only corroborate existing knowledge on the resilience of L1 diapause but also provide a practical framework for implementing a staggered plating strategy to achieve continuous availability of synchronized worm populations. However, given the potential influence of starvation on lifespan, this approach should be applied cautiously in studies involving aging or longevity.

Author contributions: S.B. and S.D. performed the experiments and analyzed the results. K.R.B. designed and supervised the experiments; wrote and edited the manuscript.

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Data availability: Data will be made available on request.

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Declaration of interests: The authors declare no competing interests.

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