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Life history traits of the gastropod-associated bacterivorous nematode *Caenorhabditis brenneri* (Nematoda: Rhabditida)

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Abstract. *Dalan L, Tañan V, Diano MA, Demayo C, Tabelin M, Sumaya NH. 2024. Life history traits of the gastropod-associated bacterivorous nematode* Caenorhabditis brenneri *(Nematoda: Rhabditida). Biodiversitas 25: 3106-3113. Caenorhabditis* species are associated with decaying plant materials and invertebrates such as terrestrial gastropods. Few *Caenorhabditis* species have been studied regarding their ecology, genetics, development, and essential life history traits (LHT). In this study, we describe the life cycle of a free-living, wild-type, bacterivorous nematode, *Caenorhabditis brenneri* Sudhaus and Kiontke 2007, associated with the terrestrial slug *Philippinella moellendorffi* Collinge 1899 and account for the effects of food density and temperature on its population dynamics by employing the hanging drop technique. The bacterial food supply was sourced from the slug cadaver and identified as *Alcaligenes faecalis* (MT012081). *C. brenneri* was fed with varying bacterial densities (10^9 and 5×10^9 cells mL^{-1}) and incubated at 20 and 25°C. The bacterial food density-temperature interaction was found to have no significant influence on the offspring production of *C. brenneri*. Moreover, the total fertility rate (TFR) and net reproductive rate (R_o) are higher in 10^9 cells mL^{-1} in both temperatures (TFR at 20°C: 139; 25°C: 169 and R_o at 20°C: 134; 25°C: 156). Whereas the alternative generation time (T_o, T_1, T) and population doubling time (PDT) are faster at 25°C in both bacterial densities, the intrinsic rate of natural increase (r_m) is faster at lower temperatures (20°C) in both bacterial densities. The average lifespan of *C. brenneri* is seven days when fed with *A. faecalis*. Offspring somatic growth (body length and width) was influenced by bacterial food density-temperature interaction with increased length and girth observed in higher bacterial density and temperature in our knowledge. This study is the first attempt to use *A. faecalis*, a wild-type bacterium from the terrestrial slug *P. moellendorffi* cadaver, as the bacterial food source for a *Caenorhabditis* LHT analysis.

Keywords: Fecundity, hanging drop, juveniles, LHT, semi-solid NGG, survivability

INTRODUCTION

Soil fauna, of which bacterivorous nematodes are part, play critical functional and ecological roles in soil ecosystems (van den Hoogen et al. 2019; Zhao et al. 2022). It was documented that they impact the community composition, including the abundance of soil microorganisms and their direct or indirect involvement in nutrient decomposition and recycling processes. With bacterial feeding and facilitating organic matter mineralization, such nematodes improve microenvironment pH (Xiao et al. 2014). Despite this crucial role in the ecosystem, the life history of soil fauna such as nematodes has remained understudied (Bardgett and van der Putten 2014).

Sudhaus and Kiontke (2007) reported that *Caenorhabditis brenneri* Sudhaus and Kiontke 2007 is a gonochoristic member species of the *Elegans* group; it is found mainly in different habitats, such as in decaying plant matter and soil, and often forms an association with invertebrates such as terrestrial gastropods with pantropical distribution (Sudhaus and Kiontke 2007; Dalan et al. 2022; Diano et al. 2022). Interestingly, *C. brenneri* has the most molecular polymorphic synonymous sites between individuals (14.1%), making them the most hyper-diverse eukaryote comparable to hyper-diverse bacteria (Dey et al. 2013). Like other free-living nematodes, *Caenorhabditis* species fed on bacteria found in their surroundings formed phoretic associations with invertebrates and reproduced in cadavers of their host organisms (Petersen et al. 2014; Slos et al. 2017; Dalan et al. 2022).

Bacteria as a food source are essential in their various life strategies, influencing reproduction, growth, virulence, and sometimes forming symbiotic relationships with nematodes. Assessment of essential life history traits (LHT) of nematodes using associated bacteria is essential for understanding nematode growth and development in controlled environments. This will provide insight into how population growth dynamics change as various factors influence them. LHT analysis is an important step in measuring the species' reproductive ability. It can be used as basic information to maximize its productivity when commercializing the species at the industrial level (Addis et al. 2016a). It also shows in detail and precision how an individual nematode species performs. Thus, such research studies are critical to natural ecosystem management, soil bioremediation, and agriculture (Zhao et al. 2022).

One way to study the LHT is by utilizing the hanging drop method, which provides a clear visualization of the

setup, giving an advantage in observing and recording the different life cycle parameters. A culture medium of Gelrite powder is the standard medium used in the hanging drop method. This medium provides high transparency, which enables researchers to facilitate convenient screening on culture plates with minimum disturbance to the setups. Moreover, nematode extraction is possible without the medium's residues adhering to nematodes (Muschiol and Traunspurger 2007). The Gelrite medium is a transparent, semi-solid, gel-like culture medium that allows clear examination of the test organisms. Individuals or pairs of nematodes were introduced into a drop of semi-solid medium containing a bacterial food source at the underside of the lid of a cell-well plate. This method of analysis was already established in EPNs using their respective symbiotic bacteria (Addis et al. 2014; Addis et al. 2016a, b, c) and on different free-living nematodes (Muschiol and Traunspurger 2007; Muschiol et al. 2009; Gilarte et al. 2015; Kreuzinger-Janik et al. 2017).

Though *C. brenneri* has the potential to be a model organism for having such hyper-diverse molecular polymorphic synonymous sites among eukaryotes (Dey et al. 2013), the information on the basic LHT is rather limited. This study aimed to unravel the basic LHT of the wild-type *C. brenneri* isolated from the cadaver of terrestrial slug *Philippinella moellendorffi* Collinge 1899, using its associated bacteria as a food source using the hanging drop method. We account for food density and temperature's effects on population dynamics. For this, the offspring production, survivability, fecundity, fertility rate, net reproductive rate, somatic growth of offspring produced, and average lifespan were assessed as influenced by bacterial density and temperature.

MATERIALS AND METHODS

Isolation and identification of the associate bacterium

Bacterial lawn from *P. moellendorffi* cadaver on an agar plate was picked and streaked aseptically onto Nutrient Agar (NA) medium M001 HIMEDIA® (0.5% peptone, 0.15% HM peptone B, 0.15% yeast extract, 0.5% NaCl, 1.5% agar; 7.4±0.2 pH at 25°C) in 90×15 mm Petri dishes and incubated at 37°C for 24 h. The resulting pure bacterial colony was selected as the food source for *C. brenneri*. The isolated bacterium was cultured in a 500 mL Erlenmeyer flask containing 100 mL of sterile Nutrient Broth TM MEDIA 350 (0.5% peptic digest animal tissue, 0.5% NaCl, 0.15% beef extract, 0.15% yeast extract; 7.4±0.2 pH at 25°C) and incubated at 37°C for 24 h with constant agitation. This served as the bacterial stock culture.

Briefly, 1 mL culture from the bacterial stock culture was loaded into a 1.5 mL Eppendorf tube and centrifuged at 10,000 rpm for 2 min to pellet the cells, and the supernatant was discarded. Genomic DNA extraction followed the manufacturer's (Dongsheng Biotech's Quick Genomic Bacteria DNA Extraction Kit N1152) protocol. The wash buffer (PE) solution was diluted with 96-100% ethanol before use. Extracted DNA was stored in a 1.5 mL Eppendorf tube and sent to Macrogen, South Korea, for PCR amplification, purification, and sequencing. The primer pair 27F 5'-AGAGTTTGATCCTGGCTCAG-3'' and 1492R 5'-GGTTACCTTGTTACGACTT-3'' (Chen et al. 2015) was used to amplify segment of 16S rDNA gene following a standard thermal profile of 94°C for 1 min pre-denaturation; 30 cycles of 94°C for 30 s denaturation; 55°C for 30 s annealing; 72°C for 1 min extension and 72°C for 10 min then cooled at 4°C final extension. Generated sequences were BLASTn searched, and all sequences were deposited in the National Centre for Biotechnology (NCBI) (Stover and Cavalcanti 2017). The obtained molecular sequences were trimmed, annotated, and subsequently submitted to NCBI Genbank for record.

Cell density and quantification of the bacterium

Quantification was done by distributing 100 mL stock bacterial culture to 20 mL centrifuge tubes. The tubes were centrifuged at 4,500 rpm for 10 min. The supernatant was discarded, and pellets were washed with sterile K-Medium (3.1 g NaCl and 2.4 KCl in 1 L), homogenized by vortex, and centrifuged for 5 min and decanted. The pellets were pooled into one 50 mL Falcon tube. Ten milliliters (10 mL) of semi-solid Nematode Growth Gelrite (NGG) medium (2.5 g peptone, 3 g NaCl, 3 g Gelrite and adjusted to 1 L; autoclaved and cooled to 55°C, 1 mL of 147 g L^{-1} suspension of $CaCl_2 \times 2\ H_2O$, 1 mL of 246. 6 gL^{-1} $MgSO_4 \times 7\ H_2O$ and 25 mL of 136 g L^{-1} KH_2PO_4 at pH 6 and 1 mL of 1 g L^{-1} cholesterol suspended in alcohol) (Dancheng Caixin Sugar Industry Co., LTD) were added to pellets and homogenized through the vortex. Two milliliters of homogenized semi-solid NGG with bacterial cells were loaded into the spectrophotometer, and the absorbance values at 600 nm were obtained and calculated. Cell densities of 10^9 and 5×10^9 cells mL^{-1} were obtained by dilution. The semi-solid NGG containing the appropriate bacterial density was distributed to several 1.5 mL Eppendorf tubes, stored in the refrigerator, and served as the food stock for LHT.

Maintenance and acclimatization of the nematode

The nematode *C. brenneri* was previously isolated from the same cadaver of *P. moellendorffi*, a terrestrial slug in the Philippines (Dalan et al. 2022). This was also the same cadaver where the pure culture of the associate bacterium used as stock culture was isolated. Before starting the LHT analysis, the nematodes were maintained by culturing them in NA plates seeded with the bacterial stock culture. Sub-cultures were obtained and maintained by transferring a block of NA containing all stages of nematode into fresh NA plates every five days. Before starting LHT analysis, *C. brenneri* cultures were acclimatized for one week on 90×15 mm Petri dishes with solid NGG medium seeded with 500 µL of bacterial stock culture and incubated at room temperature for 24 h.

Life stage synchronization of the nematode

Life stage synchronization using the hanging drop method was based on Muschiol and Traunspurger (2007) with slight modifications. Briefly, 9 drops of 15 µL semi-solid NGG (with 10^9 cells mL^{-1}) were distributed in the

inner lid of the top cover of 90×15 mm Petri dishes. One gravid female from the exponentially growing *C. brenneri* culture in solid NGG was picked and transferred into each drop to allow egg laying and hatching. Next, to avoid desiccation, the bottom pair of the Petri dishes were covered with tissue paper soaked with 3 mL of distilled water, sealed, and incubated at room temperature for 24 h. After 24 h, the added gravid females were removed, and the hatched juveniles were selected for the LHT experiment.

LHT experimental setup

The experiment started by picking nine pre-adults/J4 stage female *C. brenneri* (visible vulva) with a "nematode picker" (one bristle of toothbrush glued to the tip of a chopstick) from the previous hanging drops. It was individually introduced into fresh drops of semi-solid NGG. This was followed by picking one male *C. brenneri* to complete the pair (one male and female *C. brenneri* per drop) in semi-solid NGG drops of varying bacterial densities (10^9 and 5×10^9 cells mL^{-1}) and incubated in 20 and 25°C. The nematode pairs were transferred into new drops every 24 hours. Offspring were checked and quantified every 24 hours. If the male *C. brenneri* died, they were removed and replaced with live males from the nematode stock culture. The experiment ended with the death of the last adult female *C. brenneri*.

Determination of LHT parameters

A life table was constructed to have an accessible overview of the reproductive timetable of *C. brenneri* and to acquire the necessary parameters for analyzing LHT.

Total fertility rate (TFR) is the total number of juveniles an adult produces during its maximum lifespan

$$TFR = (\sum m_x)$$

Where:

m_x : age-specific fecundity

Net reproductive rate (R_o) is the average number of offspring an adult produces during its lifespan

$$R_o = \sum_{x=0}^{d} l_x m_x \quad (2)$$

Where:

l_x : age-specific survival probability

m_x : age-specific fecundity

The intrinsic rate of natural increase (r_m) is the rate at which an individual in a stable population increases under optimal conditions, calculated using Euler's equation

$$\sum_{x=0}^{d} e^{-rmx} l_x m_x \quad (3)$$

Where:

l_x : age-specific survival probability

m_x : age-specific fecundity

Population doubling time (PDT) is the time needed for an increasing population to double its number of individuals.

$$PDT = \ln(2)/r_m \quad (4)$$

Where:

Ln : natural logarithm

r_m : intrinsic rate of natural increase

Alternative measures of generation time ($T_0/T_1/T_h$) were T_o or cohort generation time; T_1, which is the time necessary for the increasing population to grow by a factor of R_o; and T, the mean parental age at which a new generation is produced.

$$T_o = \sum_{x=0}^{d} xl_x / \sum_{x=0}^{d} l_x m_x \quad (5)$$

$$T1 = (\ln R_o)/r_m \quad (6)$$

$$T = \sum_{x=0}^{d} x e^{-r_m x} l_x m_x \quad (7)$$

Where :

x : time in days

l_x : age-specific survival probability

m_x : age-specific fecundity

Ro : net reproductive rate

r_m : intrinsic rate of natural increase

Somatic growth assessment of juveniles

Offspring from every experimental setup were pooled into each designated 5 mL tube and fixed. Ten random offspring from every setup were picked into embryo dishes and processed into anhydrous glycerin (Ryss 2017). Solution 1 (8 parts formalin, 2 parts glycerol, and 90 parts water) was heated and dropped into the dish with the nematodes, covered for 24 h at room temperature. Solution 2 (5 parts glycerol and 95 parts 96% ethanol) was added, one drop of the solution every two hours, five times. Nematodes were fixed into slides by a paraffin wax ring with a drop of Solution 3 (glycerol) in the middle and sealed by heating. Nematodes were photographed and measured using an Olympus light microscope BX53 equipped with an Olympus DP27 microscope digital camera and Cellsens imaging software, and they were measured using ImageJ Software.

Data analysis

Life history parameters (TFR, R_o, r_m, PDT, alternative generation times, and average lifespan) of *C. brenneri* LHT were computed based on the life tables obtained. The *What-if-Analysis* function and its *goal seek* feature in Microsoft Excel were used to compute the r_m values. The mean number of offspring produced was analyzed using the Kruskal-Wallis test to determine the influence of bacterial density and temperature in offspring production. The best-fitted ANOVA model was used to determine the influence of bacterial density and temperature and the somatic growth (length and width) of *C. brenneri*. Statistical analysis was done using the RStudio program (R Core Team 2023).

RESULTS AND DISCUSSION

Identification of the associated bacterium

Molecular analysis of the 16S rDNA using BLASTn software showed that the associated bacterium from *P. moellendorffi* cadaver was *Alcaligenes faecalis* Castellani and Chalmers 1919, a Gram-negative bacillus aerobic bacterium, which was annotated as *A. faecalis* (MT012081).

Life history parameters of the nematode

Moreover, *C. brenneri* produced more offspring per adult during its maximum lifespan (TFR) and a high mean number of offspring produced during its entire lifespan (R_o) when cultured in lower bacterial density (10^9 *A. faecalis* cells mL^{-1}). In both bacterial densities, the faster generation time and population doubling time of *C. brenneri* were observed in higher temperatures (25°C). However, both bacterial densities observed high r_m at lower temperatures (20°C). The average lifespan of adult female *C. brenneri* was seven days (Table 1)

Influence of bacterial density and temperature on the nematode offspring production

Bacterial density did not significantly influence offspring production of the nematode (χ^2 (1)=0.303, p=0.5817). Likewise, the temperature did not significantly influence the offspring production of *C. brenneri* (χ^2 (1)=0.044, p=0.8345). The mean number of offspring produced did not differ significantly between treatments (Figure 1).

Fecundity and survivability of the nematode

C. brenneri is a fast-reproducing species with peaked fecundity observed 48 h after introduction to hanging drops. Fecundity also gradually declined as assessment continued in hanging drops. Regarding their survivability, adult *C. brenneri* had a stable population at 5×10^9 *A. faecalis* cells mL^{-1} until day five of assessment compared to day four at 10^9 *A. faecalis* cells mL^{-1} in both temperatures (Figure 2).

Somatic growth analysis of the nematode offspring

Morphometric analysis on the somatic growth of *C. brenneri* offspring showed that interaction between bacterial food density and temperature had a significant influence on the body length (F_1=33.983, p<0.001) and body width (F_1=41.530, p<0.001). The increased body length of hatched juveniles was observed in higher bacterial density (5×10^9 *A. faecalis* cells mL^{-1}) and higher temperature (25°C) (p<0.05). The same outcome was also observed in the body width of hatched juveniles (p<0.05), as shown in Figure 3.

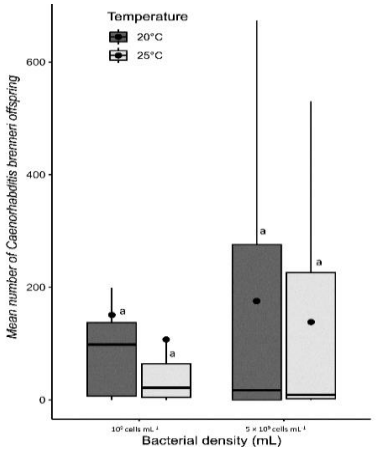

Figure 1. The mean number of *Caenorhabditis brenneri* offspring in varying bacterial density and temperature was assessed in hanging drop. Black points indicate mean, and letters signify significant differences

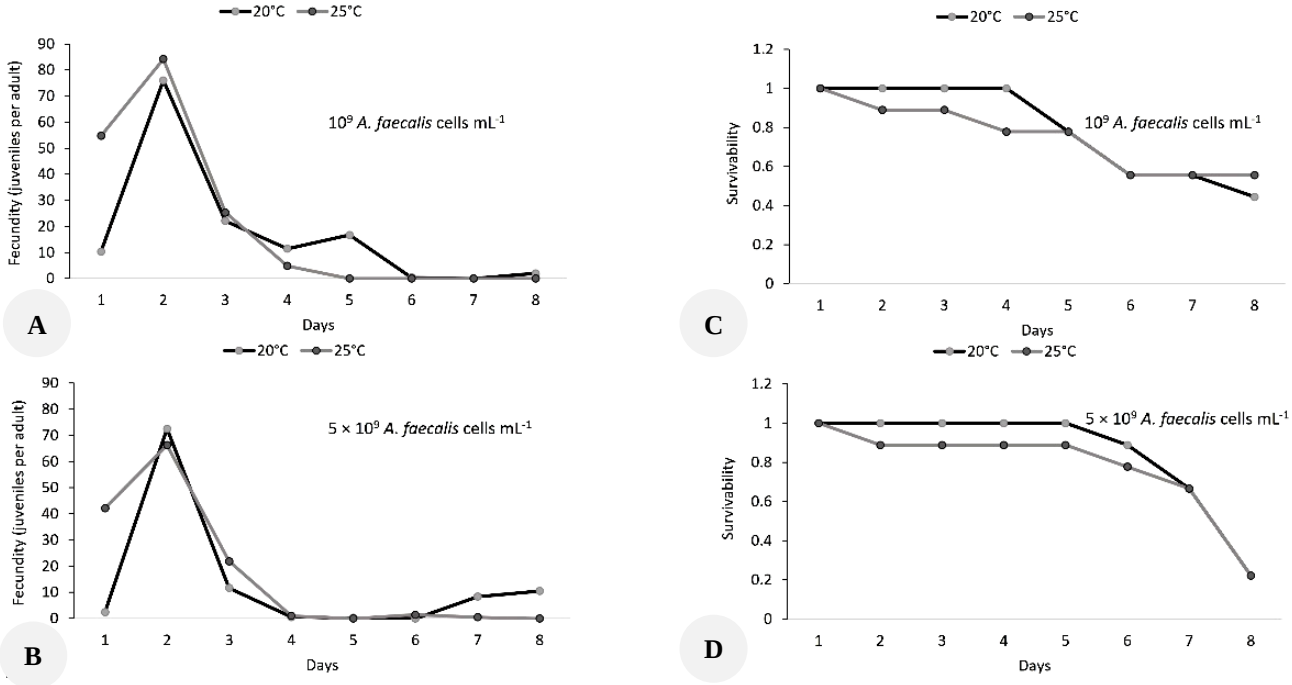

Figure 2. A-B. Fecundity; and C-D. survivability of *Caenorhabditis brenneri* as influenced by varying bacterial densities and temperatures assessed in hanging drop

Discussion

The bacterium isolated and identified from the terrestrial slug *P. moellendorffi* cadaver was *A. faecalis*, an aerobic, bacillus, Gram-negative bacterium commonly isolated from soil and water environments (Kong et al. 2014). This species is unable to degrade urea to ammonia, can produce catalase through aerobic respiration, cannot utilize citrate as a carbon source, and is not efficient in fermenting sugars. It is also sometimes found in human bodily fluids, the alimentary canal, and feces. It is generally considered non-pathogenic, though opportunistic infection does occur in the form of urinary tract infection, infection in the bloodstream, skin and soft tissue, and middle ear (Mordi et al. 2015; Tena et al. 2015; Huang 2020). Most LHT studies in hanging drops of free-living nematode utilized *Escherichia coli* Escherich, 1885 strain OP50 as the common bacterial food source and at 20°C incubation (Muschiol and Traunspurger 2007; Muschiol et al. 2009; Gilarte et al. 2015; Kreuzinger-Janik et al. 2017; Mondejar et al. 2023). In contrast, a recent study utilized an unidentified bacterium that the free-living bacterivorous nematode, *Diplolaimella stagnosa* Lorenzen 1966 carried as a food source to assess the life cycle and effects of temperature and food availability on its population dynamics (Zhao et al. 2022). The present study, however, is the first attempt to use *A. faecalis*, a wild-type bacterium isolated from the terrestrial slug *P. moellendorffi* cadaver, as the bacterial food source for *C. brenneri* LHT analysis.

The onset of reproductive maturity in *C. brenneri* is early (24 h in hanging drop) and gradually increases until 48 h. However, such a peak in the production of juveniles declines after 48 h and continues to drop until the last day. This development contrasts with other *Caenorhabditis* LHTs, such as *Caenorhabditis elegans* Maupas 1900 in hanging drop. It is observed in several studies that *C. elegans* started laying eggs and hatching within 72 h and exponentially increased to 120 h. This discrepancy could result from the difference in the utilization of cohort juveniles for the study. The cohort of juveniles used in this study has a mean age of 24 h compared with 4 h used by Muschiol et al. (2009). In this case, we started with J4 compared to their J1/J2, thus contributing to the early reproductive maturity and aging of *C. brenneri*.

Previous LHT studies implied that bacterial food density of 5×10^9 cells mL^{-1} was determined to be ideal for most of the *Caenorhabditis* species LHT as it precludes food limitation. Lower than this, such as bacterial density at 10^9 *E. coli* strain OP50 cells mL^{-1}, reportedly reduced fecundity in *Caenorhabditis briggsae* Schiemer 1982. Moreover, high bacterial density, such as 10^{10} *E. coli* strain OP50 cells mL^{-1}, resulted in reduced life expectancy in *C. elegans* (Johnson et al. 1990). In this study, offspring production, as measured in TFR and R_o of *C. brenneri*, was not influenced by the combined interaction of bacterial density and temperature even independently. The reduced fecundity observed at 10^9 bacterial cells mL^{-1} seems to vary from species to species, even from the same taxon. The fecundity of *C. brenneri* in the current study is higher (139 and 169 offspring) at lower bacterial density (10^9 bacterial cell mL^{-1}) in both temperatures. Although statistically, fecundity between the bacterial densities does not differ significantly from each other (Table 1).

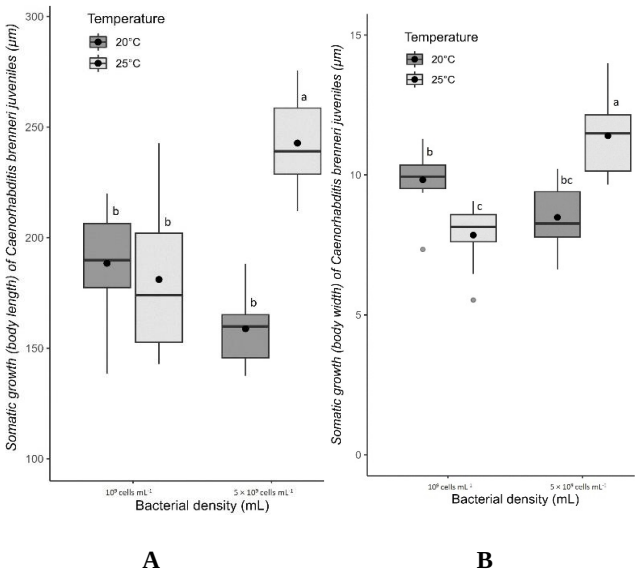

Figure 3. Somatic growth (A. Body length; B. Body width) of *Caenorhabditis brenneri* juveniles in varying bacterial density and temperature assessed in hanging drop. Black points indicate the mean, and letters indicate significant differences

Table 1. LHT parameters of *Caenorhabditis brenneri* in varying bacterial densities and temperatures were assessed in hanging drops

	10^9 *A. faecalis* cells mL^{-1}		**5×10^9 *A. faecalis* cells mL^{-1}**	
	20°C	**25°C**	**20°C**	**25°C**
n	9	9	9	9
TFR	139±23.37	169.26±29.97	106.28±22.84	133.28±23.54
R_o	134±23.53	156±27.59	95.33±23.21	122.89±21.63
T_o (d)	2.6	1.84	2.6	1.88
T_1 (d)	4.6	2.40	3.9	2.58
T (d)	13.87	9.24	15.61	9.55
PDT (d)	0.51	0.33	0.59	0.37
r_m (d^{-1})	1.37	2.09	1.18	1.87
Average lifespan (d)	7.13±2.03	6.75±1.48	7.63±2.34	7±2.06

Notes: TFR: total fertility rate; R_o: net reproductive rate; T_o, T_1, T: alternative mean generation time; r_m: intrinsic rate of natural increase; PDT: population doubling time; d: days

Table 2. LHT parameters of different nematode species were assessed in the hanging drop method

Species	***C. brenneri***				***C. elegans***		***Poikilolaimus* sp.**	***Panagrolaimus* sp.**	***Pristionchus pacificus***	***Steinernema riobrave***		***Steinernema feltiae***
Strain/origin	**Slug cadaver**				**N2**	**MY6**	**Movile Cave, Romania**		**PS312**	**Sr-12**	**Sr-HYB 19**	**EN02**
Bacteria	***A. faecalis***				***E. coli* strain OP50**					***Xenorhabdus cabanillasii***		***Xenorhabdus bovienii***
Density	10^9 cells mL^{-1}						5×10^9 cells mL^{-1}					
Temperature	20°C	25°C	20°C	25°C			20°C			25°C		
TFR	139	169.26	106.28	133.28	295	290	187	77	115	975	822	493
R_o	134	156	95.33	122.89	291	289	108	64	109	792	683	359
T_o (d)	2.6	1.84	2.6	1.88	4.79	4.42	13.36	33.71	5.71	4.04	5.46	5.61
T_1 (d)	4.6	2.40	3.9	2.58	4.13	3.86	13.5	28.36	4.08	5.92	5.33	5.35
T (d)	13.87	9.24	15.61	9.55	3.75	3.5	13.79	26.21	3.33	4.79	5.25	5.12
PDT (d)	0.51	0.33	0.59	0.37	0.5	0.46	2.25	4.21	0.62	0.58	0.58	0.64
r_m (d^{-1})	1.37	2.09	1.18	1.87	1.37	1.46	0.165	0.309	1.125	1.13	1.23	1.1
Average lifespan (d)	7.13	6.75	7.63	7	16.7	14.7	-	-	22.5	7.4	6.9	6.7
References	*This study*				(Muschiol et al. 2009)		(Muschiol and Traunspurger 2007)		(Gilarte et al. 2015)	(Addis et al. 2014)		(Addis et al. 2016a)

Note: TFR: total fertility rate; R_o: net reproductive rate; T_o, T_1, T: alternative mean generation time, *rm*: intrinsic rate of natural increase; PDT: population doubling time; d: day

This initial increase in fecundity could be attributed to the bacteria used to feed the nematodes. Commonly used bacteria in the hanging drop method are *E. coli* strain OP50 or their symbiotic bacteria (in the case of EPNs). *Escherichia coli* strain OP50 is a modified uracil auxotroph that cannot synthesize the essential nutrient uracil, resulting in the production of a thin bacterial lawn, and a biofilm formation-deficient mutant which is suitable for culturing nematodes while providing easy observation (Arata et al. 2020). The deficiency of uracil in *E. coli* strain OP50 could explain why *C. briggsae* had a reduced fecundity at 10^9 bacterial cells mL^{-1} (Schiemer 1982), unlike in *C. brenneri*, fed with a wild-type bacterium, *A. faecalis*. Therefore, as a wild-type strain bacterium, *A. faecalis* could have synthesized uracil and, in turn, provided an additional nutrient source that resulted in the high fecundity of *C. brenneri* in this study. This showed that *C. brenneri* can proliferate in food sources and temperature gradients with a considerable difference and still produce a similarly substantial number of offspring (Figure 1).

Life history parameter values in this study with conditions like those of the previous *Caenorhabditis* species LHT studies showed some differences (Table 2). Compared to the LHT of *C. elegans* (N2 and MY6), *C. brenneri* has a lower TFR and R_o (at 20°C with 5×10^9 cells mL^{-1}), which is only half the value that of *C. elegans.* This could indicate that not all *Caenorhabditis* species have the same reproductive capability. The fast population doubling time (PDT) was also observed in *C. brenneri*, the same as those of *C. elegans* (0.5 days). The intrinsic rate of natural increase (r_m) is one life history parameter widely considered the best option in showing the population dynamics of a nematode population.

Compared to lifespan and fecundity alone, r_m incorporates all survival and fecundity into a single measure. In this study, *C. brenneri* had a comparable r_m (1.18) compared to *C. elegans* (N2=1.38 and MY6=1.5) in the same condition. This suggests that the *C. brenneri* is also a fast-producing *Caenorhabditis* species. Furthermore, *C. brenneri* exhibits a boom-and-bust lifestyle as it has a short lifespan of only seven days compared to >10 days of *C. elegans*. Instead, *C. brenneri* has the same lifespan as EPNs, with an average lifespan of seven days.

A long lifespan does not always signify a favorable growth condition, as it has been shown that dietary restrictions affect fecundity and growth but increase longevity with the tradeoff of offspring fitness (Moatt et al. 2016; Mautz et al. 2020). A previous study recorded that about 50% of *Caenorhabditis remanei* Sudhaus 1974 and *C. brenneri* females survived for more than 20 days upon feeding *E. coli* strain OP50 in culture media at 25ºC (Amrit et al. 2010). Feeding nematodes with other bacteria aside from the common *E. coli* strain OP50 has also been shown to significantly alter some of the life history parameters, such as an increase in lifespan in *C. elegans* due to the bacteria's unique nutritional composition (Stuhr and Curran 2020). A study on *C. elegans* revealed that metabolically active bacterium such as *E. coli* strain OP50 significantly attract nematodes, leading to high fecundity and shorter lifespan than inactive bacteria strains (Yu et al. 2015). However, the present study's short lifespan of *C. brenneri* could also be attributed to the dietary nutritional value of *A. faecalis*, which is uncommonly used as a standard food source for lab-grown nematodes in studying growth and reproduction.

Besides offspring production, survivability, fecundity, and other population parameters, somatic growth in terms of body length and width of the resulting offspring was also assessed. It was found that bacterial food density-temperature interaction significantly affects the development of *C. brenneri* body length and width. Such increases in length and girth were all observed in higher bacterial density and temperature. These could be attributed to the nutritional composition of *A. faecalis*, a change from the usually utilized *E. coli* OP50 strain. Such high bacterial food sources and temperatures help promote an ideal nutrition and environment to produce a healthy brood of progenies.

Studies on *Caenorhabditis* LHT using the hanging drop method are few. This study showed that bacterial food density and temperature do not significantly influence the offspring production of *C. brenneri*. However, the interaction between bacterial density and temperature significantly affects the size of offspring in terms of body length and width. *C. brenneri* is a fast-producing, short-lived *Caenorhabditis* species exemplified by the higher r_m, lower PDT values, and measures of alternative generation time.

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REFERENCES

Addis T, Demissie S, Strauch O, Ehlers RU. 2016b. Influence of bacterial density and mating on life history traits of *Heterorhabditis bacteriophora*. Nematology 18 (8): 963-972. DOI: 10.1163/15685411-00003008.

Addis T, Mijuškovic N, Strauch O, Ehlers RU. 2016c. Life history traits, liquid culture production and storage temperatures of *Steinernema yirgalemense*. Nematology 18 (3): 367-376. DOI: 10.1163/15685411-00002966.

Addis T, Teshome A, Strauch O, Ehlers RU. 2014. Life history trait analysis of the entomopathogenic nematode *Steinernema riobrave*. Nematology 16 (8): 929-936. DOI: 10.1163/15685411-00002819.

Addis T, Teshome A, Strauch O, Ehlers RU. 2016a. Life history trait analysis of the entomopathogenic nematode *Steinernema feltiae* provides the basis for prediction of dauer juvenile yields in monoxenic liquid culture. Appl Microbiol Biotechnol 100: 4357-4366. DOI: 10.1007/s00253-015-7220-y.

Amrit FR, Boehnisch CM, May RC. 2010. Phenotypic covariance of longevity, immunity and stress resistance in the *Caenorhabditis* nematodes. PLoS One 5: e9978. DOI: 10.1371/journal.pone.0009978.

Arata Y, Oshima T, Ikeda Y, Kimura H, Sako Y. 2020. OP50, a bacterial strain conventionally used as food for laboratory maintenance of *C. elegans*, is a biofilm formation defective mutant. MicroPubl Bio 5: 2020. DOI: 10.17912/micropub.biology.000216.

Bardgett RD, Van Der Putten WH. 2014. Belowground biodiversity and ecosystem functioning. Nature 515: 505-511. DOI: 10.1038/nature13855.

Chen Y, Lee C, Lin Y, Yin K, Ho C, Liu T. 2015. Obtaining long 16S rDNA sequences using multiple primers and its application on dioxin-containing samples. BMC Bioinformatics 18: S13. DOI: 10.1186/11471-2105-16-S18-S13.

Dalan LB, Diano MAB, Tandingan De Ley I, Sumaya NH. 2022. First report of *Caenorhabditis brenneri* (Nematoda: Rhabditida) isolated from the cadaver of *Philippinella moellendorffi* (Stylommatophora: Ariophantidae), a terrestrial slug in the Philippines. J Helminthol 96: 1-5. DOI: 10.1017/ S0022149X22000475.

Dey A, Chan CKW, Thomas CG, Cutter AD. 2013. Molecular hyperdiversity defines populations of the nematode Caenorhabditis brenneri. Proceed Natl Acad Sci 110: 11056-11060. DOI: 10.1073/pnas.1303057110.

Diano MA, Dalan L, Rolish P, Sumaya, NH. 2022. First report, morphological and molecular characterization of *Caenorhabditis brenneri* (Nematoda: Rhabditidae) isolated from the giant African land snail Achatina fulica (Gastropoda: Achatinidae). Biologia 469-478. DOI: 10.1007/s11756-021-00972-x.

Gilarte P, Kreuzinger-Janik B, Majdi N, Traunspurger W. 2015. Life-history traits of the model organism *Pristionchus pacificus* recorded using the hanging drop method: Comparison with *Caenorhabditis elegans*. PLoS One 10: e0134105. DOI: 10.1371/journal.pone.0134105.

Huang C. 2020. Extensively drug-resistant Alcaligenes faecalis infection. BMC Infect Dis 20: 833. DOI: 10.1186/s12879-020-05557-8.

Johnson TE, Friedman DB, Foltz N, Fitzpatrick P, Shoemaker JE. 1990. Genetic variants and mutations of *Caenorhabditis elegans* provide tools for dissecting the aging process. In: Harrison DE (eds). Genetic Effects on Aging II. Telford, Inc, Caldwell, New Jersey

Kong L, Zhu S, Zhu L, Xie H, Wei K, Yan T, Wang J, Wang J, Wang F, Sun F. 2014. Colonization of *Alcaligenes faecalis* strain JBW4 in natural soils and its detoxification of endosulfan. Appl Microbiol Biotechnol 98: 1407-1416. DOI: 10.1007/s00253-013-5033-4.

Kreuzinger-Janik B, Brinke M, Traunspurger W, Majdi N. 2017. Life history traits of the free-living nematode, *Plectus acuminatus* Bastian, 1865, and responses to cadmium exposure. Nematology 19 (6): 645-654. DOI: 10.1163/15685411-00003077.

Mautz BS, Lind MI, Maklakov, AA. 2020. Dietary restriction improves fitness of aging parents but reduces fitness of their offspring in nematodes. J Gerontol Ser A 75: 843-848. DOI: 10.1093/gerona/glz276.

Moatt JP, Nakagawa S, Lagisz M, Walling C. 2016. The effect of dietary restriction on reproduction: A meta-analytic perspective. BMC Evol Biol 16: 199. DOI: 10.1186/s12862-016-0768-z.

Mondejar AJ, Paglinawan F, Tabelin C, Aguilos M, Aguilos R, Opiso E, Martinez JG, Metillo EB, Sumaya NH, Villacorte-Tabelin M. 2023. Survival, reproduction, and life history traits evaluation of H*eterocephalobellus* sp. and *Cephalobus* sp. from an artisanal and small-scale gold mine site, Davao de Oro, Philippines as bioindicators of heavy metal contamination. Philippine J Sci 152 (6A): 2031-2048.

Mordi RM, Burke ME, Odjadjare EE, Enabulele SA, Umeh OJ. 2015. Prevalence of urinary tract infections (UTI) among pregnant women in university of Benin teaching hospital (UBTH) Benin City, Nigeria. J Asian Sci Res 5: 198-204. DOI: 10.18488/journal.2/2015.5.4/2.4.198.204.

Muschiol D, Schroeder F, Traunspurger W. 2009. Life cycle and population growth rate of *Caenorhabditis elegans* studied by a new method. BMC Ecology 9: 14. DOI: 10.1186/1472-6785-9-14.

Muschiol D, Traunspurger W. 2007. Life cycle and calculation of the intrinsic rate of natural increase of two bacterivorous nematodes, *Panagrolaimus* sp. and *Poikilolaimus* sp. from chemoautotrophic Movile Cave, Romania. Nematology 9 (2): 271-284. DOI: 10.1163/156854107780739117.

Petersen C, Dirksen P, Prahl S, Strathmann EA, Schulenburg H. 2014. The prevalence of Caenorhabditis elegans across 1.5 years in selected North German locations: The importance of substrate type, abiotic parameters, and *Caenorhabditis* competitors. BMC Ecol 14: 4. DOI: 10.1186/1472-6785-14-4.

R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Ryss AY. 2017. A simple express technique to process nematodes for collection slide mounts. J Nematol 49 (1): 27-32. DOI: 10.21307/jofnem-2017-043.

Schiemer F. 1982. Food dependence and energetics of free-living nematodes. Oecologia 54: 108-121. DOI: 10.1007/BF00541117.

Slos D, Sudhaus W, Stevens L, Bert W, Blaxter M. 2017. *Caenorhabditis monadelphous* sp. n.: Defining the stem morphology and genomics of the genus *Caenorhabditis*. BMC Zool 2: 4. DOI: 10.1186/s40850-017-0013-2.

Stover NA, Cavalcanti ARO. 2017. Using NCBI BLAST. Curr Protoc Essential Lab Tech 14 (1): 11-34. DOI: 10.1002/cpet.8.

Stuhr NL, Curran SP. 2020. Bacterial diets differentially alter lifespan and healthspan trajectories in C. elegans. Commun Biol 3 (1): 653. DOI: 10.1038/s42003-020-01379-1.

Sudhaus W, Kiontke K. 2007. Comparison of the cryptic nematode species *Caenorhabditis brenneri* sp. n. and *C. remanei* (Nematoda: Rhabditidae) with the stem species pattern of the *Caenorhabditis elegans* group. Zootaxa 1456: 45-62. DOI: 10.11646/zootaxa.1456.1.2.

Tena D, Fernández C, Lago M.R. 2015. *Alcaligenes faecalis*: An unusual cause of skin and soft tissue infection. Jpn J Infect Dis 68 (2): 128-130. DOI: 10.7883/yoken.JJID.2014.164.

van den Hoogen J, Geisen S, Routh D, et al. 2019. Soil nematode abundance and functional group composition at a global scale. Nature 572:194-198. DOI: 10.1038/s41586-019-1418-6.

Xiao HF, Li G, Ming D, Hu F, Li HX. 2014. Effect of different bacterial-feeding nematode species on soil bacterial numbers, activity, and community composition, Pedosphere 24 (1): 116-124. DOI: 10.1016/S1002-0160(13)60086-7.

Yu L, Yan X, Ye C, Zhao H, Chen X, Hu F, Li H. 2015. Bacterial respiration and growth rates affect the feeding preferences, brood size and lifespan of *Caenorhabditis elegans*. PLoS One 10 (7): e0134401. DOI: 10.1371/journal.pone.0134401.

Zhao J, Zhang J, Zhu X, Lu J, Jin B, Chen H. 2022. The life cycle of the bacterial-feeding nematode *Diplolaimella stagnosa* and its population growth in response to temperature and food availability. Front Ecol Evol 10: 953608. DOI: 10.3389/fevo.2022.9536.