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HIGHLIGHTS

- Close nematode species can show contrasted growth patterns in response to temperature gradient.
- Body-size at maturity declines with increasing temperature.
- The number of eggs carried in the uterus declines with increasing temperature.
- Prevalence of early juvenile stages reduces body-mass structure with increasing temperature.

ABSTRACT

The temperature-size rule postulates that the growth rates of ectotherms increase under rising temperatures, while the sizes of these organisms at maturity decrease. However, the upper temperature-tolerance range is also typically represented by a metabolic tipping point after which growth suddenly ceases. Free-living nematodes are important members of ecosystems, but little is known about their thermal tolerance. In the present study we measured the population growth rates and body-size distributions of five species of free-living bacterivorous nematodes exposed in the laboratory to a broad range of temperatures. This allowed a determination of their different thermal tolerance ranges, even of closely related species, including *Plectus acuminatus* (thermal optimum of 20–25 °C) and *P.* cf. *velox* (10–15 °C). With the exception of *Acrobeloides nanus*, which had the broadest thermal tolerance range, the population growth of the other species declined between 25 and 30 °C. Our results were consistent with the temperature-size rule, as the body-size of the tested species at maturity decreased with increasing temperature. This reduction was accompanied by a smaller number of eggs carried by mature females. Although our study was purely experimental, it suggests that heat waves or other alterations in the thermal regime affect the population dynamics and body-size structure of nematode communities in the field.

KEY WORDS: Plectus; Acrobeloides; thermal tolerance; warming; life history; body-size distribution

1. INTRODUCTION

Increases in the mean and maximum temperatures and in the frequency of extreme climatic events are among the consequences of climate change (IPCC, 2014). Because life-history traits may be differentially affected by warming (e.g. reduced lifespan, but higher rate of offspring production), the population growth response, as the summation of several temperature-sensitive processes (Savage et al., 2004; Bingemer et al., 2016; Lindmark et al., 2018), may be the most appropriate measure of optimum temperature. Ectothermic species (i.e. poikilothermic species having the same body temperature as their environment) tend to grow quicker, but to hatch and mature at smaller body-sizes, as the temperature increases (at least within their thermal tolerance range). Consequently, temperature-mediated changes in body-size are another appropriate endpoint to consider in warming experiments (Van der Have and De Jong, 1996; Angilletta Jr et al., 2004). Changes in the body-size spectrum of a species and the multigenerational response of the life-history traits of that species to an altered temperature regime are likely to affect the functional structure of the broader community and thus the functioning of the respective ecosystem as well (e.g. in freshwater ecosystems see Woodward et al., 2010, 2016; O'Gorman et al., 2012).

Nematodes have several advantages as model organisms in studies of the response to changing temperatures, as they are ectothermic, extremely diverse, central to ecosystem functioning and highly abundant, colonizing all substrates examined thus far (Traunspurger, 2000; Yeates et al., 2009). Free-

living nematodes are intermediaries in food webs, feeding on bacteria, fungi, algae, and protists but also preyed upon by many animals, from invertebrates to vertebrates (Yeates et al., 1993; Majdi and Traunspurger, 2015). Nematode reproduction includes species able to reproduce continuously by parthenogenesis (Baldwin and Perry, 2004). Nematode population growth may be limited by physical (e.g., habitat-size, oxygen availability) or biological (e.g., resource availability) factors, but it is strongly reliant on the ambient temperature (Lee, 2002). Although much remains to be learned about the thermal physiology and ecology of free-living nematodes, each nematode species presumably has an optimal temperature range, one that favors population growth and is generally consistent with its biogeographic distribution (Anderson and Coleman, 1982; Venette and Ferris, 1997). Outside this thermal optimum, metabolic rates are altered and egg development may be delayed or cease until more favorable conditions return (Wharton, 2002; Ayub et al., 2013).

However, there are also nematode species whose habitats are characterized by temperature extremes or fluctuations. For example, the terrestrial species *Panagrolaimus davidi* can survive sub-zero temperatures (Smith et al., 2008), while a few nematode species have been found dwelling in the biofilms of mineral springs, where temperatures range from 10 to 40 °C (Ocaña, 1991). Nematode populations coping with extreme temperature fluctuations overcome the associated challenges by shifting their life-history traits (diapause, anhydrobiosis, dauer larval stage), reproduction strategy (egg dormancy, changing sex ratios) or behavior (migration, aggregation) (see reviews of Wharton, 2002; McSorley, 2003; Tahseen, 2012). As an example, adults of *Plectus parietinus* and *Plectus velox* can enter anhydrobiosis (i.e., a dry dormant stage) to survive the complete dehydration of their habitats (Sandhove et al., 2016).

Despite the ability of nematodes to thrive under a broad range of temperatures, the temperature dependence of these organisms implies that their population dynamics and community structure might be influenced by the local and global effects of climate change and by anthropogenic activities (Freckman and Virginia, 1997; Ruess et al., 1999; Yeates and Boag, 2003; Kim et al. 2013). In the last 100 years, the Earth's average surface temperature has risen by 0.85 °C, and a further increase is now ineluctable (IPCC, 2014). However, for populations of fast-reproducing but quasi-sessile species of small animals, the broader temperature extremes and fluctuations that occur at smaller spatial and temporal scales have more immediate implications for survival (Vafeiadou et al., 2018). Yet, in the case of free-living nematodes, our ability to predict the effects of temperature changes on their populations is very limited, partly because little is known about their thermal biology (but see Anderson and Coleman, 1982; Venette and Ferris, 1997). Understanding the response of nematode population growth rates to warming temperatures under standard laboratory conditions will improve predictions regarding nematode population dynamics in the field (Jager et al., 2005).

In this study, we measured the population growth and body-size structure of five widespread, free-living, bacterivorous nematode species: namely, the relatively large *Plectus* cf. *velox*, medium-sized *Plectus acuminatus*, and *Plectus aquatilis*, and minute *Plectus opisthocirculus* and *Acrobeloides nanus*, cultured under constant laboratory-controlled temperatures ranging from 10 to 35 °C. We first set out to identify the thermal optimum of each species and thus define the thermal niche of each one. Based on energy conservation rules, we predicted that larger species would achieve higher growth rates at lower temperatures and *vice versa*. Furthermore, since all of the studied nematode species populations have been cultivated for years (i.e. dozens of generations) under standard laboratory conditions at a constant temperature of 20 °C (Kreuzinger-Janik et al., 2017; Gansfort et al., 2018), we expected that each species would have an optimal growth temperature of 20 °C. Deviation from

this thermal optimum could reveal deep evolutionary roots to thermal tolerance mechanisms in the species investigated. We also predicted that nematode responses to warming would follow universal ecological rules (like the temperature-size-rule and its corollaries as formulated e.g. in Daufresne et al., 2009). For example, at the individual level an age-specific (e.g. in adults) decrease in individual body-size, and at the population-level a decrease in mean body-size due to an increase in the proportion of juveniles were expected.

2. MATERIAL AND METHODS

2.1. Nematode stock cultures

The five species of free-living bacterivorous nematodes used in this study included four semi-aquatic species from the genus *Plectus* (*Plectus acuminatus*, *Plectus aquatilis*, *Plectus opisthocirculus*, and *Plectus* cf. *velox*), obtained as wild isolates from the Lake Constance (Germany) littoral, and the small terrestrial species *Acrobeloides nanus*, was obtained as wild isolate from young soils at Berzdorf (Germany). All species have been maintained in our laboratory for several years at 20 °C in the dark on "sloppy" agar plates (1%) with a low salt content. For this study, the nematodes were fed with *Escherichia coli* strain OP50, a uracil-requiring strain used to prevent bacterial overgrowth. Bacterial densities in the nematode stock cultures were adjusted spectrophotometrically to 10^9–10^{10} cells mL^{-1}, a density determined to be optimal for many bacterivorous nematode species (see Kreuzinger-Janik et al., 2017 and references therein). Prior to the experiments, the nematodes were transferred to Petri dishes containing semi-solid gellan gum medium (see composition below) for acclimation. All laboratory procedures were carried out under sterile conditions.

2.2. Nematode growth medium

Component nematode growth gellan gum (CNGG) was used as the medium for the experiments, after Brinke et al. (2013). In addition to its lower salt content (including a lack of NaCl), CNGG medium solidifies in the presence of (divalent) cations, such as magnesium or calcium. Gelation is thus initiated by mixing cold solutions of gellan gum and salts. The advantages of CNGG is that fresh medium can be prepared at any time from previously autoclaved, separate solutions, and bacterial food is easily and homogeneously mixed into the medium if salts are added in the last step. As food, *E. coli* was added to the medium at a concentration of 1×10^9 cells mL^{-1} in. To prepare 5 mL of CNGG, a bacterial pellet containing 5×10^9 cells mL^{-1} was mixed with 4.2 mL of the gellan gum solution (1.9 g L^{-1} Gelrite®, with 1.25 mL cholesterol solution (5 mg mL^{-1} in ethanol) added after autoclaving). After the addition of 800 µL of salt solution (10 mM $MgSO_4$, 10 mM $CaCl_2$), the resulting CNGG medium was mixed for 30 s before it was added to the standard 6-well sterile multi-well plates (VWR® tissue culture plates 734-2323) used in the experiment.

2.3. Experimental design

For each nematode species and temperature condition, 5 replicates (4 mL of CNGG per well) were prepared in each 6-well plate. The sixth well was filled with 4 mL of distilled water to prevent evaporation during the course of the experiment (**Fig. A.1**). Nematodes from stock cultures were sieved on 35-µm meshes, the largest individuals (mostly adults) that did not pass through the meshes

were rinsed with Volvic® water from the sieve surface into a clean beaker. Fifty living adults were gently lifted with a mouth pipette under stereomicroscopy (magnification 40×) guidance and immediately placed in the individual wells (5 replicates × 50 nematodes × 6 temperatures × 5 species = 7500 adults; see **Fig. A.1**). After inoculation of the 50 starting adult individuals, each multi-well plate was sealed with Parafilm® and placed in the dark in temperature-controlled chambers set at the test temperatures of 10, 15, 20, 25, 30 and 35 °C (±0.2 °C). The experiment ran for 20 days with sub-samplings every 5 days.

2.4. Sub-sampling

At each sub-sampling (i.e. +5, +10, +15, and +20 days), the plates were opened under a laminar air-flow sterile bench and the amount of evaporated water in the first well was measured and, when necessary, compensated in all respective wells. Then the CNGG medium of each well was homogenized and liquefied by repeated pipetting using a 5-mL pipette set to 1 mL and with a tip widened by cutting its end. After 20 s of mixing, 1 mL was sampled and placed in a 15-mL Falcon tube. Because the chelating agent EDTA breaks the bonds in the CNGG matrix (Muschiol and Traunspurger, 2007), 4 mL of an EDTA-Rose Bengal solution (0.93 g of 0.02 M EDTA, 0.15 g Rose Bengal in 500 mL of deionized water) was added to the tube to liquefy the CNGG medium and stain the nematodes to facilitate their counting. The nematodes were killed by heating the tubes at 80 °C for 30 min and then stored for a maximum of 10 days at 4 °C in the dark before counting. One mL of CNGG with fresh *E. coli* food was then added to each well to replace the sub-sampled volume and reduce food/space limitation constraints. The plates were sealed again and incubated. Sample processing outside the climate chamber did not exceed 10 min for each plate. At day +20, the remaining CNGG medium was placed in 10 mL of EDTA solution for 30 min, before the final population was rinsed in EDTA by centrifugation and then preserved in 2% formaldehyde.

2.5. Sample analysis

Each stained sample was poured whole into a Petri dish (9.3 mm × 15 mm), under which a millimeter grid was printed. Using a stereomicroscope (40× magnification), the dish was screened and the nematodes were counted. In dishes with abundances >200 individuals, subsamples were counted. Density was expressed as the number of individuals per mL CNGG medium. At day +20, 60 calibrated photos of the population of *A. nanus* were taken and then processed with ImageJ software, to measure the maximum length and width of the population. The relatively large number of individuals in the *A. nanus* samples allowed a comparison of the size-spectrum of the population after 20 days exposure to temperatures of 20, 25 and 30 °C (N = 1732 individuals measured). Individual dry weight (ind. DW) was estimated using the formula of Andrássy (1956) and a dry:wet ratio of 0.25 (Wieser, 1960). At day +20, adult specimens of all species were removed from the samples and mounted on slides following the method of Seinhorst (1959). The number of eggs in the uterus of female individuals was counted and the maximum body length measured at 400× magnification, using a reticle in the ocular of a Leitz® Dialux 20 microscope.

2.6. Calculation of population growth rates

The development of population densities over time can be described by the exponential growth function (Ricklefs and Miller, 1999). Therefore, we plotted the densities determined in each well over time (in days) and then calculated the R^2 and the parameters of an exponential function for each replicate well. From the 150 exponential relationships (5 replicates × 6 temperature × 5 species), 90% had R^2-values > 0.5. According to the exponential growth function, the number of individuals in a population based on an initial population N_0 after *t* days can be calculated as:

$$N_t = N_0 \times e^{rt}$$

where *r* is the intrinsic growth rate of the population, also called the intrinsic rate of natural increase (Birch, 1948).

Given the artificial "mortality" induced by the removal of 25% of the medium during sub-sampling on days 5, 10, and 15, the formula had to be adapted to avoid underestimation of the growth rate (see details in Gansfort et al., 2018). After 5 days the "new" population is calculated as:

$$N_5 = N_0 \times e^{r5}$$

From that number, 25% of the individuals were removed while 75% remained in the wells and continued to grow. This resulted in the following formula expressing the number of individuals after 10 days (5 days after day 5).

$$N_{10} = N_0 \times 0.75 \times e^{r10}$$

Accordingly, after 15 and 20 days, the number of individuals could be calculated as follows:

$$N_{15} = N_0 \times 0.75^2 \times e^{r15}$$

$$N_{20} = N_0 \times 0.75^3 \times e^{r20}$$

Applying *ln* on each side allowed calculation of the intrinsic growth rate *r*:

$$r = \frac{\ln(N_{20}) - \ln(N_0) - \ln(0.75^3)}{20} \leftrightarrow r = \frac{\ln(N_{20}) - \ln(N_0)}{20} + 0.0432$$

The last transformation illustrates that artificial mortality after 20 days is adequately represented by the inclusion of a factor of 0.0432, thus allowing correction of the measured growth rate for medium and individual removal. Note that for *P. opisthocirculus*, two instead of three sub-samples were taken, because at +15 days the population had grown so slowly that renewal of a part of the medium was deemed unnecessary. Therefore, the correction of r was smaller in this case: $\ln(0.75^2)/20 = 0.0288$.

2.7. Data analysis

The influence of temperature on the growth rate of each species was compared using the non-parametric Kruskal-Wallis rank sum test (KW test) with Dunn's post-hoc comparisons and a p-value adjusted for multiple comparisons using a Holm-Bonferroni correction. Polynomial regression curves with 95% confidence intervals were fitted on population growth rate data. A KW test with a pairwise Wilcoxon signed rank test was used to test for significant differences across temperatures with respect to the number of eggs in the uterus of mature females and for differences in female body-length. For *A. nanus*, the size-structure of the population at 20, 25 and 30 °C was compared using a KW test and a Wilcoxon signed rank test. To control for the rate of false discoveries amongst the rejected

hypotheses, the p-values in the Wilcoxon comparisons were adjusted using the BH method of Benjamini and Yekutieli (2001). All statistical analyses and graphical representations were carried out using R software (R Development Core Team, 2018), including the packages *MASS*, *dunn.test*, and *vegan* for statistics and *dplyr*, *ggplot2*, and *ggpubr* for graphical representations.

3. RESULTS

3.1. Effect of temperature on the growth rate of each species

The growth rates of *Plectus acuminatus*, *Plectus aquatilis* and *Plectus opisthocirculus* followed a similar pattern with respect to temperature, with growth peaking at 20–25 °C before decreasing sharply at higher temperatures (**Fig. 1**). For those species, no individuals were found alive after 5 days of exposure to a temperature of 35 °C, and population growth was fairly low at 10 and 30 °C. The univariate results of the statistical tests, shown in the Appendix (**Table B.1**), revealed significant differences in the growth rates of the nematodes between the extreme (10 and 35 °C) and the optimum (20 and 25 °C) temperatures. In addition, the difference in the growth rate at 25 vs. 30 °C was significant for *P. acuminatus* and *P. opisthocirculus* (**Table B.1**), and marginally significant for *P. aquatilis* ($P_{adjusted}$ = 0.052). For those three species, the results suggested the existence of a metabolic tipping point between 25 and 30 °C.

The pattern observed for *Plectus* cf. *velox* differed, as this species also grew well at the colder temperatures (10 and 15 °C). For *A. nanus*, population growth peaked at 25–30 °C before collapsing at 35 °C (**Table B.1**), but this was the only species in which individuals were still found alive after 20 days of exposure to 35 °C.

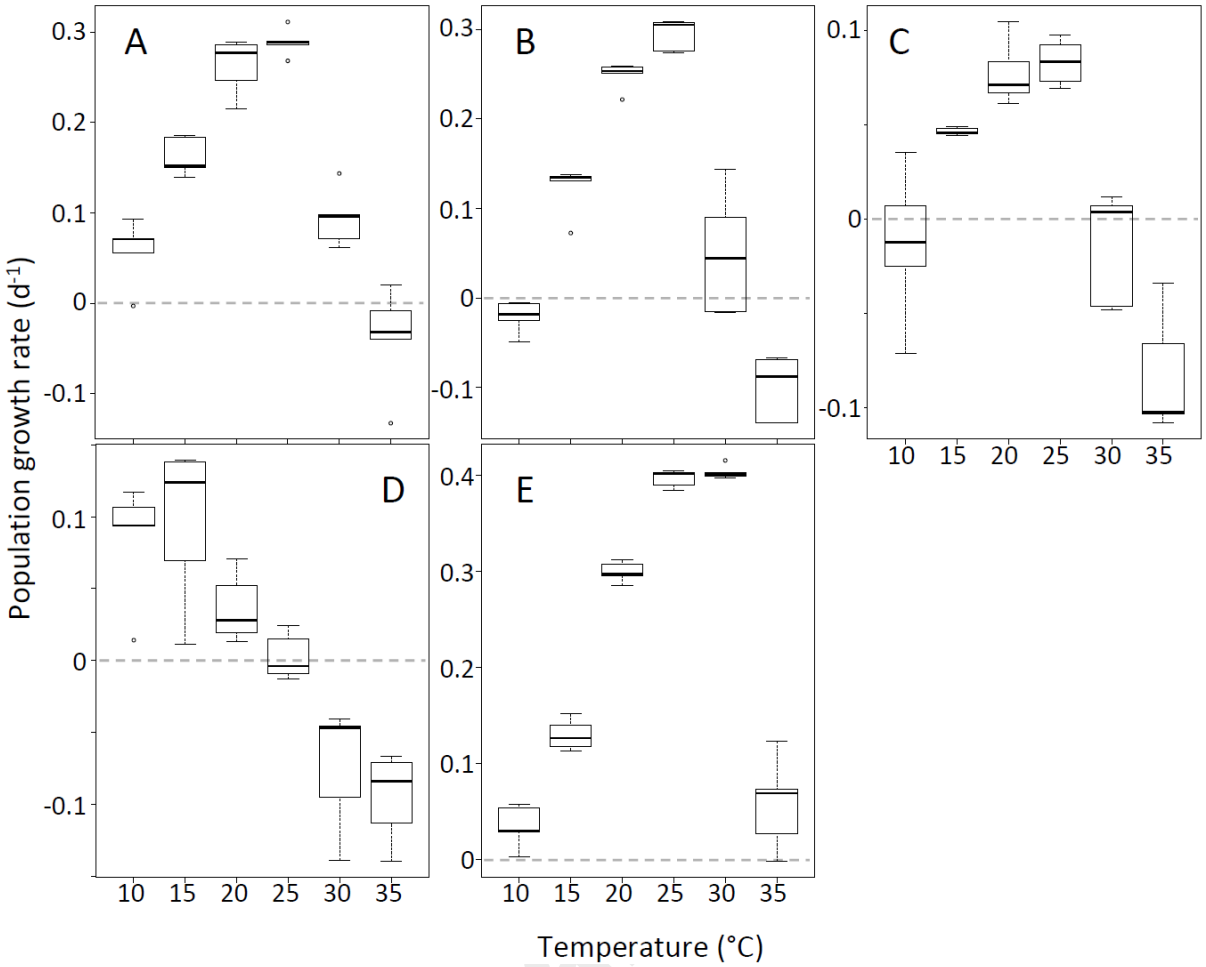

Fig. 1. Population growth rates of five species of nematodes cultured under a broad range of temperatures: (A) *Plectus acuminatus*, (B) *Plectus aquatilis*, (C) *Plectus opisthocirculus*, (D) *Plectus* cf. *velox*, and (E) *Acrobeloides nanus*. Values are medians, and boxes the interquartile range. Negative growth (i.e. population collapse) occurs under the dotted line.

3.2. Comparison of species' performance at 10–35 °C

At 10 °C, the large cryophilic *Plectus* cf. *velox* performed better than *P. aquatilis* and *P. opisthocirculus* (**Figs 2** and **B.1**). Interestingly, *P. acuminatus* also performed well at 10 °C and better than *P. aquatilis*, while the growth pattern of the two species was similar at higher temperatures (see **Table B.2** for statistical comparisons). At 15 °C, *P. opisthocirculus* stood out because of its weak growth, but this species did not grow as well as the others under all conditions tested. At 20 °C, the growth rates of *P. opisthocirculus* and *P.* cf. *velox* were far slower than those of the other species. At 25 °C, *A. nanus* had the highest growth rate, although *P. acuminatus* and *P. aquatilis* continued to grow well. At 30 °C, the growth rate of the minute thermophilic *A. nanus* peaked while that of all other species diminished. At 35 °C, as noted above, *A. nanus* was the only species still alive after 20 days and its population growth rate was still positive.

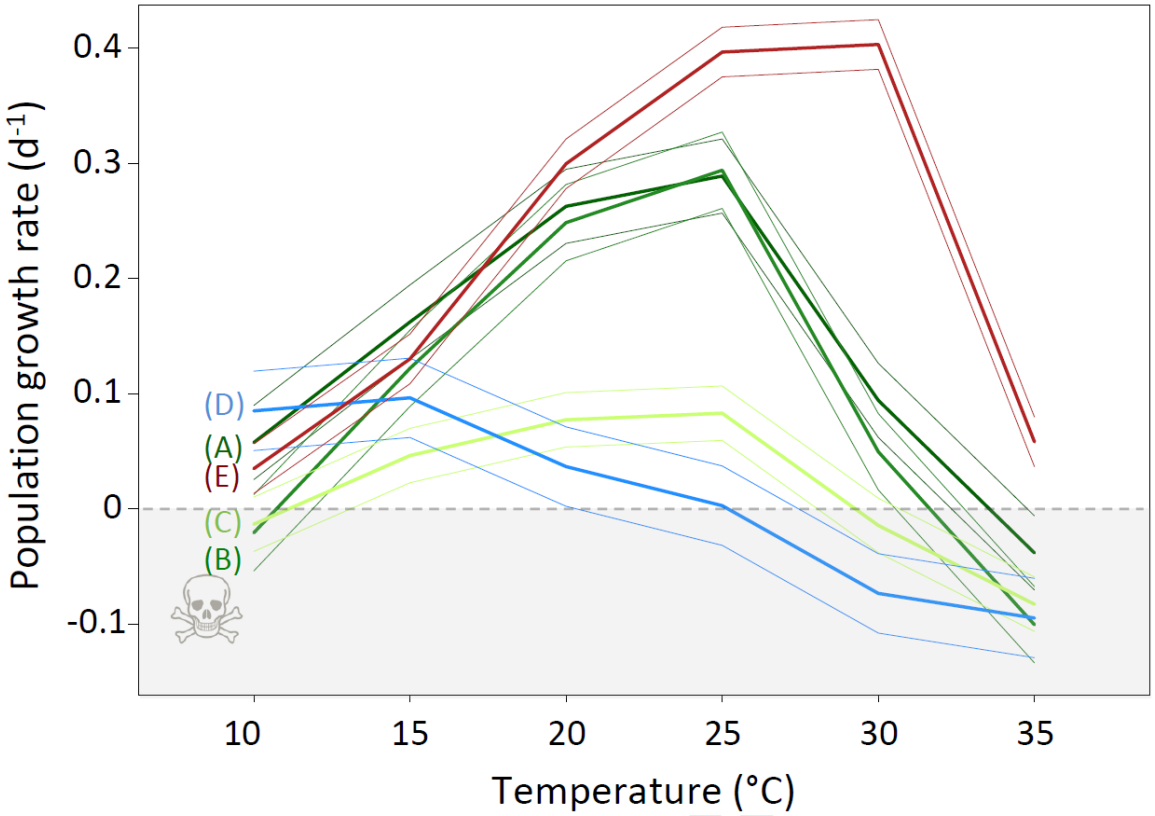

Fig. 2. Polynomial regression fit (±95% confidence interval) of the population growth rate of five species of nematodes cultured under a broad range of temperatures. (A) *Plectus acuminatus*, (B) *Plectus aquatilis*, (C) *Plectus opisthocirculus*, (D) *Plectus* cf. *velox*, and (E) *Acrobeloides nanus*. Negative growth (i.e. population collapse) occurs under the dotted line.

3.3. Effect of temperature on the number of eggs and on body-size at maturity

Both the number of eggs present in the uterus of female nematodes and the body-length of mature females allowed very small species with low numbers of eggs (*Plectus opisthocirculus* and *Acrobeloides nanus*) to be distinguished from the larger *Plectus acuminatus* and *P. aquatilis*, whose females carried many more eggs and had a body-size more than twice as long (**Figs C.1** and **C.2**). The highest number of eggs were in *Plectus* cf. *velox* females (mean of 6.96 eggs female^{-1}, max. of 15). This species was also the largest at maturity (mean body-length of 1504 µm, max. 1772 µm).
The number of eggs in the uterus of *A. nanus* females was very low under all tested conditions, whereas for the other four species the differences in the number of eggs was significant at all temperatures (KW $\chi^2 > 11.4$, $p < 0.02$). In *P. acuminatus* and *P. aquatilis*, the number of eggs peaked at 20 °C and then decreased (**Fig. 3**). In *P. opisthocirculus*, the number of eggs increased and in *P.* cf. *velox* it decreased with increasing temperature (**Fig. 3**).

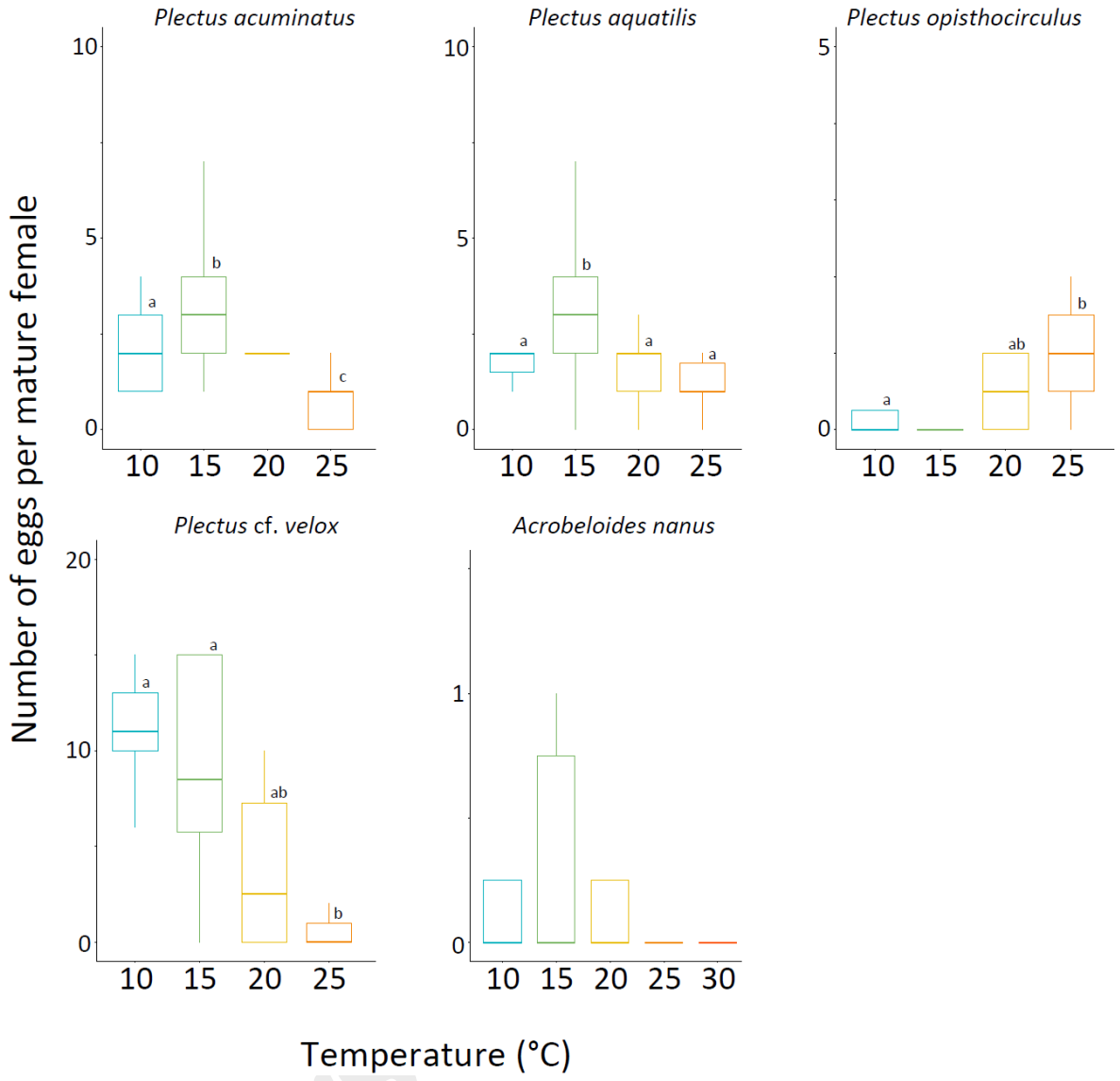

Fig. 3. Number of eggs in the uterus of mature females (N = 420) from five species of nematodes cultured under a broad range of temperatures. Different letters indicate significant differences in pairwise comparisons.

Body-length at maturity was significantly affected by temperature in all five nematode species (KW $\chi^2 > 12.1$, $p < 0.01$). The body-lengths of *P. acuminatus* and *A. nanus* tended to decrease with increasing temperature, whereas for *P. aquatilis* and *P. opisthocirculus* length at maturity decreased significantly between 25 and 30 °C (**Fig. 4**).

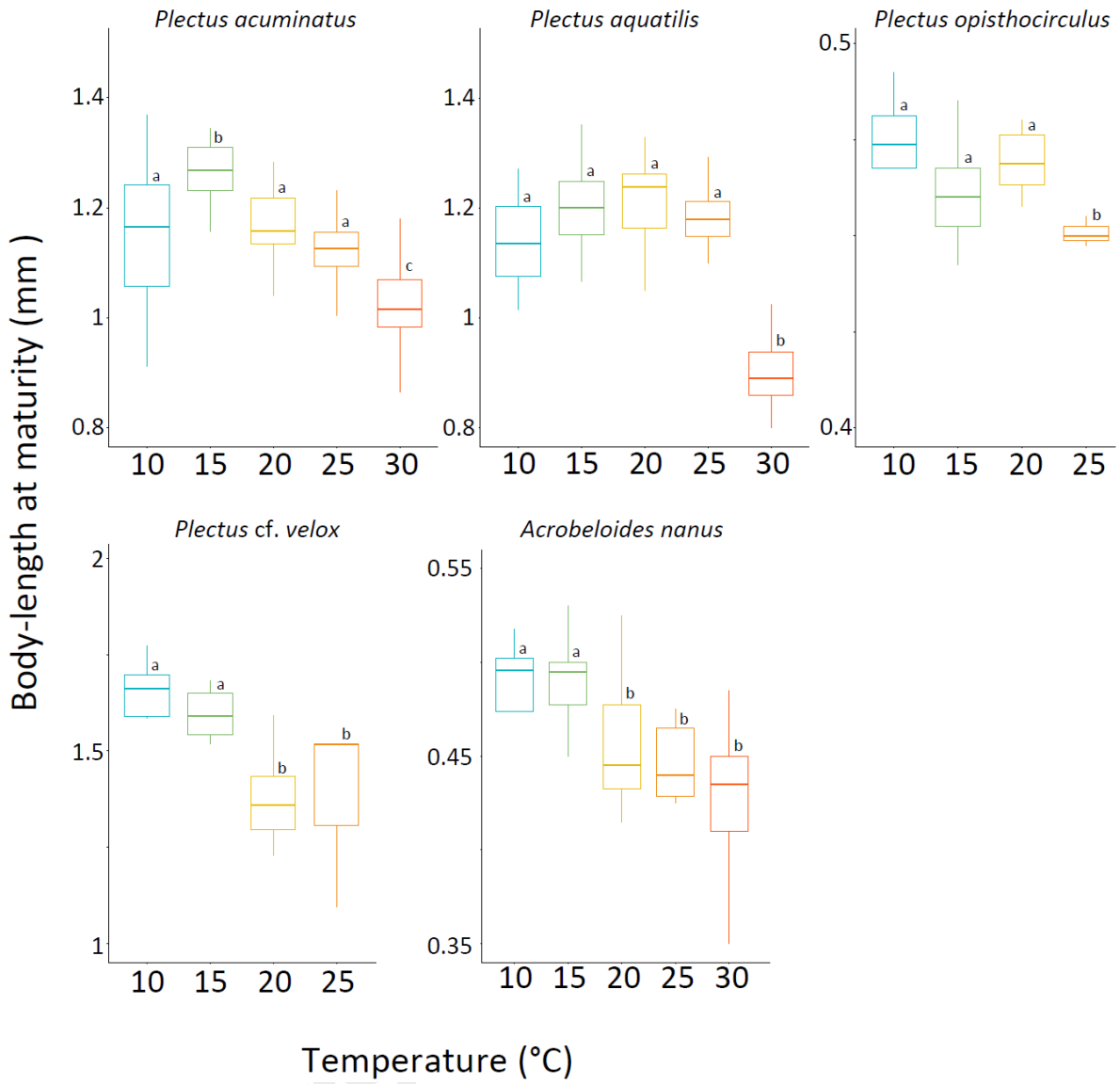

Fig. 4. Body-length of mature females (N = 420) from five species of nematodes cultured under a broad range of temperatures. Different letters indicate significant differences in pairwise comparisons.

3.4. Effect of temperature on the population size spectrum of *A. nanus*

Within a population, individual body-mass was significantly greater at 20 °C than at 25 and 30 °C (medians of 6.66, 2.91 and 3.06 ngDW ind.$^{-1}$ at 20, 25 and 30°C, respectively, KW χ^2 = 163.5, p < 0.001, see **Fig. 5**). This reduction was due to a higher proportion of early juvenile stages at 25 and 30 °C (individuals weighing roughly between 0.4 and 5 ngDW; see the changes in the population body-mass spectra in **Fig. D.1**).

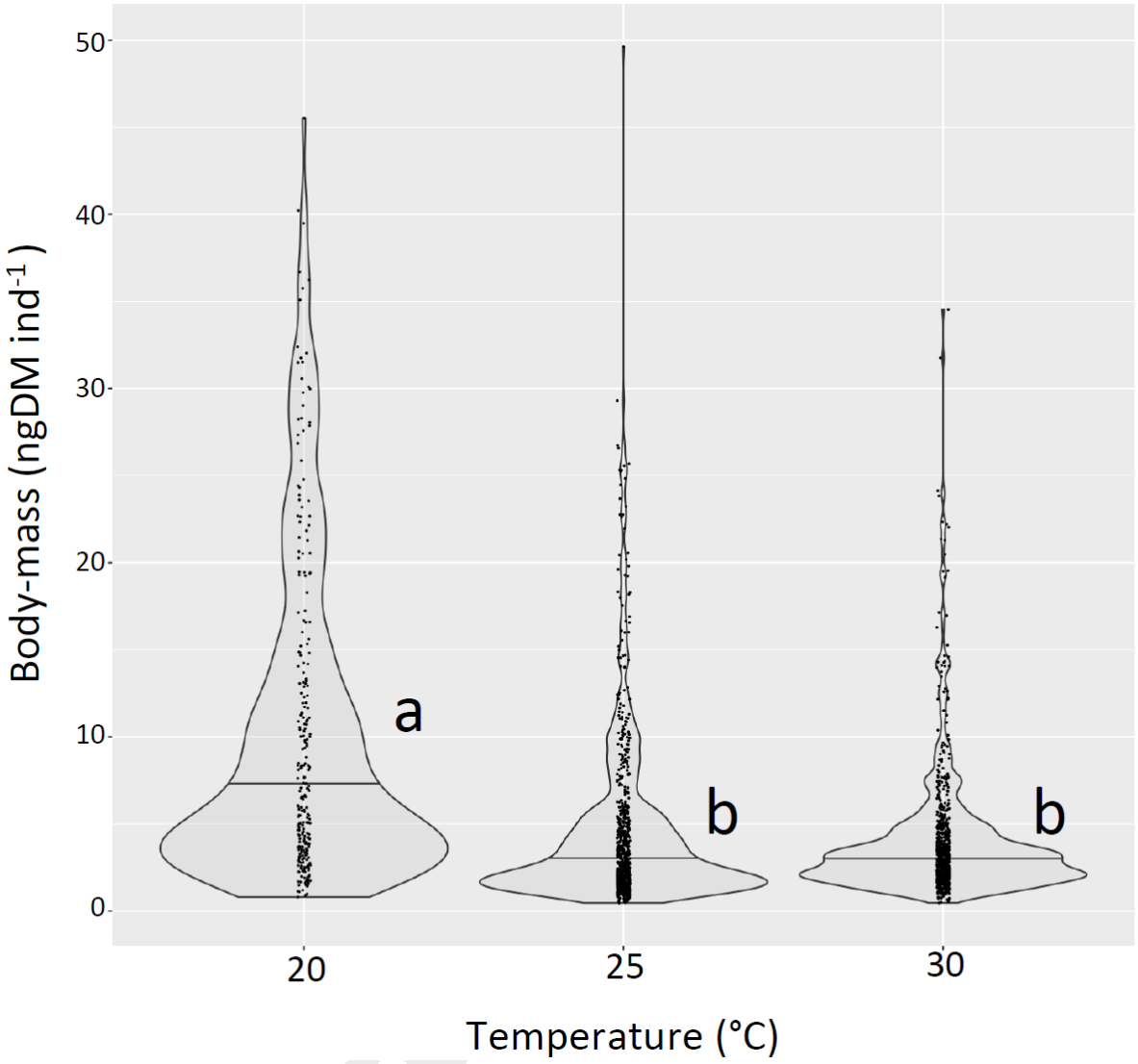

Fig. 5. Individual dry mass (DM) in populations of the nematode *A. nanus* cultured at 20, 25 and 30 °C. Violin plots show the probability density distribution on each side, as well as the median values. Points are samples. Different letters indicate significant differences in pairwise comparisons.

4. DISCUSSION

The population growth rate is an important unifying variable in ecology allowing a better understanding of the effects of environmental stress (e.g., climate change), because negative growth rates influence the boundaries of a species' ecological niche (Sibly and Hone, 2002). In nematodes and many other species, population growth reflects the sum of many temperature-sensitive processes, including changes in lifespan, rate of development, egg production, hatching success, and generation time (Pillai and Taylor, 1967; Sohlenius, 1968; Hopper et al., 1973; Trudgill and Perry, 1994; Ruess et al., 1999; Lee, 2002). Our results show that the population growth rates of different species of free-living bacterivorous nematodes may differ in their thermal optima and thermal tolerance ranges. As demonstrated in this study, this is also true for phylogenetically close species or species that have been maintained for many generations under the same standard laboratory conditions (20 °C). Tipping points for population growth between 25 and 30 °C occurred in the three species of *Plectus* (*P.*

acuminatus, *P. aquatilis* and *P. opisthocirculus*), and between 30 and 35 °C for *A. nanus*. This pattern is typical of temperature-dependent processes, as the thermal response of a species' growth rate can be interpreted in terms of enzyme kinetics, as done by Sharpe & DeMichele (1977). Those authors derived a model describing a tipping point for growth based on the sudden temperature-dependent inactivation of a hypothetical enzyme limiting major aspects of development, such as cell division and differentiation.

Our study is the first to report the temperature-related population growth rates of Plectidae (see **Table 1**). Plectidae are widely distributed in terrestrial and freshwater ecosystems, they are also frequently found in habitats subject to extreme environmental stresses, including mosses that endure dry periods, polar and alpine soils, with their cycles of freezing and thawing, and hot springs, with their very high temperatures (e.g. Ocaña, 1991; Sohlenius et al., 1995). Plectidae have unique anhydrobiotic capabilities and a longer lifespan than members of the Rhabditidae and Cephalobidae, and they produce more eggs (Sandhove et al., 2016; Kreuzinger-Janik et al., 2017). These properties could provide an advantage in cold and dry habitats. In our study, *Plectus* cf. *velox* was the largest species and the one most tolerant of cold conditions; however, the other *Plectus* species (i.e. *P. acuminatus*, *P. aquatilis* and *P. opisthocirculus*) were not particularly tolerant of either cold or very hot temperatures. Why *Plectus aquatilis* was found to persist in hot springs at 40-42 °C after Ocaña (1991), is unclear. One hypothesis is that thermal adaptation in species is different from thermal adaptation in populations (see discussion in Moens and Vincx, 2000). The tolerance of wild nematode populations might fit the local environmental conditions, and thus we recommend longer-term experiments conducted using different wild population strains to better delineate species tolerance range. Furthermore, the nematodes in our study had become acclimated to standardized media, constant temperatures, and one type of food—conditions that are quite different from the natural environment. Thus, the reported population growth rates likely represent the current characteristics of the laboratory populations rather than the full range of species' plasticity. Indeed, in the field, numerous other additional abiotic and biotic factors (e.g. moisture, oxygen concentration, food concentration and quality, presence of predators and competitors) play a role and are expected to change in synergy with changes in the temperature regime and thereby impact the population growth rate as well. Nonetheless, our measures provide a standard with which to determine the direct impact of temperature on population growth.

Table 1. Comparison of lower, optimum, and upper temperature thresholds measured in different species of free-living bacterivorous nematodes (listed by increasing temperature optima). The reported thresholds were determined based on empirically observed negative population growth rates or rapid declines in population numbers. Optima were defined from the maximum population growth rates measured across a broad range of experimental temperatures. Maximum population growth rates, reported as the maximum intrinsic rate of population growth (r_{max}), are based on data from this study (shown in bold) and from published material. The column 'Habitat' refers to the environment where the species was initially isolated from prior to laboratory rearing/culture.

Species	Low T°C	Optimum	Up T°C	Habitat	Location	T °C range tested	Culture medium, food, and data analysis methodology	r_{max} (d^{-1})	Reference
Diplolaimelloides bruciei	5	12.5	25	marine	Lynther Estuary, UK	5—30	DifcoCorne Agar. field bacteria. Measure of generation time	nd	Warwick 1981
Pelodera sp2	5	15	30	soil	CO, USA	10—40	1.5% Agar. Pseudomonas cepacia. Counts over 1-mo period	nd	Anderson & Coleman 1982
Plectus* cf. *velox	**<10**	**15**	**25**	**freshwater**	**Lake Constance, Germany**	**10—35**	**NGG. Ecoli. intrinsic growth rate. polynomial regression fit**	**0.14**	**This study**
Rhabditis cucumeris	4	17.7	24.8	soil	CA, USA	10—35	NGM. Ecoli. Schoolfield's poikilotherm model	0.9*	Venette & Ferris 1997
Mesodiplogaster lheritieri	10	20	30	soil	CO, USA	10—40	1.5% Agar. Pseudomonas cepacia. Counts over 1-mo period	nd	Anderson & Coleman 1982
Litoditis marina	15	20	30	marine	Westerschelde, The Netherlands	5—35	1% BactoAgar. field bacteria. Q10	nd	Moens & Vincx 2000
Caenorhabditis elegans	5.9	21.1	28.2	soil	CA, USA	10—35	NGM. Ecoli. Schoolfield's poikilotherm model	1.57*	Venette & Ferris 1997
Rhabditis sp.	10	24	35	soil	CO, USA	10—40	1.5% Agar. Pseudomonas cepacia. Counts over 1-mo period	nd	Anderson & Coleman 1982
Pelodera sp1	20	24	35	soil	MO, USA	10—40	1.5% Agar. Pseudomonas cepacia. Counts over 1-mo period	nd	Anderson & Coleman 1982
Caenorhabditis sp.	5	24	30	soil	CO, USA	10—40	1.5% Agar. Pseudomonas cepacia. Counts over 1-mo period	nd	Anderson & Coleman 1982
Acrobeloides sp.	5	24	35	soil	CO, USA	10—40	1.5% Agar. Pseudomonas cepacia. Counts over 1-mo period	nd	Anderson & Coleman 1982
Diplolaimelloides meyli	10	25	35	marine	Westerschelde, The Netherlands	5—35	1% BactoAgar. field bacteria. Q10	nd	Moens & Vincx 2000
Plectus acuminatus	**<10**	**25**	**34**	**freshwater**	**Lake Constance, Germany**	**10—35**	**NGG. Ecoli. intrinsic growth rate. polynomial regression fit**	**0.31**	**This study**
Plectus aquatilis	**10**	**25**	**32**	**freshwater**	**Lake Constance, Germany**	**10—35**	**NGG. Ecoli. intrinsic growth rate. polynomial regression fit**	**0.31**	**This study**
Plectus opisthocirculus	**10**	**25**	**30**	**freshwater**	**Lake Constance, Germany**	**10—35**	**NGG. Ecoli. intrinsic growth rate. polynomial regression fit**	**0.10**	**This study**
Acrobeloides nanus	<15	>25	>30	soil	Wageningen, The Netherlands	15—25	NGM. Ecoli 20°C. DEBtox fit to estimate growth rate	0.5**	Álvarez et al. 2006
Bursilla labiata	10.7	25.6	33.7	soil	CA, USA	10—35	NGM. Ecoli. Schoolfield's poikilotherm model	0.6*	Venette & Ferris 1997
Acrobeloides buetschlii	11.9	26.6	33.9	soil	CA, USA	10—35	NGM. Ecoli. Schoolfield's poikilotherm model	0.5*	Venette & Ferris 1997
Acrobeloides nanus	**<10**	**27**	**>35**	**soil**	**Berzdorf, Germany**	**10—35**	**NGG. Ecoli. intrinsic growth rate. polynomial regression fit**	**0.42**	**This study**
Panagrolaimus var NFS 25-5	<21	27	>29	soil	USA	21—29	NGG. Ecoli. Intrinsic growth rate	0.93	Ayub et al. 2013
Acrobeloides bodenheimeri	10.5	29.2	34.7	soil	CA, USA	10—35	NGM. Ecoli. Schoolfield's poikilotherm model	0.6*	Venette & Ferris 1997
Cephalobus persegnis	5.8	32.2	42.4	soil	CA, USA	10—35	NGM. Ecoli. Schoolfield's poikilotherm model	0.5*	Venette & Ferris 1997

nd not determined * approximated from graphical data and hourly rate ** max r measured at 20°C without exposure to chemicals

Overall, the rates of population growth as well as the thermal tolerance ranges and optima determined in our study are in good agreement with the results of the few other studies investigating the impact of a broad spectrum of temperatures on free-living bacterivorous nematodes (**Table 1**). Despite the different methodological approaches used by different research groups to culture nematode strains and calculate population growth rates, some agreement might emerge from comparisons of the present study with the literature. For example, the species from the family Cephalobidae, to which the genus *Acrobeloides* belongs, seem noteworthy in their capacity to tolerate relatively high temperatures (> 25 °C) (**Table 1**). This pattern matches well with compelling evidence that Cephalobidae are typical inhabitants of dry and arid soils (Steinberger et al., 1984; Freckman et al., 1987; Andrássy, 2005).

Mature females of the nematode species included in this study tended to be smaller and to produce fewer eggs at warmer temperatures. The number of eggs in the uterus was contingent upon the body-size of the female, as demonstrated by the positive linear relationship between these two parameters (R^2 = 0.36, N = 420). That nematode adults tended to be smaller with increasing temperature is in general agreement with the temperature-size-rule. According to this rule, ectothermic organisms reach a smaller size at maturity in response to warming (e.g. Huntley and Lopez, 1992; Atkinson, 1995; Daufresne et al., 2009; Gardner et al., 2011; Forster et al., 2012). This trend may be even more pronounced in aquatic habitats because the availability of dissolved oxygen is also a crucial driver of temperature-size responses (Forster et al., 2012). In the case of bacterivorous nematode populations, it is well-known that females can mature at smaller sizes under food stress (Sudhaus, 1980; Schiemer et al., 1980; Schiemer 1982; dos Santos et al., 2008). This strategy is thought to shorten the immature life period and thus the costs for attaining maturity (Schiemer et al. 1980). In field populations, a general decrease of the body-size spectrum with warming-stress would presumably have far-reaching consequences on trophic transfers since nematodes are food for many other organisms (Yeates et al., 1993; Majdi and Traunspurger, 2015). For example, an experimental field warming can dramatically affect body-size and trophic structure of soil communities in Antarctica's dry valleys as it slightly increases the density of a small bacterivorous nematode species (*Scottnema lindsayae*), while it strongly decreases the densities of a larger omnivorous-predacious species (*Eudorylaimus antarcticus*) (Freckman and Virginia, 1997). At the scale of population size-structure, the results obtained for *A. nanus* support the hypothesis of Daufresne et al. (2009), that warming induces a decrease in the mean body-size of a population due to a higher proportion of juvenile stages. In addition, our result also agreed with Jager et al. (2005) showing that *A. nanus* starts to produce eggs at a smaller size under higher temperatures. Consequently, on the other hand, populations of nematode species with a longer life cycle, such as *Plectus* spp., should be more sensitive to the effects of climate change (Ruess et al., 1999). Since body-size is a key parameter in the structuring of ecological communities (Woodward et al., 2005), the ecological role of free-living nematodes will be accordingly presumably altered.

5. CONCLUSION

Thermal pollution is one of the many anthropogenic alterations of ecosystems, and the frequency and severity of extreme climatic events such as heat waves are likely to increase with global climate warming. Ruess et al. (1999) suggested that temperature changes will alter nematode community structure, by reducing the dominance of some species and promoting that of others. In our study, populations of widespread bacterivorous nematodes varied in their responses to a broad range of temperatures, evidenced as changes in the growth and size-structure of the five species tested. These results demonstrate that changes in the thermal regime have the potential to alter the population dynamics of free-living nematodes. The implications extend to the ecosystem level, since nematodes are basal key-consumers in biofilms, soils and aquatic sediments, thereby contributing to global nutrient cycles and fueling higher trophic levels. To better forecast the response of nematode communities to changes in the thermal regime, the effect of temperature should be examined under more realistic conditions, including different culture media, different food concentrations and quality, and different conditions of coexistence with competitors and predators (see e.g. Vafeiadou et al. 2018, Gansfort et al. 2018).

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APPENDICES

Appendix A. Experiment design

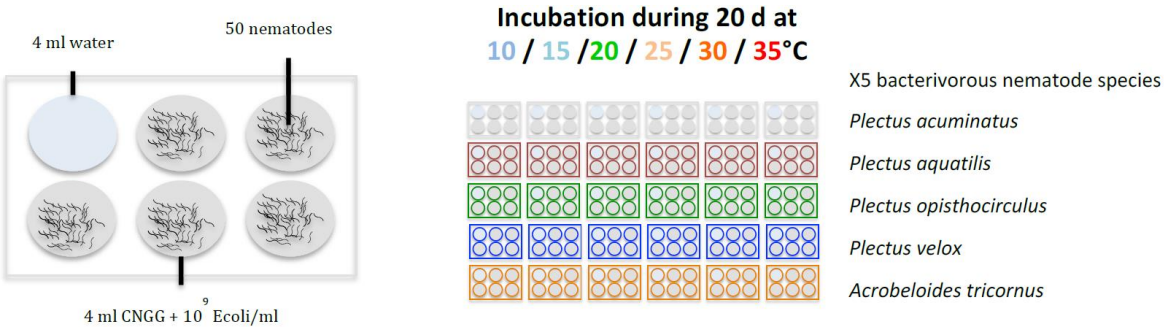

Fig A.1. Schematic representation of an experimental plate at T0 (left panel) and of the full experimental design (right panel).

Appendix B. Univariate results of the statistical tests & additional figure

Table B.1. Comparison of growth rates of each nematode species across a temperature gradient (10°C to 35°C). Bold values correspond to significant differences.

Plectus acuminatus Kruskal-Wallis chi-squared = 27.07. df = 5. p-value <0.001

"comparisons"	"Z"	"P"	p adjusted holm
10-15	-1.65	0.0492	0.3446
10-20	-2.73	0.0032	**0.0380**
15-20	-1.07	0.1406	0.7030
10-25	-3.34	0.0004	**0.0054**
15-25	-1.68	0.0457	0.3654
20-25	-0.61	0.2707	0.2707
10-30	-0.68	0.2475	0.4949
15-30	0.96	0.1661	0.6642
20-30	2.04	0.0203	0.1827
25-30	2.65	0.0039	**0.0432**
10-35	0.96	0.1661	0.4982
15-35	2.62	0.0044	**0.0437**
20-35	3.69	0.0001	**0.0015**
25-35	4.31	0.0000	**0.0001**
30-35	1.65	0.0492	0.2954

Plectus aquatilis Kruskal-Wallis chi-squared = 27.086. df = 5. p-value <0.001

"comparisons"	"Z"	"P"	p adjusted holm
10-15	-1.43	0.0754	0.4522
10-20	-2.55	0.0054	0.0591
15-20	-1.11	0.1327	0.6635
10-25	-3.44	0.0003	**0.0037**
15-25	-2.01	0.0221	0.1991
20-25	-0.89	0.1846	0.5537
10-30	-0.82	0.2043	0.4086
15-30	0.61	0.2707	0.2707
20-30	1.72	0.0423	0.2962
25-30	2.62	0.0044	0.0524
10-35	1.04	0.1487	0.5950
15-35	2.47	0.0066	0.0659
20-35	3.59	0.0002	**0.0023**
25-35	4.49	0.0000	**0.0001**
30-35	1.86	0.0309	0.2470

Plectus opisthocirculus Kruskal-Wallis chi-squared = 25.97. df = 5. p-value <0.001

"comparisons"	"Z"	"P"	p adjusted holm
10-15	-1.41	0.0779	0.5456
10-20	-2.64	0.0041	**0.0455**
15-20	-1.22	0.1110	0.5548
10-25	-2.89	0.0019	**0.0249**
15-25	-1.47	0.0704	0.5631
20-25	-0.25	0.4007	0.8014
10-30	0.00	0.5000	0.5000
15-30	1.41	0.0779	0.4677
20-30	2.64	0.0041	**0.0414**
25-30	2.89	0.0019	**0.0230**
10-35	1.13	0.1289	0.5156
15-35	2.55	0.0054	**0.0484**
20-35	3.77	0.0001	**0.0011**
25-35	4.02	0.0000	**0.0004**
30-35	1.13	0.1289	0.3867

Plectus cf. *velox* Kruskal-Wallis chi-squared = 23.694. df = 5. p-value <0.001

"comparisons"	"Z"	"P"	p adjusted holm
10-15	-0.17	0.4287	0.4287
10-20	0.75	0.2253	0.6760
15-20	0.93	0.1752	0.8758
10-25	1.65	0.0492	0.3446
15-25	1.83	0.0335	0.3013
20-25	0.89	0.1846	0.7383
10-30	3.05	0.0011	**0.0136**
15-30	3.23	0.0006	**0.0080**
20-30	2.29	0.0108	0.1075
25-30	1.40	0.0806	0.4837
10-35	3.44	0.0003	**0.0039**
15-35	3.62	0.0001	**0.0021**
20-35	2.69	0.0035	**0.0388**
25-35	1.79	0.0362	0.2899
30-35	0.39	0.3464	0.6927

Acrobeloides nanus Kruskal-Wallis chi-squared = 26.378. df = 5. p-value <0.001

"comparisons"	"Z"	"P"	p adjusted holm
10-15	-1.40	0.0806	0.4837
10-20	-2.37	0.0089	0.0888
15-20	-0.96	0.1661	0.4982
10-25	-3.62	0.0001	**0.0020**
15-25	-2.22	0.0130	0.1167
20-25	-1.25	0.1043	0.5217
10-30	-3.80	0.0001	**0.0011**
15-30	-2.40	0.0080	0.0885
20-30	-1.43	0.0754	0.5277
25-30	-0.17	0.4287	0.4287
10-35	-0.32	0.3732	0.7465
15-35	1.07	0.1406	0.5624
20-35	2.04	0.0203	0.1624
25-35	3.30	0.0005	**0.0057**
30-35	3.48	0.0002	**0.0032**

Table B.2. Comparison of the growth rate of 5 nematode species (*Acrobeloides nanus*: nan. *Plectus acuminatus*: acu. *Plectus aquatilis*: aqu. *Plectus opisthocirculus*: opi. *Plectus* cf. *velox*: vel) at each temperature. Bold values correspond to significant differences.

10°C Kruskal-Wallis chi-squared = 16.689. df = 4. p-value = 0.002

comparisons	Z	P	p adjusted holm
nan-acu	-0.64	0.259	0.519
nan-aqu	1.93	0.026	0.159
acu-aqu	2.57	0.004	**0.039**
nan-opi	1.50	0.066	0.265
acu-opi	2.14	0.015	0.110
aqu-opi	-0.42	0.333	0.333
nan-vel	-1.50	0.066	0.331
acu-vel	-0.85	0.195	0.585
aqu-vel	-3.43	0.0002	**0.002**
opi-vel	-3.00	0.001	**0.011**

15°C Kruskal-Wallis chi-squared = 16.32. df = 4. p-value = 0.002

comparisons	Z	P	p adjusted holm
nan-acu	-1.67	0.046	0.234
nan-aqu	0.30	0.381	0.381
acu-aqu	1.97	0.024	0.168
nan-opi	2.27	0.011	0.091
acu-opi	3.95	0.00003	**0.0003**
aqu-opi	1.97	0.024	0.144
nan-vel	0.81	0.207	0.621
acu-vel	2.49	0.006	0.057
aqu-vel	0.51	0.303	0.606
opi-vel	-1.46	0.072	0.288

20°C Kruskal-Wallis chi-squared = 21.43. df = 4. p-value <0.001

comparisons	Z	P	p adjusted holm
nan-acu	1.37	0.084	0.253
nan-aqu	1.71	0.042	0.214
acu-aqu	0.34	0.365	0.365
nan-opi	3.26	0.0005	**0.004**
acu-opi	1.89	0.029	0.176
aqu-opi	1.54	0.060	0.243
nan-vel	4.16	0.00001	**0.0001**
acu-vel	2.79	0.002	**0.020**
aqu-vel	2.44	0.007	0.0501
opi-vel	0.90	0.183	0.366

25°C

Kruskal-Wallis chi-squared = 21.94. df = 4. p-value <0.001

comparisons	Z	P	p adjusted holm
nan-acu	1.67	0.046	0.281
nan-aqu	1.54	0.060	0.243
acu-aqu	-0.12	0.448	0.448
nan-opi	3.22	0.0006	**0.005**
acu-opi	1.54	0.060	0.182
aqu-opi	1.67	0.046	0.234
nan-vel	4.29	0.000008	**0.00008**
acu-vel	2.62	0.004	**0.030**
aqu-vel	2.74	0.002	**0.023**
opi-vel	1.07	0.141	0.282

30°C

Kruskal-Wallis chi-squared = 20.034. df = 4. p-value <0.001

comparisons	Z	P	p adjusted holm
nan-acu	1.37	0.084	0.338
nan-aqu	2.10	0.017	0.123
acu-aqu	0.73	0.232	0.232
nan-opi	3.17	0.0007	**0.006**
acu-opi	1.80	0.035	0.177
aqu-opi	1.07	0.141	0.424
nan-vel	4.08	0.00002	**0.0002**
acu-vel	2.70	0.003	**0.027**
aqu-vel	1.97	0.024	0.144
opi-vel	0.90	0.183	0.366

35°C

Kruskal-Wallis chi-squared = 14.681. df = 4. p-value = 0.005

comparisons	Z	P	p adjusted holm
nan-acu	1.54	0.060	0.303
nan-aqu	3.24	0.0005	**0.005**
acu-aqu	1.69	0.044	0.312
nan-opi	2.62	0.004	**0.034**
acu-opi	1.07	0.141	0.564
aqu-opi	-0.62	0.266	0.799
nan-vel	3.11	0.0009	0.008
acu-vel	1.56	0.058	0.349
aqu-vel	-0.12	0.448	0.448
opi-vel	0.49	0.310	0.620

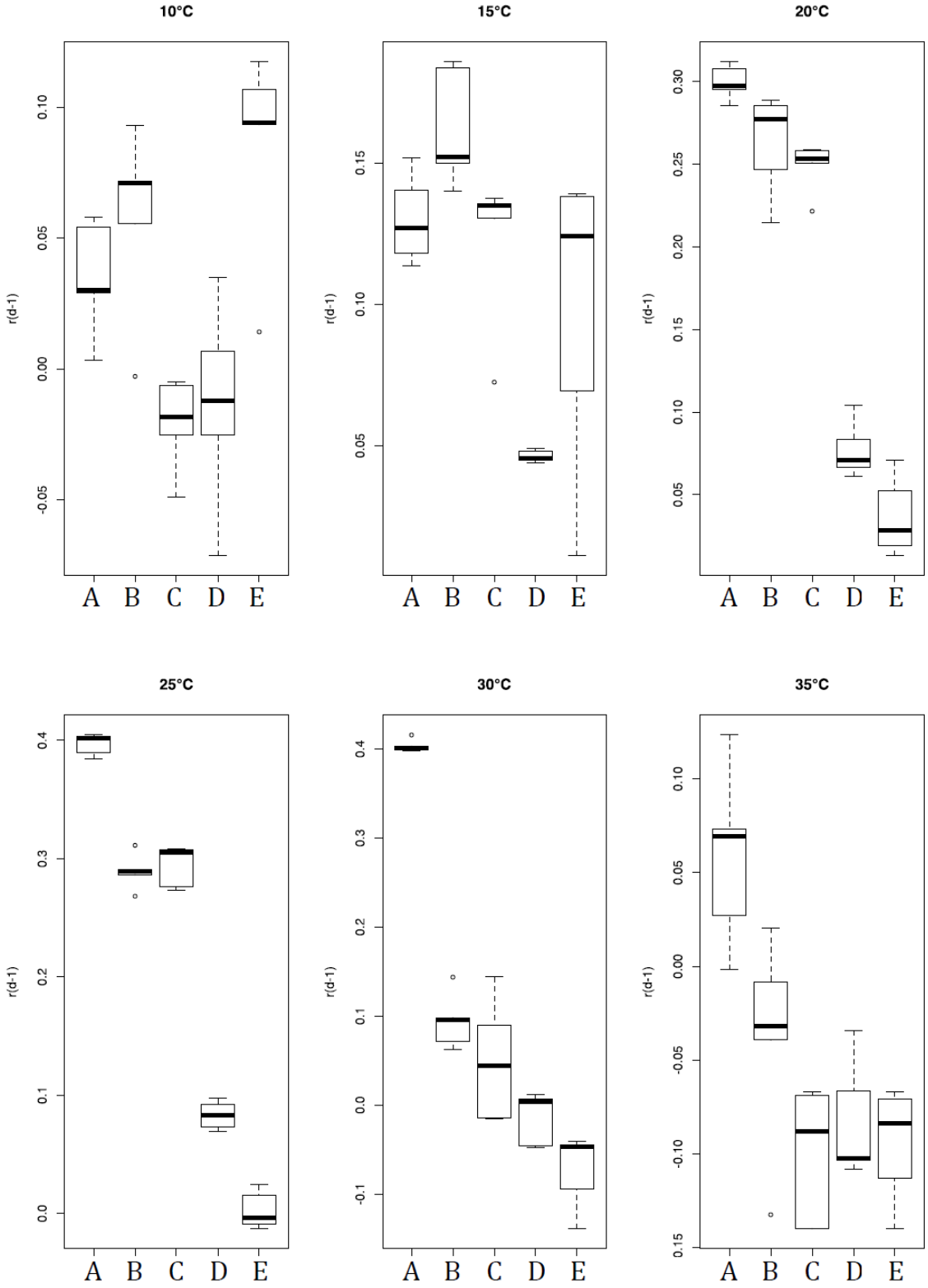

Fig B.1. Comparison of the population growth rates (*r*) of five species of nematodes cultured at different temperatures (from 10 to 35 °C). (A) *Acrobeloides nanus*. (B) *Plectus acuminatus*. (C) *Plectus aquatilis*. (D) *Plectus opisthocirculus*. and (E) *Plectus* cf. *velox*. Values are medians. boxes are interquartile range.

Appendix C. Number of eggs and size at maturity

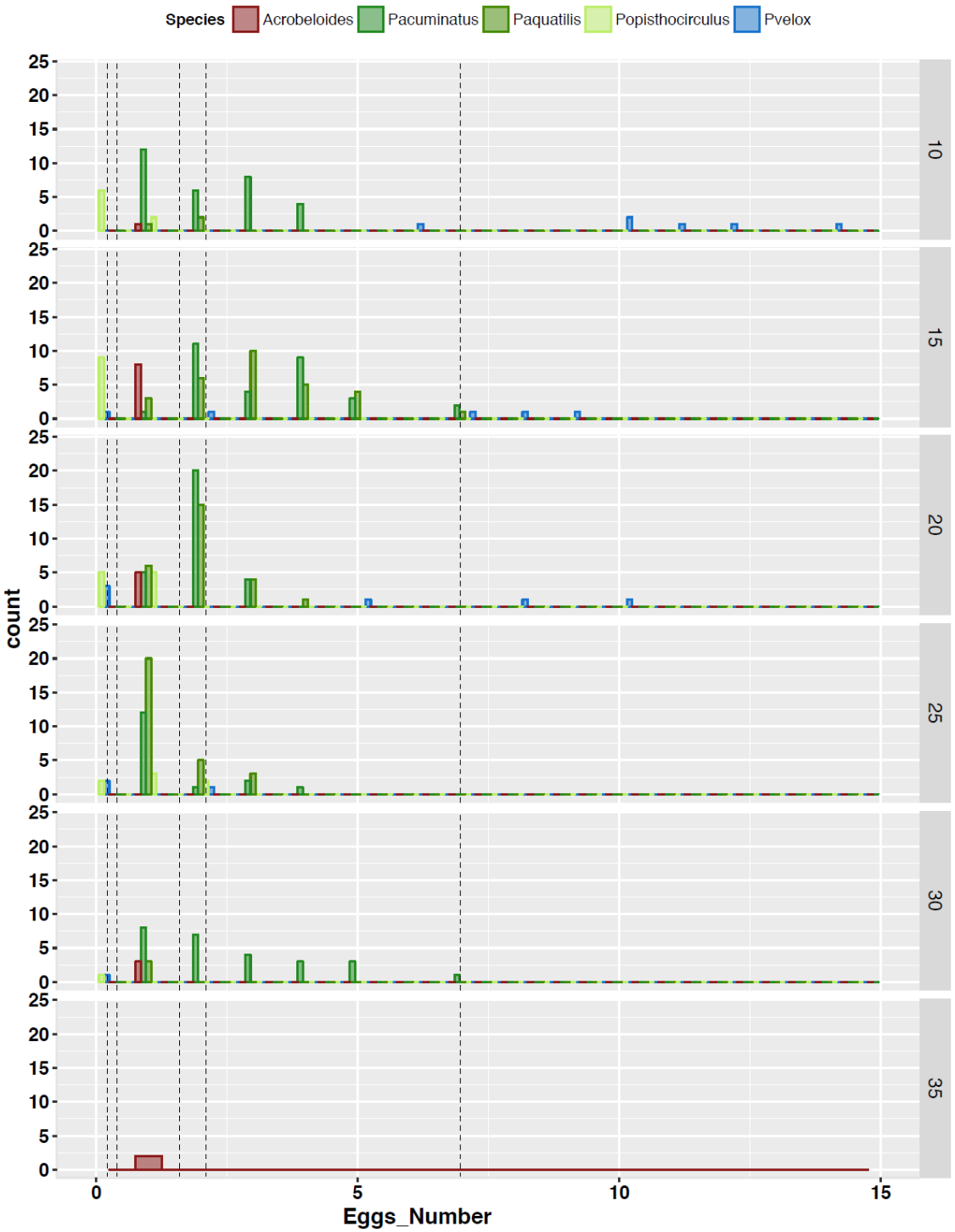

Fig C.1. Distribution of the number of eggs per mature females in five nematode species cultured under temperature conditions from 10 to 35 °C. black dotted lines showed mean number of eggs per gravide female (from the left to the right: *Acrobeloides nanus*: 0.2, *Plectus opisthocirculus*: 0.4, *Plectus aquatilis*: 1.6, *Plectus acuminatus*: 2.1, *Plectus* cf. *velox*: 6.9).

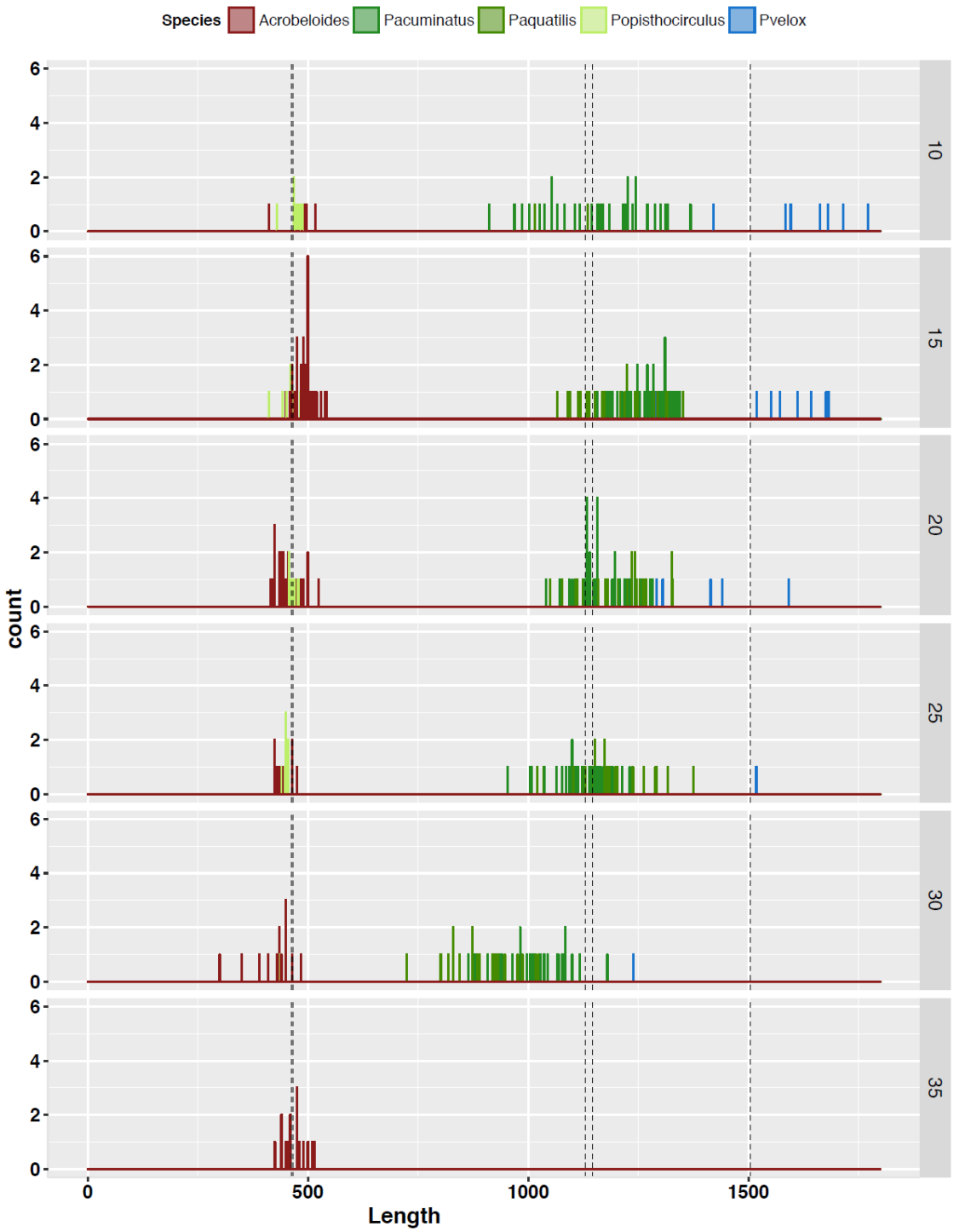

Fig C.2. Distribution of body-length (µm) of mature females in five nematode species cultured under temperature conditions from 10 to 35 °C. black dotted lines showed mean length in µm (from the left to the right: *Plectus opisthocirculus*: 462.6, *Acrobeloides nanus*: 465.8, *Plectus aquatilis*: 1130.6, *Plectus acuminatus*: 1146.7, *Plectus* cf. *velox*: 1504.5).

Appendix D. Body-mass spectra of *Acrobeloides nanus*

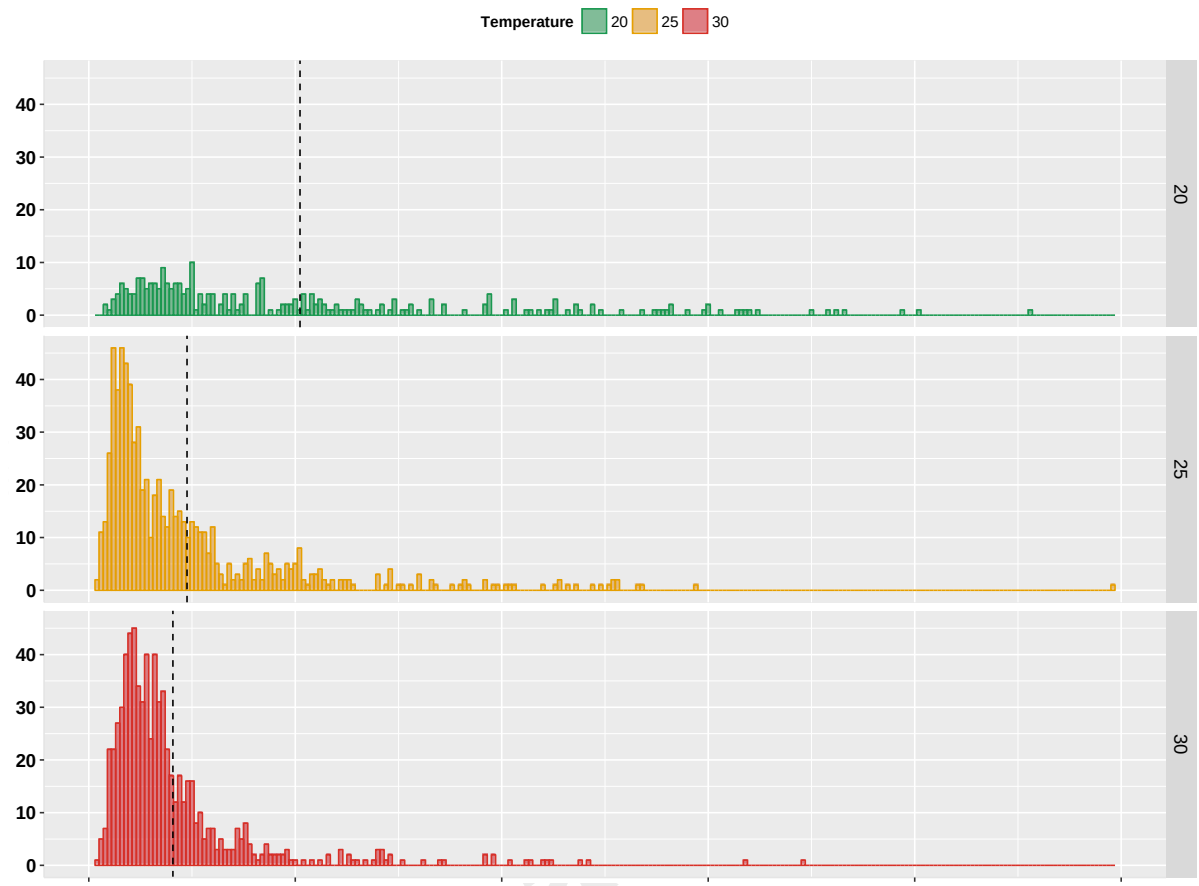

Fig D.1. Body-mass spectra of populations of *Acrobeloides nanus* cultured under temperature conditions from 20 to 30 °C. black dotted lines showed mean dry-weight in ng (from the left to the right: at 20 °C: 10.2, at 25 °C: 4.75, at 30 °C: 4.07).