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# Differential effects of food availability on population growth and fitness of three species of estuarine, bacterial-feeding nematodes

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**Abstract**

The significance of bottom-up controls on biological communities has been a long-standing topic of interest in ecology. However, before environmental effects on communities can be properly assessed, a thorough knowledge of the individual species' responses is required. We studied effects of food availability on population development and on different life-history traits in three species of bacterial-feeding nematodes, *Diplolaimelloides oschei*, *Diplolaimelloides meyli* (both Monhysteridae) and *Pellioditis marina* (Rhabditidae), which co-occur on macrophyte detritus in the Westerschelde Estuary (SW Netherlands). The bacteria *Escherichia coli* was offered in five food-availability treatments corresponding to initial cell densities from $3\times10^{10}$ cells $ml^{-1}$ to $3\times10^{7}$ cells $ml^{-1}$. The three bacterial-feeding nematode species studied here showed differential responses to food availability, which agreed with the general idea that Rhabditidae have extreme colonization abilities under very high food availability, while Monhysteridae tend to have a somewhat slower population development and comparatively lower food requirements. Several life-history traits, including juvenile mortality and development time, did not exhibit a clear food-availability dependence, but bioenergetics-related parameters did. Results on the F1 generation may, however, be affected by strong maternal effects on life-history traits of their progeny. Patterns of food-availability dependence of population increase and size at maturity were similar in *P. marina*. Both *Diplolaimelloides* species, however, exhibited a large body size at maturity but a very low population increase at the highest food availability, suggesting a trade-off between biomass and reproduction. Comparison with published data on other nematode species reveals that nematode responses to food availability as well as to other environmental factors are highly species-specific.


*Keywords:* Bacterial-feeding nematodes; Bottom-up control; Fitness; Food availability; Marine; Population development

## 1. Introduction

The significance of resources in controlling populations and communities of organisms has been a topic of interest in aquatic and terrestrial ecology for many years, with debates having long been polarized between hypotheses on predator-based ('top-down') and resource-based ('bottom-up') regulation of different trophic levels (Hairston et al., 1960; Murdoch, 1966; McQueen et al., 1989; Power, 1992; Menge et al., 1999, and many others). By now the concomitant contribution of bottom-up and top-down forces has been widely accepted (Worm et al., 2002). A majority of studies on bottom-up controls have focused on the causes and consequences of biomass variation at different trophic levels (Rosemond, 1993; Hillebrand, 2002; Hillebrand et al., 2002), while fewer studies have addressed resource effects on community assembly, composition and diversity (Menge and Sutherland, 1976; Menge et al., 2002).

Nematodes are the most abundant and species-rich taxon in marine and estuarine benthic communities, with densities in soft sediments typically ranging between $10^5$ and $10^7$ ind. $m^{-2}$ (Heip et al., 1985). The local (=sample) diversity of nematodes varies as a function of sediment texture, organic matter availability and quality, and other environmental factors, but is typically substantial, several tens of species $m^{-2}$ being common (Heip et al., 1985). Top-down control by predatory nematodes (Moens et al., 2000; Gallucci et al., 2005) and

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macrofauna (Coull, 1985, 1986; Li and Vincx, 1993), as well as bottom-up control through food availability and quality (Heip et al., 1985; Coull, 1999) have been suggested as important forces affecting density, biomass and species composition of nematode communities, but the available evidence is still scanty and does not allow for generalizations to be made. Moreover, information on food thresholds and functional responses of nematodes is still limited (Schiemer, 1985; Herman and Vranken, 1988; Moens and Vincx, 2000b).

In most aquatic sediments, it is also a rule rather than an exception to find several 'confunctional' (i.e. belonging to the same trophic guild) and congeneric species co-occurring, rendering nematode communities interesting model systems to study effects of disturbance and resource availability on community assembly (De Mesel et al., 2006). The development of this research model is, however, hampered by difficulties at manipulating and observing nematode communities in their sediment substrate. Permanent laboratory cultures of only few marine nematode species have ever been established in artificial, transparent media (Moens and Vincx, 1998), but the ones that are easy to cultivate typically (1) have short generation times and produce many progeny (Warwick, 1981; Vranken and Heip, 1983; Vranken et al., 1988; Moens and Vincx, 2000a), (2) stem from the surfaces of decomposing macrophytes, (3) are bacterial feeders, and (4) belong to only a few families, mainly Monhysteridae and Rhabditidae (Warwick, 1987; Moens et al., 1996). Since it is common to find several of these species co-occurring, these communities present interesting research models which are amenable to experimental manipulation under controlled laboratory conditions.

However, before environmental effects on (artificially assembled) communities of species can be assessed, a thorough knowledge is required on the individual species' responses. According to life-history theory, the impact of environmental stress on organisms is determined by its effect on fitness (Sibly and Calow, 1989). At the population level, fitness can be defined as the intrinsic rate of population increase (Murray, 1985; Nur, 1987), but at the individual level, different traits relating to, among others, growth, biomass, reproduction and feeding may all affect fitness (Kammenga et al., 1996, 1997; Kammenga and Riksen 1996; Schiemer, 1982a,b; Herman and Vranken, 1988; Moens and Vincx, 2000b). Schiemer (1987) argued that above food thresholds, bioenergetic traits like production and assimilation show a pronounced increase with food supply up to a level above which no or only a weak further response occurs.

The aim of the present paper is to study the effects of food availability on different life-history and bioenergetic traits and on population development of three species of bacterial-feeding nematodes, *Diplolaimelloides oschei*, *Diplolaimelloides meyli* and *Pellioditis marina*, common on several types of macrophyte detritus in the Westerschelde Estuary. Based upon information on the occurrence of these species in natural (micro)habitats, and on results from previous laboratory manipulations (Moens et al., 1996, 2006), we hypothesized that *P. marina* would require very high food levels for growth and reproduction, and would therefore only be successful at (very) high food availabilities, whereas both monhysterid nematode species would be successful in a broader range, spanning from very high to moderate food availability. We further expected pronounced effects of food availability on both bioenergetic (fecundity, individual biomass) and life-history (development time, survival) traits (Schiemer, 1987; Vranken et al., 1988).

## 2. Materials and methods

### 2.1. Nematode culture

Nematodes for our experiments were obtained from established monospecific, agnotobiotic cultures in exponential growth phase, with unidentified bacteria from the habitat as food (Moens and Vincx, 1998). The culture inocula of *D. oschei*, *D. meyli* and *P. marina* originate from the Westerschelde Estuary (SW Netherlands). All three species co-occur on decaying macroalgae and macrophytes in the Paulina saltmarsh in the polyhaline reach of this estuary. Each species had been in permanent culture under identical temperature (17 °C) and salinity (25) conditions for many generations prior to the start of our experiments.

In view of the recent discovery that *P. marina* is in fact a complex comprising different, often sympatrically occurring cryptic species (Derycke et al., 2005, 2006), we verified the identity of our laboratory population applying PCR-RFLP of the nuclear ITS region. Ten nematodes were randomly collected from the culture, DNA was extracted and the ITS region was amplified according to Derycke et al. (2005). A 5 µl aliquot of each PCR-product was digested with 0.5 µl Alu I restriction enzyme (10 units/µl RE), 1 µl 10x $Y^+$ tango buffer and 3.5 µl distilled water for 2 h at 37 °C. The digested PCR-products were subsequently submitted to electrophoresis on 2% midi agarose and bands were stained with ethidiumbromide. The *P. marina* culture used for the present experiments belongs to the Pm I lineage, which is the dominant species at the Paulina field site and in the Westerschelde Estuary in general (Derycke et al., 2005, 2006).

### 2.2. Experiments

Experiments were conducted in Petri dishes (5 cm i.d.) with 4 ml of a 0.75% bacto-agar medium, prepared with artificial seawater (ASW, Dietrich and Kalle, 1957) with a salinity of 25. The pH of the medium was buffered at 7.5–8 with Tris–HCl in a final concentration of 5 mM, which raised the salinity of the agar substrate by ca. 1.2 units.

The low nutrient content of the agar and the washing procedure of the nematodes (see below) ensured that growth of bacteria, cotransferred with the nematodes, in the experimental units was minimal. We further supplied the agar medium with 100 µl $l^{-1}$ cholesterol as a source of steroids, because nematodes are incapable of synthesizing sterols from a purely bacterial food source (Vanfleteren, 1980).

Frozen-and-thawed *Escherichia coli* (strain K12) were used as a food source. These were offered in amounts corresponding to each of five different initial cell densities ($C_1$, $C_2$,..., $C_5$,

determined before freezing through a combination of plate countings and spectrophotometric analysis of cell density): $3\times10^{10}$ cells ml$^{-1}$, $3\times10^{9}$ cells ml$^{-1}$, $1.65\times10^{9}$ cells ml$^{-1}$, $3\times10^{8}$ cells ml$^{-1}$, and $3\times10^{7}$ cells ml$^{-1}$, respectively. These different food availabilities were obtained through dilution in ASW from a stock density of $3\times10^{11}$ cells ml$^{-1}$. However, since a majority of *E. coli* cells burst open upon thawing, the food available to the nematodes was a mix of dissolved organic matter and intact as well as damaged bacterial cells. Preliminary experiments were run to ensure that nematode growth rates on *E. coli* were similar as on the bacteria present in the stock cultures from which the nematodes for the present experiments were harvested.

For experiments, adult male and female nematodes were manually picked up from stock cultures, rinsed twice in ASW to minimize transfer of bacteria, and left in ASW for 24 h at 5 °C before the start of the experiment.

All experimental treatments were performed with the three nematode species separately. Five male and five gravid female *P. marina* were randomly collected and inoculated per experimental unit. For the monhysterid species *D. meyli* and *D. oschei* we used ten males and ten gravid females; these numbers are based on previous experiments with the same or similar species (Moens and Vincx, 2000a; Vranken et al., 1988). The different numbers of *P. marina* vs the monhysterid species relate to differences in generation time and rate of population increase (Vranken and Heip, 1983; Vranken et al., 1988; Moens et al., 1996; Moens and Vincx, 2000a).

After the above described rinsing procedure, nematodes were transferred individually on the tip of a fine Tungsten wire needle to a 50 µl drop of sterile ASW on the agar substrate. 50 µl of the respective *E. coli* suspensions were added as food and spread over the agar surface. In treatments with *P. marina*, a second and equal amount of food was added on the fifth day of the experiment; in treatments with monhysterid nematodes this was done on the tenth day. There were 8 replicates per treatment and species, four of which were used to study nematode population growth, the remaining four to harvest animals for morphometric and biomass analyses.

In treatments with *P. marina*, nematode population (total numbers and number of adults) and life cycle parameters (fecundity, embryonic, postembryonic and total development time, adult sex ratio, mortality) were quantified daily during the first 11 days of the experiment and then every 48 h until the 17th day; in treatments with Monhysteridae, counts were performed every 48 h during the first 14 days and then every 92 h until 46 days of incubation. Embryonic development time was defined as the time from egg deposition to hatching. Since reproduction in all species and treatments started almost immediately after inoculation, the minimal embryonic development time was taken as the time from $T_0$ to the appearance of the first juvenile (s). Mean embryonic development time was approximated by taking the difference in the *X*-axis intercepts of the linear regressions of numbers of juveniles vs time and numbers of eggs vs time. The mean postembryonic development time was similarly approximated by taking the difference in the *X*-axis intercepts of the linear regressions of numbers of adults vs time and numbers of juveniles vs time (Moens and Vincx, 2000a). Total development time, then, is the sum of mean embryonic and mean postembryonic development time.

Approximately 30 (15 males and 15 females) F1 adult nematodes per replicate were used for morphometric and biomass analyses. These were randomly collected and preserved in a hot (70 °C), neutral formaldehyde solution (4%), on day 8 for the *P. marina* experiments and on day 24 for the *Diplolaimelloides* experiments. The timing was based on the development time of each species, and was chosen such that F1 adults of both sexes were present in sufficient numbers in all food treatments. When less than 30 F1 adult individuals were present, all adults were picked out. Nematodes were mounted on glass slides for measurements of total body length (for biomass calculations in *D. oschei*, the length of the tail was subtracted from the total body length) and maximal body width. These measurements were performed using a Quantimet 500+ image analyser. Biomass was calculated from these measurements following a slightly adapted version of Andrassy's (1956) formula:

$$\text{Biomass (in µg wet weight)} = \left(LW^2/1.7\right)N_{\text{Rd}}\cdot10^{-6}$$

where:

- $L$ = Total length (µm), omitting the elongated, filiform tail in *D. oschei*.
- $W$ = Width (µm), maximum body diameter, avoiding vulva in female.
- $N_{\text{Rd}}$ = Nematode relative density, estimated at 1.13 for marine nematodes (Somerfield et al., 2005)

### 2.3. Data analysis

Differences between treatments, time and time × treatment were analyzed using repeated measures analysis of variance (ANOVA), or with one-way ANOVA in case of morphometric and biomass data. Data were first checked for normality and homogeneity of variances using Levene's ANOVA-test on the deviation of scores and Bartlett's $\chi^2$ test. Where necessary, they were $\log_{10}(x+1)$ transformed to fit the assumptions of normality and homogeneity of variances. Pairwise a posteriori comparisons were performed using Tukey's Honest Significant Differences (HSD) test. If, after transformation, the data still did not match the criteria of normality and/or homoscedasticity, they were analysed with a Kruskal–Wallis non-parametric one-way ANOVA on the separate effects of treatment and time. All analyses were performed using the Statistica 6 software (Statsoft).

## 3. Results

### 3.1. P. marina

Eggs were visible from the first day onwards in all treatments, but their numbers remained low because of a largely ovoviviparous reproductive strategy; egg numbers tended to be higher at lower food availabilities (C3–C5), but these differences were not significant. Juveniles appeared from

Table 1
Total (i.e. for the first 14 days of the experiment for both *Diplolaimelloides* species and for the first 4 days for *P. marina*) and daily fecundity (eggs + juveniles) per F0 female at five different food availabilities

| Treatment | Total fecundity | | | Daily fecundity | | |
|---|---|---|---|---|---|---|
| | *P. marina* | *D. meyli* | *D. oschei* | *P. marina* | *D. meyli* | *D. oschei* |
| | ±1 stdev | ±1 stdev | ±1 stdev | ±1 stdev | ±1 stdev | ±1 stdev |
| C1 | 26.65±5.70 | 30.95±16.28 | 19.14±1.63 | 6.66±3.41 | 2.21±1.16 | 1.37±0.28 |
| C2 | 44.39±8.74 | 40.59±9.58 | 23.58±1.96 | 11.10±3.02 | 2.90±0.68 | 1.68±0.40 |
| C3 | 32.91±4.70 | 46.85±28.57 | 22.65±2.15 | 8.23±2.60 | 3.35±2.04 | 1.62±0.70 |
| C4 | 49.72±7.79 | 29.61±10.50 | 19.45±1.52 | 12.43±2.41 | 2.11±0.75 | 1.39±0.26 |
| C5 | 57.27±16.45 | 17.02±7.75 | 11.59±0.93 | 14.32±7.69 | 1.22±0.55 | 0.83±0.13 |

Data are means±1 stdev of four replicates.

the first day onwards in all treatments. The first F1 adult females and males appeared on the 5th day in all treatments and replicates.

Surprisingly, fecundity of F0 (parental) *P. marina* was lowest at the highermost food availability, while differences between other food treatments were small (Table 1). Total population (including all vermiform stages but not eggs) development of *P. marina* was, however, considerably higher in C1 than in all other treatments ($p<0.0001$), indicating that the food effect on fecundity was completely different between F0 and subsequent generations. Population development was also significantly higher at the secondhighest (C2) than at the lowest (C5) food availability ($p<0.04$). The interaction term treatment vs time was also highly significant ($F=11.21$; $p<0.0001$, Fig 1A), indicating that population development had different temporal dynamics at different food availabilities.

Adult population development differed significantly between treatments (interaction effect of treatment vs time: $F=5.76$; $p<0.0001$). Numbers of adults started increasing from the 5th day onwards. The highest food availability yielded the fastest (Fig. 1B) and highest (Fig. 2A) adult population development, with an abrupt increase in adult density from the 11th day onwards. At lower food availabilities, adult densities remained low throughout the experiment ($F=19.80$; $p<0.0001$, Figs. 1B and 2A). While abundances of adult *P. marina* tended to decrease with decreasing bacterial densities from the secondhighest (C2) to the lowest (C5) food availability, these differences were not statistically significant. The sex ratio was consistently female-biased and was highest at the medium food treatment (C3) with 66±23.36% (mean±1 stdev) females; however, differences between treatments were not significant (Fig. 2B).

No significant differences between treatments were found for total development time of F1 progeny. Male total development times tended to be longer than female total development times, but differences were not significant (Table 2).

Individual biomass of adult *P. marina* differed significantly between treatments ($F=38.31$; $p\leq0.0001$), which was largely due to the highest food-availability treatment (mean adult wet weight 8.99 µg compared to 2.95 µg at the lowest food availability (Fig. 3A)). Both body length and maximal body width were significantly affected by food availability. At the highest food availability, nematodes grew significantly longer ($p<0.0001$) than at all other food concentrations (Fig. 3B). Maximum body width decreased with decreasing food availability: the highest food availability differed significantly from all other treatments ($p<0.0001$), but in addition, body width of nematodes at the secondhighest food availability was significantly higher than at the lowest two food treatments ($p<0.0001$), and the medium food treatment (C3) differed

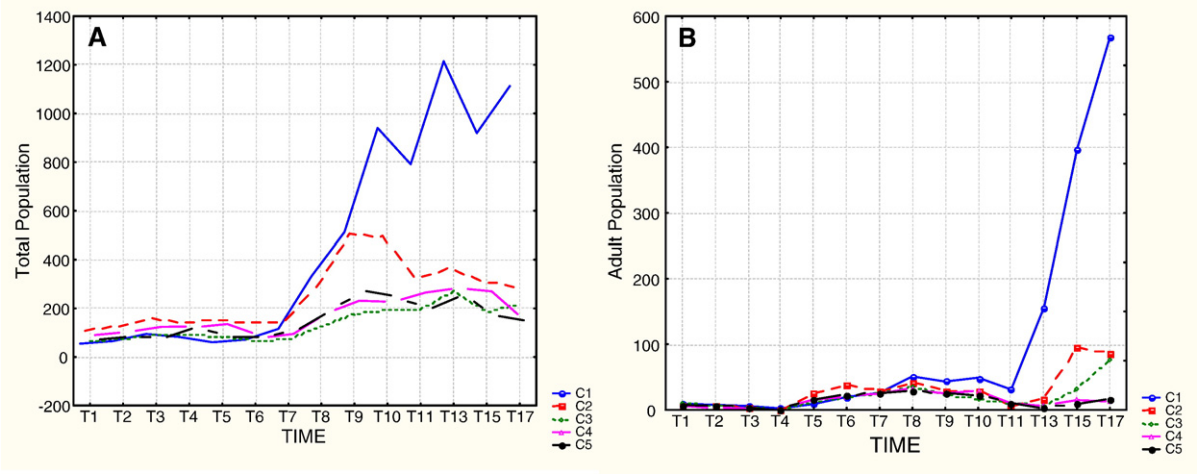


Fig. 1. A: Total *P. marina* population development, and B: *P. marina* adult population development over time at five different food availabilities. Data are averages of four replicates per treatment and time.

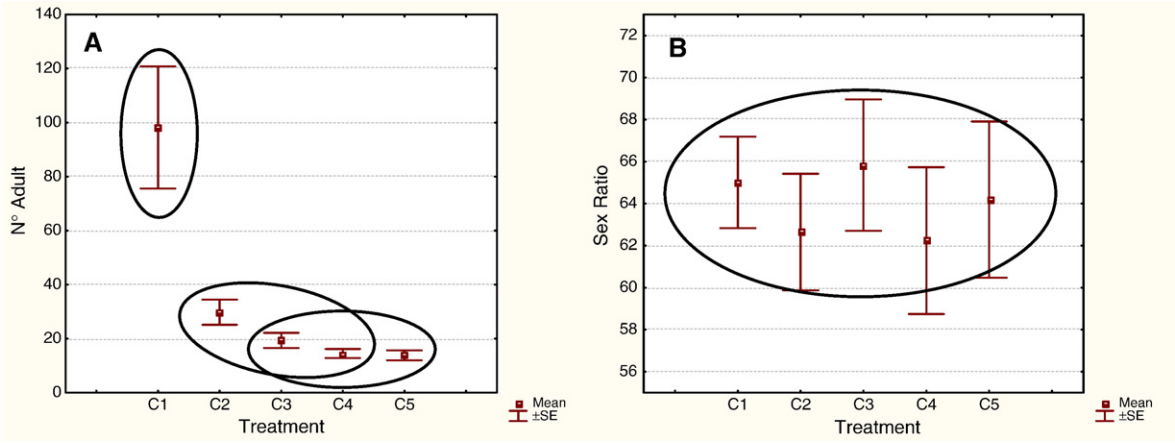


Fig. 2. The effect of food availability on A: numbers of adult *P. marina*; and B: adult sex ratio (expressed as the female/male ratio). Data are means ± 1 std error of 4 replicates per treatment averaged over the total duration (17 days) of the experiment. Values enclosed by the same circle do not differ significantly at $p<0.05$.

significantly from the lowest food treatment ($p<0.0001$, Fig. 3C *Y* axis: individual biomass).

Adult population biomass as calculated from numbers and mean individual biomass of adult *P. marina* differed significantly between treatments ($F=59.13$, $p<0.0001$), time ($F=51.49$, $p<0.0001$) and treatment vs time ($F=3.78$, $p<0.0001$). Population biomass at the highest food availability ($3\times10^{10}$ cells $ml^{-1}$) was significantly higher than at all other food treatments ($p<0.0001$), and population biomass at the secondhighest food availability (C2) was significantly higher than those at the lowest two food-availability treatments ($p<0.0001$, Fig. 3D *Y* axis: population biomass).

### 3.2. D. meyli

Egg deposition occurred from the first counting day onwards, with a high increase in numbers after 92 h at the higher food availabilities. Fecundity was highest at intermediate food availabilities (C2 and C3), but only the pairwise differences with the lowest food treatment were significant ($p<0.03$) (Table 1). The total population of *D. meyli* (including all vermiform stages but not eggs) increased continuously and monotonously with time in all treatments, densities being higher at intermediate food availabilities (C2 and C3) ($F=4.73$, $p<0.01$); the interaction term treatment vs time was also highly significant ($F=2.22$; $p<0.0001$, Fig. 4A), showing that the temporal dynamics of population development differed between food availabilities.

The first F1 adult females appeared on the 16th day in all treatments, except at the lowest food availability. The first F1 adult males appeared from the 18th to 26th day onwards, depending on the treatment. Numbers of adults abruptly decreased from 30 days onwards at intermediate food availabilities (C2 to C4), and more gradually from 34 days onwards at the highest and lowest food concentrations. Peaks in *D. meyli* adult densities occurred fairly simultaneously at all food availabilities except the lowest one, where this peak was delayed by about 4 days (Fig. 4B). Food availability had a significant effect on the average densities of adult *D. meyli* in our microcosms ($F=8.55$; $p<0.0001$, Fig. 5A). Adult densities increased with increasing food availability up to a bacterial density of $3\times10^{9}$ cells $ml^{-1}$; however, at the highest bacterial availability, adult *D. meyli* densities remained low and were comparable to the treatment with the lowest food availability. Pairwise differences were highly significant between the highest (C1) and secondhighest (C2) food availability, and between C2 and the lowest food availability (both $p<0.0001$). The adult sex ratio was highly female-biased, with up to 66 ± 12.35% (mean ± 1 stdev) females, at C3, and differed significantly between treatments ($p<0.03$, Kruskal–Wallis test, Fig. 5B).

Embryonic development time, postembryonic development time and total development time did not differ significantly between treatments, although they were on average shorter at the intermediate food availabilities (C2–C4) than at the higher- and lowermost ones (Table 3). Male postembryonic development time and total development time were considerably longer than in females ($p<0.0001$).

Biomass of individual adult *D. meyli* showed a pronounced and monotonous dependence on food availability, being highest (0.57 µg) at the highest food availability and lowest (0.25 µg) at

Table 2
Total development time of *P. marina* at five different food availabilities

| Treatment | Development time | | |
|---|---|---|---|
| | Sex | Minimum | Mean ± 1 stdev |
| C1 | ♀ | 3.85 | 4.02 ± 0.17 |
| | ♂ | 3.64 | 4.07 ± 0.35 |
| C2 | ♀ | 1.85 | 3.26 ± 0.98 |
| | ♂ | 2.97 | 4.34 ± 1.69 |
| C3 | ♀ | 3.54 | 5.01 ± 1.17 |
| | ♂ | 3.31 | 4.03 ± 0.64 |
| C4 | ♀ | 1.92 | 3.71 ± 1.66 |
| | ♂ | 3.47 | 4.56 ± 0.76 |
| C5 | ♀ | 2.22 | 2.80 ± 0.64 |
| | ♂ | 3.22 | 4.05 ± 0.91 |

Data are means ± 1 stdev of four replicates.

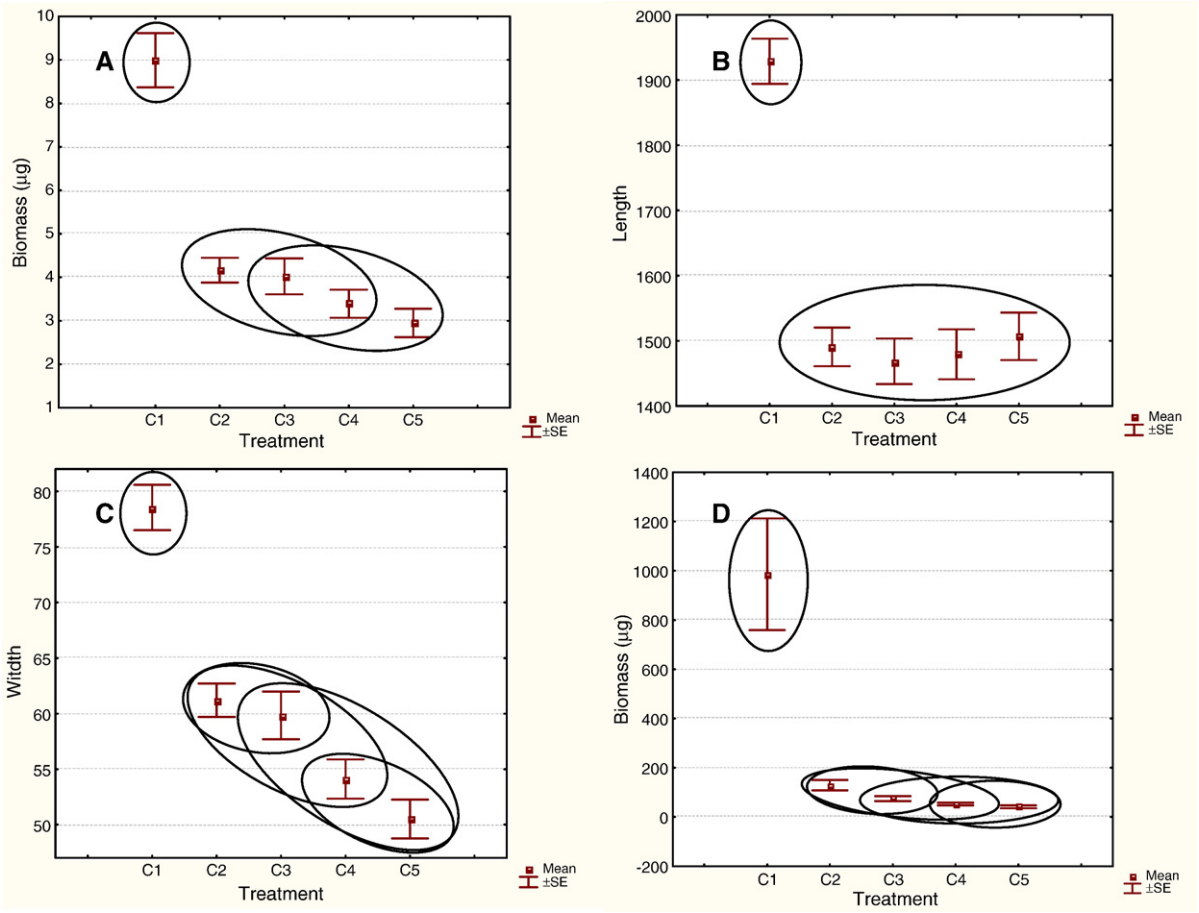


Fig. 3. A – Individual biomass (µg), B – Body length (µm) and, C – Maximum body width (µm) of *P. marina* adults on the 8th day of the experiment. D – Population biomass of adult *P. marina*. Data are means ± 1 stdev of 4 replicates per treatment.

the lowest food availability ($p<0.0001$; Kruskal–Wallis test, Fig. 6A). Nematode biomass is proportional to body length and to the square of the maximal body width. Both parameters were significantly affected by food availability: At the highest food availability, nematodes grew significantly longer ($p<0.0001$; Kruskal–Wallis test), but not wider than at lower food levels. Body width only differed significantly between the lowermost and all other food availabilities, and between C2 and C3-C4 (both $p<0.0001$, Fig. 6B and C).

Adult population biomass as calculated from numbers and mean individual biomass of adult *D. meyli* differed significantly between treatments ($F=13.86$, $p<0.0001$) and time ($F=59.01$, $p<0.0001$); the interaction factor treatment vs time was also highly significant ($F=5.85$, $p<0.0001$). Adult population

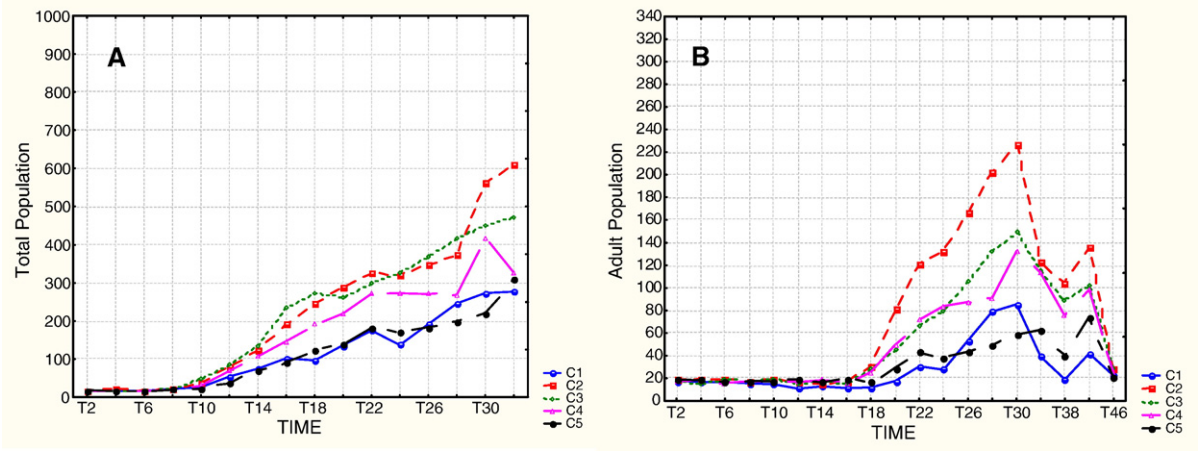


Fig. 4. A: Total *D. meyli* population development, and B: *D. meyli* adult population development over time at five different food availabilities. Data are averages of four replicates per treatment and time.

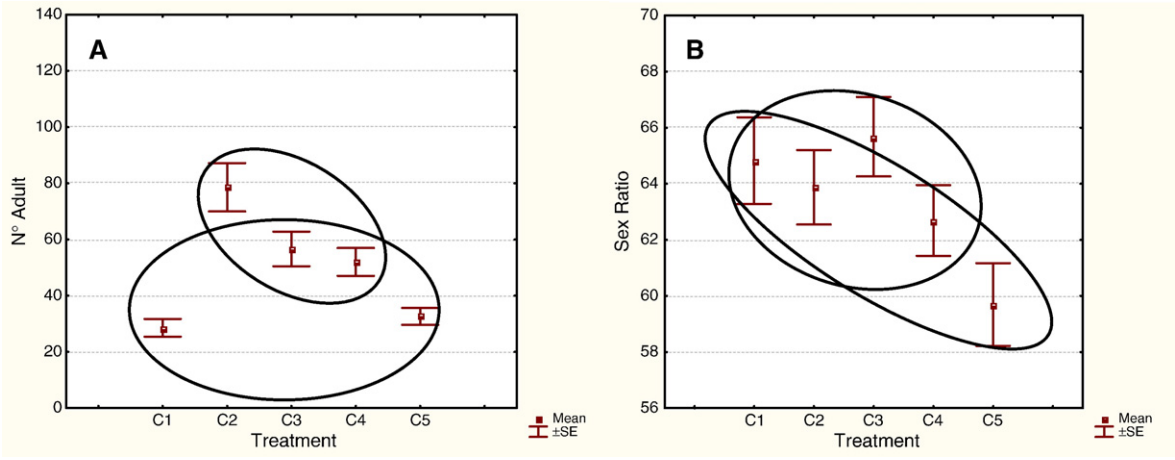


Fig. 5. The effect of food availability on A: numbers of adult *D. meyli*; and B: adult sex ratio (expressed as the female/male ratio). Data are means±std error of 4 replicates among experimental time per treatment averaged over the total duration (46 days) of the experiment. Values enclosed by the same circle do not differ significantly at $p<0.05$.

biomass was highest at the secondhighest food availability and showed a pattern that was overall more similar to the abundance vs food-availability pattern than to the individual biomass vs food-availability pattern (Fig. 6D).

### 3.3. D. oschei

Eggs appeared from the first counting day onwards in all treatments, with a steep increase in numbers after 92 h at the highest food availability. Fecundity was significantly different between treatments ($F=3.78$, $p<0.03$), the only significant pairwise difference being between C2 and C5 ($p=0.02$) (Table 1). Total population (including all vermiform stages but not eggs) development had a continuous and monotonous increase with time in most treatments, with higher numbers of individuals at intermediate food concentrations (C2–C4) ($F=4.61$, $p<0.01$); the interaction factor treatment vs time was also significant ($F=1.57$; $p=0.01$, Fig. 7A).

Numbers of adults started increasing from 16 days onwards at C2, and from 18 days onwards at the other food availabilities; they decreased again from 24 days onwards at C1–C4 and more gradually from 26 days onwards at C5. Peaks in *D. oschei* adult densities occurred fairly simultaneously at C2–C4 (Fig. 7B).

Food availability significantly impacted *D. oschei* adult densities ($F=4.33$; $p=0.03$, Fig. 8A), with a pattern similar to that in *D. meyli*. Adult densities increased, albeit discontinuously, with increasing food availability up to a bacterial density of $3\times10^9$ cells $ml^{-1}$; however, at the highest food availability, *D. oschei* densities remained low and were comparable to those at $3\times10^8$ cells $ml^{-1}$. Pairwise differences were only significant between C1 and C2 ($p<0.02$). The sex ratio was strongly female-biased in all treatments, with highest values of 71± 10.08% (mean±1 stdev) females at C3, but did not differ significantly between treatments (Fig. 8B).

Embryonic development time was significantly longer at C5 than at all other treatments ($p=0.01$; Kruskal–Wallis test). Postembryonic development time and total development time did not differ between treatments (Table 3). Male postembryonic development time and total development time were considerably slower than in females ($p<0.0001$).

Individual biomass of adult *D. oschei* showed a pronounced and monotonous dependence on food availability, with highest biomass (0.37 μg) at the highest food availability and lowest (0.13 μg) at the lowest food availability ($p<0.0001$; Kruskal–Wallis test, Fig. 9A). Both length and maximal body width were significantly affected by food availability. At the highest food

Table 3
Minimum and mean embryonic development time, postembryonic development time and total development time of *D. meyli* at five different food availabilities

| Treatment | Embryonic development time | | Postembryonic development time | | | Development time | |
|---|---|---|---|---|---|---|---|
| | Minimum | Mean±1 stdev | Sex | Minimum | Mean±1 stdev | Minimum | Mean±1 stdev |
| C1 | 2.87 | 3.16±0.34 | ♀ | 9.02 | 9.83±0.70 | 11.89 | 12.99±0.96 |
| | | | ♂ | 9.77 | 13.81±1.66 | 15.51 | 16.97±1.51 |
| C2 | 2.40 | 3.32±1.12 | ♀ | 5.38 | 7.69±1.73 | 10.30 | 11.01±0.73 |
| | | | ♂ | 9.40 | 10.89±2.17 | 12.70 | 14.20±1.90 |
| C3 | 3.12 | 4.10±1.18 | ♀ | 7.22 | 8.40±0.80 | 10.90 | 12.50±1.53 |
| | | | ♂ | 8.79 | 10.52±1.53 | 12.50 | 14.62±1.75 |
| C4 | 2.95 | 3.27±0.51 | ♀ | 6.66 | 7.89±0.87 | 11.10 | 11.16±1.28 |
| | | | ♂ | 8.70 | 9.91±1.53 | 11.70 | 13.18±2.00 |
| C5 | 2.49 | 3.14±0.79 | ♀ | 8.05 | 9.85±1.58 | 11.60 | 12.98±1.82 |
| | | | ♂ | 8.51 | 11.89±2.78 | 11.80 | 15.02±2.53 |

Data are means±1 stdev of four replicates.

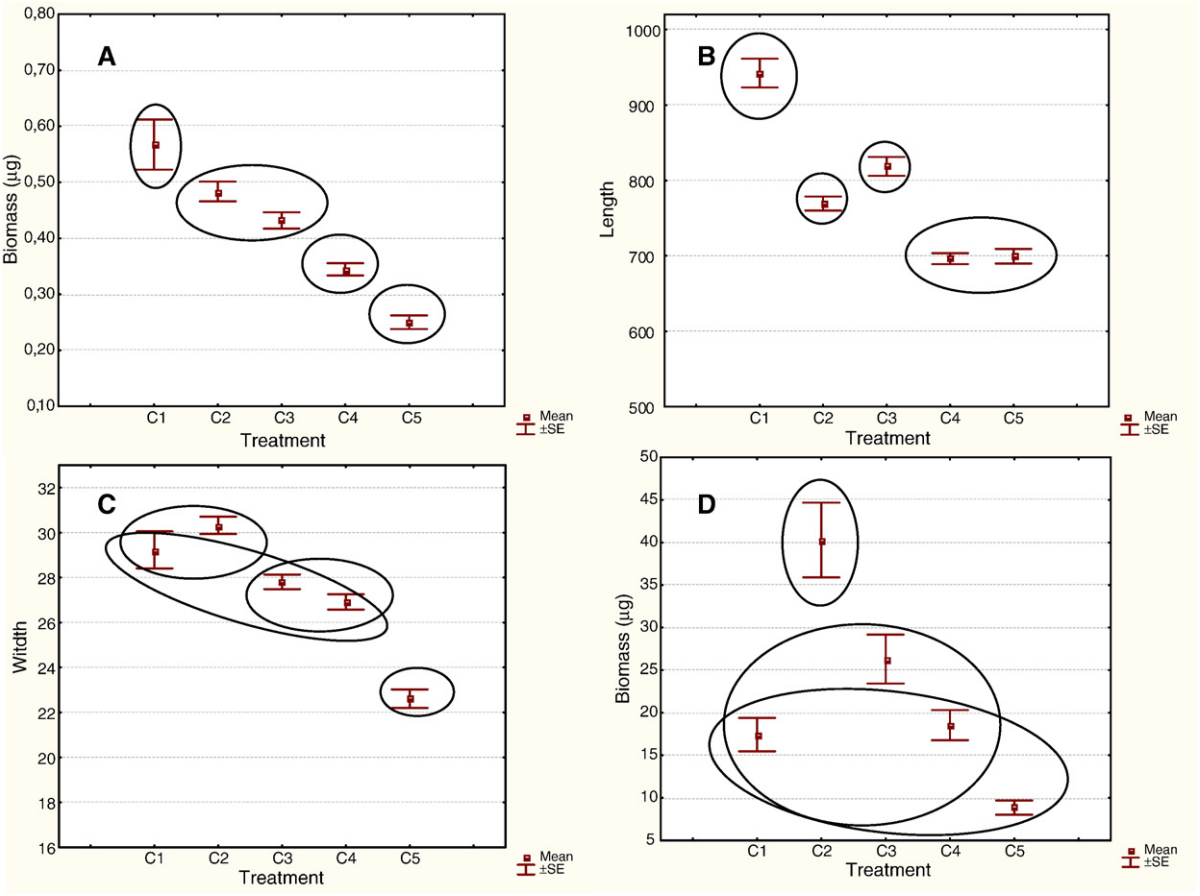


Fig. 6. A – Individual biomass (µg), B – Body length (µm) and, C – Maximum body width (µm) of adult *D. meyli* on the 24th day of the experiment. D – Population biomass of adult *D. meyli*. Data are means ± 1 stdev of 4 replicates per treatment.

availability, nematodes grew significantly longer ($p < 0.0001$) than at all other treatments (Fig. 9B). Width decreased continuously and monotonously from the highest to the lowest food availability ($p < 0.01$). Moreover, the lowest food availability had a more pronounced effect on body width than on length ($p \leq 0.0001$, Fig. 9C).

Biomass of the adult *D. oschei* population differed significantly between treatments ($F = 13.63$, $p < 0.0001$), time

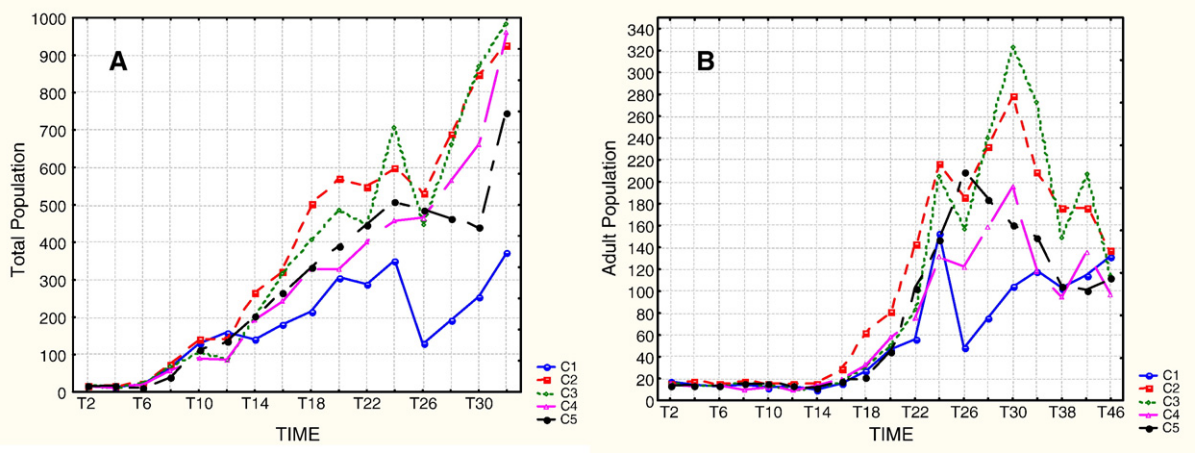


Fig. 7. A: Total *D. oschei* population development, and B: *D. oschei* adult population development over time at five different food availabilities. Data are averages of four replicates per treatment and time.

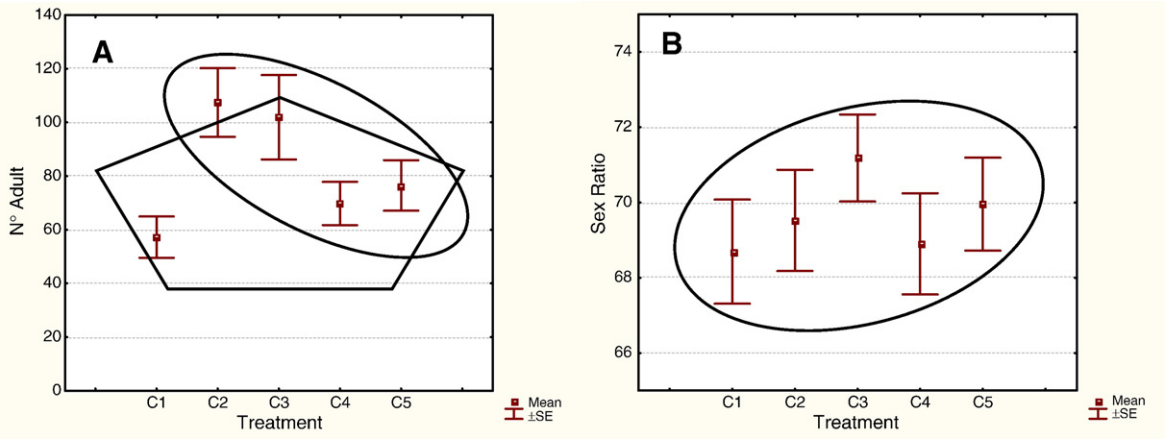


Fig. 8. The effect of food availability on A: numbers of adult *D. oschei*; and B: adult sex ratio (expressed as the female/male ratio). Data are means±std error of 4 replicates among experimental time per treatment averaged over the total duration (46 days) of the experiment. Values enclosed by the same circle do not differ significantly at $p<0.05$.

($F=221.95$, $p<0.0001$) and treatment vs time ($F=1.70$, $p<0.0001$), being higher at C2 and C3 than at all other food treatments (Fig. 9D).

## 4. Discussion

### 4.1. Food availability effects on nematode population growth

The three nematode species studied here show a differential response to food availability. The strongest difference is between the rhabditid, *P. marina*, and the two monhysterid species, the former clearly having highest fitness at the highest food availability, whereas the monhysterids peaked at intermediate food levels. The food-availability dependence of *P. marina* follows our expectation that this species requires (very) high food densities for population development. This is probably related to its feeding behaviour: rhabditid nematodes typically show a random food ingestion, which is likely to be energetically inefficient at low food densities (Vranken et al., 1988). The limited population growth of both Monhysteridae at the highest food availability is, however, somewhat counter-intuitive, the more so since food concentrations above optimal did not negatively affect life-history parameters in another monhysterid nematode, *Monhystera disjuncta* (Vranken et al., 1988). In any case, the discrepancy between rhabditid and monhysterids is a very good illustration of the distinction terrestrial nematologists make between 'enrichment opportunists' (mainly rhabditids and diplogasterids) and 'general opportunists' (mainly Cephalobidae and to some extent Plectidae) (Bongers and Bongers, 1998). The former group consists of species with extremely short generation times and large numbers of progeny (Vranken and Heip, 1983; Bongers and Bongers, 1998), but only under optimal conditions, involving very high food levels with threshold values typically close to $10^9$ bacterial cells $ml^{-1}$ (Nicholas et al., 1973; Schiemer, 1982a,b, 1983; Moens and Vincx, 2000b). Rhabditidae populations in general respond rapidly to bottom-up processes such as nutrient and organic matter loading through the stimulatory effects these have on bacterial cell density and production. Under field conditions, for instance, *P. marina* typically reaches high abundances on rotting macroalgae (Derycke et al., 2007), especially in the earlier stages of decomposition, when labile components are leaching from the algae and bacterial growth is high. However, *P. marina* populations typically decline quite fast in response to decreases in microbial activity and densities, perhaps in part through overgrazing of bacterial populations (De Mesel et al., 2004, 2006). Under food deprivation, they can form 'dauer larvae', a metabolically dormant yet motile juvenile stage which is able to survive food depletion and/or to disperse to new food-rich spots (Bongers, 1990; Bongers and Bongers, 1998; Ferris et al., 1995, 1997).

The results of the present experiments are surprising in that even at a food availability corresponding to as low as $3\times10^7$ cells $ml^{-1}$ there was still population growth of *P. marina*, albeit very limited. We have to be careful, though, when comparing the food availabilities in the present experiment with those in previous studies, because we applied 50 µl volumes of food derived from a given bacterial density. This implies that food density was not uniform over the entire surface of our microcosms, and surface-averaged food density in our microcosms was lower than the food density in the inoculum drop. This renders the (albeit low) population growth of *P. marina* at C5 in our experiments even more surprising. On the other hand, it brings the optimal feeding conditions of our experiment closer to the optimal food density for assimilation for this species (Moens et al., 2006). Furthermore, the food we offered was a mixture of dissolved organic matter, broken and intact cells and therefore only the 'starting densities' of bacteria from which food for our experiments was prepared, can be compared to the bacterial densities reported in other studies.

The two *Diplolaimelloides* species required lower food thresholds and densities than *P. marina*, which may relate to a generally more selective food uptake in Monhysteridae compared to rhabditid nematodes (Vranken et al., 1988). In nature, the species we have studied are typically abundant on more refractory detritus from vascular plants such as *Spartina*, where decomposition is slower and bacterial densities are not as

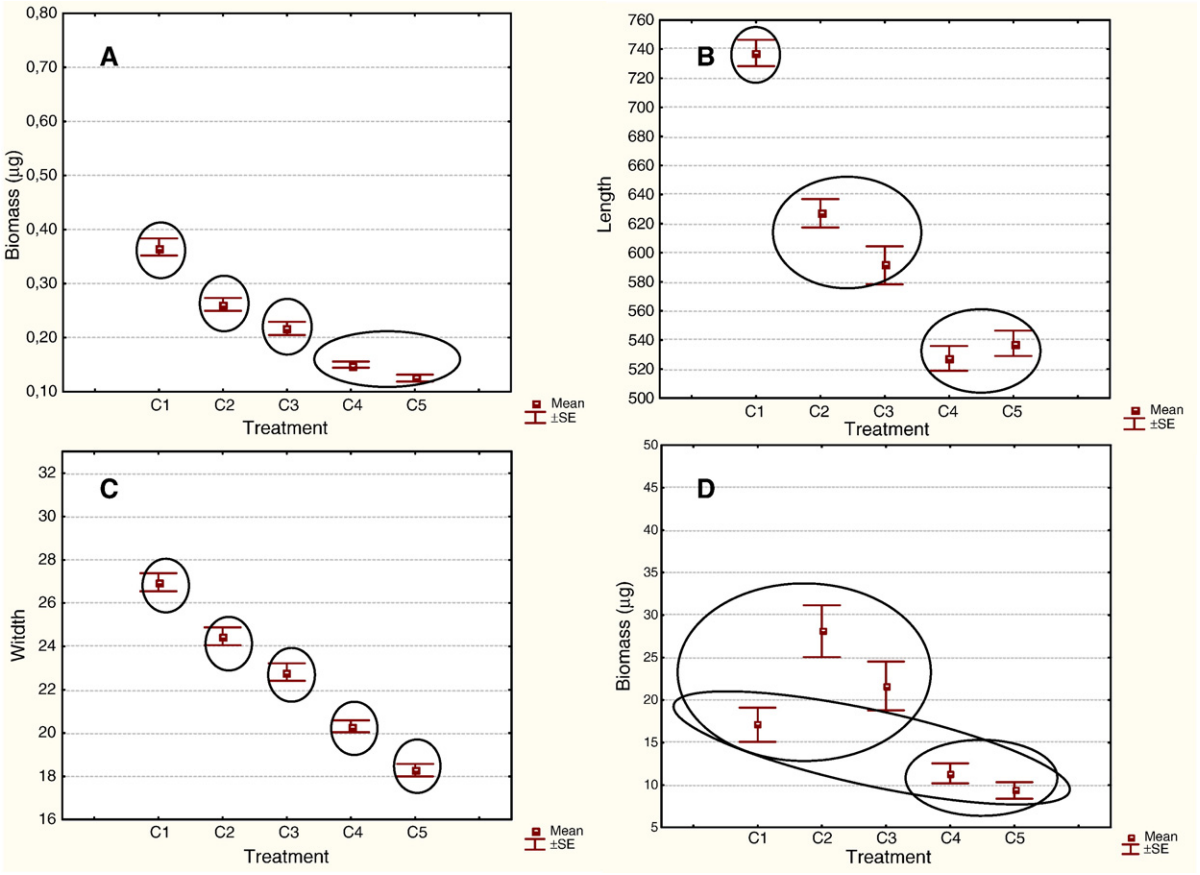


Fig. 9. A – Individual biomass (µg), B – Body length (µm) and, C – Maximum body width (µm) of adult *D. oschei* on the 24th day of the experiment. D – Population biomass of adult *D. oschei*. Data are means ± 1 stdev of 4 replicates per treatment.

high as on macroalgae (Moens and Vincx, 2000b, and references therein). Given the above-mentioned caveats when comparing food densities in the present experiment with those from previous studies, optimal food availability for population growth of *D. meyli* and *D. oschei* in the present experiment was well above the threshold for active feeding in *D. meyli* ($2.5 \times 10^8$ cells $ml^{-1}$ (Moens and Vincx, 2000b)), and for normal survival and development in *Monhystera disjuncta* (Vranken et al., 1988). Optimal bacterial densities in those studies were reached from $5 \times 10^8$ cells $ml^{-1}$. However, whereas 10 to even 200 times higher bacterial densities did not affect monhysterid 'fitness', neither positively or negatively (Vranken et al., 1988; Moens and Vincx, 2000b), population development of both *Diplolaimelloides* species in our experiment was negatively affected by excess food. This is counterintuitive, since in the absence of interspecific competition and crowding, there is no clear reason for the observed negative effect at the highest food ration. We did, however, observe a trade-off between investment in biomass and reproduction in both Monhysteridae at the highest food availability (see further), both *Diplolaimelloides* species growing larger but producing fewer offspring at the highest food availability. Moreover, the parameters determined in the studies referred to may not be adequate to assess population fitness: Moens and Vincx (2000b) determined food assimilation, which was measured over a very short (1-h) incubation. Vranken et al. (1988) focused on mortality and development times, parameters which in our experiment also did not yield significant differences between most food-availability treatments. A study on the movement of nematodes towards bacterial spots further indicated that *D. meyli* had a preference for food densities around $3 \times 10^9$ cells $ml^{-1}$ (Moens et al., 1999), in line with the present results. *D. oschei*, however, had a bimodal pattern, preferring cell densities of $>10^9$ cells $ml^{-1}$ as well as very low densities ($10^7$ cells $ml^{-1}$). Hence, different traits may yield partly conflicting information on optimal food levels.

At below-optimal food availability, population development of *D. meyli* and to a lesser extent *D. oschei* was negatively impacted. This, and the limited population growth at high food availability, narrows down the assumed broad range of food densities which could support monhysterid population growth. Hence, the fundamental niche of monhysterids with respect to food availability appears narrower than anticipated (Moens and Vincx, 2000b).

### 4.2. Life-history and bioenergetic traits

*Fitness*, when defined as the intrinsic rate of population increase ($r_m$), cannot properly be calculated from the current experiments, because they were not designed to determine age-specific mortality and reproductive output (Vranken and Heip, 1983; Kammenga et al., 1996). Hence, our assessment of

Table 4
Minimun and mean embryonic development time, postembryonic development time and total development time of *D. oschei* at five different food availabilities

| Treatment | Embryonic development time | | Postembryonic development time | | | Development time | |
|---|---|---|---|---|---|---|---|
| | Minimum | Mean±1 stdev | Sex | Minimum | Mean±1 stdev | Minimum | Mean±1 stdev |
| C1 | 2.51 | 3.62±0.98 | ♀ | 9.74 | 10.65±0.68 | 12.25 | 14.27±1.41 |
| | | | ♂ | 12.49 | 13.73±0.84 | 15.00 | 17.35±1.75 |
| C2 | 2.06 | 2.39±0.31 | ♀ | 7.84 | 9.76±1.29 | 10.33 | 12.14±1.21 |
| | | | ♂ | 11.97 | 12.79±0.79 | 14.30 | 15.17±0.95 |
| C3 | 2.31 | 2.82±0.59 | ♀ | 9.24 | 10.88±1.42 | 11.55 | 13.10±1.81 |
| | | | ♂ | 13.51 | 14.16±1.48 | 14.40 | 16.08±2.51 |
| C4 | 1.64 | 1.81±0.26 | ♀ | 9.29 | 10.33±0.89 | 10.97 | 12.13±0.88 |
| | | | ♂ | 12.07 | 13.67±1.33 | 13.75 | 15.48±1.46 |
| C5 | 2.50 | 2.61±0.09 | ♀ | 8.50 | 9.39±0.96 | 11.15 | 12.00±0.99 |
| | | | ♂ | 11.60 | 12.49±0.77 | 14.25 | 15.10±0.75 |

Data are means±1 stdev of four replicates.

nematode fitness is based on multiple traits which all contribute to the rate of population increase, such as fecundity, development time, and sex ratio. Biomass is included as a variable since population increase can also be approximated from population biomass instead of number of individuals, particularly when viewed from the angle of energy transfer (Fenchel, 1974).

The only *development-time* related parameter exhibiting a significant difference between food treatments was embryonic development time in *D. oschei* (Table 4). Postembryonic and total development time, however, were not significantly affected by food availability in any of the species studied here. These results are in agreement with observations on *Monhystera disjuncta*, where food density also had only a very minor effect on minimum generation time (Vranken et al., 1988). The absence of a strong relation with food availability in *P. marina* is, however, at odds with data on another rhabditid, *Caenorhabditis briggsae*, where minimum generation time increased threefold when bacterial densities dropped from $10^{11}$ to $2\times10^{8}$ cells $ml^{-1}$ (Schiemer, 1982b), and on the freshwater bacterial-feeding *Plectus palustris* (Schiemer et al., 1980). Development times in bacterivorous nematodes tend to be highly dependent on abiotic environmental factors such as temperature and salinity (Tietjen and Lee, 1972, 1977; Schiemer et al., 1980; Warwick, 1981; Moens and Vincx, 2000a), but apparently less so on food availability. Embryonic development time of F1 in our experiments was likely more determined by the feeding history of the females than by the food availability surrounding the deposited eggs. This is supported by observations that food supply affects egg size (Schiemer, 1982a). The lack of food availability effects on postembryonic development time, however, may suggest that juvenile maturation (postembryonic development) also still relies importantly on energy reserves transferred from female to progeny (Herman and Vranken 1988; Moens and Vincx, 2000a,). Further, it is interesting to note that in both monhysterid species studied here, males had longer development times than females, irrespective of food availability. This agrees well with a previous study on *D. meyli*, where male development times were longer than female development times over a range of temperatures and salinities (Moens and Vincx, 2000a), but is at odds with observations on *Monhystera disjuncta*. In the latter species, however, the type of food offered differentially affected male and female development times (Vranken et al., 1988).

*Juvenile mortality* has been found to be a highly sensitive life-cycle trait with respect to different kinds of pollution (Kammenga and Riksen, 1996, Kammenga et al., 1997). It also increased strongly at *E. coli* densities below $10^{9}$ cells $ml^{-1}$ in *C. briggsae* (Schiemer, 1982b). In our experiment, however, F1 juvenile mortality was negligible in all treatments and species.

*Sex ratio* did not vary significantly between food treatments for any of the species studied here. There was generally a strong bias in favour of females (62%≤), which may be linked to the generally faster maturation of females in our experiments. For *P. marina*, moderately (Vranken, 1985; Moens and Vincx, 2000a) to strongly (66–75% females, Tietjen et al., 1970) female-biased sex ratios have been reported in North Sea+ Westerschelde and in a North-American population, respectively. Note, however, that these populations may actually have been different cryptic species. In contrast, *Diplolaimelloides bruciei* (Warwick, 1981) and *D. meyli* under optimal temperature conditions (Moens et al., 1996) had a 66% male dominance. This is at odds with the sex ratio of 2:1 in favour of females, as found in the present experiment. However, sex ratios in these nematodes vary importantly with environment, including e.g. temperature and salinity (Moens and Vincx, 2000a), and at least part of the discrepancy between this and previous studies may be explained by differences in the abiotic regime.

Hence, in agreement with our hypothesis, food availability had rather minor effects on life-history parameters such as development time and survival. Several bioenergetic traits, however, were significantly affected by food availability. Trends in *fecundity* of parental adults resembled those of total population development reasonably well in both *Diplolaimelloides* species, but not in *P. marina*, probably as a result of feeding history – parental individuals were harvested from stock cultures where food was plentiful. Indeed, food availability effects on population growth of *P. marina* became much more pronounced from the second generation onwards, highlighting that fecundity of F1 adults was higher at the highest food availability.

Fecundity of our *P. marina* inoculum was generally low when compared to published values on this species (up to 600 eggs female$^{-1}$ (Vranken and Heip, 1983)); that of *D. meyli* and *D. oschei* was, however, comparable to previous reports on *D. meyli* (Moens and Vincx, 2000a). Because parental individuals (a) were not followed from the beginning of their reproductive period, and (b) deposited substantial numbers of eggs during the 24 h incubation in ASW prior to their inoculation into the experimental microcosms, the fecundity reported here should not be considered as absolute values.

It is difficult to explain the different trends in monhysterid *individual biomass* and population development vs food availability. Both monhysterid species grew larger but showed a reduced fecundity and population development at the highest food availability. *M. disjuncta* exhibited even much more pronounced food-density effects on individual adult biomass, log(body mass) being a linear function of log(bacterial density). Its reproductive output, however, remained constant at surplus food densities (Vranken et al., 1988). Trade-offs between investment in biomass and reproduction are common in invertebrates (Bennett and Boraas, 1989; Ernsting et al., 1993; Clarke, 1993). In planktonic cladocerans like *Daphnia*, larger mothers are expected to either produce more or larger progeny (Lampert, 1993; Boersma, 1995). Our data show that the former definitely does not hold in the two *Diplolaimelloides* species. We did not measure egg sizes and hence cannot assess the latter option. However, the rationale for producing larger offspring is that these are typically more resistant to starvation and hence have better chances for survival. Juvenile mortality in our experiments was negligible in all food treatments. Resistance to starvation would seem more relevant under conditions of food limitation and/or crowding, both of which were more likely to apply to other than the highest food-availability treatments. The causes for the observed trade-offs between biomass and reproduction in both *Diplolaimelloides* species at high food availabilities remain unclear. In contrast, food availability effects on *P. marina* adult biomass paralleled effects on population development, nematodes growing larger at the highest food availability. Food availability also affected size at maturity in *C. briggsae*, but following a more gradual pattern (Schiemer, 1982a). The observation that *P. marina* had equally short development times at lower food densities, but a smaller size at maturity, may be interpreted as a strategy to cut down the metabolic costs required to attain sexual maturity (Schiemer et al., 1980).

## 5. Conclusions

The three bacterial-feeding nematode species studied here showed differential responses to food availability, which agree with the general idea of Rhabditidae having extreme colonization abilities under very high food availability, while Monhysteridae tend to have a somewhat slower population development and comparatively lower food requirements. Several life-history traits, including juvenile mortality and development time, did not exhibit a clear food-availability dependence, but bioenergetic traits like fecundity and body size did. Care has to be taken, though, when extrapolating from results on the F1 generation to subsequent generations, because feeding history of the parental adults may strongly affect life-history traits of F1 individuals. A complete understanding of food availability effects on life-history traits and fitness therefore requires that effects be tested in subsequent generations, which is very time-consuming and technically difficult because of overlap between subsequent generations. A population assay, focusing on total and adult population development, may be the easiest way to approach food effects on fitness in these nematode species. Comparison with published data on confamiliar nematode species further reveals that responses to food availability as well as to other environmental factors are very species-specific and may relate to responses in different life-history traits. The present results can be used to generate hypotheses on total community development as well as on community composition in multispecies assemblages under different conditions of food availability.

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