ELSEVIER

Journal of Experimental Marine Biology and Ecology
243 (2000) 115–135

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.nl/locate/jembe

Temperature and salinity constraints on the life cycle of two brackish-water nematode species

Tom Moens*, Magda Vincx

University of Gent, Biology Department, Marine Biology Section, K.L. Ledeganckstraat 35, B-9000 Gent, Flanders, Belgium

Received 12 January 1999; received in revised form 20 July 1999; accepted 29 July 1999

Abstract

The present study investigates the influence of salinity and temperature on the life history of two estuarine bacterivorous nematode species, *Pellioditis marina* and *Diplolaimelloides meyli*, isolated from the mesohaline zone of the Westerschelde Estuary, SW Netherlands. Gravid females and adult males were inoculated in petri dishes containing agar layers of nine (for *P. marina*) or five (for *D. meyli*) different salinities, from almost freshwater to higher than marine, and incubated at a temperature of 20°C, to study the impact of salinity; agar layers with a salinity of 20‰, incubated under each of six different temperatures from 5 to 30°C, served to study the effect of temperature. Daily and total fecundity, development time and sex ratio were quantified, and preadult mortality was estimated. The results are compared to those of a partner study on the influence of salinity and temperature on respiration, assimilation and scope for production in the same nematode species. Salinity had relatively minor effects on fecundity, development times and sex ratio in both species, but strongly impacted juvenile viability at the extremes of the salinity range: at salinities close to 0 and 40‰, preadult mortality was more than 80% in *P. marina*; it was 100% at 5‰ in *D. meyli*. Both species had an (near) optimal fitness at salinities of 10 to 30‰. Temperature had a pronounced influence on both nematodes over the entire range studied. *Diplolaimelloides meyli* still reproduced and matured at temperatures exceeding 30°C, while *P. marina* had an upper temperature limit for reproduction of 25°C. Development times of *D. meyli* were more temperature-dependent than those of *P. marina*: the mean development time from adult to adult for the latter nematode ranged from 2 days at 25°C to 7 days at 9°C. The development time of *D. meyli* increased from 7 days at 25–30°C to 63 days at 10°C, temperature below which no reproduction occurred. Female-biased sex ratios were found in *D. meyli* at low temperatures and in *P. marina* under optimal salinity conditions. The life history results largely agree with the predicted scope for production, but discrepancies were found near the extremes of the abiotic range of both species. It is emphasized that the ranges observed are characteristic of populations, not of species; they may to an extent have been influenced by culture conditions. A comparison of

*Corresponding author. Fax: +32-9-264-5344.
E-mail address: tom.moens@rug.ac.be (T. Moens)

0022-0981/00/$ – see front matter
PII: S0022-0981(99)00113-6

the present results with literature data on other *P. marina* populations demonstrates that some populations of this species may still reproduce successfully under conditions which are lethal to other populations, raising the question as to whether cryptic species rather than populations of a single species are involved.

Keywords: Nematodes; Temperature; Salinity; Reproduction; Population development

1. Introduction

Nematodes are the numerically dominant metazoan meiofauna in salt marshes, with densities up to 16 000/10 cm^{-2} in the sediment and on surface litter (Teal and Wieser, 1966; Montagna and Ruber, 1980; Reice and Stiven, 1983; Alkemade et al., 1993). Their abundance on standing live and dead macrophytes is less well established, but densities on *Spartina anglica* in a brackish temperate tidal marsh were of the order of a few hundred to more than 10 000 individuals g^{-1} dry weight, with the highest average densities on brown leaves and old stems and the lowest on living green biomass (Alkemade et al., 1994). Some nematode taxa generally have a low average abundance in the benthos, but abound on leaves or other macrophyte detritus deposited onto the sediment or still attached to the standing plant (e.g. Hopper, 1970; Warwick, 1981; Bouwman et al., 1984; Alkemade et al., 1993, 1994). These taxa specifically comprise members of the nematode family Monhysteridae and of the order Rhabditida (Warwick, 1987). The latter is composed mainly of freshwater, soil and insect-parasitic species, but also comprises a few brackish-water, marine and halotolerant species.

Although many marine nematodes have conservative life strategies (Warwick, 1980), these Aufwuchs species are usually characterised (under optimal conditions) by a short generation time, a high reproductive capacity, and a relatively broad temperature and salinity tolerance. The impact of salinity on growth and reproduction has been studied in only five brackish-water nematode species (Tietjen et al., 1970; Tietjen and Lee, 1972, 1977; Warwick, 1981; Vranken, 1985), three of which Monhysteridae. Temperature influences have been the subject of more studies pertaining to a dozen species (see Heip et al., 1985; Vranken et al., 1988).

The Aufwuchs habitats frequented by monhysterid and rhabditid nematodes are highly unstable: in a tidal environment, they are subject to daily fluctuations in salinity and temperature. Superimposed on these daily variations are seasonal fluctuations, which at the site where nematodes for the present study were isolated span a range of average daily temperatures from <0 to >25°C, and salinity changes which are relatively minor (8 to 21) in the river water itself, but which may be more pronounced in intertidal habitats such as in shallow gullies and puddles and on Aufwuchs substrates. The Aufwuchs habitats are typically short-lived, and a variable microbial flora may be associated with different phases in the detrital decay. The quality of the detritus itself, and of detritus–bacterial aggregates, changes over the decay process (Tenore et al., 1984). All these factors may affect the performance of the nematodes associated with macrophyte detritus — their grazing rates, production, and respiration — and as such

quantitatively determine the functional role of the populations studied under their extant environmental regimes. They may also determine the relative success of one Aufwuchs species compared to others (Schiemer, 1982).

In a partner paper, we report on the impact of temperature, salinity and food density on assimilation and respiration rates in two nematode species isolated from the mesohaline reach of the Westerschelde Estuary (Moens and Vincx, 1999). *Diplolaimelloides meyli* (Monhysteridae) and *Pellioditis marina* (Rhabditidae) responded sharply to temperature and food level, yet were less affected by salinity in the range of almost oligohaline to marine. A scope for production (somatic growth + reproduction) was calculated for different temperature and salinity regimes. Here, we report on the influence of the same two abiotic variables on reproduction and life history characteristics in both species, and test whether the observed abiotic influences on the scope for production are reflected in patterns of fecundity, generation time, preadult mortality, and sex ratio.

2. Materials and methods

2.1. Nematode culture

Nematodes were isolated from small macrophyte stands in a tidal flat station in the mesohaline reach of the Westerschelde Estuary (station WO22, see Moens and Vincx, 1997), and established in monospecific, agnotobiotic cultures with unidentified bacteria from the habitat as the food. A 1% agar, composed of bacto and nutrient agar in a weight/weight ratio of 4/1, was used as substrate. A more detailed outline of culture procedures for these and related species is given elsewhere (Moens and Vincx, 1998).

2.2. Experiments

For experiments, adult male and female nematodes from a culture in exponential growth phase were manually transferred to 1% agar layers (see above) in 5-cm diameter petri dishes. In experiments with *P. marina*, two males and eight females were inoculated per petri dish; with *D. meyli*, five males and five females were used. Agar was prepared with artificial seawater (ASW, Dietrich and Kalle, 1957) of 40‰, diluted to the desired salinity (0, 5, 10, 15, 20, 25, 30, and 35‰) with deionised water. The pH of the medium was buffered at 7.5–8 with TRIS (trihydroxymethylaminomethane)–HCl in a final concentration of 5 mM. Due to the addition of the buffer and to the salt content of the agar substrate, the true salinity in the medium was ~1.2‰ higher than the above values.

Bacteria (isolate BDM1 for *D. meyli* and isolate BPM1 for *P. marina*) were isolated from nematode stock cultures by inoculation of μl-aliquots, devoid of nematodes, in liquid heart infusion broth medium with a salinity of 20‰. Bacteria to be used as food in the present experiments were harvested by centrifugation, washed three times in sterile ASW, and eventually resuspended in ASW of the respective experimental salinities. A few drops of such bacterial suspensions ($\geq 10^9$ cells ml^{-1}; cell densities were

determined spectrophotometrically with reference to absorption vs. cell density curves determined beforehand for each strain separately), containing a mix of several strains, two of which together comprised ca. 90% of total numbers, were spread on the surface of agar layers and allowed to grow overnight at 25°C, to ensure an adequate food availability at the start of the experiment. A few more drops of bacterial suspension were added once (for *D. meyli*) or twice (for *P. marina*) a week during the experimental incubation to prevent food depletion.

Five replicate petri dishes of each of these salinities were incubated at 20°C in the dark. Numbers of eggs, juveniles, adult males and females, as well as of dead adults and juveniles were counted daily (in *P. marina*) or every second day (for *D. meyli*) up to the full maturation of the first progeny in at least four replicate petri dishes. Adults were counted regularly thereafter to study fluctuations in sex ratio. The adults inoculated at the start of the experiment were not removed from the experimental dishes.

Similarly, five replicate agar layers with a salinity of 20‰ were inoculated with nematodes and incubated in the dark at each of the following temperatures: 5, 10, 15, 20, 25, and 30°C. The same life cycle characteristics were studied as in the salinity experiment.

2.3. Data analysis

Differences between treatments were analysed using one-way analysis of variance (ANOVA). Data were first checked for normality and homogeneity of variances (using both Levene's ANOVA-test on the deviation of scores and Bartlett's χ^2). Where necessary, they were $\log_{10}$-transformed to meet these assumptions. Pairwise a posteriori comparisons were performed using Tukey's Honest Significant Differences (HSD) test. If after transformation, the data did not match the criteria of normality and/or homoscedasticity, they were analysed with a Kruskall–Wallis non-parametric ANOVA, and a posteriori multiple comparisons were performed following the procedure outlined in Conover (1980, Chapter 5.2). All parametric analyses, as well as Kruskall–Wallis tests, were performed using the Statistica 5.1 software.

Sex ratios were compared to the null hypothesis of equal numbers of males and females using replicated *G*-tests for goodness of fit (Sokal and Rohlf, 1995). Heterogeneity *G* (G_H) was calculated as a measure of heterogeneity among replicates. Pooled *G* (G_P) tested the goodness of fit for the pooled data over all experimental replicates, and the sum of G_H and G_P tested whether the data as a whole fitted the expected 1:1 male:female distribution.

Curve fitting followed standard least squares procedures with 100 up to 300 iterations, and was performed using the solver function in Excel 7.0 for Windows.

3. Results

The effect of salinity on the total fecundity in *P. marina* is shown in Fig. 1A. Our definition of total fecundity and of other life cycle traits studied is given in Table 1.

The data presented on the fecundity of *P. marina* are not representative of the true

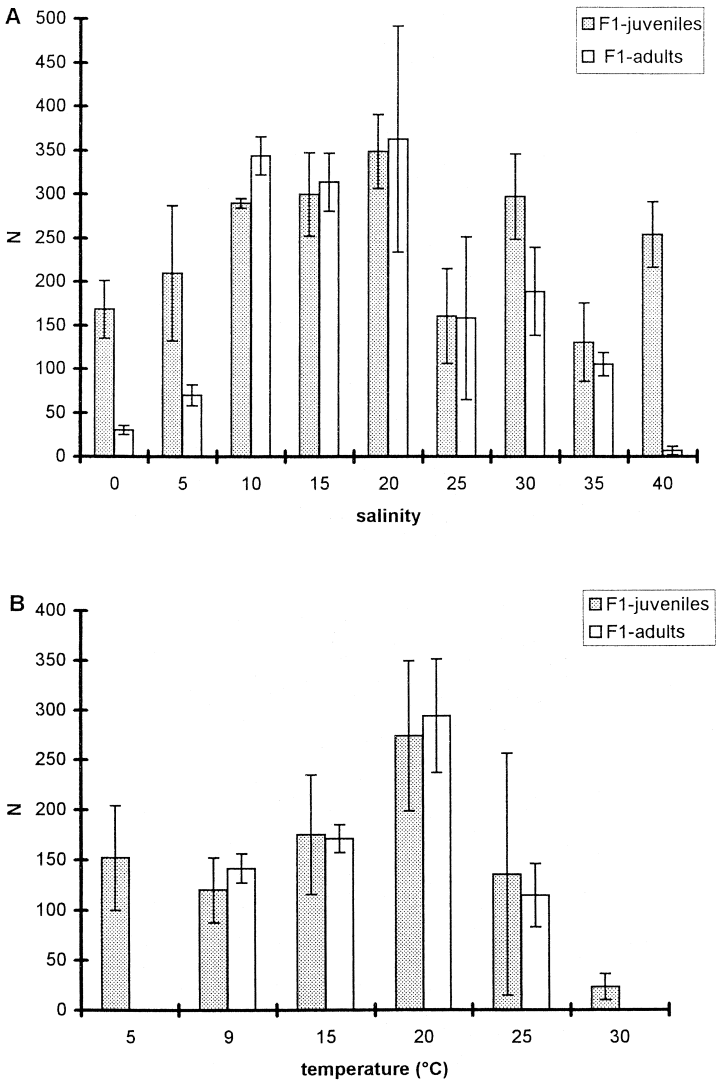
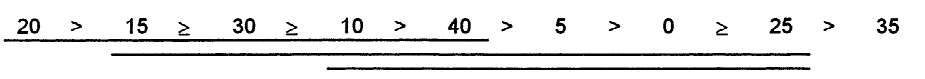


Fig. 1. Total fecundity and total number of F1-adults of *Pellioditis marina* at different salinities (A) and temperatures (B). *N* = number of progeny per eight females. Averages ± 1 S.D. of four or five replicates are shown.

reproductive capacity of the population, since adult females during reproduction rather than virgin females were used as an inoculum. *Pellioditis marina* has a relatively short reproductive period, and the highest reproductive output under favourable conditions is during the second and third day after maturation (Vranken and Heip, 1983). The highest number of progeny was produced at a salinity of 20‰, i.e. the salinity at which stock cultures were kept. Total fecundity at different salinities ranked as:

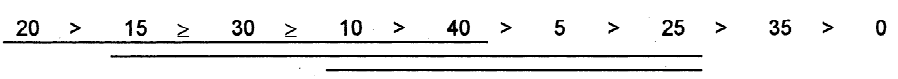
Table 1
Some definitions of life cycle traits as used in this study

	Pellioditis marina	*Diplolaimelloides meyli*
Total fecundity	Number of progeny produced over the interval from the start of the experiment to the maturation of the first F1-offspring to adults	
Daily fecundity	The average daily per capita offspring production over the same time interval as for total fecundity when this did not cover the whole reproductive period, or over the interval until the maximum number of F1-progeny was attained	
Minimum development time (MDT)	The interval from the start of the incubation until the appearance of the first F1-adult	The distance between the *x*-axis intercepts of the linear regressions of egg production and appearance of F1-adults vs. time (see text)
Mean development time (ADT)	The interval between the appearance of the 50th percentile of F1-progeny and the appearance of the 50th percentile of F1-adults	
Minimum postembryonic development time (MPD)	Equals the minimum development time	The distance between the *x*-axis intercepts of the linear regressions of egg hatch and the appearance of adults vs. time
Mean postembryonic development time (APD)	Equals the mean development time	The interval between the appearance of the 50th percentile of F1-juveniles and the appearance of the 50th percentile of F1-adults
Minimum embryonic development time (MED)	Not determined, since egg development is intrauterine	The distance between the *x*-axis intercepts of the linear regressions of egg production and egg hatch vs. time
Mean embryonic development time (AED)	Not determined, since egg development is intrauterine	The interval between the production of the 50th percentile of F1-eggs and the appearance of the 50th percentile of F1-juveniles



where $>$ and $\geq$ indicate average differences larger and smaller than 5%, respectively. Underlined groups of data did not differ significantly at $P<0.05$ (Tukey's HSD-test on untransformed data).

As for total fecundity, daily fecundity during the reproductive interval was highest at 20‰ (Table 2). Using the above annotation, salinities ranked in the following order:



Discrepancies between total and daily fecundity related to the duration of the reproductive interval.

Females averaged just over 50% of the adult population at salinities from 5 to 30‰ (Table 2). The sex ratio was significantly different from 1:1 in favour of females at 10 and 15‰ ($P<0.01$), but not at the other salinities in this range. Significant differences ($P<0.05$, Kruskall–Wallis ANOVA) in % females at different salinities were due to the lower and higher values at 0 and 40‰, with respective female proportions of less than

Table 2
Daily fecundity and sex ratio of *Pellioditis marina* as a function of salinity and temperature

	Daily fecundity		% Females	
	Mean	S.D.	Mean	S.D.
Salinity				
0	5.26	1.03	31.43	7.98
5	11.87	5.95	46.77	10.03
10	18.12	0.33	59.99	3.40
15	18.75	2.99	60.51	1.95
20	21.81	2.64	57.94	3.65
25	10.04	3.41	50.09	4.16
30	18.62	3.05	49.77	4.88
35	8.19	2.81	72.33	24.01
40	15.92	2.34	70.30	20.46
Temperature (°C)				
5	1.86	0.59	n.d.[a]	n.d.[a]
9	3.28	1.36	50.95	1.23
15	12.38	3.16	48	0.79
20	18.27	5.03	49.93	2.60
25	9.04	8.06	56.3	5.11
30	4.64	2.62	n.d.	n.d.

[a] n.d., not determined.

one third and more than three quarters of the adult population ($P<0.05$, non-parametric a posteriori test). Since none of the replicates at these two salinities ever contained more than 20 adults, the significance of these deviations is doubtful. The sex ratio averaged over all replicates at all salinities did not differ from 1:1 ($P>0.05$).

Data on the minimum development time of *P. marina* are exact to within 1 day, as only one count per day was performed. The minimum development time was 3 days, except at the higher- and lowermost salinity, where it slightly exceeded 4 days (Table 3). There were no differences between the sexes, except at the highest salinity, where the minimum development time of males was 5 days, compared to 4 in females (data not shown). The mean development time of *P. marina* showed only minor deviations compared to the minimum development time (Table 3). Only at salinities of 5 and 40‰ was a difference between the sexes indicated, with a slightly shorter and longer mean development time for males, respectively (data not shown).

It was impossible to derive an exact value for preadult mortality from the present experiments, because (a) carcasses of dead juveniles decayed rapidly (residence time less than 2 days on average), and (b) cohorts overlapped. Nevertheless, the proportion of F1-adults to total F1-offspring, as well as the daily counts of dead juveniles, are instructive. They strongly suggest that juvenile mortality was well below 10% in the salinity range of 10 to 35‰, and was negligible in the range of 15 to 30‰. At 40‰, less than 5% of the F1-offspring matured, although juvenile mortality remained low over the course of the experiment. At the lowest two salinities, juvenile mortality did increase. The maximal observed mortality — i.e. the highest single count of dead juveniles divided by the total number of F1-offspring — was 34.35 ± 4.68 (mean$\pm$1 standard

Table 3
Minimum and mean development time of *Pellioditis marina* as a function of salinity and temperature. The means of four replicate incubations are given, with the replicate variability as the standard deviations of the replicate observations

	Minimum development time		Mean development time	
	Mean	S.D.	Mean	S.D.
Salinity				
0	4.33	0.58	3.83	0.76
5	3	0	4.33	0.29
10	3	0	2.83	0.29
15	3	0	3.17	0.29
20	3	0	3.25	0.43
25	3	0	3.08	0.14
30	3	0	3.25	0.25
35	3	0	3.33	0.29
40	4.5	0.84	3.83	0.29
Temperature (°C)				
5	n.d.[a]	n.d.	n.d.	n.d.
9	6.8	0.45	7.1	0.22
15	4	0	3.8	0.45
20	3	0	2.6	0.55
25	2.6	0.55	2	0
30	n.d.	n.d.	n.d.	n.d.

[a] n.d., not determined.

deviation of four replicates) and 14.13±2.59% at salinities of 0 and 5‰, respectively. The corresponding proportions of F1-progeny that matured to adults were 18.44±5.43 and 36.13±10.42%.

Total fecundity in *P. marina* was influenced by temperature (Fig. 1B), yet only at 20 and 30°C, respectively, were significantly elevated and decreased production values found compared to the other temperatures ($P<0.05$, non-parametric multiple comparisons test). At 30°C, nearly all offspring emerged during the first day after inoculation, while at the other temperatures, a plateau was indicated after 3 (15–25°C), 7.5 (9°C) and 16.5 (5°C) days. This resulted in the daily fecundity values shown in Table 2. Daily fecundity, then, ranked with temperature as:

20 > 15 > 25 > 30 > 10 > 5

The percentage females was highest (56%) at 25°C (Table 2), but the sex ratio did not differ significantly from 1:1 at any of the temperatures investigated ($P>0.05$).

No juveniles had matured into adults after a 21-day incubation at 5°C, although the exponential model (see below) predicted minimum and mean development times of 12.11 and 14.75 days, respectively. In a parallel experiment performed at 4°C, the minimum development time was ~24 days (Moens, unpublished). At the other

temperatures, the minimum development time ranged from 2.6 days (mean of five replicate incubations) at 25°C to 6.8 days at 9°C (Table 3). Mean development times were always close to the minimum development times. The temperature dependence of minimum and mean development time between 9 and 25°C was adequately described by the exponential equations $y = 59.54T^{-0.98964}$ (for minimum development time) and $y = 109.02T^{-1.24275}$ (for mean development time) ($r^2 \geq 0.97$).

Only at 25°C was there an indication that males had slightly shorter minimum and mean development times than females (data not shown). Preadult mortality was low (probably less than 5%) at all but the highest temperature. At 30°C, nearly all juveniles emerged from the adult females during the first day following inoculation, but none survived beyond 5 days of incubation.

The effect of salinity on the total fecundity in *D. meyli* (Fig. 2A) was small and statistically non-significant ($P = 0.092$, Kruskall–Wallis ANOVA). As in *P. marina*, the fecundity shown for *D. meyli* is not representative of its true reproductive capacity, which averages nearly threefold the values reported here (Moens, unpublished). The daily per capita egg production, on the other hand, was affected by salinity. Using the above annotation, daily fecundity varied as follows with salinity:

10 > 5 > 20 > 30 > 40

The reproductive period of *D. meyli* at 5‰ was considerably shorter than at higher salinities.

Salinity had a significant influence on the sex ratio of *D. meyli* ($P < 0.05$, Kruskall–Wallis ANOVA), with females comprising 45 (at 10‰) to 59% (at salinities of 30 and 40‰) of the adult population (Table 4). Only at 40‰ was the sex ratio significantly different from 1:1, in favour of females ($P \ll 0.01$).

The minimum hatching time was estimated from linear regressions of cumulative egg production and cumulative appearance of juveniles. For these regressions, we omitted the last 5% of the cumulative curves, as well as the first 5–10% when there was evidence of an acclimatation period (i.e. eggs at salinities of 30 and 40‰ and at temperatures of 10 and 15°C). The remaining part of the cumulative curves of egg production, appearance of juveniles and appearance of adults was fairly linear (coefficient of determination $r^2 > 0.90$ in most cases). The intercepts with the *x*-axis were calculated from the regression equations, and taken as the time where the first egg was deposited, hatched, or where the first adult appeared. The influence of salinity on minimum and mean embryonic development time, postembryonic development time and development time is shown in Table 5. The minimum embryonic development time showed only minor differences between salinities of 10 and 30‰, but increased at 40‰. Mean postembryonic development time, on the other hand, differed between salinities of 10 to 20‰ on the one hand and of 30 to 40‰ on the other. As a result, the minimum development time gradually increased at increasing salinities from 20‰ onwards. Salinity significantly impacted the mean embryonic development time, mean postembryonic development time and mean development time ($P < 0.005$, one-way ANOVA on $\log_{10}$-transformed data). Each of these took significantly longer at 40‰ compared to

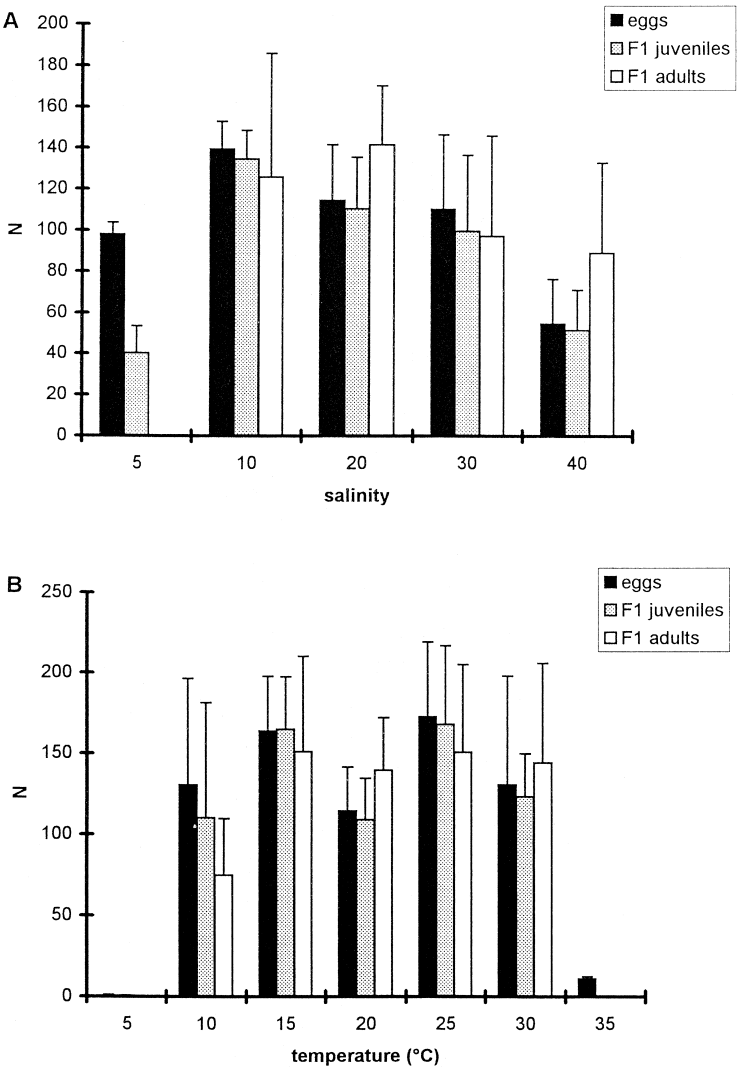


Fig. 2. Total fecundity and total number of F1-adults of *Diplolaimelloides meyli* at different salinities (A) and temperatures (B). N = number of progeny per five females. Averages ± 1 S.D. of four or five replicates are shown.

the other salinities ($P < 0.05$, Tukey's HSD-test). The time needed for the deposition of 50% of the eggs was also significantly longer at 30‰ compared to salinities of 10–20‰ ($P < 0.05$, Tukey's HSD-test).

Contrary to the total fecundity, the total number of F1-juveniles was significantly impacted by salinity ($P < 0.0005$, one-way ANOVA on $\log_{10}$-transformed data). The ranking of salinities was:

Table 4
Fecundity and sex ratio of *Diplolaimelloides meyli* as a function of salinity and temperature

	Daily fecundity		% Females	
	Mean	S.D.	Mean	S.D.
Salinity				
5	3.35	0.59	n.d.[a]	n.d.
10	3.77	0.85	44.92	6.08
20	2.40	0.38	51.13	4.83
30	1.64	0.44	59.02	11.78
40	0.59	0.39	58.89	0.73
Temperature (°C)				
5	0	0	n.d.	n.d.
10	0.64	0.31	75.89	8.31
15	1.32	0.27	60.67	5.56
20	2.18	0.43	51.13	4.83
25	4.28	1.08	56.57	7.82
30	4.19	1.25	48.51	10.34

[a] n.d., not determined.

Table 5
Minimum and mean development time of *Diplolaimelloides meyli* as a function of salinity and temperature. The means of four replicate incubations are given, with the replicate variability as the standard deviations of the replicate observations

Development time					
Salinity	5	10	20	30	40
Mean embryonic development time	3.50±1.12	3.37±0.85	3.62±0.48	4.25±1.55	7.25±1.32
Mean postembryonic development time	n.d.[a]	6.87±1.11	6.87±1.03	7.00±1.35	13.12±1.44
Mean development time all adults	n.d.	10.25±0.65	10.50±1.00	11.25±1.19	20.37±1.38
Mean development time males	n.d.	11.50±0.41	11.62±0.48	11.50±1.68	21.62±0.95
Mean development time females	n.d.	8.87±0.85	9.12±1.03	11.12±1.70	19.12±1.65
Minimum embryonic development time	4.54	3.76	4.32	4.32	7.32
Minimum postembryonic development time	n.d.	6.15	5.90	8.18	10.41
Minimum development time all adults	n.d.	9.92	10.22	12.50	17.73
Minimum development time males	n.d.	11.10	11.20	12.70	19.15
Minimum development time females	n.d.	9.09	8.13	12.48	16.35
Temperature (°C)	10	15	20	25	30
Mean embryonic development time	25.62±4.64	7.87±1.31	6.0±0	2.50±0.41	3.33±0.58
Mean postembryonic development time	38±3.83	12.50±0.58	5.25±0.96	5.12±0.85	4.50±0.58
Mean development time all adults	63.62±3.14	20.37±0.75	11.25±0.96	7.62±0.85	7.67±0.58
Mean development time males	n.d.	20.72±0.85	11.62±0.48	8.25±1.19	8.75±0.65
Mean development time females	n.d.	19.85±1.25	9.12±1.03	7.44±0.97	7.5±0.41
Minimum embryonic development time	9.40	6.61	4.32	2.99	2.90
Minimum postembryonic development time	42.20	16.23	5.90	4.85	3.97
Minimum development time all adults	51.60	22.84	10.22	7.08	6.87
Minimum development time males	53.90	23.06	11.20	7.77	8.04
Minimum development time females	51.28	22.57	8.13	6.11	5.33

[a] n.d., not determined.

10 > 20 > 30 > 40 > 5

The discrepancy between total fecundity and total number of F1-juveniles reflects egg mortality. Using the same criteria as for *P. marina*, no significant preadult mortality was found for *D. meyli* at any of the salinities tested, except the lowest. Preadult mortality at a salinity of 5‰ was 100%, and was fairly equally distributed over egg and juvenile mortality. Most juveniles died during the first larval stage, the remainder during the second.

The total fecundity of *D. meyli* over the interval studied was close to 30 per female at all temperatures except at 5°C, where no egg deposition occurred. Differences in total fecundity, in total numbers of F1-juveniles or of F1-adults between the different temperatures (Fig. 2B) were not significant ($P>0.05$, one-way ANOVA on untransformed data).

Daily fecundity ranged from 0.6 eggs female^{-1} day^{-1} at 10°C to 4.3 eggs female^{-1} day^{-1} at 25°C. Ranking fecundity with temperature gave the following result:

25 > 30 > 20 > 15 > 35 > 10

The sex ratio of *D. meyli* was significantly influenced by temperature (Table 4). The proportion of females decreased from 76% at 10°C to 48.5% at 30°C; 10°C differed significantly from all other temperatures in this respect ($P<0.05$, Tukey's HSD-test). The sex ratio differed significantly from 1:1 at 10 and 15°C ($P \ll 0.01$) in favour of females. At the other temperatures, males and females were equally represented.

The minimum embryonic development time of *D. meyli* ranged from 9.4 days at 10°C to 2.9 days at 30°C (Table 5). The data fitted the allometric equation $y = 126.37T^{-1.12}$ ($r^2>0.99$). The minimum postembryonic development time ranged from 42.3 days at 10°C to 2.9 days at 30°C and conformed well to the allometric relation $y = 12\,225.22T^{-2.46}$ ($r^2>0.99$). Minimum development times ranged from 51.7 to 5.5 days ($y = 6243.42T^{-2.08}$, $r^2>0.98$). They were systematically shorter for females (51.3 to 5.3 days) than for males (53.9 to 8.0 days).

Mean embryonic development times of *D. meyli* ranged from 25.6 days at 10°C to 2.5 days at 25°C; mean postembryonic development times from 38 to 4.5 days, with the shortest value now at 30°C; and mean development times from 63.6 to 7.6 days, again with a small difference between females and males. Allometric relations between temperature and mean embryonic development time, mean postembryonic development time and mean development time are given in Table 6.

Preadult mortality was negligible at all temperatures except 35°C, where up to 20% egg mortality and 100% juvenile mortality were observed. An exogenous contaminant in the cultures incubated at this temperature was, however, in part responsible for this effect, as we were able to rear a complete generation of *D. meyli* in a control experiment at the same temperature (Moens, unpublished). However, here too, preadult mortality was high ($\geq$50%).

Table 6
a and *b* values in the allometric relation $y = aT^b$ relating development times to temperature, with *T* as the absolute temperature (T_{abs}) in the left column and as the effective temperature (T_{eff}) in the right column. The effective temperature equals T_{abs} − the estimated temperature at which the development time becomes infinite. All coefficients of determination were ≥0.95

	Development time	a ($T = T_{abs}$)	b ($T = T_{abs}$)	a ($T = T_{eff}$)	b ($T = T_{eff}$)
Diplolaimelloides meyli	AED	6497.8	−2.41	87.3	−1.12
	APD	13 316.4	−2.55	137.3	−1.17
	ADT	19 734.8	−2.49	224.9	−1.15
	MED	126.4	−1.12	39.8	−0.86
	MPD	12 225.2	−2.46	445.8	−1.60
	MDTall	6243.4	−2.08	352.3	−1.32
	MDTmales	6470.6	−2.08	288.4	−1.22
	MDTfemales	9499.1	−2.27	725.8	−1.68
Pellioditis marina	ADT	109.0	−1.24	39.2	−0.95
	MDT	59.5	−0.99	26.6	−0.76

4. Discussion

Despite an important structuring role of salinity on nematode species diversity and community structure along estuarine gradients (see Heip et al., 1985, 1995 for reviews), only a few studies have experimentally addressed the impact of salinity on individual species or populations. In a partner paper, salinity was shown to have a relatively minor impact compared to temperature on metabolic rates of *P. marina* and *D. meyli* (Moens and Vincx, 1999). A scope for production was calculated, predicting the highest production values for both species in the range of salinities from 10 to 30‰. The scope at salinities of 5 and of 35‰ (to 45‰ in *D. meyli*) was lower (ca. 40% on average in *D. meyli*, ca. 20% in *P. marina*), though still significantly positive. Under freshwater conditions, tested solely with *P. marina*, assimilation and respiration were in balance, suggesting no energy was available for production. At first glance this seems to be contradicted by the fairly high reproductive output of female *P. marina* in freshwater agar. Only a small percentage of the juveniles produced in freshwater agar did, however, mature, and subsequently failed to give rise to an F2-generation. This suggests that progeny was produced and temporarily survived on energy reserves already present in the female inoculum at the start of the experiment. Moreover, the difference between true freshwater conditions as used for the respiration and assimilation measurements and a salinity of 1.2‰ as in the 'freshwater' agar may be significant to estuarine nematode populations (Heip et al., 1985). A reproductive output similar to that in freshwater agar was found at a salinity of 40‰. Here too, only few juveniles matured and the F1-adults did not reproduce.

Total fecundity in *D. meyli* was highest at 10 to 30‰, and comparatively lower at the lower- and higher-most salinities of the tested range. Fecundity and rate of population increase of its congener *D. bruciei* at 26‰ were twice those at lower (9 and 17.5‰) and higher (35‰) salinities (Warwick, 1981). As in *P. marina*, the reduced egg production interval of *D. meyli* at a salinity of 5‰ suggests that experimental animals may have partly used energy reserves, already present at the onset of the experiment, for

reproduction. At the lower-most salinity (5‰), however, preadult mortality was 100%, while it was negligible at the highest salinity (40‰). In *D. bruciei*, no maturation to the adult stage was observed at a salinity of 1.75 (Warwick, 1981).

Few studies have hitherto investigated marine or brackish-water nematode mortality as a function of salinity. Preadult mortality of *Monhystera denticulata* reached a maximum of 65% at a high/high combination of temperature and salinity (Tietjen and Lee, 1972), this was at a salinity of 39‰. Although they are likely to have high ecological relevance, the present study did not look for combined effects of temperature and salinity, and may be better compared to the results of Vranken (1985) who, next to testing different combinations of temperature and salinity, also studied the impact of salinity at a fixed optimum temperature in the species *Diplolaimella dievengatensis* (note that the original study erroneously identified this nematode as *Monhystera microphthalma*). Juvenile mortality, then, was close to 10% in the salinity range from 11 to 30‰, and did not vary with salinity. Egg mortality, however, did, and was much higher (48–63%) at the extremes of the tested range than at an optimal salinity of 20‰. Recently, Forster (1998) noted adult mortalities of 10–35% during up to a 48-h exposure to a salinity of 3.33‰ in three nematode species from a coastal environment. In a fourth species, *Daptonema oxycerca*, the same salinity induced a 70% mortality within 10 min of incubation, increasing to 90% after 48 h. These results are remarkable, as some of the species studied by Forster have previously been recorded in oligohaline environments, down to salinities of 0.5–1‰ (Heip et al., 1985). This suggests a strong capacity for adaptation of nematode populations to local salinity regimes, as is further indicated by the lesser sensitivity to abrupt changes in salinity of intertidal vs. subtidal nematodes (Forster, 1998). The general salinity independence of life cycle (this study) and ecophysiological (Moens and Vincx, 1999) processes in a salinity range of 5 to 35‰ in *P. marina* and *D. meyli* probably reflects the range of seasonal and daily salinity fluctuations the present populations face in their natural habitat, and thus equally suggests localized adaptation.

Although *D. meyli* eggs and juveniles were both subject to a high mortality at a salinity of 5‰, adults incubated under these conditions were still alive and behaved actively at the end of the experiment, i.e. after 3 weeks. The tolerance of low salinities is therefore clearly (st)age-dependent; in agreement with observations on pesticide-induced stress in short-lived animals (Meyer and Boyce, 1994), the nematodes appear particularly sensitive in the juvenile stages. *Pellioditis marina* and *D. meyli* adults were inactivated when exposed to unbiased (the salinity in the 'freshwater agar' was still 1.2‰) freshwater conditions, but survived for a few hours (*P. marina*) to (exceptionally) more than 1 day (*D. meyli*).

Exposure of the four coastal species studied by Forster (1998) to a hypertonic environment (66.7‰) did not result in increased mortality. In our experiments, salinities above 35‰ (marine) caused a slightly elevated adult mortality and more than a 90% preadult mortality in *P. marina*. *Diplolaimelloides meyli*, on the other hand, was not affected.

Contrary to *Monhystera denticulata* and *C. germanica* (Tietjen and Lee, 1972, 1977), the development times of *P. marina* were not strongly impacted by salinity. Only at the lower- and higher-most salinity was a 50% increase compared to the other salinities

noted. *Diplolaimelloides meyli* exhibited a slightly more complicated response of development time to salinity. Whereas embryonic development times were not affected by salinity, except at 40‰, postembryonic development times were longer as salinities increased above 20‰. Consequently, the total development time from adult to adult was shortest at 10 to 20‰, then increased with increasing salinity. A decrease in salinity from 26 to 9‰, or an increase to 35‰, had a comparable impact on *D. bruciei*, both lengthening the minimum generation time by less than 50% (Warwick, 1981).

From the foregoing, it can be deduced that the salinity boundaries for normal reproduction of the present *P. marina* population approximate 5‰ at the lower and 35‰ at the higher end. This is a rather narrow range compared to that of another *P. marina* population studied by Tietjen et al. (1970), which reproduced over a range of salinities from 0 to 80‰, with an optimum between 45 and 55‰. It also differs from the salinity range of a tropical coastal *P. marina* population, which tolerates salinities down to 15‰ and up to at least 45‰ (Moens and Vincx, unpublished). In view of the significant morphological variation among *P. marina* populations, their different abiotic preference and tolerance ranges raise the question as to whether they may represent cryptic species rather than populations of a single species. *Diplolaimelloides meyli* reproduces at higher salinities than does *P. marina*, but its lower salinity limit for normal reproduction is close to the oligo-mesohaline boundary. Its tolerance of low salinities is comparable to that of the related *D. bruciei* studied by Warwick (1981), juveniles of which did not mature at a salinity of 1.75‰ but did at 8.95‰, and to that of *Diplolaimella schneideri*, which was maintained at 6‰ (Chitwood and Murphy, 1964). Both of these species stemmed from brackish environments. The salinity range of *Monhystera denticulata* (Tietjen and Lee, 1972) and of *Chromadorina germanica* (Tietjen and Lee, 1977), isolated from nearly marine environmental salinities, was more restricted: *C. germanica*, e.g., did not survive at a salinity of 6.5 nor of 52‰, while its generation time at 13‰ was almost twice that at salinities of 26–39‰ (i.e. under optimal temperature conditions). Finally, the reproductive potential of *Eudiplogaster pararmatus* at 17°C showed only minor variation in the salinity range from 0.5 to 5‰, but decreased steeply when salinity was further increased (Romeyn et al., 1983).

The salinity ranges for reproduction found in the present study roughly reflect the natural ranges of both species in the Westerschelde Estuary. *Diplolaimelloides meyli* is common on *Spartina anglica* detritus in the entire meso- and polyhaline, and can sometimes be found on *Phragmites australis* detritus in the oligohaline, at an average salinity of 5–10‰, although it is doubtful whether the species actually reproduces there (Moens, unpublished). *Pellioditis marina* covers the entire meso- and polyhaline reach of the estuary, and is fairly common in sheltered coastal habitats of the North Sea too (Vranken, 1985; Moens, unpublished). In a recent survey, it was not found in the oligohaline (Moens, unpublished) of the Westerschelde Estuary. Consequently, its natural reach is more restricted than suggested by our culture data, which indicate it can cope with salinities as low as 5‰ or even less. This absence may relate to competition with other, halotolerant rhabditids, especially of the genus *Panagrolaimus*, which are abundant in this zone. On the other hand, the agreement between the salinity optimum observed for both nematodes in this study and the average extant salinity at the site where they were originally isolated, may in part have been enhanced by adaptation to

culture conditions. Both populations had been in continuous culture for at least 4 years prior to the onset of the present experiments, at a temperature of 15 to 20°C and a salinity of 15 to 30‰. While the results of similar culture experiments over the course of this period do not indicate adaptation to either salinity or temperature in the present *D. meyli* population, a *P. marina* population from Zanzibar did adapt both its upper temperature tolerance and its generation time at lower temperatures to the culture conditions over a considerably shorter culture period (Moens and Vincx, 1998). The possibility therefore remains that patterns and ranges observed in this study are partly deviant from those of the original populations.

The trends of total and daily fecundity and egg production interval between 10 and 30°C for *D. meyli* are largely corroborated by the pattern of scope vs. temperature (Moens and Vincx, 1999). In this temperature interval, no significant preadult mortality was observed. Only during prolonged (several days) incubations at 35°C did high egg, juvenile, and — to a lesser extent — adult mortality occur. The total fecundity of *P. marina* was highest at 15 to 20°C, whereas the highest scope was found at 25°C (Moens and Vincx, 1999); furthermore, reproduction was impaired at 30°C, while the scope for production at this temperature was still significantly positive (Moens and Vincx, 1999). The differences between predicted and realized production result from differences in the time-scale at which the processes were measured: hours for respiration and assimilation measurements, days for life cycle experiments. *Pellioditis marina* can cope with temperatures above 25°C for a few hours, during which its activity is impaired only to a limited extent. Prolonged exposure, however, inactivates or even kills this nematode: a temperature of 30°C induced 100% preadult mortality, and even at 25°C was preadult mortality higher than at lower temperatures, suggesting that 25°C is close to the upper limit for normal reproduction of the present *P. marina* population.

This temperature dependence pattern is again not a characteristic of the species but of the population studied. A subtropical mangrove population of *P. marina* reproduced successfully at up to 35°C, although the increased variability among replicate incubations at this temperature was interpreted as an indication of stress (Hopper et al., 1973). A population from the NE coast of the United States reproduced in a range from 10 to 38°C, although mortality above 35°C was high (Tietjen et al., 1970). A population isolated from coastal waters at Zanzibar, East Africa, also reproduced successfully at 35°C, although its upper limit for successful reproduction decreased to just under 30°C after having been cultivated for several months at 25°C (Moens, unpublished). It is particularly striking that some populations of this species may still reproduce at temperatures which other populations of the same species cannot even survive for more than a few hours.

Most previous studies dealing with the influence of temperature on the reproduction of marine or brackish-water nematodes have found an increase of fecundity and/or rate of increase with temperature (see Heip et al., 1985; Vranken et al., 1988, for reviews) up to a maximum which is usually situated a few degrees below the upper temperature (tolerance) limit of the species (Hopper et al., 1973). This upper tolerance limit may to some extent be correlated to the upper average temperatures in the species' or population's natural habitat (Hopper et al., 1973; Sudhaus, 1980). Moreover, Sudhaus (1980) found an influence of maintenance temperature on the upper temperature

tolerance of some rhabditid nematodes. This upper limit may be around 35–37°C in nematodes from mangrove environments in Florida (Hopper et al., 1973). Nematodes from temperate zones have upper lethal temperatures of the order of 25–30°C in *Geomohystera disjuncta*, *Theristus pertenuis* (Gerlach and Schrage, 1971) and *Neochromadora poecilosomoides* (Vranken, 1985), of 30–35°C in *C. germanica* (Tietjen and Lee, 1977), and of ≥35°C in *Monhystrella parelegantula*, *D. dievengatensis* (Vranken, 1985) and the present *D. meyli* population. Only in one of these species (*M. parelegantula*) mortality did not increase at temperatures above 30°C.

Although several life cycle aspects (mortality, fecundity, ...) already hint at deteriorating environmental conditions as temperature approaches its upper (lethal) limit (see above), this is not always the case for nematode development times, which may be at their shortest at temperatures only just below the upper tolerance limit (see, e.g., Hopper et al., 1973; Moens et al., 1996a). In the present study, *P. marina* had its shortest development time at 25°C and did not mature at 30°C. In *D. meyli*, development times were lowest and nearly constant at 25–30°C, and apparently did not increase at 35°C (Moens, unpublished). Within the temperature interval of 9 to 25°C for *P. marina* and of 10 to 30°C for *D. meyli*, solutions to the power relation $y = aT^b$ adequately described the influence of temperature on development times. Table 6 lists *a* and *b* values for both species; *b* equals the slope of a (natural) logarithmic plot of development time vs. temperature, and is thus a measure of temperature dependence. It is twice as high in *D. meyli* as in *P. marina*, which agrees with the Q_{10}-values for respiration and assimilation in these species (Moens and Vincx, 1999). The discrepancy in temperature dependence between minimum embryonic development time and the other development times in *D. meyli* (Table 6) relates to the exclusion of the lag in reproduction, which probably resulted from temperature acclimatation upon transfer from 20 (stock cultures) to 10°C. This also explains why the mean postembryonic development time at this temperature was shorter than the minimum postembryonic development time.

The values recorded for *D. meyli* and *P. marina* fall in the range of published *b* values for other marine and brackish-water nematodes (see Warwick, 1981; Heip et al., 1985; Vranken and Heip, 1986, for reviews), which span values from −0.67 in *Geomonhystera disjuncta* to −3.52 in *Diplolaimella* sp.; *b* values of other *Diplolaimelloides* species were close to −2, as for *D. meyli* (Hopper et al., 1973; Warwick, 1981), but were strongly dependent on the culture conditions (Warwick, 1981). For a subtropical *P. marina* population, *b* was −1.59 (Hopper et al., 1973), considerably higher than in the present study, whereas Bergholz and Brenning (1978) reported a *b* of −0.66 for a temperate population of this species.

The allometric relation $y = aT^b$ is a simplification of Bèlehràdek's equation $y = a(T - \alpha)^b$ where α represents the biological zero. If α does not equal 0°C, as implicitly assumed in the power function, then *b* and α will to an extent be correlated, and species with a high α will automatically have a high *b* (Vranken, 1985). We have therefore recalculated our development time data with effective rather than absolute temperatures, assuming the biological zero for *D. meyli* and *P. marina* to be close to 7 and 3°C, respectively. The resulting *b* values are shown in Table 6, and are systematically smaller than those calculated with the absolute temperatures. When compared to the effective *b* values of the species studied by Vranken (1985) and Warwick (1981), the *b* of *D. meyli*

is close to that of the related species *D. bruciei* (−1.10, assuming $\alpha = 7.5$), *Diplolaimella dievengatensis* (−1.05) and *Monhystera parva* (−1.29), but considerably smaller than that of *Monhystrella parelegantula*, a nematode showing no signs of adverse conditions when constantly exposed to a temperature of 35°C (Vranken, 1985). The *b* value of *P. marina* is among the lowest observed.

The sex ratio in *P. marina* did not vary importantly with temperature or salinity, and did not differ significantly from 1:1, except at mesohaline salinities, where a female dominance ($\leq$60%) was noted. Previously, a small but insignificant female dominance in a coastal North Sea population of this species was observed (Vranken, 1985), while Tietjen et al. (1970) found a pronounced female dominance of 66–75% for a North American population. Sex ratio data on laboratory-reared populations of brackish-water and marine nematodes are scanty. They concern ~15 species, a majority of which had sex ratios not significantly different from 1:1 (Hopper and Meyers, 1966; Tietjen, 1967; Tietjen et al., 1970; Tietjen and Lee, 1972, 1973; Heip et al., 1978; Warwick, 1981; Vranken, 1985). Deviations from this ratio may favour females (*M. filicaudata*: 95% females, Tietjen, 1967; *P. marina*, see above; *G. disjuncta*: 70% females, Vranken, 1985), or males (*Oncholaimus oxyuris*: 60% male dominance, Heip et al., 1978; *D. bruciei*: sex ratio of 2:1 in favour of males, Warwick, 1981). A similar 2:1 ratio in favour of males was found for *D. meyli* in mixed agar cultures with *P. marina* under optimal temperature conditions, whereas at low temperature (10°C), females comprised nearly 75% of the adult population (Moens et al., 1996c). The female predominance at 10°C (and at high salinities) was corroborated by the present study, but the male predominance at 25°C was not.

Except for the respiration measurements, which are fairly easy and rapid provided large quantities of nematodes are available (Moens et al., 1996b), the methods employed in delineating the abiotic ranges of these nematodes in this and a partner study (Moens and Vincx, 1999) are labour-intensive and time-consuming. In toxicity studies, it is common practice to evaluate the impact of toxicants by focusing on one or a few life cycle traits which are considered to be the most sensitive (e.g. preadult mortality, daily fecundity). It has recently been argued, however, that small effects on less sensitive traits may more importantly impair the fitness of organisms than comparatively larger effects on more sensitive traits, fitness being defined in relation to the intrinsic rate of population increase (Kammenga et al., 1996, 1997a,b; Kammenga and Riksen, 1996). The present results equally suggest that there is no single life history trait upon which a straightforward assessment of optimal or suboptimal environmental conditions can be judged. With respect to salinity, preadult mortality appeared to be the most sensitive trait, but it could not be used to assess, e.g., effects of lowered temperature. The energetics-related parameters, respiration and assimilation, similarly cannot be used without supporting life history data: high respiration rates, e.g., may indicate favourable as well as stressful conditions (Moens and Vincx, 1999, and references therein). There thus appears to be no rapid and simple way to determine environmental ranges of free-living aquatic nematodes, except by focusing on the rate of increase of populations with a stable age distribution (Warwick, 1981), which may require the use of liquid media in large incubators rather than of agar media in small petri dishes. Obviously, in doing so, the driving traits behind the observed rates of increase remain largely obscured.

Finally, the data presented in this and a partner paper (Moens and Vincx, 1999) advocate the use of experiments at different time scales to arrive at a better understanding of the impact of environment on the performance of nematode populations. Preliminary experiments with the cultivation of subsequent generations under conditions near the extremes of the abiotic ranges observed here, indicate that prolonged exposure may further limit the actual range of a population (Moens, unpublished). Vice versa, further research is needed on the impact of episodes of extreme conditions (e.g. a 3-h, non-lethal, exposure to a temperature of 35°C in *P. marina*) on the subsequent performance of nematodes.

Acknowledgements

The first author benefitted from a grant as aspirant with the Fund for Scientific Research Flanders during most of this study. This work received financial support from the University of Gent (Belgium) through contract BOF 98-03, 12050398.

References

Alkemade, R., Wielemaker, A., Hemminga, M.A., 1993. Correlation between nematode abundance and decomposition rate of *Spartina anglica* leaves. Mar. Ecol. Prog. Series 99, 293–300.

Alkemade, R., Wielemaker, A., Herman, P.M.J., Hemminga, M.A., 1994. Population dynamics of *Diplolaimelloides bruciei*, a nematode associated with the salt marsh plant *Spartina anglica*. Mar. Ecol. Prog. Series 105, 277–284.

Bergholz, E., Brenning, U., 1978. Studies on the reproductive cycles of marine nematodes (*Rhabditis marina* and *Prochromadora orleji*). Wiss. Z. Univ. Rostock 27, 393–398.

Bouwman, L.A., Romeyn, K., Kremer, D.R., van Es, F.B., 1984. Occurrence and feeding biology of some nematode species in estuarine aufwuchs communities. Cahiers de Biologie Marine 25, 287–303.

Chitwood, B.G., Murphy, D.G., 1964. Observations on two marine monhysterids — their classification, cultivation and behavior. Trans. Am. Microsc. Soc. 83, 311–329.

Conover, W.J., 1980. In: 2nd ed, Practical Non-parametric Statistics, John Wiley, New York.

Dietrich, G., Kalle, K., 1957. Allgemeine Meereskunde. Eine Einführung in die Ozeanographie, Gebrüder Borntraeger, Berlin.

Forster, S.J., 1998. Osmotic stress tolerance and osmoregulation of intertidal and subtidal nematodes. J. Exp. Mar. Biol. Ecol. 224, 109–125.

Gerlach, S.A., Schrage, M., 1971. Life cycles in marine meiobenthos. Experiments at various temperatures with *Monhystera disjuncta* and *Theristus pertenuis* (Nematoda). Mar. Biol. 9, 274–280.

Heip, C.H.R., Goosen, N.K., Herman, P.M.J., Kromkamp, J., Middelburg, J.J., Soetaert, K., 1995. Production and consumption of biological particles in temperate tidal estuaries. Oceanogr. Mar. Biol. Annu. Rev. 33, 1–149.

Heip, C., Smol, N., Absillis, V., 1978. Influence of temperature on the reproductive potential of *Oncholaimus oxyuris* (Nematoda Oncholaimidae). Mar. Biol. 45, 255–260.

Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol.: Annu. Rev. 23, 399–489.

Hopper, B.E., 1970. *Diplolaimelloides bruciei* n.sp. (Monhysteridae: Nematoda), prevalent on marsh grass, *Spartina alterniflora* Loisel. Can. J. Zool. 48, 573–575.

Hopper, B.E., Fell, J.W., Cefalu, R.C., 1973. Effect of temperature on life cycles of nematodes associated with the mangrove (*Rhizophora mangle*) detrital system. Mar. Biol. 23, 293–296.

Hopper, B.E., Meyers, S.P., 1966. Aspects of the life cycle of marine nematodes. Helgoländer wissenschaftliche Meeresuntersuchungen 13, 444–449.

Kammenga, J.E., Busschers, M., Van Straalen, N.M., Jepson, P.C., Bakker, J., 1996. Stress induced fitness reduction is not determined by the most sensitive life-cycle trait. Func. Ecol. 10, 106–111.

Kammenga, J.E., Riksen, J.A.G., 1996. Comparing differences in species sensitivity to toxicants: phenotypic plasticity versus concentration-response relationships. Environ. Toxicol. Chem. 15, 1649–1653.

Kammenga, J.E., Korthals, G.J., Bongers, A.M.T., Bakker, J., 1997a. Reaction norms for life-history traits as the basis for the evaluation of critical effect levels of toxicants. In: Van Straalen, N.M., Løkke, H. (Eds.), Ecological Principles For Risk Assessment of Contaminants in Soil, Chapman and Hall, London.

Kammenga, J.E., Van Koert, P.H.G., Koeman, J.H., Bakker, J., 1997b. Fitness consequences of toxic stress evaluated within the context of phenotypic plasticity. Ecol. Appl. 7, 726–734.

Meyer, J.S., Boyce, M.S., 1994. Life historical consequences of pesticides and other insults to vital rates. In: Kendall, R.J., Lacher, Jr. E.J. (Eds.), Wildlife Toxicology and Population Modeling. Integrated Studies in Agroecosystems. SETAC Special Publication, CRC, Boca Raton, pp. 349–364.

Moens, T., Coomans, A., De Waele, D., 1996a. Effect of temperature on the in vitro reproduction of *Aphelenchoides rutgersi*. Fundam. Appl. Nematol. 19, 47–52.

Moens, T., Vierstraete, A., Vanhove, S., Verbeke, M., Vincx, M., 1996b. A handy method for measuring meiobenthic respiration. J. Exp. Mar. Biol. Ecol. 197, 177–190.

Moens, T., Vierstraete, A., Vincx, M., 1996c. Life strategies in two bacterivorous marine nematodes: preliminary results. Pubblicazioni della Stazione Zoologica di Napoli I Mar. Ecol. 17, 509–518.

Moens, T., Vincx, M., 1997. Observations on the feeding ecology of estuarine nematodes. J. Mar. Biol. Assoc. UK 77, 211–227.

Moens, T., Vincx, M., 1998. On the cultivation of free-living estuarine and marine nematodes. Helgoländer Meeresuntersuchungen 52, 115–139.

Moens, T., Vincx, M., 1999. Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: assessing niches from food absorption and respiration experiments. J. Exp. Mar. Biol. Ecol. (in press).

Montagna, P.A., Ruber, E., 1980. Decomposition of *Spartina alterniflora* in different seasons and habitats of a northern Massachusetts salt marsh, and a comparison with other Atlantic regions. Estuaries 3, 61–64.

Reice, S.E., Stiven, A.E., 1983. Environmental patchiness, litter decomposition and associated faunal patterns in a *Spartina alterniflora* marsh. Estuarine Coastal Shelf Sci. 16, 559–571.

Romeyn, K., Bouwman, L.A., Admiraal, W., 1983. Ecology and cultivation of the herbivorous brackish-water nematode *Eudiplogaster paramatus*. Mar. Ecol. Prog. Series 12, 145–153.

Schiemer, F., 1982. Food dependence and energetics of free-living nematodes. I. Respiration, growth and reproduction of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. Oecologia 54, 108–121.

Sokal, R.R., Rohlf, F.J., 1995. In: 3rd ed, Biometry, WH Freeman, New York.

Sudhaus, W., 1980. Vergleichende Untersuchungen zur oberen Grenztemperatur saprobionter Nematoden der Gattung *Rhabditis*. Nematologica 26, 75–82.

Teal, J.M., Wieser, W., 1966. The distribution and ecology of nematodes in a Georgia salt marsh. Limnol. Oceanogr. 11, 217–222.

Tenore, K.R., Hanson, R.B., McClain, J., Maccubbin, E., Hodson, R.E., 1984. Changes in composition and nutritional value to a benthic deposit feeder of decomposing detritus pools. Bull. Mar. Sci. 35, 299–311.

Tietjen, J.H., 1967. Observations on the ecology of the marine nematode *Monhystera filicaudata* Allgén, 1929. Trans. Am. Microsc. Soc. 86, 304–306.

Tietjen, J.H., Lee, J.J., 1972. Life cycles of marine nematodes. Influence of temperature and salinity on the development of *Monhystera denticulata* Timm. Oecologia 10, 167–176.

Tietjen, J.H., Lee, J.J., 1973. Life-history and feeding habits of the marine nematode *Chromadora macrolaimoides* Steiner. Oecologia 12, 303–314.

Tietjen, J.H., Lee, J.J., 1977. Life history of marine nematodes. Influence of temperature and salinity on the reproductive potential of *Chromadorina germanica* Bütschli. Mikrofauna Meeresboden 15, 263–270.

Tietjen, J.H., Lee, J.J., Rullman, J., Greengart, A., Trompeter, J., 1970. Gnotobiotic culture and physiological ecology of the marine nematode *Rhabditis marina* Bastian. Limnol. Oceanogr. 15, 535–543.

Vranken, G., 1985. Een autoecologische studie van brakwaternematoden. Ph.D. thesis, University of Gent, Belgium, 282 + 202 pp.

Vranken, G., Heip, C., 1983. Calculation of the intrinsic rate of natural increase, r_m, with *Rhabditis marina* Bastian 1865 (Nematoda). Nematologica 29, 468–477.

Vranken, G., Heip, C., 1986. The productivity of marine nematodes. Ophelia 26, 429–442.

Vranken, G., Herman, P.M.J., Heip, C., 1988. Studies of the life-history and energetics of marine and brackish-water nematodes. I. Demography of *Monhystera disjuncta* at different temperature and feeding conditions. Oecologia 77, 296–301.

Warwick, R.M., 1980. Population dynamics and secondary production of benthos. In: Tenore, K.R., Coull, B.C. (Eds.), Marine Benthic Dynamics, University of South Carolina Press, Columbia, pp. 1–24.

Warwick, R.M., 1981. The influence of temperature and salinity on energy partitioning in the marine nematode *Diplolaimelloides bruciei*. Oecologia 51, 318–325.

Warwick, R.M., 1987. Meiofauna: their role in marine detrital systems. In: Moriarty, D.J.W., Pullin, R.S.V. (Eds.), Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Center for Living Aquatic Resources Management, Manila, pp. 282–295.