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ASPECTS OF THE LIFE-CYCLE OF MARINE FREE-LIVING NEMATODES.

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ABSTRACT

The life-cycle of several species of marine nematodes has been studied in laboratory conditions. Temperature has a significant influence on egg development and on the development time of both males and females. Temperature dependent developmental acceleration is highly different among the species studied. The marine species Monhystera disjuncta has a small temperature dependent acceleration. The development of Monhystrella parelegantula on the contrary is extremely dependent on temperature. The optimum temperature of the latter species is 35C.

There exists a highly significant correlation ($p < 0.01$) between egg size and the embryonic developmental period on the one hand and between minimum generation time T_{min} and weight at sexual maturity M_s on the other hand. The latter relationship can be described with an allometric function:
 $T_{min} = 1.530 M_s^{0.647}$ at 20C, with T_{min} in days and M_s in ng dry weight.

The mean number of generations for the species studied up to now is estimated at 8 per year. For each species separately, the annual number of juvenile periods $D(t)$ can be estimated from the wet weight ww at sexual maturity with the allometric function
 $D(t) = 7.566 ww^{-0.503}$ with ww in μg .

Weight specific production of the juveniles is constant due to the exponential somatic growth in this stage. The weight specific productivity of the adults is highly dependent on age, except for Monhystera disjuncta where it is constant. It peaks immediately after the adult stage has started. This initial maximum is followed by a steep decline with ageing. Weight specific reproductivity is nearly constant among the species studied. It varies between 40% of the total productivity in Diplolaimella spec.1 and 77% in the parthenogenetic species Monhystrella parelegantula. The productivity/biomass ratio (P/B) per year is much higher than 9 and varies between 22 for Monhystrella parelegantula and 66 for Monhystera disjuncta.

Introduction

The importance of free-living marine nematodes in aquatic environments is generally recognized (Platt and Warwick, 1980; Heip *et al.*, 1982; Heip *et al.*, in press and Nicholas, 1984). However information on their life-cycles is still very scarce. Existing data on development time, reproductivity and longevity have been summarized by Zaika and Makarova (1979) and Heip *et al.* (in press). Most data on life-histories have been obtained from laboratory conditions and this will remain unavoidable, especially for the small and moderately large species such as *Monhystrella parelegantula* (Vranken *et al.*, 1981) and *Diplolaimelloides brucei* (Warwick, 1981), species which are able to realize very high developmental rates in the summer. The impact of temperature (T) on development time (T_{min}) can be described by a simple power equation (Warwick, 1981; Heip *et al.*, 1982) : $T_{min} = aT^b$, where a and b are constants. b is a measure of temperature-dependency. In this paper we will examine whether the value of b is correlated with geographic distribution and whether b relates to taxonomic position.

From literature it is known that large species tend to develop more slowly than small ones. For example, the large oncholaimid *Oncholaimus oxyuris* which has an adult weight of 20 μg , has a generation time of 114 days at 20° C (Heip *et al.*, 1978), whereas the generation time of the small monhysterid *Diplolaimella* spec. 1 (wet weight = 0.5 μg) is only 10 days at the same temperature (Vranken *et al.*, 1984a). Similar discrepancies in development rate between eggs of different size is known (Gerlach and Schrage, 1971; Heip *et al.*, 1978 and Vranken *et al.*, 1981). In this paper we will study the relationship between development time (T_{min}), embryonic development time (E_{min}) on the one hand and biomass on the other and discuss the scatter around the regression line relating T_{min} and biomass.

Direct production estimates from the field require frequent sampling with intervals of two or three days. For meiobenthic populations of the open sea such a sampling frequency is much too expensive. The use of indirect production estimates is therefore completely justifiable. Two of these short-cut methods, which have been reviewed by Heip *et al.* (in press) are of special interest for this study. A first approach consists of calculating the annual production biomass ratio (P/B ratio) from the empiric relationship between wet weight at sexual maturity (Banse and Mosher, 1980) and P/B. The other uses the life-cycle turnover concept (life-cycle P/B) of Waters (1969). In the latter method, the life-cycle turnover, which has a modal value of three in nematodes (Gerlach, 1971; Herman *et al.*, 1984; Heip *et al.*, in press; Vranken, 1985), has to be multiplied by the annual number of generations the population realizes in the

field. Again, field studies can only provide us with information for the long-lived, slowly developing species (Smol *et al.*, 1980). For the fast-developers the number of generations has to be determined from laboratory experiments. The relationship between the annual number of generations and the adult biomass is investigated. Finally the partitioning of production over the different life-history stages is analyzed.

Material and methods

The nematode species studied were isolated from the Sluice Dock of Ostend, a marine lagoon near the Belgian coast and from the Dievengat, a poly-meso-haline pond, situated in a Polder in northwestern Belgium. Meiobenthic organisms and detritus were extracted from the mud using Barnett's method (1968), and collected on a sieve (mesh width = 38 μ m). Hereafter the mud containing the organisms is placed in petri-dishes filled with unenriched bacto-agar (0.8 %) made up with brackish water. Bacto-agar was used to cultivate the nematodes. The great advantage of this material is that the animals can be observed individually during their entire life. Two enrichments have been used successfully: the Vlasblom-medium (Vranken *et al.*, 1984a) and modified Killian medium (von Thun, 1966). During the experiments the food for the bacterial-feeders (*Diplolaimella* spec. 1, *Monhystera disjuncta*, *Monhystrella parelegantula*) consisted out of a mixture of unidentified bacteria which were given in excess. The herbivorous nematode-species (*Chromadora nudicapitata*, *Monhystera parva* and *Paracanthochus caecus*) were grown on the following mixture of diatoms: *Navicula peregrina*, *Nitzschia ovalis*, *Cocconeis scutellum*, *Cyclotella* sp. and *Melosira* sp., supplemented with the green algae *Dunaliella salina*. The experiments were done in small vented petri-dishes (\emptyset : 35 mm). The bacterial-feeders were grown in the dark, the herbivorous species under continuous lighting. Salinity was measured with a Goldberg refractometer. For more details concerning the culture techniques and for a definition of some life-history features discussed in the present work, we refer to Vranken *et al.*, 1984 a & b.

Results and Discussion

a. Influence of temperature on development rate

Here we will present the results of an extensive study of the influence of temperature on development time (T_{\min}), which is defined as the time-period between egg-deposition and the moment when 50 % of the female offspring become gravid. Time zero is taken as the mid-point of the interval in which the eggs are deposited. Temperature has a profound influence on T_{\min} as is shown in Fig. 1a & b for males and females of *Diplolaimella* sp. 1 grown at a constant salinity of 20 ‰. The full line is the power equation, $T_{\min} = at^D$ and the dotted line is a

quadratic function, the use of which will not be discussed here. Development times of the other species together with complete statistics are given by Vranken (1985). b-values obtained for female and male development times and for the embryonic development are shown in table 1. From these data it is obvious that within each species, *Monhystrella parelegantula* excluded, the temperature dependency of the duration of the three stages studied, does not differ substantially. Hence both sexes have the same temperature dependent development acceleration.

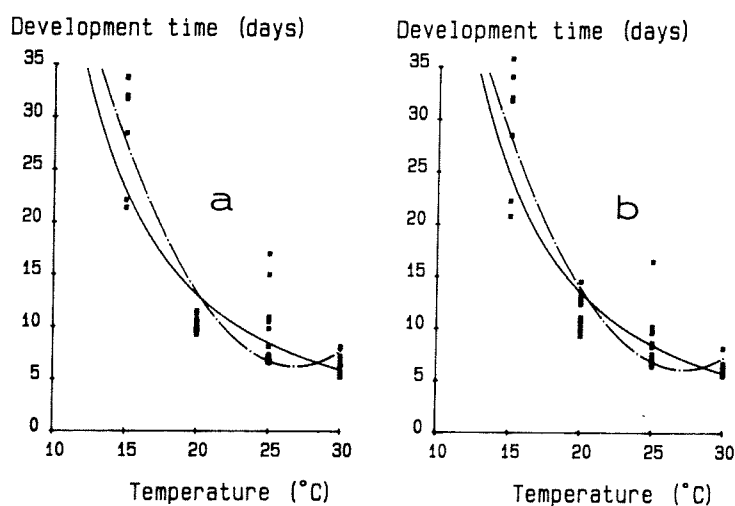


Fig. 1. *Diplolaimella* spec. 1 : Duration of development time (days) until adulthood in relation with temperature (°C) at 20 ‰ salinity under laboratory conditions (a : females; b : males).

Table 1. b-values of the power equation. The error of b, given between brackets, is calculated according to Sokal and Rohlf, 1981 (♀♀ we = gravid females, ♂♂ : males, E min : embryonic development)

Species	Sal. (‰)	b (♀♀ we)	b (♂♂)	b (E min)
<i>Diplolaimella</i> sp. 1	20	-1.96 (0.49)	-2.10 (0.35)	-2.25 (0.36)
	30	-2.01 (0.09)	-1.99 (0.13)	-2.15 (0.42)
	11	-1.67 (0.41)	-1.76 (0.40)	-1.92 (0.46)
<i>M. parelegantula</i>	30	-3.11 (0.44)	--	-2.07 (0.21)
<i>M. parva</i>	30	-1.74 (0.21)	-1.78 (0.22)	-1.76 (0.29)
<i>C. nudicapitata</i>	30	-1.49 (0.25)	-1.48 (0.24)	-1.45 (0.20)
<i>M. disjuncta</i>	30	-0.84 (0.05)	-0.82 (0.05)	-0.76 (0.06)

Further it is shown with *Diplolaimella* sp. 1 that salinity has no influence on the value of b , whereas it has a profound influence on development time itself. Among the species on the contrary, there exists large differences in temperature-dependency. The marine monhysterid *Monhystera disjuncta* has a much lower b than the highly opportunistic brackish water species *Monhystrella parelegantula*. The latter species also occurs in hot water springs and in inland salinas where temperature can rise above 30° C. Its biological zero (T_0 , temperature below which no development occurs) is $\pm 14^\circ$ C and therefore it has to realize a high developmental acceleration in the favourable season to guarantee its survival in the temporary habitats where it lives. In fig. 2 all b -values for the nematode species cultivated up to now are compiled. The b -values are highly heterogeneous ($F = 7.3$; $df = 18,48$; $P < 0.001$). The bars are 95 % comparison intervals (Gabriels' T-method, Sokal & Rohlf, 1981). b 's with non-overlapping intervals are significantly different from each other. *Monhystera disjuncta* has a much lower value than the other species as well, most of which are estuarine or brackish water forms. The tropical species (*Haliplectus dorsalis*, *Oncholaimus* sp., *Diplolaimella ocellata*, *Diplolaimelloides* sp. and *Pellioditis marina*) cultured by Hopper *et al.* (1973) have statistically higher b -values than those inhabiting temperate regions. The monhysterids with the exception of *Monhystrella parelegantula*, have intermediate b -values. Temperature-dependency of development time of *Monhystrella parelegantula* is as high as that of the warm-water species. Its optimum temperature is $\pm 35^\circ$ C which is even higher than that of the warm-water species (Hopper *et al.*, 1973). Temperature appears to have a minor effect on the development of *Monhystera disjuncta*. This species is able to reproduce at temperatures as low as 0° C (Gerlach and Schrage, 1971), observations which are confirmed from the field as the species was found in Antarctica (Viglierchio, 1974). *Pellioditis marina* has an intermediate b -value (-1.59). The species has already a short development time in its lower temperature range and therefore its acceleration rate is limited. The chromadorids have smaller b -values than the monhysterids and much smaller values than the warm-water species. It may be concluded that the warm-water species have the highest b -values and that there appears to exist a relationship between temperature-dependency and geographic occurrence.

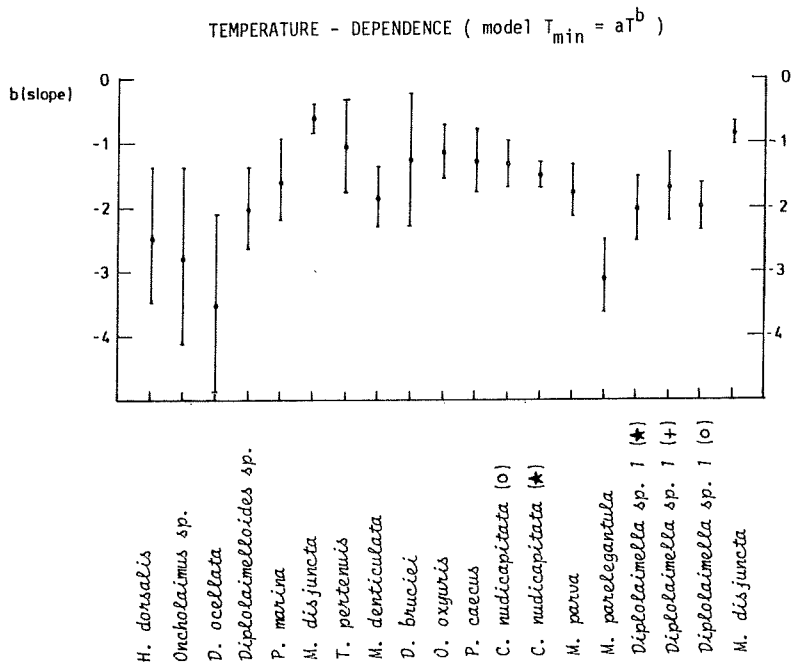


Fig. 2. Values of b in the power equation $T_{\min} = aT^b$. References are given in Heip *et al.* (in press). The b -value for *Pellioiditis marina* was calculated by using the data of Hopper *et al.*, 1973. (+) = 11 ‰ S; (0) = 20 ‰ S and (*) = 30 ‰ S; b -values of *Paracanthochus caecus* and *Chromadora nudicapitata* (0) are unpublished data of D. Van Brussel.

b. Effect of body weight on T_{\min} , E_{\min} and $D(t)$

In general small species have short life-cycles while large species mature later, having longer life-cycles. Therefore a positive relationship between minimum generation time (T_{\min}) and biomass is to be expected. The relationship between T_{\min} at 20° C and biomass (dry weight) at sexual maturity is given in fig. 3. There exists a weak but significant correlation ($r = 0.325$; $0.05 > P > 0.01$; $n = 41$) between T_{\min} and biomass. The relationship can be described by

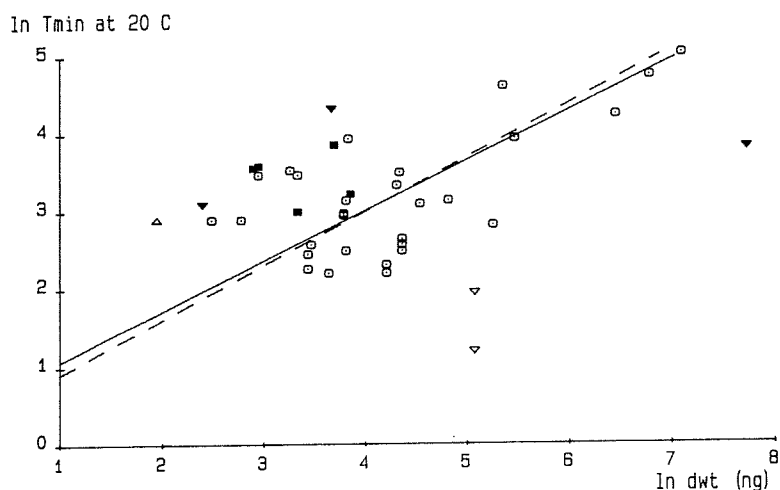


Fig. 3. Relationship between T_{\min} (days) at 20° C and biomass at sexual maturity (dry weight in nanogram). The full line is a GM-regression using all data : $T_{\min} = 1.53 \text{ dwt}^{0.65}$; the dashed line is a GM-regression using the circles only : $T_{\min} = 1.24 \text{ dwt}^{0.69}$. See text for more explanation.

the following equation : $T_{\min} = 1.530 \text{ dwt (ng)}^{0.647} (\text{SE} = 0.098)$ (GM-regression, model II). In the computations the generation time of *Chromadorita tenuis* (Jensen, 1983) is included. This is the reason why a slightly different equation is obtained as the one proposed by Heip *et al.* (in press). The scatter around the line has been attributed to suboptimal cultivation (species labeled as ■), to differences in acceleration rate and basal temperature (T_0) between species inhabiting different climates (▼) and to the nature of the temperature response of extreme opportunists (∇ and Δ). For a more detailed analysis with a full argumentation for each species separately, we refer to Heip *et al.* (in press). After omitting these points a much higher correlation coefficient $r = 0.654$

($P < 0.01$) is obtained. Using all data in the literature the correlation between embryonic development time (E_{\min}) and egg weight, presented in fig. 4, is 0.612 ($P < 0.01$; $n = 32$). The correlation is $r = 0.676$ ($P < 0.01$) with *Pellioditis marina*, an euryhaline saprobic terrestrial form, excluded. Data for *Chromadorita tenuis* (Jensen, 1983) and *Pontonema vulgare* (Malakhov, 1974) were included.

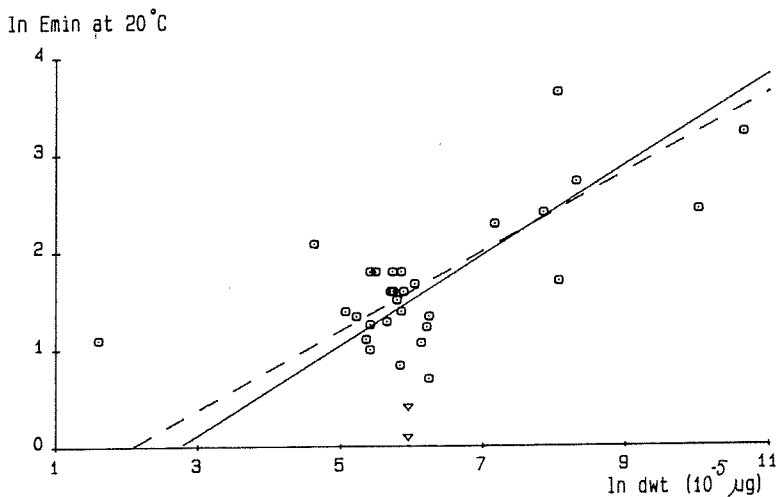


Fig. 4. Relation between E_{\min} (days) at 20°C and egg-weight (dry weight in 10^{-5} μg). The full line is a GM-regression using all data : $E_{\min} = 0.28 \text{ dwt}^{0.46}$; the dashed line is a regression with *Pellioditis marina* (∇) excluded : $E_{\min} = 0.43 \text{ dwt}^{0.41}$.

Again development rate is highly correlated with biomass.

A high correlation between the number of annual generations $D(t)$ and biomass is therefore to be expected. Existing estimations of $D(t)$ both from the field and the laboratory have been summarized previously (Heip *et al.*, in press). Here we added data listed in table 2. For all species studied up to now the mean annual generation number is 8.2 (95 % CI = 2.5; $n = 30$) which is considerable higher than Gerlach's number 3.

Table 2. Number of annual generations $D(t)$ of nematode species, estimated either from the field (F) or laboratory experiments (L)

Species	$D(t)$	Reference
<i>Pontonema vulgare</i> F	0.5	Malakhov, 1974
<i>Atrochromadora denticulata</i> L	14	Garcia, 1982
<i>Monhystera</i> sp. L	14	id.
<i>Monhystera parva</i> L	14	id.
<i>Monhystera multisetosa</i> L	17	id.
<i>Diplolaimella</i> spec. 1	10	this study
<i>Chromadora nudicapitata</i> ^o L	13	id.
<i>Monhystera parva</i> L	16	id.
<i>Monhystera disjuncta</i> L	23	id.
<i>Monhystrella parelegantula</i> L	6.3	id.
<i>Paracanthonus caecus</i> L	3.5	Van Brussel, unpublished
<i>Chromadora nudicapitata</i> ^h L	10	id.
<i>Mesacanthion diplochma</i> F	1	Lorenzen, 1974

^o : Sluice Dock population; ^h : Dievengat population

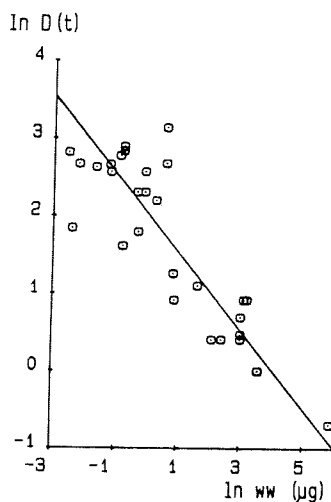


Fig. 5. Relation between $D(t)$ (maximum number of annual generations) and wet weight (ww in μg) at sexual maturity.

The relation between $D(t)$ and wet weight (μg) at sexual maturity (M_s) is presented in fig. 5. There exists a high significant correlation between the two ($r = -0.876$; $P < 0.01$; $n = 30$). It can be described by the following GM-

regression : $D(t) = 7.566 M_s^{-0.503}$ (SE = 0.046). Warwick (1984) proposed for both meio- and macrofauna an optimum weight. For the meiofauna it is approximately 2.56 μg . A nematode species having this weight realizes approximately 5 generations per year. A number significantly less than the mean number obtained in this study, but 1.6 times higher than Gerlach's number 3. However the present data indicate that some species realize far more cycles as has been assumed up to now. Nevertheless it still remains premature to speculate upon a modal value for the meiobenthic community, mainly because the error induced by extrapolating development rates from laboratory assays to the field is not known as yet.

c. Productivity

Somatic growth during the juvenile stage of the five species studied is exponential. Consequently weight specific growth during this period is constant and the juvenile P/B equals the growth rate G in the exponential growth equation: $W_t = W_0 e^{Gt}$, where W_t is fresh weight at time t, W_0 and G are constants obtained by linear least squares regression. The following juvenile P/B's are obtained :

species	T (°C)	Juvenile P/B (Joule. Joule ⁻¹ . day ⁻¹)
<i>Monhystera disjuncta</i>	12	0.21
<i>Diplolaimella</i> spec. 1	20	0.36
<i>Monhystrella parelegantula</i>	30	0.36
<i>Monhystera parva</i>	12	0.20
<i>Chromadora nudicapitata</i>	12	0.17

Fecundity is much higher than 20 eggs per female (Gerlach, 1971; Zaika and Makarova, 1979). In optimum conditions *Diplolaimella* sp. 1 produces approximately 300 eggs; *M. disjuncta* approximately 200 eggs; *M. parva* 170 eggs; *Monhystrella parelegantula* 50 eggs and *Chromadora nudicapitata* (Sluice Dock population) deposits more than 400 eggs during her life-time. Initial daily fecundity is constant in all species studied. With *Diplolaimella* sp. 1 and *M. disjuncta* daily fecundity is highly dependent on temperature. It ranges between 2.5 eggs per day at 15° C and 13.6 eggs per day at 30° C with *Diplolaimella* sp. 1 and between 2.7 and 9.2 eggs per day respectively at 3 and 7° C with *M. disjuncta*.

Daily weight specific productivity (calculated as the birth rate) is dependent on temperature. With *Diplolaimella* sp. 1 daily P/B is a linear function of temperature : $P/B \text{ (day}^{-1}\text{)} = -0.23 + 0.023T$ ($r^2 = 0.96$; $n = 4$). With *M. disjuncta* the daily P/B varies between 0.06 day⁻¹ at 3° C and 0.29 day⁻¹ at 17° C. Again it is a linear function of temperature : $P/B \text{ (day}^{-1}\text{)} = 0.006 + 0.016T$ ($r^2 = 0.98$; $n = 3$). The daily P/B of *C. nudicapitata* at 12° C equals 0.135 day⁻¹, that of *M. parva* is 0.16 day⁻¹ at 12° C and the daily P/B of *M. parelegantula* is 0.31 day⁻¹ at 30° C.

When the weight of hatchlings is considered as reproductive production

(Herman *et al.*, 1984), the adult P/B, calculated as the sum of the weight-specific reproductivity (sE) and the weight-specific somatic growth (sP), equals 0.20 day⁻¹ with *M. disjuncta* (12° C, 30 ‰ S); 0.21 day⁻¹ with *Diploelaimella* spec. 1 (20° C, 20 ‰ S); 0.23 day⁻¹ with *M. parva* (12° C, 30 ‰ S); 0.08 day⁻¹ with *Monhystrella parelegantula* (30° C, 30 ‰ S) and 0.24 day⁻¹ with *Chromadora nudicapitata* (12° C, 30 ‰ S). The adult weight-specific productivity is highly dependent on age, except with *M. disjuncta* where the individuals (at least in agnotobiotic cultures) stop growing once they have reached the adult stage. As a result of prolonged somatic growth in the adult stage, the adult P/B peaks immediately after the moment of maturing. Hereafter the adult P/B decreases strongly with ageing. Weight-specific reproductivity, situated on population level, is fairly constant and varies between 40 % with *Diploelaimella* spec. 1 and 70 % with the parthenogenetic *Monhystrella parelegantula* of the total productivity. When the negative production in the egg-stage is considered (fresh eggs are heavier than hatchlings) this reduces to 33 % in *Diploelaimella* spec. 1 and 46 % in *M. parelegantula*. The relationship between body weight at sexual maturity (M_s in kcal) and the annual P/B is highly significant ($r = -0.90$; $P < 0.01$; $n = 30$). The weight-dependency (model I regression) of the annual P/B is -0.44 (95 % CI = ± 0.08) a value not different from -0.37 as proposed by Banse and Mosher (1980). The weight-specific productivity of nematodes is much lower than in other invertebrates (0.05 versus 0.65 as calculated by Banse and Mosher, 1980). Nevertheless yearly P/B is much higher than 9 in the species studied. The yearly P/B is 22 for *Monhystrella parelegantula*, 27 for *Diploelaimella* spec. 1, 49 for *Chromadora nudicapitata*, 52 for *Monhystera parva* and 66 for *Monhystera disjuncta*.

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