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Effects of nutritional quality on the reproductive biology of *Archegozetes longisetosus* (Actinotrichida, Oribatida, Trhypochthoniidae)

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Abstract

The parthenogenetic trhypochthoniid oribatid mite *Archegozetes longisetosus* serves as a model organism. Numerous studies have investigated different aspects of its life history and nutritional biology, yet several results remain contradictive. To clarify effects of nutrition on life history parameters, we set up a large scale experiment with ten food resources of different origins and nutritional composition (animal, bacterial, fungal and herbal). Generally, food influenced all life history parameters. The number of offspring ranged from 0 to 106 individuals per female, while the developmental time and body mass varied in a range of 32 up to 88 days and 3 to 43 µg dry weight, respectively. The number of offspring per female was correlated to the C/N-ratio and thus the availability of nitrogen in the food, while the body mass was correlated to the C/P-ratio and C/Ca-ratio (for early juvenile instars). The developmental time did not respond to any measured nutritional parameter.

Keywords Nutrient ecology | life history | macroelements | mites | development

1. Introduction

Archegozetes longisetosus Aoki (1965) is among the most studied soil microarthropods, and certainly the best investigated oribatid mite species (Heethoff et al. 2013). It is found on continents and islands throughout the tropical regions of the world (Subias 2004) and seems to be a panphytophagous mite, mostly feeding on fungi, algae and decomposed litter (e.g., Beck 1967, Haq 1978, 1982). *Archegozetes longisetosus* predominantly occurs in anthropogenically disturbed habitats, e.g., organic trash, debris, compost or frequently timbered forest patches (see Heethoff et al. 2013). Such disturbed habitats differ from natural forests in their availability of biomass and macronutrient composition, e.g. more bacteria/animal remains and higher amounts of nitrogen (Borken et al. 2002, Bastida et al. 2008). This nutritional heterogeneity and potentially fast turnover of resources in its' preferred habitat may explain why *A. longisetosus* appears to be an opportunistic feeder in laboratory feeding experiments (Heidemann et al. 2011, Heethoff & Scheu 2016, Brückner et al. 2018a).

Published life history studies (e.g., Haq 1978, Haq & Adolph 1981, Honciuc 1996, Seniczak 1998, 2006, Seniczak et al. 2016) on *A. longisetosus*, which also discussed the direct or indirect effects of food, are conflicting. While Estrada-Venegas et al. (1999) claimed that food had no influence on the development of *A. longisetosus* individuals from a Mexican population, specimens of the well-defined laboratory strain *A. longisetosus* ran (Heethoff et al. 2007), showed considerable response of life history parameters, food processing and body size to different food (Seniczak 1998, Smrz & Norton 2004, Seniczak et al. 2016). Comparing algae, lichens and tree bark, Seniczak (1998) found an influence of food on the number of offspring (48–102

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individuals per female), developmental time (32–45 days), mortality (4–21 %), certain morphometrical features (e.g. body length: 884–1119 µg) and also sclerotization. According to this study (Seniczak 1998) higher amounts of total protein may cause a higher reproductive output, shorter developmental times and lower mortality. This, however, would be in contrast to the results of Seniczak et al. (2016), where napa cabbage (26.3 % total protein) always performed worse when compared to algae (15.5 % total protein). Hence, it is hitherto not possible to decipher the effects of nutritional composition of food on *A. longisetosus*.

To elucidate the influences of nutritional quality on the reproductive biology of *A. longisetosus* we setup a no-choice feeding experiment using ten different food resources from different origins (animal, bacterial, fungal, herbal; Tab. 1) and recorded life history parameters and body masses over a period of three months. We investigated i) the differences in life history parameters caused by different food along a complete ontogenetic sequence (from egg to adult), and ii) how macroelement composition (C, N, P, Ca) can help to understand these differences.

2. Materials and methods

2.1. Experimental setup

Archegozetes longesitosus ran (Heethoff et al. 2007) were reared at approx. 28°C and 80–85 % relative humidity in constant darkness on one out of ten resources for several generations (approx. 18 month). Detailed information about the ten resources of animal, bacteria, fungal and herbal origin can be found in Table 1. Fresh food and water was provided *ad libitum* three times a week. For each resource, specimens were cultured in three separate plastic boxes (100 × 100 × 50 mm) grounded with 2 cm mixture of plaster of Paris/activated charcoal mixture (9:1).

At the start of the experiment we randomly selected 25 specimens per resource from the original cultures (25 replicates × 10 resources = 250 experimental boxes in total), and individually placed them into small culture boxes (45 × 40 × 35 mm; grounded with the plaster of Paris mixture). The 250 mites could lay eggs for ten days (the adult individuals were removed then) and the same food and water was provided *ad libitum* three times a week. Every box was checked on a daily base and we counted the number all eggs, larvae, protonymphs, deutonymphs, tritonymphs and adults for a period of up to 12 weeks (new eclosed adults were removed to avoid new egg deposition). For each replicate we removed one individual per juvenile instar. These specimens and the initially used mothers were subsequently dried at 60°C until weight constancy (approx. 3 days) to determine their body mass (dry weight) with a microbalance (Mettler Toledo, XS3DU, Columbus, USA; with 0.1 µg). The body masses of dried eggs and larvae were always < 1 µg, thus we could not reliably determine their individual weight.

2.2. Analyses of carbon, nitrogen, phosphorus and calcium

For carbon (C) and nitrogen (N) analyses dried resource powders were weighed into tin capsules

Table 1. Summarizing table of the ten resources offered to *Archegozetes longisetosus* including food classificant, supplier and macronutrient composition. Higher ratios indicate a larger proportion of carbon (C = carbon) compared to the other elements (N = nitrogen, P = phosphorus, Ca = calcium).

classification		supplier	elemental analyses		
			C/N	C/P	C/Ca
animal	blood	Common Baits, Rosenfeld, Germany	3.3	541.6	866.6
	bone	Canina Pharma, Hamm, Germany	4.2	4.4	2.0
bacterial	spirulina	Interaquaristik, Biedenkopf, Germany	4.3	38.7	690.1
fungal	fungi	Arche Naturprodukte, Hilden, Germany	11.3	53.6	836.2
	yeast	Rapunzel Naturkost, Legau, Germany	7.5	51.1	417.9
herbal	chlorella	Naturya, Bath, UK	5.4	38.6	192.8
	hemp	Naturya, Bath, UK	5.6	26.2	264.1
	lupine	Govinda Natur, Neuhofen, Germany	7.0	79.1	305.8
	pollen	Ascopharm, Wernigerode, Germany	13.6	119.5	544.4
	wheat	Naturya, Bath, UK	12.4	183.9	176.3

(5±1 mg) and subsequently measured by an elemental analyzer (EA 1108 Elemental Analyzer, Carlo Erba, Milan, Italy). Acetanilide (Merck, Darmstadt, Germany) was used as an external standard. For phosphorus (P) and calcium (Ca) analyses 10 ± 1 g of the dried resource powders were digested using microwave-assisted pressure decomposition and subsequently measured via inductively coupled plasma atomic emission spectroscopy (ICP-AES). P and Ca analyses were performed by LUFA Nord-West (Oldenburg, Germany) according to DIN standard EN 15621:2012. C, N, P and Ca amounts were calculated based on external standards and the initial dry weight and expressed as C/N, C/P and C/Ca ratios.

2.3. Statistical analyses

The developmental time [days] for each mother's offspring was calculated as weighted arithmetic mean (developmental time= $\sum [d_i * p_i]$; where d_i is the day and p_i is the proportion of new adult specimens on d_i). The counted numbers [N] of eggs, larvae, proto-, deuto-, tritonymphs and adults, as well as the eclosion/hatching time [day] and the body mass [µg] across all resources were analyzed with Kruskal-Wallis tests (Kruskal & Wallis 1952). As post-hoc tests we used Mann-Whitney-U multiple pairwise comparisons (Mann & Whitney 1947). The differences in the number of eggs, larvae, proto-, deuto-, tritonymphs and adults within one resource were analyzed with Friedman tests (Friedman 1937) and pairwise Wilcoxon rank-sum tests (Wilcoxon 1945) to access differences between an instar pair. We used Spearman's rank coefficient (Spearman 1904) to explore whether the nutritional quality (expressed as C/N, C/P and C/Ca ratios) of the resources was correlated with the means of total number [N], eclosion time [day] or dry weight [µg]. Since only one individual reached the adult stage in the blood meal treatment, we had to exclude it from some analyses (but see results). Type I error accumulation for all analyses was corrected with the false discovery rate (Benjamini & Hochberg 1995). Additionally, we performed ordinary least squares regressions to analyse the i) increase in dry body mass [µg] from protonymph to adult and ii) the effects of mite density on body mass (see Seniczak 2006). Finally, we analysed if there is a trade-off between the total number of offspring [N] and the total development time [d] of a mothers offspring across the resources. To standardize both variables to a comparable scale we normalized both as for total number and for total development time and plotted means ± standard errors as xy-scatter. All statistics were performed with PAST 3.16 (Hammer et al. 2001) and R 3.3.2 (R Core Team 2016).

3. Results

3.1. Macroelement composition

The food offered to *Archegozetes longisetosus* differed in its macroelemental composition (Tab. 1). For example, while blood meal was extremely rich in organic N, it lacked P and Ca. Other foods like fungi, wheat and pollen had much less N, but comparatively more C, yet their proportion of P/Ca was very variable (Tab. 1).

3.2. Life history

The ten resources resulted in strong differences in the number of individuals (= offspring) across all instars of *A. longisetous* (Fig. 1A; Egg: $N = 219$, $df = 9$, $\chi^2 = 106.40$, $P < 0.0001$; Larva: $N = 217$, $df = 9$, $\chi^2 = 106.70$, $P < 0.0001$; Protonymph: $N = 196$, $df = 9$, $\chi^2 = 124.80$, $P < 0.0001$; Deutonymph: $N = 192$, $df = 9$, $\chi^2 = 129.50$, $P < 0.0001$; Tritonymph: $N = 183$, $df = 9$, $\chi^2 = 134.50$, $P < 0.0001$; Adult: $N = 173$, $df = 9$, $\chi^2 = 134.7$, $P < 0.0001$). Within each instar, blood meal (0.7 indviduals/female) and spirulina (5.6 indviduals/female) fed mites had the lowest number of offspring, while especially wheat grass (55.4 indviduals/female) animals had the highest number of offspring (see post-hoc comparisons Fig. 1A and Tab. 2 for detailed numbers). Similar to the offspring number, also eclosion/hatching times of the different instars across all food resources were significantly different (Fig. 1B; Larva: $N = 200$, $df = 8$, $\chi^2 = 41.48$, $P < 0.0001$; Protonymph: $N = 186$, $df = 8$, $\chi^2 = 38.42$, $P < 0.0001$; Deutonymph: $N = 185$, $df = 8$, $\chi^2 = 39.04$, $P < 0.0001$; Tritonymph: $N = 181$, $df = 8$, $\chi^2 = 49.29$, $P < 0.0001$; Adult: $N = 171$, $df = 8$, $\chi^2 = 41.13$, $P < 0.0001$). Mites fed with blood meal were excluded from these analyses because only one individual finally hatched to an adult and thus no statistical evaluation was possible (see also Tab. 2 for detailed numbers). Generally, the patterns of eclosion/hatching times were quite stable across all instars, just the initial period before eggs hatched was different (Fig. 1B, Tab. 2). Afterwards, the time pattern became quite similar (see post-hoc tests Fig. 1B), with hemp (18–49 days) and yeast (19–48 days) leading to the earliest hatching and bone (27–68 days), fungi (24–71 days) and spirulina (38–75 days) to the latest hatching times. As for the number of individuals, also the eclosion/hatching time of the other resources gradually differed from each other (Fig. 1 post-hoc results, Tab. 2). Also the number of eggs, larvae, proto-, deuto-, tritonymphs and adults within one resource differed, indicating some degree of mortality of the juvenile instars (Fig. 2; see also Friedman tests and Wilcoxon post-hoc results

in Tab. 2). For some food resources the mortality was relatively high (e.g., yeast ~50%, chlorella ~ 90% and fungi ~85%, Figure 2), while for others the offspring population was comparatively stable (e.g., wheat ~20% or lupine ~25%, Fig. 2). Also the dry weights (= body masses) of protonymphs (Fig. 3A, Tab. 3), deutonymphs (Fig. 3B, Tab. 3), tritonymphs (Fig. 3C, Tab. 3) and adults (Fig. 3D, Tab. 3) of *A. longisetosus* differed across all ten food resources (see Kruskal-Wallis tests in Tab. 3). While blood meal fed mites always were the lightest individuals (1.5–8.3 µg), specimens fed on yeast (8.3–24.5 µg) and on chlorella (6.8–28.1 µg) were the heaviest. Mites gained weight during their development, but the growth rate differed among resources (see slopes of body mass regressions in Tab. 3). Additionally, we found no relationship between mite density (Fig. 1, 2; Tab. 2) in the culture boxes and the offsprings' body mass (Fig. 3, Tab. 3) for any instar (OLS-regression: P_{Pro}= 0.16, P_{Deu}= 0.79, P_{Tri}= 0.96, P_{Adu}= 0.90).

3.3. Influence of nutrient quality on life history and reproductive traits

The numbers of individuals (= offspring) across all instars were always positively correlated to the C/N ratio in the food resources, but never to C/P or C/Ca (Tab. 4). The eclosion/hatching time was not correlated to any nutritional parameter, while the dry weights (= body masses) of the later instars of *A. longisetosus* (deuto-/

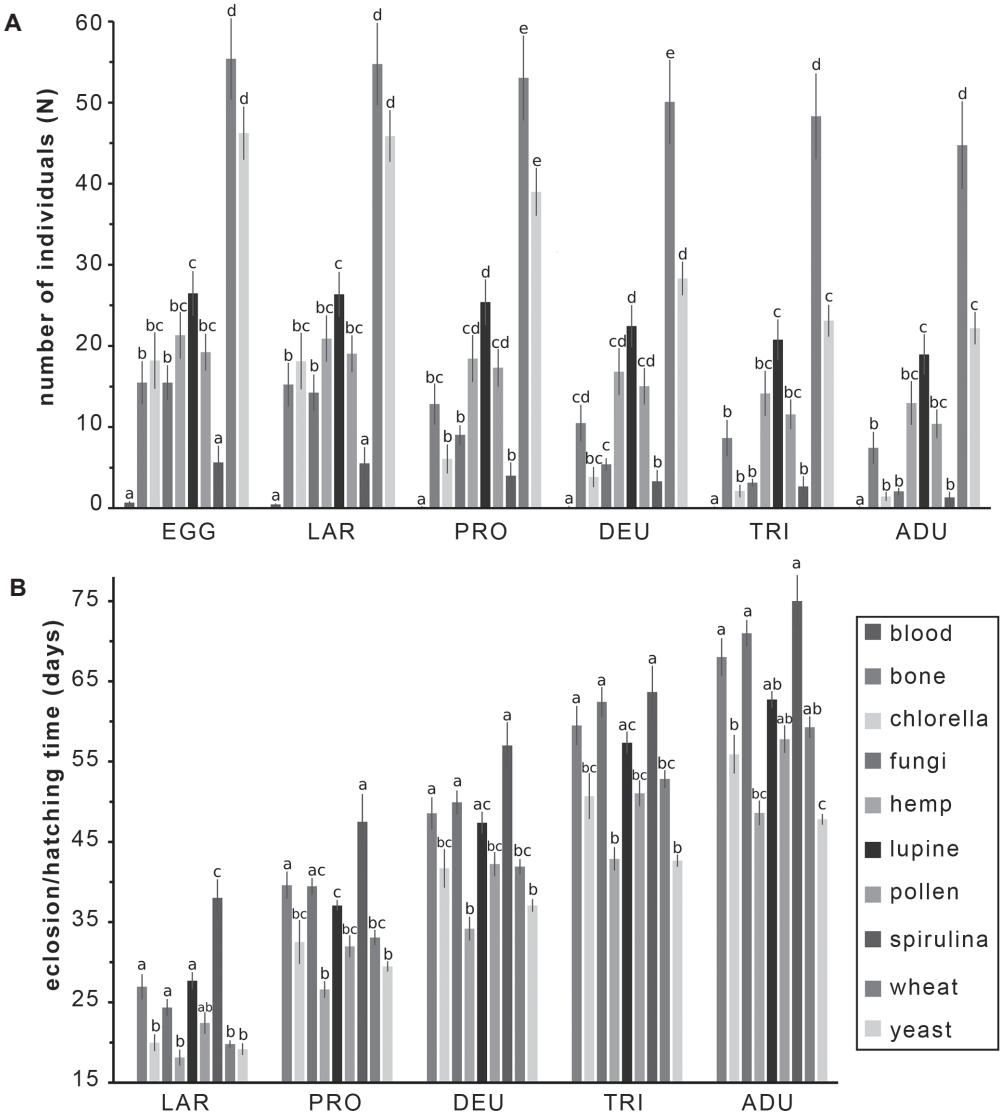

Figure 1. Number of individuals originated by one female (**A**) and eclosion/hatching time of her offspring, calculated as weighted mean (**B**) across the ten resources (see legend in **B**) and ontogenetic instars of *Archegozetes longisetosus*. Different letters indicate significant differences ($P < 0.05$) of pairwise Mann-Whitney-U tests within each instar. Bars represent means, error bars indicate standard errors. Blood meal was excluded in (**B**).

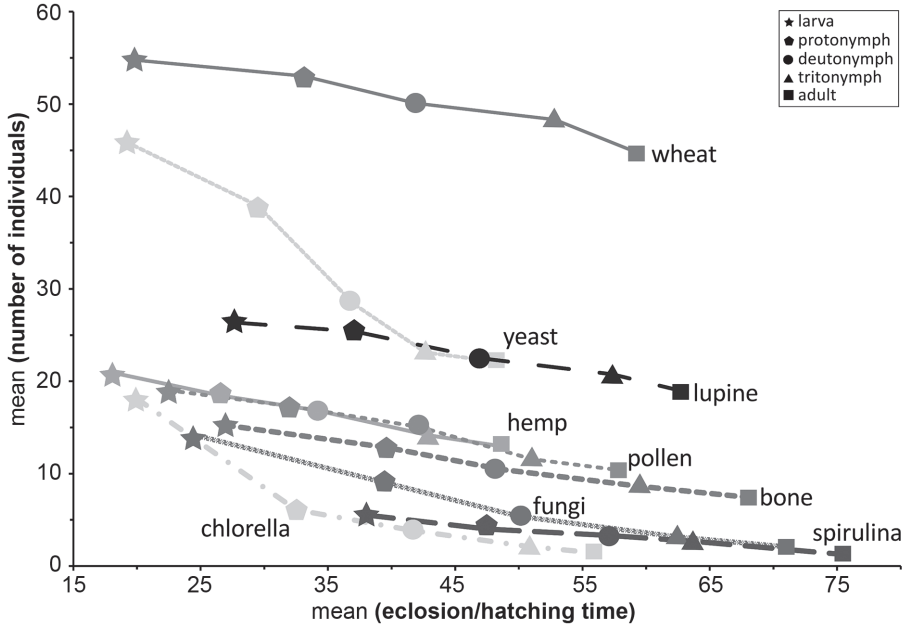

Figure 2. Survival curves of *Archegozetes longisetosus* cultured on nine different resources (blood meal was excluded) over a period of approximately 80 days.

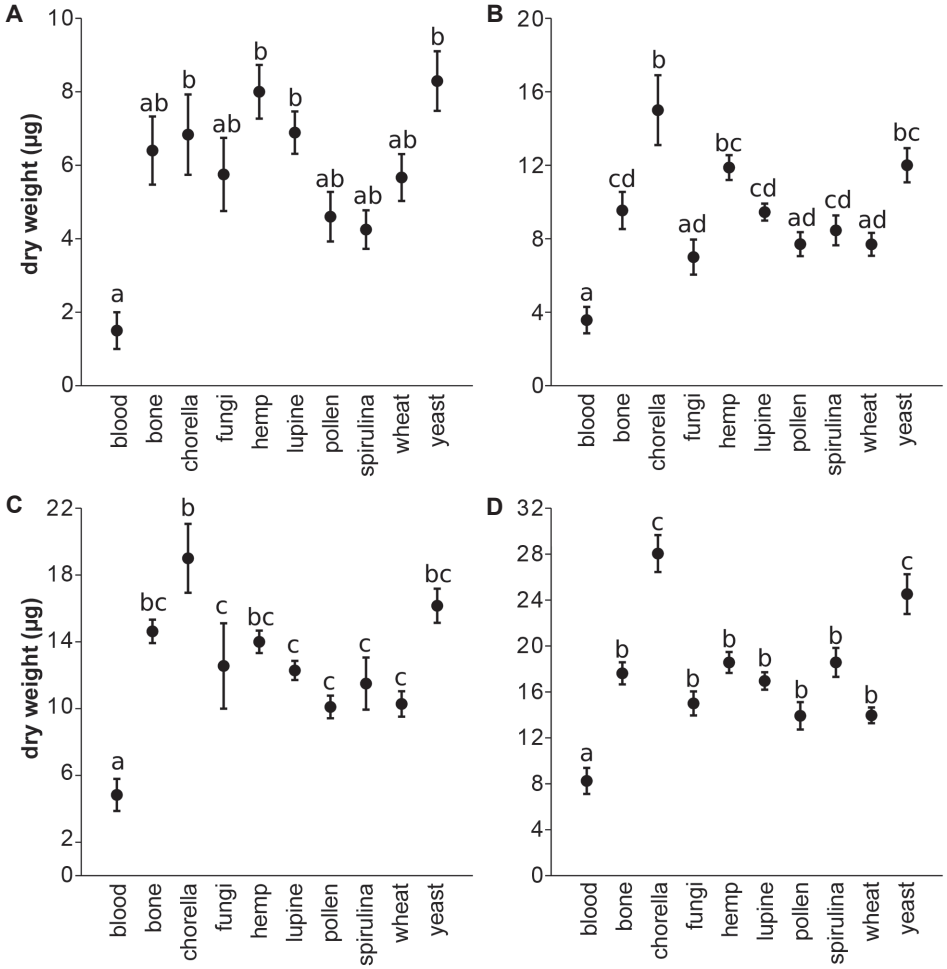

Figure 3. Mean dry weight of *Archegozetes longisetosus* proto- (**A**), deuto- (**B**), and tritonymphs (**C**), as well as adults (**D**), kept on different resources. Different letters indicate significant differences ($P < 0.05$) of pairwise Mann-Whitney-U tests within one ontogenetic instar. Circles represent means, error bars indicate standard errors.

Table 2. Life-history characteristics – the number of offspring per female (**A**) and the eclosion time (**B**) – of *Archegozetes longisetosus*. Reported numbers are means ± standard deviations. Friedman tests denote differences in the number of individuals within one food resource across all instars. Different letters indicate significant differences ($P < 0.05$) of pairwise Wilcoxon signed-rank tests across the instars of one resource (= post-hoc analyses of Friedman tests). *** = $P < 0.001$, NA = not applicable.

	egg	larva	protonymph	deutonymph	tritonymph	adult	
(A) NUMBERS							**Friedman test**
blood	0.68 ± 0.19	0.48 ± 0.11	0.16 ± 0.07	0.08 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	NA
bone	15.48 ± 2.64a	15.24 ± 2.68a	12.84 ± 2.53b	10.48 ± 2.27c	8.64 ± 2.24d	7.44 ± 1.96e	$\chi^2_{5,143}$= 89.69 ***
chlorella	18.20 ± 3.47a	18.12 ± 3.48a	6.08 ± 1.78b	3.84 ± 1.25c	2.12 ± 0.78d	1.44 ± 0.56e	$\chi^2_{5,143}$= 106.68 ***
fungi	15.48 ± 2.14a	14.24 ± 2.24b	9.04 ± 1.20c	5.40 ± 0.76d	3.16 ± 0.48e	2.08 ± 0.42f	$\chi^2_{5,149}$= 100.42 ***
hemp	21.32 ± 2.88a	20.92 ± 2.85a	18.44 ± 2.90b	16.84 ± 2.87c	14.16 ± 2.76d	12.96 ± 2.71e	$\chi^2_{5,143}$= 108.44 ***
lupine	26.48 ± 2.74a	26.36 ± 2.78a	25.40 ± 2.81b	22.44 ± 2.60c	20.76 ± 2.50d	18.96 ± 2.48e	$\chi^2_{5,149}$= 74.12 ***
pollen	19.24 ± 2.28a	19.04 ± 2.26a	17.32 ± 2.31b	15.04 ± 2.24c	11.56 ± 1.84d	10.40 ± 1.77e	$\chi^2_{5,143}$= 90.32 ***
spirulina	5.64 ± 2.06a	5.52 ± 2.02a	4.00 ± 1.63b	3.32 ± 1.37c	2.68 ± 1.27d	1.32 ± 0.68e	$\chi^2_{5,65}$ = 34.88 ***
wheat	55.40 ± 5.00a	54.76 ± 5.06a	53.04 ± 5.23a	50.08 ± 5.19b	48.32 ± 5.30c	44.76 ± 5.41d	$\chi^2_{5,149}$= 59.49 ***
yeast	46.24 ± 3.27a	45.88 ± 3.18a	39.00 ± 2.95b	28.32 ± 2.07c	23.12 ± 1.97d	22.20 ± 2.01d	$\chi^2_{5,149}$= 121.67 ***
(B) ECLOSION TIME							
blood	-	20.93 ± 3.48	24.46 ± 4.00	26.00 ± 6.48	27.33 ± 4.24	31.00 ± 0.00	
bone	-	26.95 ± 1.58	39.60 ± 1.71	48.56 ± 2.00	59.51 ± 2.45	68.03 ± 2.35	
chlorella	-	19.99 ± 1.05	32.52 ± 2.73	41.69 ± 2.40	50.70 ± 2.85	55.91 ± 2.41	
fungi	-	24.36 ± 1.04	39.47 ± 1.02	49.92 ± 1.47	62.44 ± 1.85	71.00 ± 1.62	
hemp	-	18.13 ± 0.99	26.61 ± 1.04	34.19 ± 1.47	42.88 ± 1.46	48.61 ± 1.54	
lupine	-	27.69 ± 1.07	37.07 ± 0.69	47.38 ± 1.39	57.35 ± 1.39	62.74 ± 1.05	
pollen	-	22.42 ± 1.37	31.97 ± 1.38	42.24 ± 1.50	51.06 ± 1.62	57.80 ± 1.72	
spirulina	-	38.03 ± 2.30	47.51 ± 3.44	57.01 ± 2.87	63.66 ± 3.29	75.01 ± 3.24	
wheat	-	19.82 ± 0.46	33.08 ± 0.95	41.91 ± 0.93	52.83 ± 1.11	59.28 ± 1.34	
yeast	-	19.17 ± 0.73	29.48 ± 0.63	37.07 ± 0.80	42.66 ± 0.76	47.81 ± 0.68	

Table 3. Body mass of ontogenetic instars of *Archegozetes longisetosus*. Note that eggs and larvae were too small for individual weighing. Kruskal-Wallis tests denote differences among food resources within instars, while the body mass regression describe the growth of *A. longiseotsus* within one resource across the series of ontogenetic instars. Reported numbers are means ± standard deviations. Significant P-values are in **bold**; *** = P < 0.001, ** = P ≤ 0.01.

	protonymph	deutonymph	tritonymph	adult	body mass regression
blood	1.50 ± 0.50	3.57 ± 1.76	4.83 ± 3.18	8.25 ± 3.77	*slope*: 2.15, r^2 = 0.96, p = **0.018**
bone	6.40 ± 1.85	9.54 ± 3.50	14.63 ± 2.71	17.62 ± 4.29	*slope*: 3.88, r^2 = 0.99, p = **0.005**
chlorella	6.83 ± 3.62	15 ± 5.02	19.00 ± 6.84	28.05 ± 7.01	*slope*: 6.77, r^2 = 0.98, p = **0.009**
fungi	5.75 ± 2.63	7.00 ± 3.31	12.56 ± 7.23	15.00 ± 4.44	*slope*: 3.33, r^2 = 0.94, p = **0.027**
hemp	8.00 ± 2.19	11.88 ± 2.69	14.00 ± 2.77	18.57 ± 4.28	*slope*: 3.38, r^2 = 0.98, p = **0.009**
lupine	6.89 ± 2.38	9.45 ± 2.01	12.29 ± 2.57	16.95 ± 3.48	*slope*: 3.30, r^2 = 0.97, p = **0.011**
pollen	4.60 ± 2.52	7.71 ± 2.61	10.10 ± 2.95	13.92 ± 5.84	*slope*: 3.04, r^2 = 0.99, p = **0.004**
spirulina	4.25 ± 1.74	8.45 ± 2.57	11.50 ± 4.12	18.57 ± 4.55	*slope*: 4.60, r^2 = 0.97, p = **0.016**
wheat	5.67 ± 2.85	7.70 ± 2.93	10.28 ± 3.73	13.96 ± 3.35	*slope*: 2.75, r^2 = 0.98, p = **0.009**
yeast	8.29 ± 3.89	12.00 ± 4.55	16.16 ± 5.00	24.52 ± 8.49	*slope*: 5.29, r^2 = 0.95, p = **0.021**
	$\chi^2_{9,126}$ = 26.84 **	$\chi^2_{9,152}$ = 52.91 ***	$\chi^2_{9,156}$ = 61.38 ***	$\chi^2_{9,196}$ = 90.08 ***	

Table 4. Correlative relationships (ρS) of the nutritional composition (C/N, C/P, C/Ca) to the number of offspring, the eclosion times and dry weights of *Archegozetes longisetosus*. Significant P-values after false-discovery rate correction are denoted in **bold**.

	C/N	C/P	C/Ca
TOTAL NUMBER			
eggs	0.67, p = **0.032**	0.10, p = 0.79	-0.52, p = 0.13
larva	0.64, p = **0.047**	0.07, p = 0.85	-0.56, p = 0.09
protonymph	0.66, p = **0.038**	0.07, p = 0.82	-0.52, p = 0.13
deutonymph	0.66, p = **0.038**	0.08, p = 0.83	-0.51, p = 0.12
tritonymph	0.65, p = **0.043**	0.09, p = 0.80	-0.45, p = 0.19
adult	0.66, p = **0.037**	0.08, p = 0.83	-0.52, p = 0.13
ECLOSION TIME			
larva	-0.35, p = 0.36	0.02, p = 0.95	0.28, p = 0.46
protonymph	-0.42, p = 0.25	0.07, p = 0.84	0.12, p = 0.74
deutonymph	-0.20, p = 0.58	0.08, p = 0.81	0.40, p = 0.27
tritonymph	-0.25, p = 0.52	0.05, p = 0.88	0.28, p = 0.42
adult	-0.25, p = 0.51	0.05, p = 0.87	0.28, p = 0.46
DRY WEIGHT			
protonymph	0.16, p = 0.65	-0.53, p = 0.12	-0.65, p = **0.043**
deutonymph	-0.13, p = 0.73	-0.75, p = **0.013**	-0.63, p = **0.047**
tritonymph	-0.12, p = 0.72	-0.79, p = **0.006**	-0.59, p = 0.072
adult	-0.22, p = 0.53	-0.77, p = **0.009**	-0.36, p = 0.31

tritonymph and adults) were negatively correlated to C/P of the food (Tab. 4). Furthermore, the body masses of the earlier instars (proto-/deutonymphs) were negatively correlated to the C/Ca ratios (Tab. 4).

3.4. Reproductive output and developmental time

To investigate a possible trade-off between the number of offspring (= reproductive output) and the day until eclosion (= developmental time), we needed to transform both variables to project them on the same scale (see materials and methods). Both parameters were normalized as N' (output) and d' (speed). Thus, N' equals one for the sample replicate with the highest number of offspring and zero for the lowest number of offspring; reciprocally, d' equals one for the lowest developmental time and consequently lower d' represent faster development. Generally, we found no obvious trade-off between reproductive output and time (Fig. 4). *A. longisetosus* specimens fed on wheat grass had the highest N', but only an intermediate d'; on the other hand hemp and yeast cultured mites had low d'-values, yet only an intermediate-low reproductive output. The number of offspring N' in chlorella, fungi and spirulina treatments was very low, and the latter two also had the longest development d'.

4. Discussion

Overall, nutrient composition had an effect on the life history and reproductive biology of *A. longisetosus* across all instars (Fig. 1 and 2; Tab. 1–3), thus supporting the results of previous studies by Seniczak (1998) and Seniczak et al. (2016). Surprisingly, Estrada-Venegas et al. (1999) observed no effects of diet on life history of *A. longisetosus*, although dietary altered life histories are common among animals (e.g., Rushton & Hassall 1983, Boggs 1992, Elser et al. 1996, Jensen et al. 2011). Furthermore, several studies (Haq & Prabhoo 1977, Haq & Adolph 1981), suggested that *A. longisetosus* mainly feds on decomposed leaves, moss and certain soil fungi, but reject algae, yeast, lichens, seeds, vegetative tissue/bark of higher plants as well as food of animal origin in laboratory assays. Instead, our and others results (e.g., Honciuc 1996, Seniczak 1998, Smrz & Norton 2004, Seniczak 2006, Heethoff et al. 2007, Heidemann et al. 2011), clearly

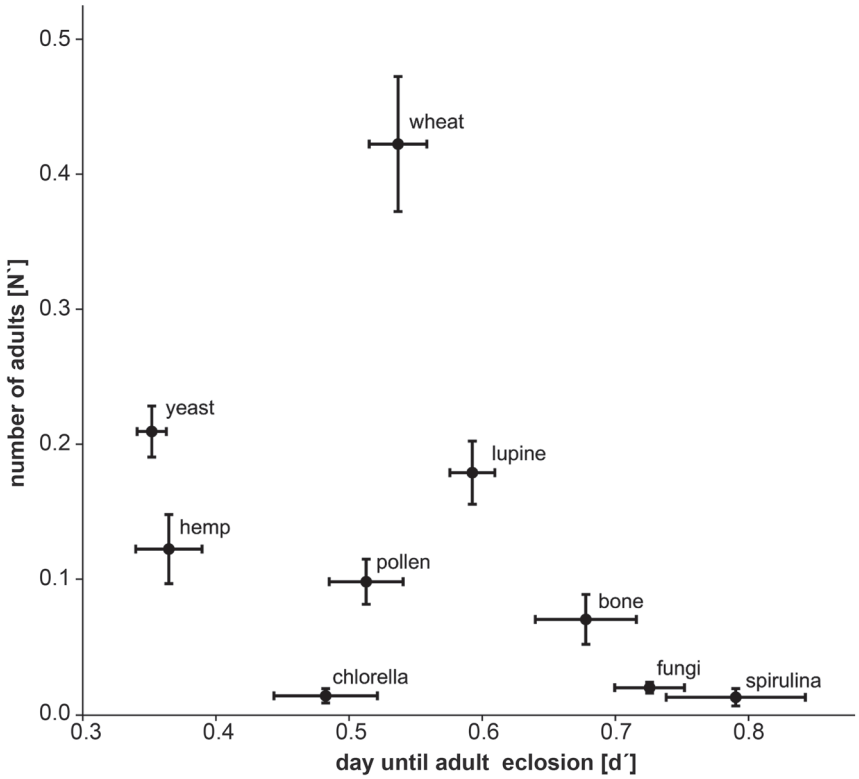

Figure 4. Comparison of the normalized number of adults (N'= reproductive output) and day until adult eclosion (d'= developmental time) of *Archegozetes longisetosus*. Higher N' and d' indicate more reproductive output and longer developmental time, respectively. Circles represent means, error bars indicate standard errors in both directions. Blood meals was excluded from this analysis.

demonstrate that *A. longisetosus* feeds and survives on various resources (Tab. 2) from different origins (animal, bacterial, fungal, herbal; Tab. 1). Hence, *A. longisetosus* may be classified as a broad opportunistic feeder (see also Heethoff & Scheu 2016), yet certain preferences for distinct resources, possibly related to olfactory signals of the food seem to be an innate characteristic of the mite (Brückner et al. 2018a, Brückner et al. 2018b). For instance, *A. longisetosus* appears to prefer food resources with a high amount of fat and thus uses fatty acids as a potential cue to find its food (Brückner et al. 2018a). We could demonstrate an adaptive value of this resource choice for chemical defense (i.e. food with high calories results in a faster regeneration of defensive secretions; Brückner & Heethoff 2018). However, there seems to be no connection of olfactory based food selection and life history, because *A. longisetosus* e.g. preferred lupine powder over wheat grass (Brückner et al. 2018b; this issue). Although *A. longisetosus* survived on every food we offered, there was a tremendous difference in all life history and reproductive traits across the resources. While some food led to high numbers of offspring (e.g. wheat, yeast, lupine, hemp or pollen), others just enabled the mites to survive (e.g., spirulina, chlorella and fungi) or were not able to foster stable reproduction (blood meal). The eclosion times and thus the developmental speed of the instars were strongly influenced by food, showing differences of about three (juvenile instars) to four (adults) weeks among the resources. This mechanism has also been studied in other arthropods groups (e.g., Barbosa & Capinera 1977, Gebhardt & Stearns 1988, Jiménez-Cortés et al. 2012), yet mostly the effects of diet are less pronounced compared to *A. longisetosus*. The strong influence of diet on body mass was expected, since animals generally show altered growth of their body (and fat storage) in response to dietary changes and composition (Case 1979, Demment & Van Soest 1985, Robinson & Redford 1986). While Seniczak (2006) found a density dependency of *A. longisetosus'* body size (smaller individuals at higher densities), we could not confirm this relationship in our experiments. In fact, we found that food indeed induced different densities (Fig. 1A), but the body mass never increased or decreased with lower or higher animal density across all investigated instars. The mites in our experiments had an unlimited access to food ('*ad libitum* conditions') and were hence not influenced by any nutritional shortages. Limited resource access, on the other hand, can lead to limited growth in animals as well as plants (e.g., Cohen 1971, Kozłowski 1992, Heino & Kaitala 1999) and might explain the effects observed in *A. longisetosus*. Since Seniczak (2006) did not clearly state whether the mites were cultured under *ad libitum* conditions or not, it is not possible to confirm or reject this idea.

In general, the number of offspring per female, as well as the size, but not the developmental time were influenced by macronutrients (C/N-, C/P- and C/Ca-ratios; Tab. 4). The correlation of the C/N-ratio – a proxy for nitrogen containing substances like amino acids – to the number of offspring of *A. longisetosus* is conclusive, because the availability of these substances is a prime regulator of growth and reproductive output in animals (e.g., Zanotto et al. 1993, Behmer et al. 2002, Fagan et al. 2002, Lee et al. 2003). As indicated by our data (Tab. 1 and 2), however, too high amounts of nitrogen (as indicated by a low C/N-ratio) as well as too low nitrogen (as indicated by a high C/N-ratio) are not optimal for high numbers of offspring. Hence, it seems evident that nitrogen requirements in *A. longisetosus* are not linear, but rather have an optimum – a phenomenon also quite common in the nutritional biology of other animals (e.g., Davis 1975, Ramsay & Houston 2003, Raubenheimer et al. 2005). A nitrogen optimum could also explain the results of Seniczak (1998) and Seniczak et al. (2016): tree bark and lichen protein contents were too low to yield in substantial offspring, *Protococcus* algae had an optimal amount of protein and could sustain the most offspring, while the protein content in napa cabbage appeared to be too high, yielding in a lower number of offspring again. As stated before, nutritional optima are quite common and can be explained by Bertrand's rule (Mertz 1981): at a low level of nutrients the benefits gained by more nutrients increase until a phase of equilibrium (= optimum), nutrients beyond are associated with increasing costs for the regulatory mechanisms yielding in disadvantage which are higher than the benefits gained by higher nutrient levels. While originally proposed for micronutrients, Raubenheimer et al. (2005) have shown that this principle also applies to macronutrients like carbohydrates, protein and P-content. Correspondingly, the same principle may not only apply to the C/N-ratio, but also to the correlation of C/P-ratio and body mass in *A. longisetosus*. However, the P-contents in the food resources we used were unevenly distributed and thus a potential P-optimum remains to be elucidated. Yet, we found that lower C/P-ratios (= higher P) had a positive effect on body mass; probably because nucleic acid and even more importantly ATP – the energy fueling the growth of all organisms – contains phosphorus (e.g., Call et al. 1978, Klausmeier et al. 2004). Interestingly, C/Ca-ratios were correlated to the body mass of earlier juvenile instars, which suggests that higher amounts of Ca are somehow important for the earlier life stages of *A. longisetosus*. Calcium-depended biomineralization (Norton & Behan-Pelletier 1991) would explain this pattern, however since *A. longisetosus* lacks this form of cuticular modification (Pachl et al. 2012), the positive influence of Ca on earlier instars remains unexplained. Developmental times in arthropods not only depend

on available nitrogen (Nestel et al. 2003), but also appear to be heavily influenced by the available energy of a food resource – stored as fat or carbohydrates (Florkin & Scheer 1970, Canavoso et al. 2001, Arrese & Soulages 2010). In invertebrates, a trade-off between the number and developmental time of offspring is common (Simmons 1987, Nunney 1996, Olofsson et al. 2009), however, we did not find that for *A. longisetosus* (Fig. 4). Instead, we found that certain resources (especially hemp, wheat, yeast) were able to sustain both – a relatively high reproductive output with a moderately long development time. This lack of constraints together with previously discussed results suggest that *A. longisetosus* requires resources of a distinct nutritional spectrum to sustainably stabilize its life history and reproductive energy budget. Thus, some resources – in our study for example blood meal or spirulina – may have a too imbalanced nutritional composition, yielding low reproductive rates, long developmental times and smaller offspring.

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6. Authors' contributions

AB and MH designed the research; AB, RS and KW performed the experiment, AB performed chemical analyses; AB analyzed the data; AB and MH wrote the paper. All authors discussed and approved the final manuscript.

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