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ORIGINAL PAPER

Changes in the population structure of the lineage 'Nevada' belonging to the *Brachionus plicatilis* species complex, batch-cultured under different feeding regimes

Venetia Kostopoulou · Helen Miliou · George Katis · George Verriopoulos

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Abstract Two experiments were conducted examining the population structure of *Brachionus* 'Nevada' under feeding conditions commonly applied in hatcheries, using 4-day rotifer batch cultures. In the first experiment two diets were supplied: yeast with *Tetraselmis suecica* (treatment A) or Culture Selco® with *T. suecica* (treatment B). The second experiment (treatments C, D, E) differed in the phytoplankton quantity used (20 times higher): treatment C was analogous to A and treatments D and E to B. Initial rotifer density differed among treatments and was about 200 individuals ml^{-1} in A, C and E, and 60 individuals ml^{-1} in B and D. Multivariate analysis discriminated A and C from B, D and E. In treatments A and C, a 24-h cycle in ovigerous females, immature individuals and E/F ratio was observed, showing a high reproductive rate. Treatments B, D and E displayed a 48-h cycle in the aforementioned parameters, indicating a lower reproductive rate. The latter treatments had a significantly higher number of females with multiple eggs for most of the samplings, compared to A and C, except for treatment E until 40 h of sampling. Specific growth rate was significantly higher in treatments B and D (Culture Selco® diet) compared to A and C (yeast diet), while treatment E had intermediate rates. Initial rotifer density influenced the abundance of females with multiple eggs, but resulted in slight variations in growth rate and population structure. The type of dry food greatly affected the population structure of rotifers, leading to significant differences in the growth rate.

Keywords *Brachionus* · Feeding regime · Population density · Population structure · Rotifer

V. Kostopoulou (✉) · G. Katis · G. Verriopoulos
Department of Zoology-Marine Biology, Faculty of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 157 84, Greece
e-mail: vkostop@biol.uoa.gr

H. Miliou
Laboratory of Applied Hydrobiology, Faculty of Animal Production, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece

Abbreviations

ANOVA	Analysis of variance
E/F ratio	Egg female ratio
ppt	Parts per thousand
r	Intrinsic growth rate
SEM	Standard error of grand mean

Introduction

The rotifer, *Brachionus plicatilis* O.F. Müller 1786 (Rotifera), is commonly used as food for fish larvae during the initial period of their life (Lubzens et al. 2001). The mass culture of high quality rotifers is a critical factor in fish larval production (Dhert et al. 2001; Lubzens et al. 2001). For this reason, a lot of research focused in defining the conditions that enhance the growth of rotifers through the study of population dynamics (Theilacker and McMaster 1971; Hirayama et al. 1979; Gatesoupe and Luquet 1981; James et al. 1983). Subsequently, new ways were devised towards the improvement of mass culture production efficiency, namely high density cultures (Yoshimura et al. 1996; Suantika et al. 2000), use of rotifer cysts (Hagiwara 1994), condensed phytoplankton diets (Navarro and Yúfera 1998) and application of probionts (Hirata et al. 1998; Rombaut et al. 1999). All aforementioned studies quantify effects at the population level, using mainly the intrinsic rate of increase as a measure of fitness.

Such a measure represents population dynamics but does not provide information about the individual's life history characteristics. That is, growth, maturation, reproduction and survivorship, which are processes that describe the phases of an animal's life cycle. A lot of researchers have investigated life history parameters of rotifers, using mainly individual cultures to test the effects of temperature, salinity, phytoplankton quantity and quality, starvation, and preconditioning of the medium (Korstad et al. 1989; Miracle and Serra 1989; Schmid-Araya 1991; Cabrera et al. 1993; Serra et al. 1994; Yoshinaga et al. 1999, 2000, 2003). One way to combine both types of studies-growth rate at the population level and life history at the individual level—is to try to incorporate a more detailed analysis at the population level. The analysis of population structure would provide such an insight of the processes and mechanisms of population change. We are aware of one such study (Yoshinaga et al. 2001), that has examined changes in population structure during growth of phytoplankton-fed rotifers.

In the present study, the population structure of rotifer batch cultures was examined, under conditions routinely used in a Greek hatchery (Octapus S.A.). Notwithstanding the high-technology systems that have been developed (mentioned earlier), European hatcheries still rear rotifers in the simplest and less complicated batch culture system. On such a commercial scale, very few modifications or improvements have been realized and problems still arise relating to the constant production of rotifers. To overcome this, hatcheries exchange rotifer cultures (Dhert et al. 2001). This can prove problematic owing to the rapidly changing taxonomic status of *B. plicatilis*: this species is actually a complex of at least nine genetically divergent lineages (Gómez et al. 2002), difficult to differentiate morphologically (Hagiwara et al. 2001; Campillo et al. 2005; Kotani et al. 2005). The result is an unknown species composition in the mass cultures.

The mass culture of rotifers in hatcheries is constrained by time and cost. In that respect, two types of feeding regimes have been established; baker's yeast and microparticulate diets. The former is cheaper and less labor intensive but poor in nutritional quality (Hirayama and Funamoto 1983; Moretti et al. 1999). The microparticulate diets are nutritionally boosted (Dhert et al. 2001 for Culture Selco®), but deteriorate the quality of the culture medium (Coutteau and Sorgeloos 1997). In order to overcome the aforementioned constraints, an adequate quantity of phytoplankton is combined with yeast and a lesser one with microparticulate diets, for improving the nutritional value of the diet (Hirayama and Watanabe 1973; Hirayama and Funamoto 1983; Hirayama 1987; Lubzens et al. 1989; Lie et al. 1997) and the quality of the culture medium (Øie et al. 1994; Dhert et al. 2001), respectively. The feeding regimes used here combine phytoplankton with either baker's yeast or Culture Selco®. Due to the different amount of phytoplankton added in the two diets—the starting population of rotifers being arithmetically equal—differences ensued in the resultant initial rotifer densities. A laboratory experiment was also conducted, in order to investigate the effect of initial rotifer density and phytoplankton quantity on population structure.

The objective of our research was to evaluate possible changes in the reproductive strategy of rotifers under routine hatchery feeding regimes, and to examine which of the factors under study influenced the population structure of rotifers in batch culture.

Materials and methods

Materials

The strain (isolate K with lorica length of ovigerous female (mean ± standard error): 238.5 ± 0.6 μm; $n = 385$) used in the present study was identified on the basis of mitochondrial gene COI sequencing and was named according to the nomenclature in Gómez et al. (2002). It belongs to *B.* 'Nevada' (GenBank accession number AM180752), a newly described lineage of the *B. plicatilis* species complex. B. 'Nevada' has been grouped in the *B. plicatilis* s.s. clade (Gómez et al. 2002). Its presence has been confirmed in several hatcheries around Europe (Papakostas et al. in press).

The feeding regime included the microalga *Tetraselmis suecica* (LB 2286), the microparticulate compound diet Culture Selco® (INVE N.V., Belgium), and baker's yeast *Saccharomyces cerevisiae*. *T. suecica* was batch cultured in the medium described by Walne (1966) and modified by Laing (1991). All cultures were performed at 25°C and 35 ppt salinity under constant illumination and aeration (Moretti et al. 1999). The culture medium consisted of diluted seawater that was UV-treated or filtered (Whatman GF/C filter) and autoclaved (121°C, 10 min), depending on the volume of the experimental culture.

A pre-experimental culture of a parthenogenetic population of *B.* 'Nevada' was maintained under these experimental conditions for several generations, using *T. suecica* as food.

Experimental set up

Two experiments were performed using different culture volumes; the first experiment (Experiment 1) was conducted in polyester tanks of 2.5 m^3 capacity, and the second (Experiment 2) in 500 ml glass conical flasks. The working volumes can be seen in Table 1. In both experiments, the rotifers were batch cultured for a total duration of 4 days

Table 1 Management of rotifer cultures with respect to the diet offered in the five experimental treatments (Diet YP: yeast and *T. suecica*, Diet CSP: Culture Selco® and *T. suecica*)

	Treatment				
	A	B	C	D	E
Experiment	1	1	2	2	2
Diet	YP	CSP	YP	CSP	CSP
T. suecica cells (rotifer^{-1} day^{-1}×10^2)	1	0.2	20	4	4
Initial volume	0.5 m^3	1.7 m^3	50 ml	170 ml	50 ml
Final volume	2.0 m^3	2.3 m^3	200 ml	230 ml	200 ml
Volume of *T. suecica* added daily	0.5 m^3	0.2 m^3	50 ml	20 ml	50 ml
Initial rotifer density (ind ml^{-1})	200	60	200	60	200
Initial rotifer total quantity	10^8	10^8	10^4	10^4	10^4

(96 h). In tanks (Experiment 1), the initial rotifer population was approximately 100 million rotifers, while in flasks (Experiment 2), it was 10,000 rotifers (Table 1). Food consisted of either yeast and phytoplankton (*Tetraselmis suecica*) (diet YP) or Culture Selco® and phytoplankton (*T. suecica*) (diet CSP).

The first experiment included two treatments, A and B (three replicates each): populations A were fed on diet YP, and populations B on diet CSP (Table 1). The second experiment consisted of three treatments, C, D and E (three replicates each). Populations C and D were analogous to populations A and B respectively, in terms of diet type and initial rotifer density. In the case of populations E, the diet type of treatment D was combined with the initial rotifer density of treatment C. The daily amount of phytoplankton per rotifer in the second experiment was set to be twenty times higher compared to the first.

The starting culture volume was higher in treatments B and D, resulting in a lower initial rotifer density (app. 60 ind ml^{-1}), compared to the other treatments (app. 200 ind ml^{-1}-Table 1). The phytoplankton volume added daily to treatments A, C and E was 2.5 times higher compared to treatments B and D. The quantity of phytoplankton offered daily was adjusted according to the rotifer density of that particular day, resulting in a constant feeding ratio per rotifer. Phytoplankton was added every day between 10:00 and 12:00, while yeast or Culture Selco® were added in five equal fractions at 13:00, 15:00, 17:00, 22:00, 2:00 and 5:00. The mean daily amount of yeast and Culture Selco® were adjusted according to rotifer density and were based on the instructions given by Moretti et al. (1999) and Suantika et al. (2000).

All animals used in the study reproduced asexually; no sexual (mictic) females or males were observed.

Data collection

In the first experiment, rotifer samples for analysis (10 ml) were harvested from the mass cultures. Pre-experimental trials showed that the distribution of rotifers in the culture tanks was homogeneous. In the second experiment, a sample of 2 ml was taken.

The sampling of rotifers started on day 0 at 16:00 (‘0 h’) and ended on day 4 at 8:00 (‘88 h’). The intermediate samples were collected twice daily and were classified as follows: day 1 morning (‘16 h’), day 1 afternoon (‘24 h’), day 2 morning (‘40 h’), day 2 afternoon (‘48 h’), day 3 morning (‘64 h’), day 3 afternoon (‘72 h’). The rotifers were preserved in 4% buffered formalin. Phytoplankton culture density was determined daily, using a Neubauer haemocytometer, and diluted so that the added volume provides a

constant feeding ratio per rotifer, based on the latter's morning density measurement (Table 3).

Water quality parameters of cultures were monitored daily. Total ammonia, nitrates and nitrites were measured with a HACH spectrophotometer (model DR-2400). Un-ionized ammonia was calculated from total ammonia, temperature, pH and salinity values according to Bower and Bidwell (1978). The values (mean ± standard error) of pH, dissolved oxygen, total ammonia, un-ionized ammonia, nitrates and nitrites were almost constant throughout the experimental period (pH = 7.40±0.02, DO = 6.9±0.6 mg l^{-1}, NH_4^+ = 0.52±0.05 mg l^{-1}, NH_3 = 6±0.6× 10^{-3} mg l^{-1}, NO_3^- = 5.12±0.26 mg l^{-1}, NO_2^- = 0.32±0.03 mg l^{-1}).

Data analysis

To analyze the population structure, the preserved rotifer samples were inspected under an inverted microscope (ZEISS) and the rotifers were classified as: immature females, non-ovigerous females, females with one egg, females with multiple eggs, females with sac (i.e. external membrane of the egg after hatching) and post-reproductive females. The number of loose eggs was also recorded. The abundance (%) of each life cycle phase was estimated as its density divided by the total density and multiplied by 100.

The intrinsic growth rate (r) of rotifer populations was calculated as follows:

$$r = (\ln Q_n - \ln Q_{day0})/t$$

where $\ln Q_n$ is the natural log of the total quantity of rotifers on day n (Table 2), $\ln Q_{day0}$ is the natural log of the total quantity of rotifers on day 0, t is time in days.

The egg female ratio (E/F) was estimated as:

$$\text{E/F} = \frac{\text{Number of loose eggs} + \text{Number of eggs produced by ovigerous females}}{\text{Number of non-ovigerous and ovigerous females}}$$

Population structure was analyzed using the multivariate technique of Cluster analysis based on the Bray–Curtis dissimilarity index, after square root transformation of data. The dendrograms were formed with the grouping average method, and Anosim was used for testing the significance of grouping. In order to determine discriminating life cycle phases, the Simper analysis was performed (Primer 5) (Clarke and Warwick 1994). In addition, the multivariate technique of Correspondence Analysis was applied (Statistica 5.1).

One- and two-way analyses of variance (ANOVA) were performed on square root transformed life cycle data (%) and untransformed growth rate and E/F ratio values.

Table 2 Mean values of rotifer total quantity for all treatments at each sampling time

Day	Sampling time (h)	Treatment				
		A ($\times 10^3$)	B ($\times 10^3$)	C	D	E
1	16	124,450	130,520	14,075	9803	8880
1	24	135,950	146,540	18,137	11,864	14,335
2	40	138,400	165,850	16,383	14,825	14,295
2	48	169,690	225,530	15,187	28,175	17,340
3	64	186,940	242,020	21,025	28,175	20,025
3	72	212,500	255,110	21,500	29,542	20,720
4	88	211,200	323,720	23,422	42,665	29,440

Two-way ANOVA was used to identify the main effects and interaction of factors; treatment and sampling time. One-way ANOVA was applied to log transformed density data. Comparison of means was conducted with LSD multiple range test (Statgraphics plus 4). Differences were considered significant at $P < 0.05$. Untransformed data is presented in the tables.

Results

All the analyses were performed on the abundance (%) of the whole data set, excluding the samples of the first day of culture (day 0). The reason for the latter is that at the beginning of the culture, the population structure has not yet been influenced by the provided food. According to the dendrogram (Fig. 1), the following three groups could be identified: the first group (Group1), containing samples of treatments A and C, obtained at 24, 48 and 72 h (afternoon samples), was discriminated from the rest of the samples at 20% dissimilarity level (Anosim, $R = 0.8$, $P < 0.1\%$). Simper analysis identified females with multiple eggs, loose eggs, females with sac and females with one egg as the life cycle phases contributing most (69.55%) to the observed dissimilarity (Dissimilarity/Standard deviation index = 2.25, 1.85, 1.62 and 1.55 respectively). The second group (Group 2) consisted of the samples of treatments A and C, collected at 16, 40, 64 and 88 h (morning samples). The second group was discriminated at 14% dissimilarity level (Anosim, $R = 0.5$, $P < 0.1\%$) from the third group (Group 3), which contained the samples of treatments B, D and E. Simper analysis identified immature individuals, non-ovigerous females, post reproductive females and loose eggs as the life cycle phases to which most (60.54%) of the observed dissimilarity could be attributed (Dissimilarity/Standard deviation index = 1.88, 1.60, 1.48 and 1.43 respectively).

The scatterplot of the first two axes scores of Correspondence Analysis (Fig. 2), which explained 74% of the variance of the data, shows the distribution of samples (Fig. 2a) in relation to rotifer life cycle phases (Fig. 2b). Group 1 samples were placed closer to females with sac and furthest away from loose eggs and females with one or multiple eggs. Group 2 samples were located near loose eggs, non-ovigerous and post reproductive females. Group 3 was nearer females with multiple eggs and immature individuals. In particular, Group 3 samples obtained at 48 h were closer to immature individuals, whereas those of treatments B and D sampled at 40 and 88 h, approached females with multiple eggs. Samples of treatment E collected at 24 and 40 h were closer to the samples of Group 2.

Two-way ANOVA confirmed the significant effect of the factors tested, namely treatment and sampling time on the life cycle phases. The only exceptions were the effect of treatment on loose eggs and females with sac and sampling time on post reproductive females (Table 4). The fact that, interaction of the two factors was significant in all life cycle phases implies that the pattern observed in the treatments was not consistent throughout the experimental period. Interaction plots were designed in order to look at the temporal pattern in the abundance (%) of the life cycle phases (Figs. 3 and 4). In most life cycle phases, the temporal patterns of treatments A and C followed a diurnal cycle (24-h cycle): immature individuals and females with sac increased at 24, 48 and 72 h (Group 1), whereas loose eggs, females with one egg and females with multiple eggs showed exactly the opposite pattern (i.e. were higher at 16, 40, 64 and 88 h-Group 2). In the case of treatments B, D and E, loose eggs showed an increase at 24 and 72 h. Immature individuals increased to reach a peak at 48 h, where after it decreased. Females with one egg were higher at 24 h and showed a lesser

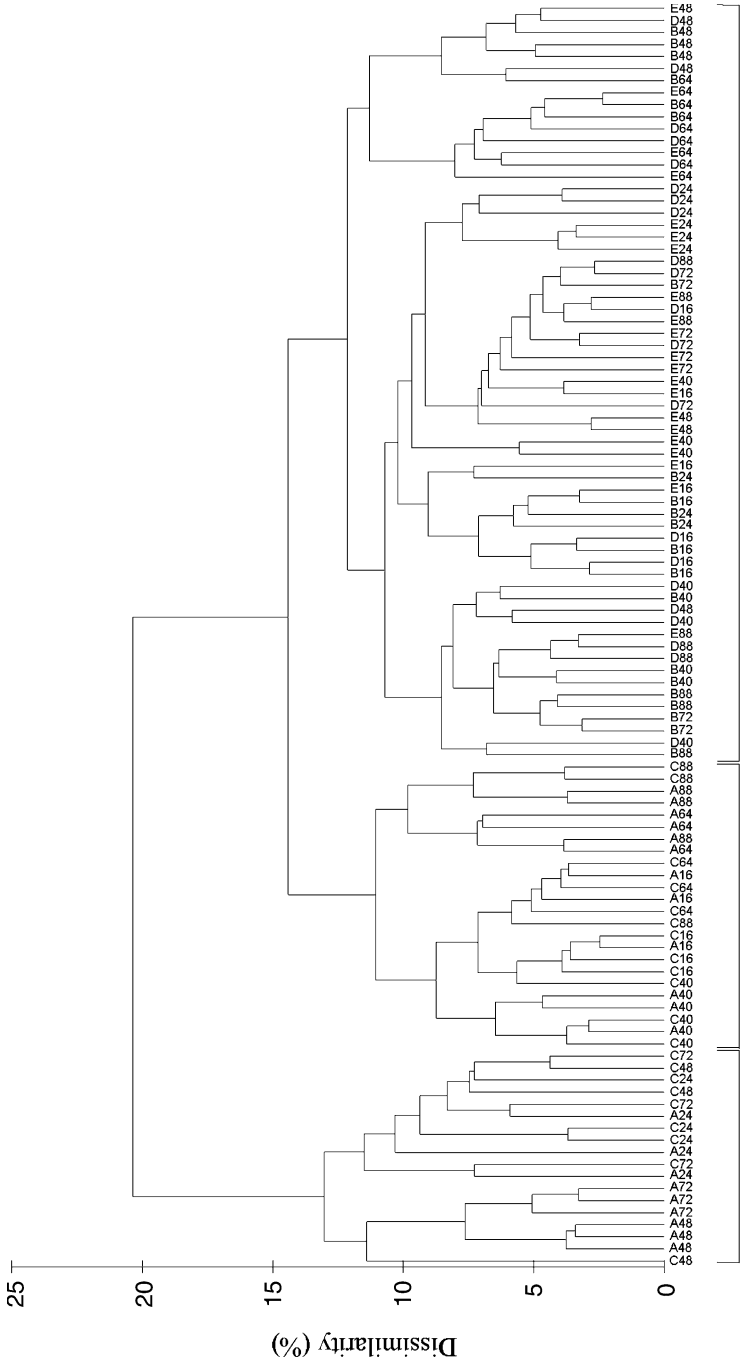

Fig. 1 Dendrogram showing the samples of treatments A, B, C, D and E, based on the abundance (%) of rotifer life cycle phases, obtained at 16, 24, 40, 48, 64, 72 and 88 h. Three replicates for each combination of treatment and sampling time

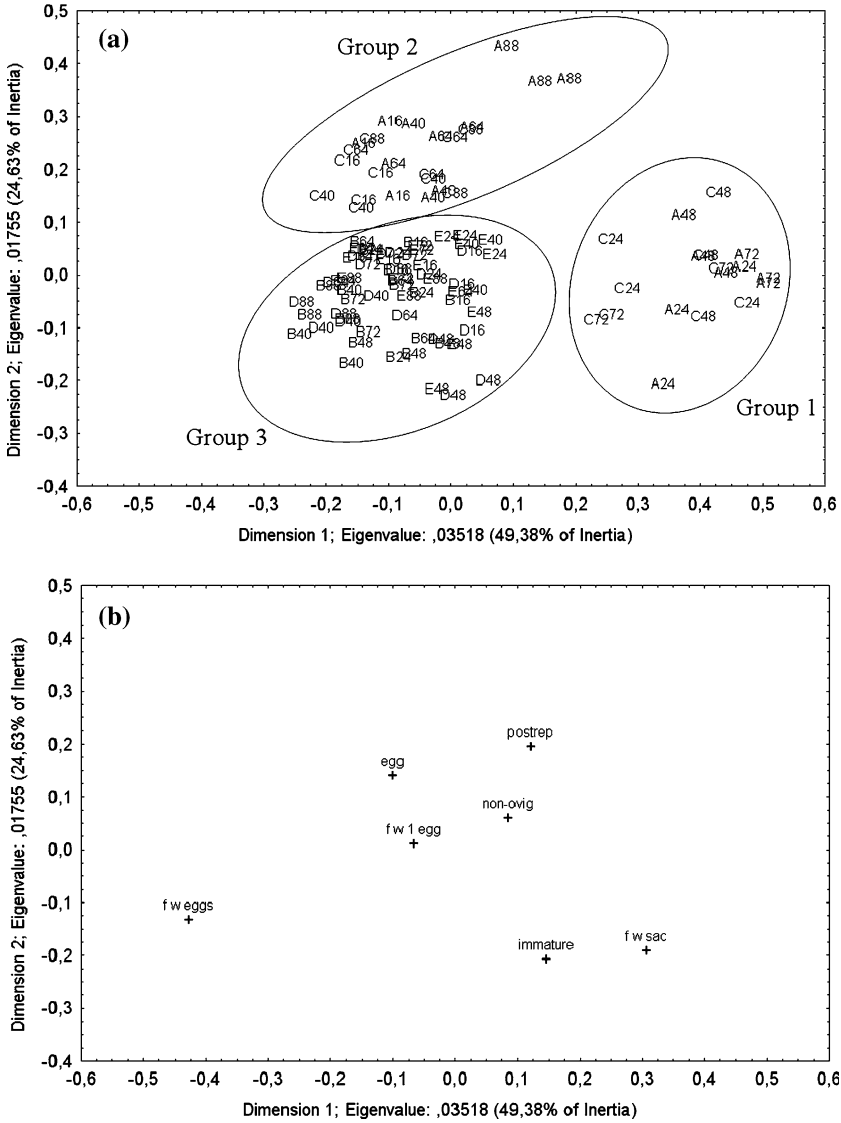

Fig. 2 Correspondence analysis showing (**a**) the samples of treatments A, B, C, D and E based on (**b**) the abundance (%) of rotifer life cycle phases (egg: loose eggs, immature: immature individuals, non-ovig: non-ovigerous females, f w sac: females with sac, f w 1 egg: females with one egg, f w eggs: females with multiple eggs, postrep: post reproductive), obtained at 16, 24, 40, 48, 64, 72 and 88 h. Three replicates for each combination of treatment and sampling time

increase towards the end of the sampling period (from 72 h onwards). Females with multiple eggs showed two peaks, one at 40 h and one at 88 h. Treatment E followed the pattern of B and D after 48 h. Overall, the population structure at 16, 24 and 40 h resembled that of 64, 72 and 88 h respectively, indicating a 48-h cycle for treatments B, D and E.

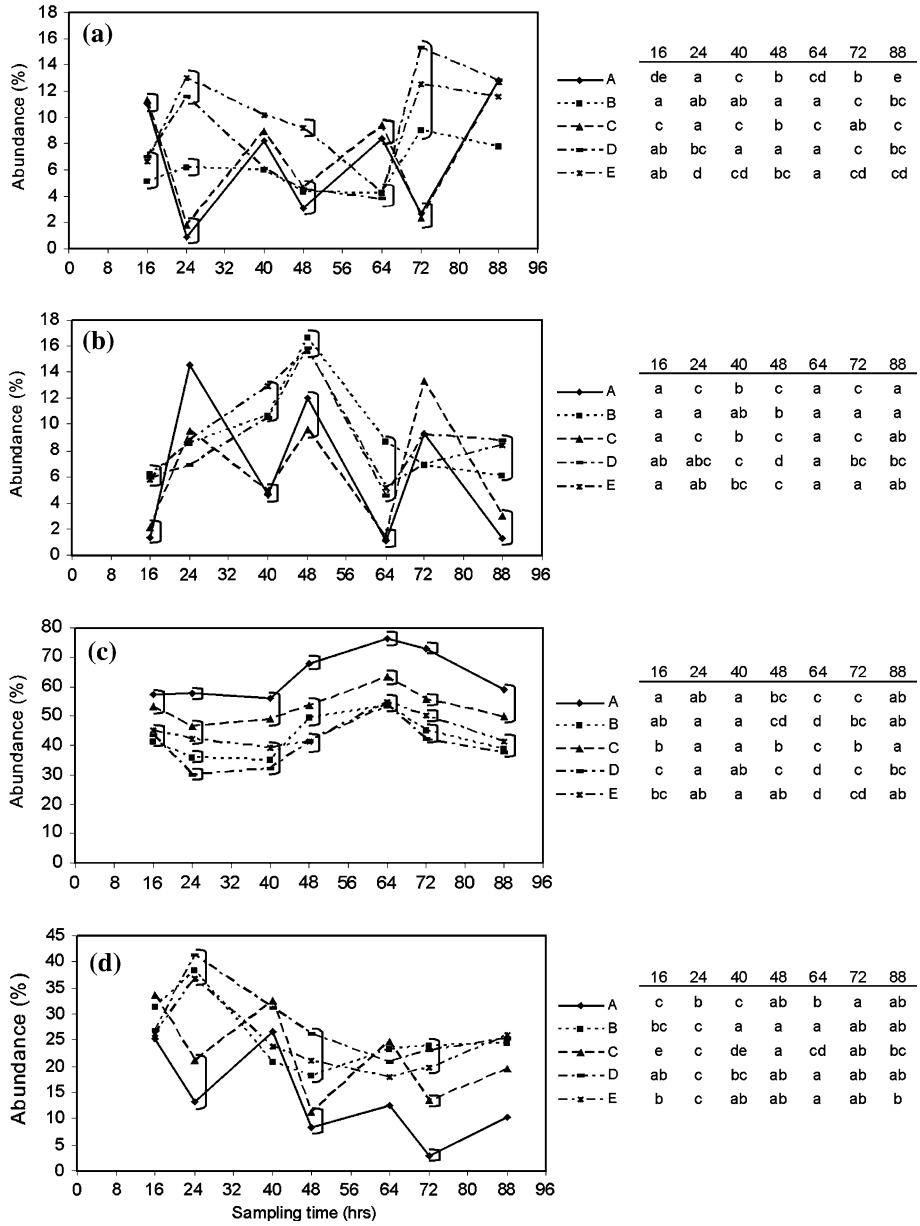

Fig. 3 Interaction plots showing the temporal variation in the abundance (%) of: (**a**) loose eggs, (**b**) immature, (**c**) non-ovigerous females and (**d**) females with one egg for each treatment (A, B, C, D, and E). The brackets inside the graph denote significant differences within each sampling time, whereas the letters in the tables on the side show differences within each treatment ($P < 0.05$)

Amongst treatments (Figs. 3 and 4), significant differences were observed at 16 and 64 h in loose eggs, non-ovigerous, post-reproductive females (significantly higher in treatments A and C) and immature individuals (significantly higher in B, D and E). At

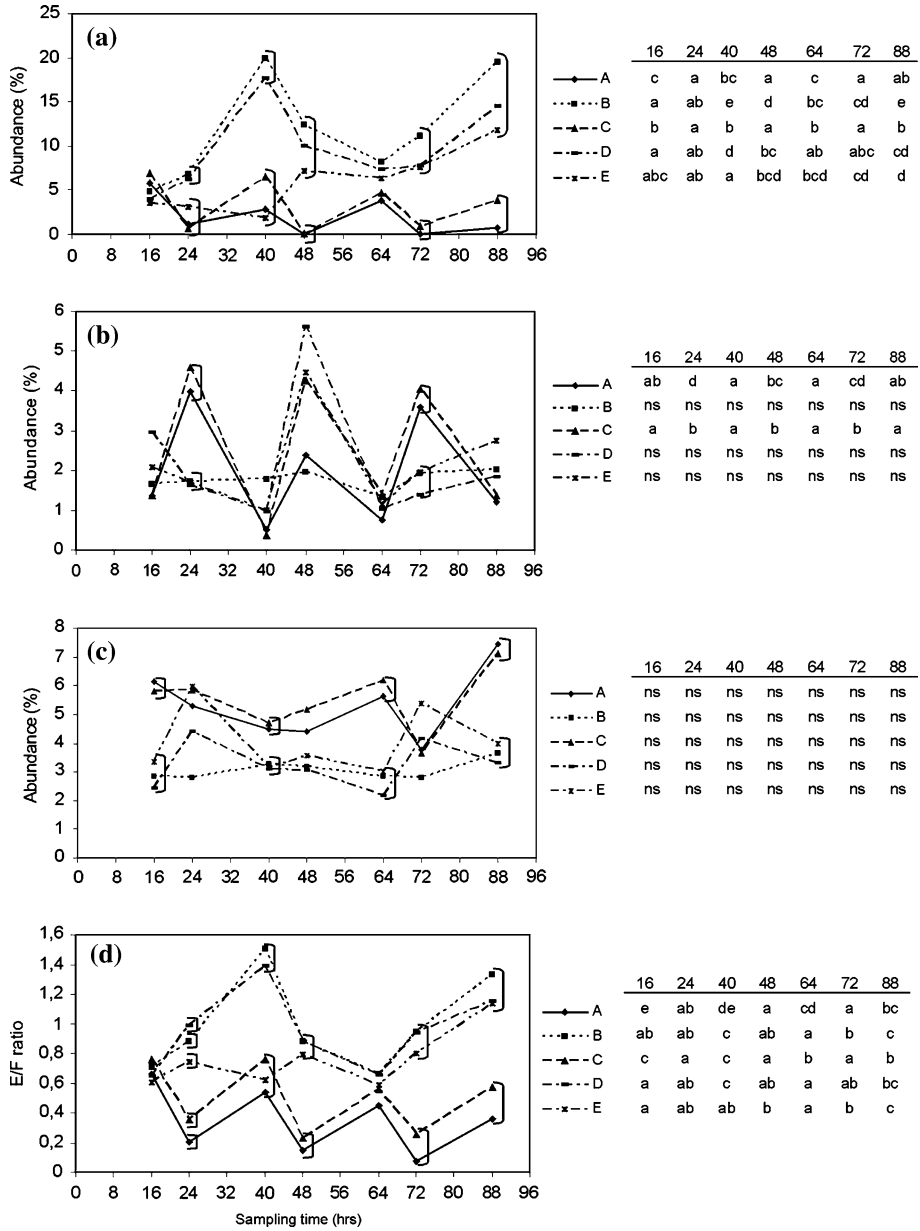

Fig. 4 Interaction plots showing the temporal variation in the abundance (%) of: (**a**) females with multiple eggs, (**b**) females with sac, (**c**) post-reproductive females and (**d**) E/F ratio for each treatment (A, B, C, D, and E). The brackets inside the graph denote significant differences within each sampling time, whereas the letters in the tables on the side show differences within each treatment ($P < 0.05$)

24 and 72 h, loose eggs, females with one egg and females with multiple eggs were significantly higher in B, D and E treatments, compared to A and C, whereas females with sac were significantly higher in the latter treatments. Treatment E was grouped with A and

Table 3 Mean values of rotifer density (individuals ml^{-1}) for all treatments at each sampling time (SEM: standard error of grand mean)

Day	Sampling time (h)	Treatment					SEM
		A	B	C	D	E	
1	16	248.9^{b}	76.8^{a}	281.5^{b}	57.7^{a}	177.6^{b}	14.9
1	24	135.9^{b}	77.1^{a}	181.4^{b}	62.4^{a}	143.3^{b}	13.2
2	40	141.8^{b}	87.3^{a}	163.8^{b}	78^{a}	142.9^{b}	10.9
2	48	113.1	107.4	101.2	134.2	115.6	11.3
3	64	124.6	115.2	140.2	134.2	133.5	13.1
3	72	106.2	110.9	107.5	128.4	103.6	10.9
4	88	105.6	140.7	117.1	185.5	147.2	12.9

Means within a row having a different letter in superscript are significantly different ($p < 0.05$)

C in the case of females with multiple eggs at 24 h. At 40 and 88 h, immature and females with multiple eggs were significantly higher in treatments B, D and E (except for females with multiple eggs at 40 h), than in A and C; non-ovigerous and post reproductive females were significantly higher in treatments A and C compared to the other treatments. At 48 h, immature individuals, females with one egg and females with multiple eggs were significantly higher in B, D and E, than in A and C. In addition, at 48 h, loose eggs were significantly higher in treatment E compared to the other treatments.

The density of rotifers was significantly higher during the first 40 h of sampling in treatments A, C and E, compared to B and D (Table 3, $P < 0.05$). After that time, no statistical differences in densities were observed.

The growth rate was significantly higher in treatments B and D, compared to A and C, whereas treatment E showed intermediate levels (Fig. 5). No significant interaction was found between treatment and sampling time. For the E/F ratio the effects of both factors and their interaction were significant (Table 4). According to the interaction plot (Fig. 4d), the E/F ratio was significantly higher in treatments B and D compared to A and C in all but 16 and 64 h of sampling. Treatment E followed the pattern of B and D, except for 40 h of sampling, where it was grouped together with treatments A and C.

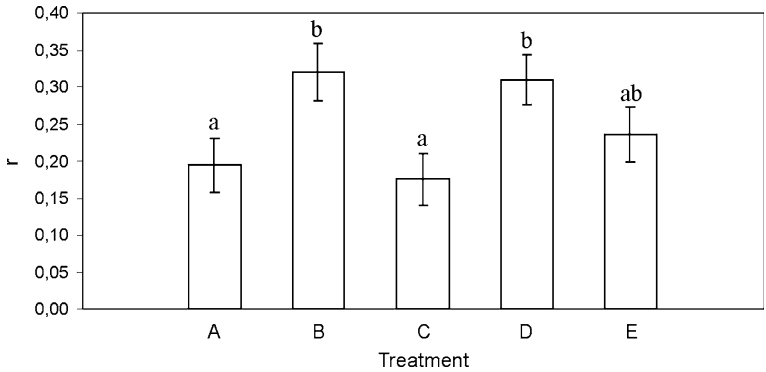

Fig. 5 Mean values (± standard error) of growth rate (r) in treatments A, B, C, D and E. Different letters indicate significant differences ($P < 0.05$)

Table 4 Two-way ANOVA applied to the abundance (%) of life cycle phases and E/F ratio, according to sampling time (16, 24, 40, 48, 64, 72 and 88 h) and treatment (A, B, C, D and E) (SEM: standard error of grand mean)

	Sampling time (h)							Treatment					P_h	P_t	P_hP_t	SEM
	16	24	40	48	64	72	88	A	B	C	D	E				
Loose eggs	8.1	6.5	7.9	5.0	5.5	9.1	11.2	6.3	6.0	7.6	8.6	9.7	**	ns	*	0.5
Immature	4.3	9.8	8.7	14.0	4.3	9.1	5.7	6.7	9.0	6.3	8.8	9.0	***	***	***	0.3
Non-ovigerous females	48.2	42.5	42.3	50.7	59.0	52.0	44.3	62.8	42.4	52.7	39.7	44.5	***	***	***	0.9
Females with 1 egg	28.5	30.0	26.9	17.0	20.3	17.5	21.5	15.1	25.9	22.2	27.8	24.5	***	***	***	0.7
Females with multiple eggs	4.9	3.5	9.6	5.8	6.0	5.8	10.5	2.1	11.9	3.4	9.7	6.0	***	***	***	0.5
Females with sac	1.8	2.8	0.8	3.7	1.1	2.6	1.9	1.9	1.7	2.4	2.2	2.2	***	ns	**	0.1
Post reproductive females	4.2	4.9	3.8	3.8	3.8	3.9	4.9	5.1	3.1	5.4	3.2	4.1	ns	***	*	0.2
E/F ratio	0.68	0.63	0.96	0.58	0.58	0.60	0.91	0.34	0.99	0.50	0.95	0.75	***	***	***	0.02

*0.05%, **0.01%, ***0.001%, ns: non significant

P: significance level; P_h for sampling time effects; P_t for treatment effects; P_hP_t for interaction of both factors

Discussion

The rotifer populations under study exhibited temporal variations in their structure, indicating two different reproductive patterns. Populations fed on yeast and phytoplankton (treatments A and C) were characterized by diurnal variations (24-h cycle) in females with one egg, females with multiple eggs and immature individuals. This diel periodicity in egg laying and hatching points towards a high rate of reproduction (defined as offspring/day of reproductive life). Populations fed on Culture Selco® and phytoplankton (treatments B and D) showed variations in the abovementioned rotifer life cycle phases in a 48-h cycle, reflecting a lower rate of reproduction. The latter populations also contained a comparatively higher number of females with multiple eggs, for most of the samplings, indicating a higher reproductive fraction in the population and an increased fecundity. These reproductive patterns have been associated with lifespan in rotifers (Snell and King 1977; King 1982; Kirk 1997; Yoshinaga et al. 2000, 2001, 2003): individuals with lower rates of reproduction have longer lifespans, whereas individuals reproducing at a higher rate do so over fewer age classes and have shorter lifespans. Such life history adjustments can have direct consequences on an organism's fitness. According to the present study, individuals fed on Culture Selco® and phytoplankton produced more offspring, yielding a significantly higher growth rate. This can be attributed to the increased fecundity and longer lifespan, even though the rate of reproduction was lower compared to individuals fed on yeast and phytoplankton. The higher growth rate attained with Culture Selco® and phytoplankton compared to yeast and phytoplankton has been recorded in previous studies using similar food types (Hirayama and Watanabe 1973; Hirayama and Funamoto 1983; James et al. 1983; Nyonje and Radull 1991; Suantika et al. 2000).

Rotifer density has been known to be a reproduction-inhibiting factor (James et al. 1983). An increase in initial density (treatment E) resulted in decreased abundance of females with multiple eggs, compared to that in populations of similar diet and lower initial density (treatment D), during the first 40 h of culture. The decrease in the abundance of females carrying multiple eggs could be a response to higher initial rotifer density, as has been suggested by Yoshinaga et al. (2001). After 40 h of culture, rotifer density was similar in all treatments and the abundance of females with multiple eggs (treatment E) was similar to that of populations having the same diet (treatment D). However, the initial restriction in the number of females with multiple eggs influenced the growth rate. Even so, the reproductive pattern in treatment E can be related to that of individuals with low reproductive rates (treatments B and D).

The increased phytoplankton quantity offered per rotifer (treatments C and D) did not alter the reproductive pattern in populations fed on yeast and phytoplankton (A vs. C), and those fed on Culture Selco® and phytoplankton (B vs. D). An increase in phytoplankton quantity has been shown to have a positive effect on rotifer growth rates (Theilacker and McMaster 1971; James et al. 1983; Rezeq and James 1987; Schmid-Araya 1991), but in this study no such effect was observed. It is possible that the quantity of phytoplankton in the first experiment was sufficient and a further increase did not have an additional positive effect on the growth rate of rotifers during the studied time interval. This also confirmed that the higher rate of reproduction of treatment A was not associated to the higher quantity of phytoplankton offered, compared to treatment B.

The E/F ratio followed the temporal pattern of females with multiple eggs (24- and 48-h cycle). In particular, the E/F ratio of treatments fed on Culture Selco® and phytoplankton at

40 and 88 h was at the uppermost range of reported values (Snell et al. 1987; Yúfera and Pascual 1989; Korstad et al. 1995; Yúfera et al. 1997).

Variations observed in rotifer population structure in this study, reflecting two reproductive patterns, could be attributed mainly to the quality of dry food provided. Compared to yeast, Culture Selco® is a diet rich in lipids, especially in n-3 highly unsaturated fatty acids (Hirayama and Funamoto 1983; James et al. 1987; Dhert et al. 2001). Culture Selco® seems to contribute to an increased abundance of gravid females, especially those bearing multiple eggs, thus contributing towards an increased fecundity. The role of diet lipids in egg formation has been documented in cladocerans (Goulden et al. 1982; Tessier et al. 1983; Acharya et al. 2005) and inferred in rotifers (Olsen et al. 1993; Øie and Olsen 1997).

In conclusion, batch-cultured rotifers showed different reproductive patterns, under the feeding regimes studied. Plasticity in rotifer life history parameters has been well documented in relation to food availability and population density (Yoshinaga et al. 2000, 2001, 2003). This study indicates that the type of dry food plays an important role in the reproductive strategy and growth rate of rotifers. The observed temporal differences in rotifer life cycle phases could also be associated to variations in size (Lubzens et al. 2001) and biochemical composition (Scott and Baynes 1978; Yúfera and Pascual 1989), with important consequences for fish larval performance. Thus, the changes in population structure and growth rate, recorded in the present study, should be taken into consideration in the management of rotifer mass cultures.

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