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Abstract

A detailed study of the life history of the rotifer *Brachionus plicatilis* was done at 20 °C, 20 ppt salinity, and 90 mg C l^{-1} food concentration. Rotifers were grown individually in culture plate wells (150 µl culture volume) and fed *Isochrysis galbana* Tahiti, *Tetraselmis* sp., *Nannochloris atomus*, or a 1 : 1 mixture (weight) of two of the algae. Observations were made every 2–8 hr and rotifers were sized and transferred to new food daily. A total of 19 different parameters were compared. Rotifers fed *Isochrysis* averaged 21 offspring per female, a 6.7 day reproductive period, a lifespan of 10.5 days and a mean length of 234 µm. After *Isochrysis*, the foods giving the highest growth, survival, and reproduction in decreasing order were *Isochrysis* + *Nannochloris*, *Nannochloris*, *Isochrysis* + *Tetraselmis*, *Tetraselmis* + *Nannochloris*, and *Tetraselmis*. Although the small volume culture system used in this study seems appropriate for studying life history of *B. plicatilis*, the results cannot always be directly applied to larger cultures.

Introduction

Brachionus plicatilis Müller is a euryhaline rotifer commonly used as initial food for a variety of marine fish larvae. Several reviews have discussed its biological characteristics and its importance in aquaculture (Walker, 1981; Lubzens, 1987, 1989).

Although much is known about the biology of *B. plicatilis*, relatively few researchers have investigated the life history of this rotifer. The only detailed studies of the life history of individually cultured *B. plicatilis* were done by Ruttner-Kolisko (1972), who tested the influence of temperature and salinity on several important life history parameters, and King & Miracle (1980), who tested the influence of temperature on rates of survivorship and reproduction in three clones of *B. plicatilis*. Most other studies of various aspects of the life history of *B. plicatilis* have investigated the influence of food and temperature on population growth rates (Snell *et al.*, 1983; Yufera, 1987).

More information on the growth, survival, and reproduction of *B. plicatilis* fed different diets is needed before optimization of the culture of this rotifer can take place. Mathematical models of the production process of live feed for marine fish larvae (e.g., Slagstad *et al.*, 1987) might also be refined by this information.

This study examines the size at birth, size of first egg, size at first newborn, maximum size, juvenile period, time to last egg, time between egg-laying, egg development time, total number of eggs, total number of eggs hatching, percent eggs hatching, maximum number of eggs carried at one

time, time to first newborn, time to last newborn, time between newborn, reproductive period, lifespan, post-reproductive period, and percent of lifespan post-reproductive of individually cultured *B. plicatilis* fed single and mixed diets of *Isochrysis galbana* Tahiti, *Tetraselmis* sp., and *Nannochloris atomus*.

Methods

Brachionus plicatilis were obtained from the Institute of Marine Research in Bergen, Norway. Separate cultures were maintained in 4 l beakers with continuous aeration at 20 °C and 20 ppt salinity. Sea water was obtained from direct pipeline supply to the laboratory from the nearby fjord, diluted to the desired concentration, and filtered through a Whatman GF/C glass-fiber filter. Rotifer cultures were daily fed one of the following algae: *Isochrysis galbana* Tahiti, *Tetraselmis* sp., and *Nannochloris atomus* (hereafter called Iso, Tetra and Nanno, respectively), or a 1 : 1 mixture (weight) of two of the algae: Iso + Tetra, Iso + Nanno, and Tetra + Nanno. Algae were obtained from the culture collections at the Institute of Microbiology and Plant Physiology in Bergen (Iso and Nanno) and the Laboratory of Biotechnology, University of Trondheim (local strain of Tetra; species unknown).

Algal cultures were grown in semi-continuous cultures diluted at 0.33 day^{-1} using f media (Guillard 1975) and constant light (160 μEin m^{-2} sec^{-1}). About 500 ml (13%) of the water in the rotifer cultures was removed each day and replenished by new sea water with algae. Rotifer cultures were physiologically adapted to the experimental conditions for a minimum of 10 days prior to the life history experiments to assure complete acclimation to the respective food (Korstad *et al.*, 1989). In another life history study, we determined that there are no significant differences among all life history parameters measured for four generations (F_1, F_2, F_3, and F_4 generations), indicating that 10 days acclimation is sufficient.

Twelve egg-bearing females were randomly selected from each stock culture and individually micro-pipetted into 150 μl tissue culture plate wells (Nunclon) containing the same algal culture at a concentration of 90 mg C l^{-1}. These concentrations were determined spectrophotometrically using an established carbon versus absorption relationship. These rotifers were observed every 1–2 hr under a Wild dissecting microscope at 25 × magnification, and as soon as neonates appeared, the mother was removed. Thereafter, observations were made every 2–8 hr for the appearance of eggs and neonates which were counted and removed. Rotifers were sized (under 50 × magnification) and transferred to new food daily. Between observations, the culture plates were kept inside a large beaker immersed in a water bath at 20 °C. Light intensity was approximately 6 μEin m^{-2} sec^{-1} (12L : 12D).

A separate experiment was done to study the low survival and reproduction of *B. plicatilis* which were fed *Tetraselmis*. Individual rotifers were cultured under the same conditions as the first study. Four groups of six rotifers each were fed Iso diluted with different solutions to a final concentration of 60 mg C l^{-1}. One group was diluted with filtrate from dense cultures of Tetra (Tetra grown in stock culture at a concentration of 240 mg C l^{-1} and filtered through a Whatman GF/C filter). Another group was diluted with Tetra filtrate at a concentration of 90 mg C l^{-1} filtered as above and a third group was diluted with f media to check if some compound in the media not metabolized by Tetra was toxic to *Brachionus*. A fourth group was diluted with 20 ppt sea water (control). All rotifers were observed and transferred to new food daily as in the other study.

Differences between means for each life history parameter were analyzed with one-way ANOVA. Significant differences ($p<0.05$) were further analyzed with the Least Significant Difference multiple comparisons test (Statgraphics, Statistical Graphics Corp.).

Results

A total of 19 different parameters were determined for each rotifer (Table 1). Size at birth, time to first newborn, and post-productive period were not significantly different between foods. Other life history traits showing significant dietary effects included size at first egg, size at first newborn, and maximum size, all of which were larger for rotifers fed Iso and Iso + Nanno. Rotifers were smallest when fed Tetra. Rotifers fed Nanno and Iso + Nanno reached reproductive maturity in the shortest time, followed by animals fed Iso, Tetra + Nanno, Tetra, and Iso + Tetra. Time to last egg and last newborn, reproductive period, and lifespan were longest for rotifers fed Iso and shortest for those fed Tetra and Tetra + Nanno. Percent of lifespan post-reproductive was lowest for animals fed Iso, Iso + Tetra, and Iso + Nanno and highest for those fed Tetra, Nanno, and Tetra + Nanno.

Rotifers fed Iso had the highest reproductive

Table 1. Life history parameters for *Brachionus plicatilis* fed different algae. When one-way ANOVA for diet effects had significance values of $p < 0.05$, a Least Significance Difference (LSD) multiple comparison test was done. Numbers under Multiple Comparison indicate sample means which are similar (same number) or different (different number).
A. Size parameters.

Food species		Size at birth (μm)	Size at first egg (μm)	Size at first newborn (μm)	Max. size (μm)
Iso:	N =	12	12	12	12
	mean =	130.00	190.00	207.50	234.17
	SE =	1.18	0.00	1.25	4.32
Tetra:	N =	12	5	0	11
	mean =	128.18	174.00	–	165.45
	SE =	1.73	2.19	–	4.52
Nanno:	N =	12	12	10	12
	mean =	128.33	189.17	202.00	208.33
	SE =	1.08	0.80	1.90	3.50
Iso + Tetra:	N =	9*	4	4	8
	mean =	126.67	187.50	197.50	183.75
	SE =	1.57	2.17	6.50	7.06
Iso + Nanno:	N =	12	12	12	6
	mean =	128.33	194.17	210.00	230.00
	SE =	1.08	1.42	2.04	6.24
Tetra + Nanno:	N =	12	7	0	8
	mean =	125.83	184.29	–	180.00
	SE =	1.42	2.75	–	6.12
Anova:	p =	0.37	<0.001	0.014	<0.001
LSD multiple comparison:					
Iso		–	1	1	1
Tetra		–	2	–	2
Nanno		–	1	2	3
Iso + Tetra		–	1	3	4
Iso + Nanno		–	3	1	1
Tetra + Nanno		–	4	–	4

* Only 9 newborn were obtained.

Table 1. (continued). B. Egg parameters.

Food species		Time to first egg (juvenile period) (days)	Time to Last egg (days)	Time between egg-laying (days)	Egg devel. time (days)	Total number eggs	Total number hatching (R_0)	Percent hatching	Max. no. eggs carried at one time
Iso:	N =	12	12	12	12	12	12	12	12
	mean =	1.39	8.11	0.33	0.41	22.42	21.17	0.93	4.00
	SE =	0.07	0.32	0.02	0.04	1.46	1.66	0.03	0.26
Tetra:	N =	5	5	1	0	12	12	12	0.12
	mean =	1.80	2.21	2.04	–	0.50	0	0	0.50
	SE =	0.09	0.29	–	–	0.19	0	0	0.19
Nanno:	N =	12	12	11	10	12	12	12	12
	mean =	1.23	4.92	0.79	1.97	5.75	3.08	0.41	2.50
	SE =	0.09	0.98	0.10	0.32	1.28	1.12	0.07	0.22
Iso + Tetra:	N =	4	4	4	4	9	9	9	9
	mean =	1.84	4.26	0.39	0.78	3.22	3.00	0.39	1.56
	SE =	0.19	0.44	0.02	0.21	1.39	1.37	0.15	0.59
Iso + Nanno:	N =	12	11	12	11	11	11	11	12
	mean =	1.19	6.73	0.42	0.42	17.36	16.00	0.92	4.33
	SE =	0.04	0.57	0.07	0.02	1.62	1.56	0.02	0.27
Tetra + Nanno:	N =	7	7	4	0	12	12	12	12
	mean =	1.57	2.20	0.89	–	1.00	0	0	0.92
	SE =	0.09	0.26	0.07	–	0.29	0	0	0.25
Anova:	p =	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD multiple comparison:									
Iso		1	1	1	1	1	1	1	1
Tetra		2	2	2	–	2	2	2	2
Nanno		3	3	3	2	3	3	3	3
Iso + Tetra		2	3	1	3	4	3	3	4
Iso + Nanno		3	4	1	1	5	4	1	1
Tetra + Nanno		4	2	3	–	2	2	2	2

output both in terms of eggs and newborn. Those fed Iso and Iso + Nanno had the highest percentage of viable eggs and carried the most eggs at one time. Rotifers fed Tetra and Tetra + Nanno produced a few eggs but none were viable. Time between egg-laying, egg development time, and time between newborn were shortest for animals fed Iso, Iso + Nanno, and Iso + Tetra.

Percent survival was highest for rotifers fed Iso, followed by Iso + Nanno, Nanno, Iso + Tetra, Tetra, and Tetra + Nanno (Fig. 1). Rotifers fed Iso lived a minimum of 8.5 days and a maximum of 12.5 days, whereas rotifers fed Tetra and Tetra + Nanno lived a minimum of about 1.0 day and a maximum of 5.4 days.

The effects of Tetra filtrate on reproductive rate are presented in Table 2. The highest reproduction was achieved with rotifers fed a diet of Iso cells and Tetra filtrate and lowest for the control (sea water) and f media dilutions. Differences between the dilute and concentrated Tetra filtrate were not statistically significant.

Table 1. (continued). C. Newborn, reproduction, lifespan, and post-reproductive parameters.

Food species		Time to first newborn (days)	Time to last newborn (days)	Time between newborn (days)	Repro. period (days)	Life-span (days)	Post-repro. period (days)	Percent of lifespan post-repro.
Iso:	N =	12	12	12	12	12	12	12
	mean =	2.25	8.88	0.36	6.72	10.46	2.35	0.22
	SE =	0.07	0.39	0.04	0.34	0.41	0.39	0.03
Tetra:	N =	0	0	0	12	12	5	4
	mean =	–	–	–	0.17	2.47	1.65	0.39
	SE =	–	–	–	0.16	0.40	0.46	0.08
Nanno:	N =	10	10	7	12	12	12	12
	mean =	2.66	5.47	1.06	3.70	7.92	3.00	0.39
	SE =	0.42	1.08	0.20	0.99	1.11	0.70	0.05
Iso + Tetra:	N =	4	4	4	9	9	4	4
	mean =	2.81	5.07	0.33	1.08	4.40	1.52	0.24
	SE =	0.31	0.44	0.11	0.48	0.60	0.60	0.07
Iso + Nanno:	N =	12	11	11	11	11	12	11
	mean =	1.96	7.21	0.36	5.54	8.87	1.96	0.20
	SE =	0.05	0.59	0.02	0.56	0.86	0.76	0.05
Tetra + Nanno:	N =	0	0	0	12	12	7	7
	mean =	–	–	–	0.37	2.71	2.71	1.29
	SE =	–	–	–	0.16	0.32	0.32	0.28
Anova:	p =	0.12	0.006	<0.001	<0.001	<0.001	0.58	0.049
LSD multiple comparison:								
Iso		–	1	1	1	1	–	1
Tetra		–	–	–	2	2	–	2
Nanno		–	2	2	3	3	–	2
Iso + Tetra		–	2	1	4	4	–	1
Iso + Nanno		–	3	1	5	3	–	1
Tetra + Nanno		–	–	–	2,4	2	–	2

Discussion

The life history parameters of rotifers fed Iso in our study are similar to those for *B. plicatilis* fed excess *Dunaliella* at 20 °C reported by Ruttner-Kolisko (1972), indicating that the food value of these two algae is similar. Walker (1981) analyzed Ruttner-Kolisko's (1972) data and found that the rotifers spent approximately one-third of their lifespan in the post-reproductive stage at all temperatures tested. In our study, however, we observed a wider range, averaging about 22 percent for rotifers fed Iso, Iso + Tetra, and Iso + Nanno and about 38 percent for rotifers fed the other algal combinations.

The three clones of *B. plicatilis* used by King & Miracle (1980) had a wider range of mean lifespan at 20 °C than those used in our study. Clone SP had the longest lifespan (16.5 days), while rotifers fed Iso, which had the longest lifespan in our study, lived 11 days. Clones LA and MC in King & Miracle's study had fairly short lifespans (6–8.5 days), which were similar to rotifers fed all other algal types in our study (2.5–8.9 days).

Yufera (1987) measured the effect of algal diet and temperature on egg development time in two

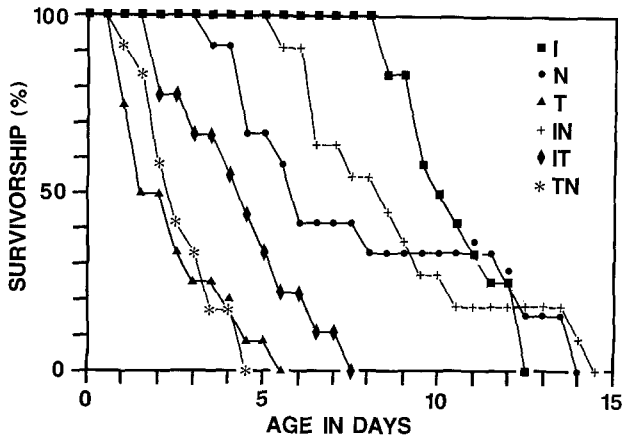

Fig. 1. Survivorship (fraction surviving over time) of *Brachionus plicatilis* fed Iso (I), Tetra (T), Nanno (N), Iso + Tetra (IT), Iso + Nanno (IN), and Tetra + Nanno (TN).

strains of *B. plicatilis*. He found the slowest development time in rotifers fed *Nannochloropsis gaditana*, which was most similar to rotifers fed *Nannochloris atomus* in our study.

Snell *et al.* (1983) studied the effects of unialgal and mixed diets of *Chlorella* sp., *Dunaliella tertiolecta*, and *Schizothrix calcicola* on the reproductive rate of *B. plicatilis*. They found that the reproductive rate was an average of 2.7 times higher on the mixed diet than on either unialgal diet. Other researchers have reported similar findings with yeast and algae as food (Hirayama & Watanabe, 1973; Yufera & Pascual, 1980). Yufera and Pascual, however, found highest growth rates on rotifers fed *Tetraselmis suecica*. The results from our study are quite different from those reported above. Rotifers fed Tetra and Tetra + Nanno had the lowest reproductive

Table 2. Reproductive rate of *Brachionus plicatilis* fed a diet of Iso diluted to 60 mg C l^{-1} with 20 ppt sea water (Control), *f* Media, dilute Tetra filtrate (DTF), and concentrated Tetra filtrate (CTF). See text for details. Numbers represent mean ± (SE); n = 6 for each treatment. Description of statistical analyses same as for Table 1.

Dilution	Total no. eggs	Newborn R_0	Percent hatching
Control	7.50 (1.45)	3.33 (1.10)	38 (14)
f Media	6.17 (1.04)	2.33 (0.38)	38 (3)
DTF	16.00 (1.65)	13.00 (1.43)	81 (2)
CTF	17.33 (3.04)	15.17 (2.69)	89 (2)
Anova: p =	0.002	<0.001	<0.001
LSD multiple comparison:			
Control	1	1	1
f Media	1	1	1
DTF	2	2	2
CTF	2	2	2

rates, while those fed only Iso had the highest reproductive rate.

The results of the experiment testing the effect of Tetra filtrate on rotifers suggest that Tetra cells, but not filtrate, have an inhibitory effect on rotifers. However, we have also maintained larger volume aerated batch cultures of *B. plicatilis* fed the same algae used in this study (Korstad *et al.*, in prep.). Rotifers had somewhat similar growth rates on each food, indicating that the effects of Tetra are different in the culture plate wells than in the larger volume batch cultures. Tetra cells are motile but tend to readily attach to culture vessel surfaces, while Iso cells tend to remain motile (personal observation). Relatively more Tetra cells may therefore have been attached to the walls of the culture plate wells than in the batch culture vessels because of the larger surface-to-volume ratio in the smaller containers. This might account for the lower life history values for *B. plicatilis* fed Tetra in our study.

We have observed *B. plicatilis* feeding on Iso and Tetra. They seem to be able to ingest Iso cells more readily than Tetra. Whether this is because Iso cells move rapidly in the water while Tetra cells tend to adhere to surfaces is not clear. The Iso cells are also smaller than Tetra (approximately 11 and 250 μm^3, respectively), which may affect ingestion. Another possibility is that Tetra may be assimilated with lower efficiency than Iso. It's also probable that different species of Tetra may have differences in affinity to attachment, nutritional quality, ingestion by *Brachionus*, and other characteristics. Tetra has been commonly used as a nutritious food for *B. plicatilis* by many researchers (Trotta, 1983; Okauchi & Fukusho, 1984; Fukusho *et al.*, 1985).

Snell *et al.* (1983) reported that the enhancement of the reproductive rate of *B. plicatilis* fed a combination of *Chlorella* end *Schizothrix* was not dependent on ingestion of the *Schizothrix* cells. The reproductive rate of *B. plicatilis* increased in direct proportion to the amount of the *Schizothrix* filtrate added to the *Chlorella*. Further investigation revealed that the enhancing factor was a heat labile substance deactivated at 100 °C. Although we did not investigate the effects of heating on the Tetra filtrate in our study, our results were different. Reproductive rate of *Brachionus* did not significantly increase from the dilute to the concentrated Tetra filtrate. There was, however, a nearly five-fold increase in R_0 between rotifers fed Iso in control (sea water) and *f* media treatments and those in the two Tetra filtrate treatments. Rotifers may possibly have obtained extra nutritional benefits from the chemicals released by the Tetra cells and present in the filtrate.

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