Vol. 193: 125–133, 2000 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published February 28

Life history and fatty acid composition of the marine rotifer *Synchaeta cecilia valentina* fed different algae

Rafael Oltra[1,*], Rafael Todolí[1], Teresa Bosque[1], Luis M. Lubián[2], Juan C. Navarro[3]

[1]Departamento de Microbiología y Ecología, Facultad de Ciencias Biológicas, Universitat de València, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain

[2]Instituto de Ciencias Marinas de Andalucía (CSIC), Campus Río San Pedro s/n, 11510 Puerto Real (Cadiz), Spain

[3]Instituto de Acuicultura Torre de la Sal (CSIC), Ribera de Cabanes, 12595 Torre la Sal (Castellón), Spain

ABSTRACT: A strain of the marine rotifer *Synchaeta cecilia valentina*, isolated from 'El Hondo de Elche', a Spanish Mediterranean coastal lagoon, was cultured in the laboratory in 20 ml test tubes, at 20°C and 25 ppt salinity and fed with the algae *Tetraselmis* sp., *T. chuii*, *Cryptomonas* sp., *Rhodomonas salina* and *R. baltica*. The effects of these 5 algal diets and 3 food levels (2.1, 3.2 and 4.2 µg ml^{-1} dry wt) on life history traits of this rotifer were studied in life tables performed with replicated individual cultures of 1 ml, at the temperature and salinity mentioned above. Algal diet had a significant effect on both average lifespan (*LS*) and net reproductive rate (R_0), but food concentration only had an effect on R_0. *Tetraselmis* sp. provided the best results: *LS* ranging from 4.4 to 6 d, R_0 from 10.9 to 12.8 offsping female^{-1}, and intrinsic growth rate (r) from 0.84 to 1.12 d^{-1}. *S. cecilia valentina* also grew with the remainder of the algae, except with *R. baltica*. With this alga the rotifer grew only in 500 ml cultures performed at a higher food concentration (5.55 µg ml^{-1} dry wt). The effect of the algal diet on the fatty acid composition of the rotifers was analysed to infer its nutritional value according to the changes induced by diet. Saturated fatty acids made up 34 to 40% of total fatty acids, whereas monoenes accounted for 15 to 23%, and polyunsaturated fatty acids constituted 20 to 29%. Main fatty acids were: 16:0 (which ranged from 17 to 22%), 18:0 (10 to 12%), and 18:1 (8.5 to 12%). In certain algal treatments (both *Tetraselmis* species), the linolenic acid 18:3n-3 was moderately abundant (5 to 9%). Eicosapentaenoic acid, 20:5n-3, was markedly lower in rotifers fed with *R. baltica* (less than 3%), whereas docosahexaenoic acid, 22:6n-3, was found in a higher proportion in *Cryptomonas* sp. (4.9%) and *R. salina* (3.4%) treatments. The nutritional value deduced from the lipid composition was similar to that reported in the literature for other rotifers such as *Brachionus plicatilis*.

KEY WORDS: Life table · Growth rate · *Tetraselmis* · *Cryptomonas* · *Rhodomonas* · Fatty acids

INTRODUCTION

Culture of marine rotifers is difficult and has only been possible with a small number of species, among them *Encentrum linnhei* (Schmid-Araya 1991), *Synchaeta hutchingsi* (Brownell 1988), *S. cecilia* (Egloff 1986, 1988) and *S. cecilia valentina* (Oltra & Todolí 1997) as well as the mixohaline species *Brachionus plicatilis-B. rotundiformis* (Rezeq & James 1987, Maruyama et al. 1997), both considered as strains belonging to the same species until recently (Segers 1995). This is clearly different from freshwater rotifer species, where a number of species have been cultured (Pourriot 1965, May 1987).

The culture of a new species of marine rotifer would be interesting not only for the new knowledge this would bring in terms of the biology of the species, but also due to the possible application of its cultures to aquatic ecotoxicology and aquaculture, in which the only species used up to date is *Brachionus plicatilis-B.*

*E-mail: rafael.oltra@uv.es

rotundiformis (Lubzens et al. 1989, Snell & Janssen 1995).

In a recent paper, Oltra & Todolí (1997) reported the isolation of and first attempts at culturing the marine rotifer *Synchaeta cecilia valentina*. This species has been found in several harbours, salines and Mediterranean coastal lagoons of the Valencian region (Spain), within a broad range of temperatures (15 to 28°C) and salinities (18 to 40 ppt) at densities of up to 4800 ind. l^{-1} (X. Armengol-Díaz & R. Oltra unpubl. obs.). *S. cecilia valentina* has been cultured in the laboratory at 20 and 24°C, from 20 to 37 ppt salinity, and fed with the alga *Tetraselmis suecica* at concentrations of 15 000 and 25 000 cells ml^{-1}. The longest average lifespan (5.6 d) and highest number of offspring per female (9.2) were obtained at 20°C and 25 ppt, it being fed at low algal concentration.

The first aim of the present work was to carry out life table analyses of *Synchaeta cecilia valentina* cultured under optimal conditions of temperature and salinity, using 5 algal species as food: *Tetraselmis* sp., *T. chuii*, *Cryptomonas* sp., *Rhodomonas salina* and *R. baltica*. These algae were selected after initial attempts at feeding with the algae *Isochrysis galbana*, *Pavlova lutheri* and *Nannochloropsis oculata* proved to be unsuccessful.

Nutritional 'status' of the rotifer is critical if it is to serve as a nutrient source in aquaculture. One limiting factor in the use of live prey in aquaculture is the presence of adequate amounts of essential fatty acids for marine fish larvae. These are polyunsaturated fatty acids such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), which are often absent or present in low amounts in the live prey commonly used as larval food. Both fatty acids have been found in varying amounts in the rotifer *Brachionus plicatilis*, depending on the algal food used in the cultures (Ben Amotz 1987, Frolov et al. 1991). Therefore, the second aim of this work was to study the fatty acid profiles of *Synchaeta cecilia valentina* cultured with the different algal diets.

Table 1. Food level and its equivalent algal cell concentration (cells ml^{-1}) for the 5 microalgae assayed

Alga	Food level (µg ml^{-1} dry wt)			µg/10^6 cells
	2.10	3.15	4.20	
Tetraselmis sp.	12 100	18 100	24 200	174.1[a]
T. chuii	11 500	17 300	23 000	182.4
Cryptomonas sp.	13 700	20 600	27 500	152.8
Rhodomonas salina	18 000	27 000	36 000	112.9
R. baltica	22 700	34 000	45 400	92.6

[a]Lubián & Yúfera (1989)

MATERIALS AND METHODS

The rotifer *Synchaeta cecilia valentina* was kept in the laboratory in 20 ml test tubes at 20°C, 25 ppt salinity, 12 µE m^{-2} s^{-1} light intensity with a 12:12 h light:dark photoperiod, and fed with the algae *Tetraselmis* sp., *T. chuii*, *Cryptomonas* sp., *Rhodomonas salina* and *R. baltica*. Life table analyses were performed on individual rotifer cultures of 1 ml using these 5 algal diets and 3 food levels (2.10, 3.15 and 4.20 µg ml^{-1} dry wt), at the same temperature, salinity, light intensity and photoperiod as described above. Algae were provided by the Culture Collection of Marine Microalgae, of Instituto de Ciencias Marinas de Andalucía (CSIC). Table 1 shows the cellular concentrations equivalent to the respective food level for each alga tested. Culture medium for rotifers and algae was prepared with filtered and sterilized seawater of 38 ppt (Spanish Mediterranean coast) diluted to 25 ppt and fertilized according to Guillard & Ryther (1962). Algae were grown in 500 ml Erlenmeyer flasks at 20°C and 80 µE m^{-2} s^{-1} light intensity (12:12 h light:dark photoperiod), without aeration. Cultures were renewed daily to maintain a continuous exponential growth phase.

The procedure used for each of the 15 experimental assays was as follows: Pre-experimental cultures were maintained under the 'experimental' conditions for at least 1 wk in 20 ml test tubes at low population density (<5 ind. ml^{-1}). From these cultures, approximately 100 egg-bearing females were selected at random and placed in glass wells (2 females ml^{-1}). After 12 h, 60 newborn females were isolated and cultured individually throughout their lifetime in 24-well plates with 1 ml of fresh culture medium in each well. Offspring were counted every 24 h and mothers were transferred to new trays with freshly prepared medium and food. Algal concentrations in exponentially growing cultures were determined with a haemocytometer and diluted to the desired food level. After daily adjustment of food level, the algae, all of which are motile, were homogenously distributed in culture wells. Rotifers moved through the volume without showing a preference for a particular site. Just before the renewal of the medium, a small percentage of the algae, approximately 10%, remained sedimented at the bottom. This low settling was observed in all algal treatments and had no apparent incidence in the performance of the cultures.

From survival and fecundity schedules, the following demographic parameters were calculated: average lifespan (*LS*), average number of female descendants per female lifetime (R_0), average age of

offspring production (T_c), intrinsic rate of population increase (r) [calculated as $r = \ln R_0 / T_c$], age-specific survival (l_x), age-specific fecundity (m_x = newborns per female) and age-specific mortality (q_x = deaths/living females) (Carey 1993). The effects of algae and food level on *LS* and R_0 were examined using a factorial design for a univariate ANOVA.

For lipid analyses, triplicate 500 ml cultures of *Synchaeta cecilia valentina* were grown under the same conditions and with the same algal foods. Cultures with *Tetraselmis suecica* were also grown, since this alga has been used successfully to feed *S. cecilia valentina* (Oltra & Todolí 1997). An air pump that worked for 15 min each hour was used to keep the algae suspended in the cultures. No continuous aeration was provided, in order to avoid the risk of damaging the rotifers since their resistance is unknown. The initial algal concentration was 3.15 µg ml^{-1} dry wt, with the exception of *Rhodomonas baltica* (5.55 µg ml^{-1}, 60 000 cells ml^{-1}). Initial rotifer density was 1 ind. ml^{-1}. When the density was higher than 50 ind. ml^{-1}, the cultures were filtered through a Nytal 45 µm mesh, washed with diluted sterilised marine water (25 ppt) and transferred to vials filled with chloroform:methanol (2:1, v:v) with 0.01% (w:v) butylated hydroxytoluene (BHT). The vials were capped after flushing with nitrogen and stored at −80°C until the fatty acid analyses were performed.

Lipid and fatty acid analyses were carried out following the procedures described in Navarro et al. (1992). Since rotifers were stored in solvent once harvested from the cultures, no data are available for the absolute percentage of lipids. Methyl esters were injected in a Fisons 8000 series gas chromatograph fitted with an on-column injector, a Tracksil capillary column (30 m, 0.25 mm, 0.15 µm film thickness) and a flame ionisation detector. Helium was used as the carrier gas. Oven temperatures were programmed from 50 to 180°C at 40°C min^{-1} and from 180 to 220°C at 3°C min^{-1}, and then were held at 220°C for 20 min. Peaks were identified and characterised by comparison with known standards.

The most discriminating variables, i.e., those fatty acids common to all algal treatments giving significant differences after univariate ANOVA (<14:0, 14:0, 16:1, 20:1, 20:4n-6, 22:6n-3), were entered in a discriminant analysis model (Navarro et al. 1995) using the algal treatments as the discriminating variable. Prior to the analysis, percentage data were log transformed.

RESULTS

The effects of algal diet and food concentration on the survival and age-specific fecundity of *Synchaeta cecilia valentina* are shown in Fig. 1. The maximum lifespan of the rotifer is longer when cultured with the algae *Tetraselmis* sp., *T. chuii* and *Rhodomonas baltica* (up to 11 d) than when cultured with *R. salina* (up to 9 d) or with *Cryptomonas* sp. (up to 7 d). The lowest values for age-specific fecundity (m_x) appear with *R. baltica*: maximum values from 0.3 to 1, depending on the food concentration. These values are, moreover, obtained at late ages (Days 7 to 9) when the number of surviving females is low, which implies a low number of new borns. When feeding with the algae *Cryptomonas* sp., *T. chuii*, and *Tetraselmis* sp., the m_x values obtained are progressively greater and appear at earlier ages (Days 1, 2, 3, 4). The highest values are for the alga *Tetraselmis* sp., with which a maximum range from 2.4 to 4.5 is reached. Age-specific mortality (q_x) tends to be low on first days as long as the age-specific survival l_x reaches high values and increases at late ages. This tendency can be clearly appreciated with the *Tetraselmis* sp. treatment and to a lesser extent with *T. chuii*. More variations were registered with the rest of the algae due to the high mortalities which occurred at early ages.

In general, the maximum lifespan is longer with the intermediate food level, and shorter when the food concentration is low. With the higher food concentration, the lifespan is either reduced by 1 or 2 d with respect to the medium concentration (*Tetraselmis* sp., *T. chuii*, *Cryptomonas* sp., *Rhodomonas baltica*) or presents the same values (*R. salina*). The m_x values tend to increase along with the food concentration (except with the alga *Cryptomonas* sp.), and the maxima occur earlier (with the exception of the alga *R. baltica*).

In Table 2, the average lifespan (*LS*) and average number of offspring per female (R_0) are shown for each of the 15 assays. It can be seen that the effect of food type is highly significant for both parameters (Table 3). *LS* is longer in cultures with the algae *Tetraselmis* sp. (from 4.4 to 6 d) and *T. chuii* (from 3.7 to 5.1 d) than with the others. The lowest values are for the alga *Cryptomonas* sp. (1.6 to 2.0 d). R_0 is also higher with both *Tetraselmis* species: over 10 in all the assays with *Tetraselmis* sp. and from 3.3 to 6.8 with *T. chuii*. This parameter is greatly reduced, and less than 1, in all treatments with *Rhodomonas baltica*, which indicates a negative growth in *Synchaeta cecilia valentina*.

The effect of food concentration, especially on *LS*, is less clear (Tables 2 & 3). With the algae *Rhodomonas salina*, *Cryptomonas* sp. and *Tetraselmis chuii*, both *LS* and R_0 increase as the food concentration increases in the density range used, whereas with *R. baltica* and *Tetraselmis* sp. *LS* decreases when the algal concentration increases and R_0 reaches its maximum values with the intermediate food concentration.

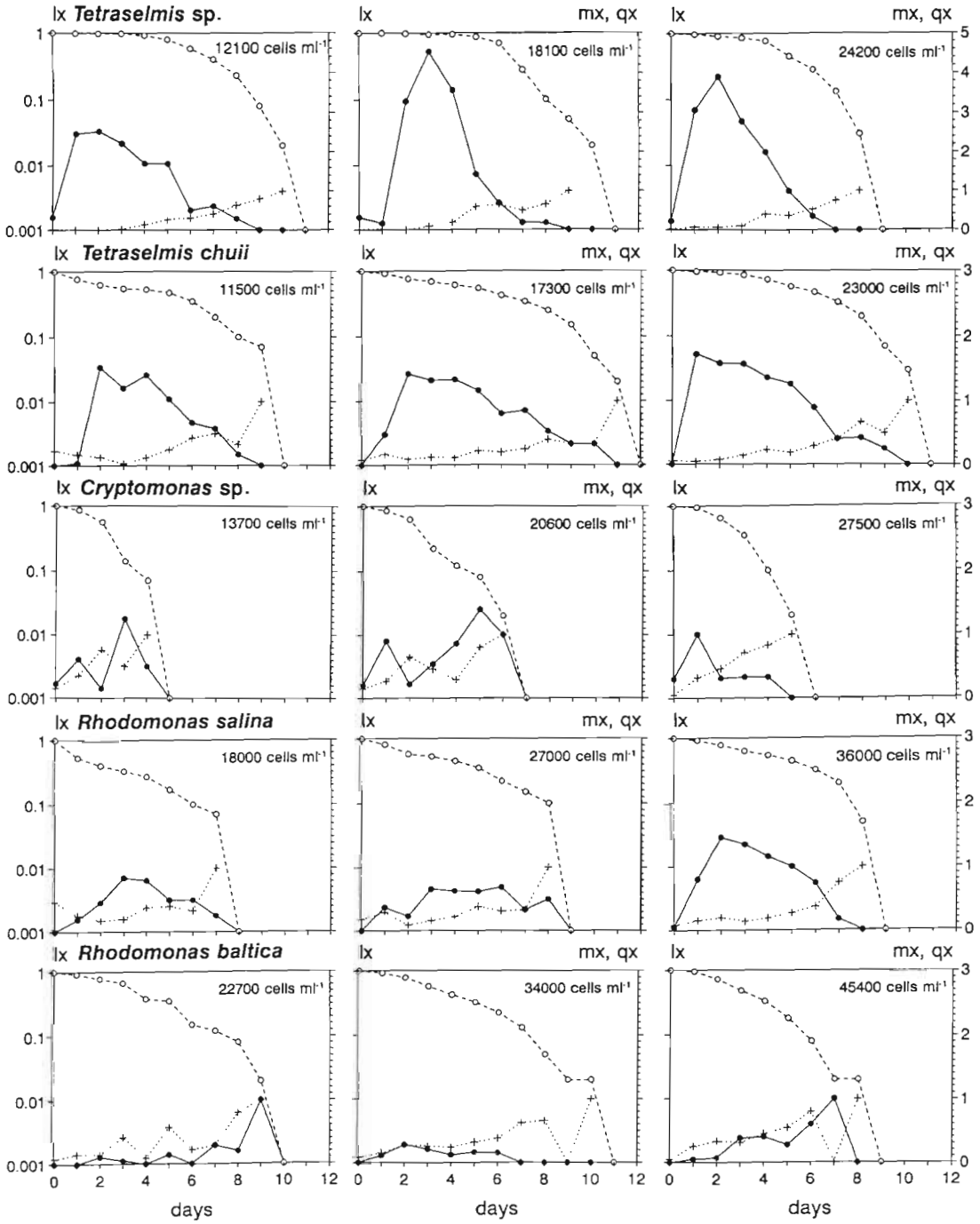

Fig. 1. Age-specific survival (l_x, ○), fecundity (m_x, ●) and mortality (q_x, +) for *Synchaeta cecilia valentina* fed with different algae at several food concentrations. Note that treatment with *Tetraselmis* sp. has a different scale for m_x and q_x

Fig. 2 shows the variations of R_0, cohort generation time (T_c) and intrinsic rate of population increase (r). The lowest values of T_c appear with *Cryptomonas* sp., which also gives the lowest LS values. *Tetraselmis* sp. also offers low values of T_c, indicating that offspring appear early. *Rhodomonas baltica* shows the highest values (except with the medium food concentration) in concordance with the increase in age-specific fecundity at the end of life (Fig. 1). The variations in T_c relative to food concentration are not so clear. It does, however, seem that reproduction of *Synchaeta cecilia valentina* is earlier (lower T_c values) with intermediate or high concentrations.

r varies together with R_0. The greatest values are obtained with *Tetraselmis* sp. (0.84 to 1.12 d^{-1}), and the lowest with *Rhodomonas baltica* (−0.18 to −0.33 d^{-1}).

Table 2. Average lifespan (*LS*) and average number of descendants per female (R_0), with their respective standard errors (SE), for *Synchaeta cecilia valentina* grown at different food levels on 5 different algae

Alga	Food level (µg ml^{-1} dry wt)	n	*LS*	SE	R_0	SE
Tetraselmis sp.	2.10	60	6.0	0.3	10.9	0.4
	3.15	60	4.9	0.2	12.8	0.5
	4.20	60	4.4	0.2	11.3	0.7
Tetraselmis chuii	2.10	60	3.7	0.4	3.3	0.4
	3.15	59	4.7	0.4	4.7	0.5
	4.20	60	5.1	0.3	6.8	0.5
Cryptomonas sp.	2.10	60	1.6	0.1	1.0	0.2
	3.15	60	1.9	0.2	1.4	0.2
	4.20	60	2.0	0.1	1.6	0.2
Rhodomonas salina	2.10	60	2.8	0.3	0.9	0.2
	3.15	60	3.2	0.3	1.5	0.2
	4.20	60	3.9	0.3	4.2	0.4
Rhodomonas baltica	2.10	60	3.6	0.3	0.2	0.1
	3.15	60	3.4	0.3	0.6	0.1
	4.20	60	2.8	0.2	0.5	0.1

Table 3. Univariate ANOVA for *Synchaeta cecilia valentina* average lifespan (*LS*) and average number of offspring per female (R_0) with respect to the 5 different algal treatments and the 3 different food levels

Effect	df	*LS*		R_0	
		F	p	*F*	p
Alga (A)	4, 884	60.9	0.000	181.3	0.000
Food level (F)	2, 884	2.1	0.121	29.3	0.000
A × F interaction	8, 884	7.7	0.000	7.0	0.000

R. salina and *Cryptomonas* sp. present similar values for R_0 at low and intermediate food concentrations although the lower T_c obtained with the alga *Cryptomonas* sp. makes its *r* greater.

Table 4 shows the percentage of the fatty acids from the total lipid of rotifers fed the different algal treatments. Saturated fatty acids accounted for 34 to 40% of total fatty acids, whereas monoenes represented 15 to 23%, and polyunsaturated fatty acids (PUFA) constituted 20 to 29%. Main fatty acids were 16:0 (which ranged from 17 to 22%), 18:0 (10 to 12%), and 18:1 (8.5 to 12%). In certain groups (*Tetraselmis* spp.) 18:3n-3 was moderately abundant (5 to 9%). Eicosapentaenoic acid, 20:5n-3, was markedly lower in *Rhodomonas baltica* fed rotifers (less than 3%), whereas 22:6n-3 was found in higher proportion in *Cryptomonas* sp. (4.9%) and *R. salina* (3.4%). *T. chuii* showed a high proportion of shorter chain fatty acids (<14). In closer detail, 14:0 was higher in rotifers cultured with *Rhodomonas* spp. and *Cryptomonas* sp., 16:0 higher when *T. suecica* and *Tetraselmis* sp. were used as food, 16:1 and 16:3 were higher in rotifers fed *R. baltica*, as was 20:1 in those cultured with *T. chuii*. *Cryptomonas* sp. increased the proportion of n-3 long-chain PUFA such as 22:5n-3 and 22:6n-3. In general, n-6 fatty acids were less variable than n-3 among the different dietary groups.

The first discriminant function explained more than 72% of total variance with a significance level of 0.0116, whereas both first and second discriminant functions explained a cumulated percentage of variance over 90%, although the second function was only significant at $p < 0.15$. The centroids for these first 2 discriminant functions are plotted in Fig. 3. The discriminant analysis model easily separated the algal treatments corresponding to the 2 main groups of algae: *Tetraselmis* spp. versus *Cryptomonas-Rhodomonas* spp. in the first discriminant function.

DISCUSSION

Synchaeta cecilia valentina showed positive growth when fed *Tetraselmis* sp., *T. chuii* and *Crytomonas* sp. at the 3 experimental concentrations. With the alga *Rhodomonas salina* the rotifer grew only with the 2 highest food concentrations. Although no growth was obtained with any of the experimental concentrations of *R. baltica*, the rotifer grew in 500 ml cultures at 5.55 µg ml^{-1} dry wt, which suggests that this microalga can also be used as food when provided at adequate concentrations. All these algae, together with *T. suecica* (Oltra & Todolí 1997), are, until now, the best-known food for use in the culture of *S. cecilia valentina*.

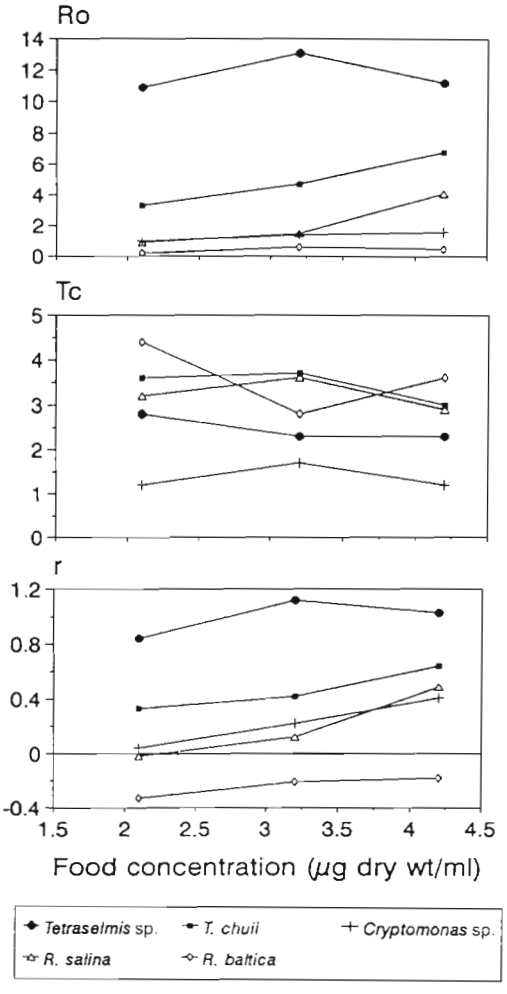

Fig. 2. Effect of algae type and food concentration on net reproductive rate (R_0), cohort generation time (T_c) and intrinsic growth rate (r) of *Synchaeta cecilia valentina*

The Cryptophyceae algae seem to give the best results for culturing species of the *Synchaeta* genus, as they do in fresh water (Stemberger 1981, May 1987, Gilbert & Schreiber 1998) and as in previous research work in salt water (Egloff 1988). The marine species *S. cecilia valentina* does not, in fact, survive in the long term with cultures of only 1 alga, unless it is *Cryptomonas* sp. or another unindentified cryptophyte (strain WH2), which resulted in 2 positive assays out of a total of 37 with algae from 7 groups (Egloff 1988). *S. hutchingsi*, also a marine species, has been cultivated with a mixture of diatoms and flagellates (among others the alga *Tetraselmis chuii*), but not with algal monocultures (Brownell 1988). Notwithstanding this, it has been possible to cultivate *S. cecilia valentina* successfully not only with cryptophyte algae cultures, but also with cultures of chlorophytes (*Tetraselmis* spp.).

The r values obtained with *Tetraselmis* sp. averaged 1.00 d^{-1}, with a maximum of 1.12 d^{-1}, and are higher than those obtained with other algae (Table 5, Fig. 2). Rotifers cultured with *Tetraselmis* sp. also showed the highest lifespan and net reproductive rate. The r values can be considered high when compared with values obtained for other rotifer cultures in salt water at the same temperature (20°C). Egloff (1986) obtained r of 0.63 d^{-1} feeding *S. cecilia valentina* with *Heterocapsa pygmaea* (23 ppt salinity). With the species *Brachionus plicatilis* r values range between 0.1 and 0.5 d^{-1} in life tables at 24 ppt (Miracle & Serra 1989), as in mass cultures for aquaculture (Lubzens et al. 1989, Caric et al. 1993).

Nevertheless, the average lifespan of *Synchaeta cecilia valentina* is clearly lower than that of other rotifers cultured at the same temperature. Serra et al. (1994) obtained values of 8.1 and 9.9 d for *Brachionus plicatilis* fed *Tetraselmis suecica* (24 ppt) and Schmid-Araya (1991) reported values from 11.6 to 16.7 d (18 ppt) for the same rotifer fed on *Brachiomonas submarina* and from 11.5 to 12.4 d for the rotifer *Encentrum linnhei* cultured under the same conditions.

When compared with the cultures using *Tetraselmis* sp. as food, the r values decrease with *T. suecica*, *T. chuii*, *Cryptomonas* sp. and *Rhodomonas salina*. The average value of R_0 decreases in a similar way to r except in the cultures fed with *Cryptomonas* sp. The average R_0 obtained with this alga is 1.4, lower than the value obtained for *R. salina*, although the generation time is also short (1.4 d), which increases the average value of r slightly with respect to *R. salina*.

The selection of the assayed food concentrations was based on preliminary assays in which it was evident that *Synchaeta cecilia valentina* shows a high sensitivity to excessive food concentrations. At 20°C and using *Tetraselmis suecica* as food, R_0 reduces when the algal concentration increases by 66% (2.10 to 3.49 µg ml^{-1} dry wt), whereas exactly the opposite happens at 24°C (Oltra & Todolí 1997). Optimum thresholds of food concentration have been observed in several freshwater species of rotifers (Stemberger & Gilbert 1985) as well as in the euryhaline species *Brachionus plicatilis* and *Encentrum linnhei* (Schmid-Araya 1991).

Food concentration does not have a significant effect on *LS*, but does on R_0 (Table 3). With some algae, *LS* increases with food concentration (*Rhodomonas salina*, *Cryptomonas* sp., *Tetraselmis chuii*), whereas a decrease is produced with others (*R. baltica*, *Tetraselmis* sp.) (Table 2). R_0 tends to increase with food concentration (Table 5). *Tetraselmis* sp. produces the best results at medium food concentration (higher R_0 and r, and lower T_c). From this point of view, *Tetraselmis* sp. is the most adequate diet. The remainder of the algae produce an increase in R_0 and a decrease in T_c, with r increasing at higher food concentrations. Whether r would continue to increase at higher food concentrations has yet to be studied.

Table 4. Main fatty acids (FA) from the total lipids of *Synchaeta cecilia valentina* cultured with different microalgae. Values are expressed as percentages of total fatty acids (mean of 3 replicates). nd: not detected; 0.0: mean values <0.09. PUFA: polyunsaturated fatty acids; HUFA: PUFA longer than 20 C

FA	Algal treatment											
	Tetraselmis suecica		*Tetraselmis chuii*		*Tetraselmis* sp.		*Cryptomonas* sp.		*Rhodomonas salina*		*Rhodomonas baltica*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<14	1.2	0.9	3.1	1.7	2.9	0.7	1.5	0.3	0.5	0.0	1.4	0.2
14:0	2.2	0.4	1.8	0.3	2.1	0.4	3.3	0.4	3.8	0.3	2.9	0.2
14:1	0.4	0.0	0.6	0.2	1.3	0.7	0.7	0.2	0.9	0.1	0.8	0.2
15:0	1.5	0.6	1.2	0.6	1.0	0.1	1.7	0.2	1.4	0.2	2.0	0.5
15:1	0.3	0.1	0.3	0.0	0.3	0.1	0.4	0.0	0.4	0.1	0.5	0.2
16:0	22.2	0.1	19.8	0.8	21.0	1.5	19.9	1.0	19.8	1.5	16.8	1.4
16:1[a]	5.1	2.1	2.5	0.8	2.7	0.8	3.8	0.3	3.9	0.7	7.6	2.4
16:2	0.3	0.1	0.2	0.2	0.1	0.0	0.4	0.0	0.2	0.1	0.5	0.2
17:0	1.0	0.1	1.2	0.3	1.1	0.4	1.4	0.0	1.3	0.0	1.2	0.3
16:3	0.6	0.5	0.4	0.2	0.3	0.1	0.7	0.1	0.6	0.1	1.0	0.3
16:4	nd		nd		nd		0.2	0.1	0.2		nd	
18:0	12.0	1.8	11.5	2.8	12.9	2.0	11.7	0.7	11.4	0.1	9.6	1.0
18:1[a]	10.8	0.7	9.5	0.3	10.8	2.5	8.5	1.9	10.7	0.5	12.2	3.4
18:2n-6	6.0	3.7	3.8	0.5	4.2	0.2	2.8	0.7	4.1	1.5	5.4	2.6
18:3n-6	0.2		nd		nd		nd		nd		nd	
18:3n-3	4.8	1.4	9.1	5.0	6.9	3.8	3.3	1.5	2.8	0.1	2.0	1.2
18:4n-3	3.3	0.8	1.7	1.0	1.7	1.0	3.4	0.9	2.8	0.0	2.3	1.8
20:0	0.8	0.2	0.6	0.2	0.6	0.1	0.7	0.0	0.6	0.0	0.6	0.2
20:1[a]	1.8	0.4	2.1	0.8	1.6	0.5	1.2	0.2	0.6	0.0	0.6	0.3
20:2n-6	1.0	0.4	1.9	0.4	1.7	0.3	1.4	0.2	1.9	0.5	1.9	1.3
20:3n-6	0.4	0.2	0.7	0.3	0.6	0.4	0.6	0.1	0.6	0.0	0.4	0.1
20:4n-6	1.0	0.6	0.8	0.1	0.9	0.3	0.7	0.8	0.3	0.3	0.2	0.0
20:3n-3	0.9	0.4	1.6	1.1	1.1	0.6	1.0	0.4	0.7	0.0	0.3	0.3
20:4n-3	1.0	0.3	0.7	0.4	1.0	0.5	1.1	0.5	1.1	0.2	0.9	1.1
20:5n-3	6.8	2.0	5.7	2.9	4.4	1.7	7.6	2.0	5.0	0.0	2.6	1.7
22:0	0.8	0.5	0.5	0.2	0.7	0.3	0.7	0.1	0.8	0.5	0.5	0.1
22:1[a]	0.9	0.3	1.1	0.5	0.9	0.3	0.5	0.1	0.7	0.5	1.6	1.1
22:2n-6	0.9	0.3	0.5	0.2	0.5	0.2	0.4	0.0	0.2	0.1	0.2	0.1
22:4n-6	nd		nd		nd		nd		0.3		0.1	
22:5n-6	0.1		0.3		0.4	0.3	0.3		1.0		nd	
22:5n-3	0.2	0.1	0.2	0.1	0.1	0.1	0.6	0.2	0.4	0.0	0.6	0.1
22:6n-3	0.7	0.3	0.7	0.3	1.1	0.1	4.9	1.5	3.4	0.1	1.8	1.2
Saturates	40.5	2.6	36.6	5.0	39.4	3.3	39.3	0.9	39.1	2.6	33.7	2.6
Monoenes	19.2	2.2	16.2	0.6	17.5	3.4	15.0	2.2	17.2	0.9	23.3	5.8
PUFA	28.0	2.5	28.3	10.0	24.9	7.5	28.9	6.4	24.8	0.5	20.0	5.7
n-3	17.8	5.2	19.9	10.5	16.2	7.6	21.9	6.6	16.2	0.1	10.3	7.3
n-6	9.3	2.2	7.9	0.2	8.3	0.4	5.8	0.8	7.7	0.3	8.2	1.9
HUFA n-3	9.6	3.1	9.0	4.7	7.7	2.8	15.2	4.5	10.6	0.2	5.9	4.4
HUFA n-6	3.3	1.4	4.1	0.6	4.1	0.6	3.0	0.8	3.6	1.1	2.8	1.2
dha/epa	0.1		0.1		0.3		0.6		0.7		0.7	

[a]May contain other monoenes

There are, also, other aspects that require further studies. One of these is the contradictory result obtained in the cultures of *Synchaeta cecilia valentina* with *Rhodomonas baltica* under different conditions in 500 ml cultures. One possible explanation for this may lie in the greater concentration of food in the 500 ml cultures, and the use of aeration to keep the alga suspended. Another related aspect is whether all the algae were equally available for the rotifers. We have found no reasons to think otherwise considering that all the algae assayed are motile, that daily renewal of food involves necessarilly homogenization and that no irregularities were detected in any of the treatments. Besides, the results of the 500 ml cultures followed the same trends as those of the life tables, i.e., better growth with algae of *Tetraselmis* genus than with *Cryptomonas* and *Rhodomonas*.

The fatty acid composition of the rotifers fed different algal diets showed high variability within the same dietary treatment. This, together with the fairly in-

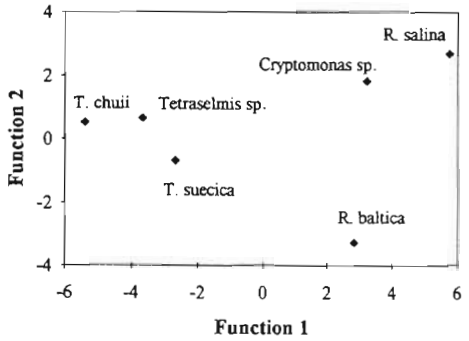

Fig. 3. Centroids for first 2 discriminant functions of fatty acid data from total lipid of the rotifer *Synchaeta cecilia valentina* cultured with different microalgae

significant differences found among the dietary groups, resulted in few statistically significant differences for many fatty acids. Even though the fatty acid composition of algal species is variable and depends on many phenotypic factors, some trends can be identified. Generally speaking, the main difference between Chlorophyceae and Cryptophyceae is the higher concentration of n-3 PUFA in the latter. Both *Cryptomonas* sp. and *Rhodomonas* spp. are rich in 18:4n-3 and 18:3n-3 (23 to 31%), and moderate in 20:5n-3 (8 to 12%) and 22:6n-3 (4 to 7%). Their 16:0 level is lower than in *Tetraselmis* species, which have higher 16:4n-3 (16%), high 18:3n-3 (18 to 22%) and moderate 20:5n-3 (4%), together with very low, if any, 22:6n-3 (Renaud et al. 1999, see also Pohl & Zurheide 1979). To a certain extent, this is reflected in the fatty acid profile of the rotifers fed both kinds of algae.

It has been postulated that the fatty acid composition of rotifers resembles that of their algal food (Ben-Amotz et al. 1987, Frolov et al. 1991). This may be true in general terms, but a metabolic transformation by the rotifers has also been suggested (Frolov et al. 1991). A deeper analysis of some of the data available reveals a particularly striking lack of correspondence between some of the percentages of certain fatty acids in the algal food and in the rotifers. A good example can be found in the data reported by Ben-Amotz et al. (1987) regarding the fatty acid profiles of *Brachionus plicatilis* when cultured with different algal species and the corresponding fatty acid profiles of the microalgal feed species. *Chaetoceros gracilis*, which had 4.1% of their total fatty acids as 20:5n-3, produced levels of 2.8% of the same fatty acid in the rotifers, whereas *Nannochloropsis salina*, rich in 20:5n-3 (14.8%), produced rotifers with only 6.3% of the same fatty acid. *Isochrysis galbana*, relatively rich in 22:6n-3 (3.4%), produced rotifers with the highest 22:6n-3 proportion, but other algae lacking this fatty acid (*Chaetoceros gracilis*) also induced the presence of 22:6n-3 (1%) in the rotifers. The same lack of direct correlation can be found for rotifers fed other algae for different fatty acids in the same work and in others (Frolov et al. 1991).

Considering these facts, together with the abundant variability among replicate cultures of the same microalgal fed species, it seemed to us to be more accurate to use a multivariate chemometric method (see Navarro et al. 1995) to study the effect of algal food. The use of chemometric techniques such as the multivariate discriminant analysis used here does, however, produces clear separation between the rotifers fed on the 2 main algal groups: Chlorophyceae (*Tetraselmis* species) and Cryptophyceae (*Rhodomonas* and *Cryptomonas* species). This effect is clear in Fig. 3 with the presence of 2 main groups of rotifers fed the 2 main classes of algae.

Table 5. Mean values of demographic parameters (average lifespan, *LS*, average number of offspring per female, R_0, cohort generation time [T_c] and intrinsic growth rate [r]) for each experimental alga and food concentration. Table includes results from previous assays with *Tetraselmis suecica* at the same temperature and salinity (Oltra & Todolí 1997)

	n	*LS*	SE	R_0	SE	n	T_c	SE	r	SE
Alga										
Tetraselmis sp.	180	5.1	0.1	11.7	0.3	3	2.5	0.1	1.00	0.07
T. chuii	179	4.5	0.2	4.9	0.3	3	3.4	0.2	0.46	0.07
T. suecica[a]	144	4.2	0.3	7.7	0.5	2	3.0	0.1	0.68	0.02
Cryptomonas sp.	180	1.8	0.1	1.4	0.1	3	1.4	0.1	0.22	0.08
R. salina	180	3.3	0.2	2.2	0.2	3	3.2	0.2	0.20	0.12
R. baltica	180	3.3	0.2	0.4	0.1	3	3.6	0.4	−0.24	0.03
Food concentration (μg ml^{-1} dry wt)										
2.10	300	3.3	0.2	3.3	0.3	5	2.8	0.5	0.33	0.18
3.15	299	3.6	0.1	4.2	0.3	5	2.8	0.3	0.33	0.20
4.20	300	3.6	0.1	4.9	0.3	5	2.6	0.4	0.48	0.18

[a]Mean values of 2 tests (n = 72 in each) made with food concentration of 2.10 and 3.49 μg ml^{-1} dry wt, respectively

In summary, it has been possible to culture the marine rotifer *Synchaeta cecilia valentina* with 5 different monoalgal diets. The effect that these diets and different food levels have on several demographic parameters of this rotifer has been determined. *Tetraselmis* sp. provided the best results. The comparison of our data on fatty acids in *S. cecilia valentina* fed different algal food with other information available on the fatty acids of *Brachionus plicatilis* cultured with microalgal feed species leads us to conclude that cultures of both rotifers should have similar nutritional value regardless of their marine 'versus' mixohaline ecological origin.

Acknowledgements. This study was supported by Spanish Culture and Education Ministry, project MAR98-0871-C02-01/02. We thank A. Gómez and M. Serra for their suggestions.

LITERATURE CITED

Ben Amotz A, Fishler R, Schneller A (1987) Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar Biol 95:31–36

Brownell CL (1988) A new pelagic marine rotifer from the southern Benguela, *Synchaeta hutchingsi*, n. sp., with notes on its temperature and salinity tolerance and methods of culture. Hydrobiologia 162:225–233

Carey JM (1993) Applied demography for biologists. Oxford University Press, New York

Caric M, Sanko-Njire J, Skaramuca B (1993) Dietary effects of different feeds on the biochemical composition of the rotifer (*Brachionus plicatilis* Müller). Aquaculture 110:141–150

Egloff DA (1986) Effects of *Olisthodiscus luteus* on the feeding and reproduction of the marine rotifer *Synchaeta cecilia*. J Plankton Res 8:263–274

Egloff DA (1988) Food and growth relations of the marine microzooplankter, *Synchaeta cecilia* (Rotifera). Hydrobiologia 157:129–141

Frolov AV, Pankov SL, Geradze KN, Pankova SA, Spektorova LV (1991) Influence of the biochemical composition of food on the biochemical composition of the rotifer *Brachionus plicatilis*. Aquaculture 97:181–202

Gilbert JJ, Schreiber DK (1998) Asexual diapause induced by food limitation in the rotifer *Synchaeta pectinata*. Ecology 79:1371–1381

Guillard RL, Ryther JH (1962) Studies of marine plankton diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve). Can J Microbiol 8:229–239

Lubián LM, Yúfera M (1989) Colección de cepas de microalgas del Instituto de Ciencias Marinas de Andalucía (CSIC). In Yúfera M (ed) Acuicultura intermareal. Inst Cien Mar Andalucía, Cádiz, p 69–78

Lubzens E, Tandler A, Minkoff G (1989) Rotifers as food in aquaculture. Hydrobiologia 186/187:387–400

Maruyama I, Nakao T, Shigeno I, Ando Y, Hirayama K (1997) Application of unicellular algae *Chlorella vulgaris* for the mass-culture of marine rotifer *Brachionus*. Hydrobiologia 358:133–138

May L (1987) Culturing freshwater, planktonic rotifers on *Rhodomonas minuta* var. nannoplanctica Skuja and *Stichococcus bacillaris* Nägeli. J Plankton Res 9:1217–1223

Miracle MR, Serra M (1989) Salinity and temperature influence in rotifer life history characteristics. Hydrobiologia 186/186:81–102

Navarro JC, Amat F, Sargent JR (1992) Fatty acid composition of coastal and inland *Artemia* sp. populations from Spain. Aquaculture 102:219–230

Navarro JC, McEvoy LA, Amat F, Sargent JR (1995) Effects of diet on fatty acid composition of body zones in larvae of the sea bass *Dicentrarchus labrax*: a chemometric study. Mar Biol 124:177–183

Oltra R, Todolí R (1997) Effects of temperature, salinity and food level on the life history traits of the marine rotifer *Synchaeta cecilia valentina*, n. subsp. J Plankton Res 19: 693–702

Pohl P, Zurheide F (1979) Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Hoppe HA, Levring T, Tanaka Y (eds) Marine algae in pharmaceutical science. Walter de Gruyter, Berlin, p 473–523

Pourriot R (1965) Récherches sur l'écologie des Rotifères. Vie Milieu Suppl 21:1–224

Renaud SM, Thinh L, Parry DL (1999) The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture 170:147–159

Rezeq TA, James CM (1987) Production and nutritional quality of the rotifer *Brachionus plicatilis* fed marine *Chlorella* sp. at different cell densities. Hydrobiologia 147:257–261

Schmid-Araya JM (1991) The effect of food concentration on the life histories of *Brachionus plicatilis* (O F M) and *Encentrum linnhei* Scott. Arch Hydrobiol 121:87–102

Segers H (1995) Nomenclatural consequences of some recent studies on *Brachionus plicatilis* (Rotifera, Brachionidae). Hydrobiologia 313/314:121–122

Serra M, Carmona MJ, Miracle MR (1994) Survival analysis of three clones of *Brachionus plicatilis* (Rotifera). Hydrobiologia 227:97–105

Snell TW, Janssen CR (1995) Rotifers in ecotoxicology: a review. Hydrobiologia 313/314:231–247

Stemberger RS (1981) A general approach to the culture of planktonic rotifers. Can J Fish Aquat Sci 38:721–724

Stemberger RS, Gilbert JJ (1985) Body size, food concentration, and population growth in planktonic rotifers. Ecology 66:1151–1159

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: June 9, 1999; Accepted: September 22, 1999
Proofs received from author(s): February 11, 2000