Marine Biology (2001) 139: 975–979
DOI 10.1007/s002270100632

D.J.S. Montagnes · S.A. Kimmance · G. Tsounis
J.C. Gumbs

Combined effect of temperature and food concentration on the grazing rate of the rotifer *Brachionus plicatilis*

Received: 6 April 2001 / Accepted: 8 May 2001 / Published online: 28 June 2001

Abstract We developed a predictive relationship to determine the grazing rate of *Brachionus plicatilis* at given temperatures and food concentrations; this function could be applied to experimental culturing and aquaculture practices. Grazing experiments were conducted at temperatures between 5°C and 40°C and at food concentrations, of the flagellate *Isochrysis galbana*, ranging between ~0 and 10^6 ml^{-1}. In total, 136 grazing rates were determined, using the prey depletion method, for rotifers acclimated to treatments for 0.5 or 4 h. The response of grazing rate to temperature and food concentration was described using a model that combined a rectangular hyperbolic function for food concentration and a sigmoidal function for temperature. Using non-linear curve-fitting methods an equation was obtained: $G = (45 \times F)/(159000 + F) \times 0.94/(1 + 219000 \times T^{-4.35})$, where G is the grazing rate (flagellates rotifer^{-1} min^{-1}), F is the food concentration (flagellates ml^{-1}), and T is temperature (°C). The equation indicates a maximum grazing rate of ~35 prey rotifer^{-1} min^{-1}, above ~4×10^5 prey ml^{-1} and 25°C.

Introduction

Rotifers are an excellent food source for many fish larvae (Lubzens 1987); they may also be major grazers of primary producers (e.g. Hernroth 1983). Specifically, the rotifer *Brachionus plicatilis* has contributed to the successful hatchery production of more than 60 marine finfish and 18 crustacean species (Dhert 1996), and in many countries the success of mass production of marine fish larvae is largely dependent on the availability of *B. plicatilis* (Pillay 1990). The genus *Brachionus* has a cosmopolitan distribution, in fresh and saline waters, and it is easy to culture; thus *Brachionus* provides a model experimental organism for practical and theoretical studies of a trophic or ecophysiological nature (Starkweather 1980; Rothhaupt 1990a, b).

Considerable data exist for the genus *Brachionus* (e.g. Starkweather 1980; Lubzens 1987), and much of this work has examined the grazing rate of *B. plicatilis* (e.g. Rothhaupt 1990a, b; B. Hansen et al. 1997; Navarro 1999). Like that of many planktonic organisms, the grazing of *B. plicatilis* is influenced by food concentration (B. Hansen et al. 1997) and temperature (Hirayama and Ogawa 1972; Scott and Baynes 1978; Dhert 1996). Both food concentration and temperature vary under natural conditions and can be controlled when maintaining rotifer cultures. Thus, knowledge of the combined impact of these two factors is needed, for example, for researchers growing experimental organisms, for efficient aquaculture practices, and ideally for ecosystem modelling. However, data are lacking on the combined effect of temperature and food concentration on the grazing rate of *B. plicatilis*.

Independently, temperature and food concentration are two of the main factors affecting the grazing rate of rotifers (Hirayama and Ogawa 1972; Galkovskaja 1987; Dhert 1996; Navarro 1999). Temperature affects biological rates directly by altering the rate of chemical reactions, and indirectly by altering viscosity and diffusion (Cossins and Bowler 1987). Food concentration affects feeding by determining the rate at which a grazer encounters food items (Begon et al. 1986). This study examined the combined effects of ambient temperature and concentration of the prey species *Isochrysis galbana*, which is a good food source for *B. plicatilis* (Scott and Baynes 1978; Hoff and Snell 1987). Our objective was to develop a predictive relationship that will allow the

Communicated by J.P. Thorpe, Port Erin

D.J.S. Montagnes (✉) · S.A. Kimmance · G. Tsounis
J.C. Gumbs
Port Erin Marine Laboratory, University of Liverpool,
School of Biological Sciences, Port Erin,
Isle of Man IM9 6JA

E-mail: dmontag@liv.ac.uk
Tel.: +44-1624-831008
Fax: +44-1624-831001

grazing rate of *B. plicatilis* to be determined at any given temperature and food concentration. Such a relationship could be applied to culturing practices. However, this study is also one aspect of our ongoing work to determine general relationships between temperature and other abiotic and biotic factors on rate processes and composition of plankton (e.g. Weisse and Montagnes 1998; Montagnes and Franklin, submitted; Montagnes et al., submitted); such information will ultimately be applicable to ecosystem modelling.

Materials and methods

Algae and rotifers

The flagellate *Isochrysis galbana* (~5–10 μm, 7–20 pg C cell^{-1}, 1–3 pg N cell^{-1}, Montagnes et al. 1994; Montagnes and Franklin, submitted) was grown in batch cultures, in f/2 medium (Guillard 1975). Flagellates were maintained in exponential growth phase in semi-continuous cultures, at 15–18°C, at an irradiance of ~50–100 μmol photons m^{-2} s^{-1}, on a 14:10 light:dark cycle. Amictic female rotifers, *Brachionus plicatilis* (200–300 μm), were obtained from the Larval Rearing Centre at Port Erin Marine Laboratory, where they were maintained at 20–23°C at saturating food levels, on a mixed diet of *Nannochloropsis occulata* and yeast (Fermipan, Gist-Brocades, The Netherlands).

Experiments

Prior to the experiment, rotifers were collected onto a 45-μm mesh and rinsed with filtered seawater (Whatman GF/C) to remove flocculent material and ciliates that occasionally contaminate cultures, to remove prey from the stock cultures, and to concentrate rotifers. The rotifers were then resuspended in filtered seawater (32 ppt), and their abundance was determined by replicate counts using a 1-ml counting chamber, after fixation with 2% Lugol's iodine.

All experiments were conducted in filtered seawater (32 ppt). To initiate experiments, rotifers were added to 250-ml conical flasks containing 100–200 ml of filtered seawater with *I. galbana* at various prey concentrations. These flasks were suspended in controlled water baths at experimental temperatures. Final rotifer concentration in the experimental flasks was 50–100 ml^{-1}. Prior to experiments, rotifers were acclimated to experimental conditions for 0.5 or 4 h (see Figs. 1, 2). A total of 136 such incubations were prepared at various food concentrations and temperatures (see Figs. 1, 2); no replication was conducted at any one temperature–food-concentration combination.

After the acclimation period, samples were taken approximately every 10 min for 0.5–1 h. At each interval flasks were gently shaken, and 1 ml was removed. Prey concentrations were determined using a Coulter Counter (Multisizer II, Fla., USA), fitted with a 70-μm aperture.

To determine if flagellate growth or mortality occurred during the experiments, controls containing only *I. galbana* were established. These flasks experienced the same experimental conditions as the ones containing *I. galbana* and rotifers. These incubations indicated that *I. galbana* numbers did not significantly change over the experimental period when rotifers were not present (regression analysis $\alpha = 0.05$).

Grazing rate calculations

Grazing rates were determined by estimating the reduction in food concentration within an experimental flask over time, using least squares linear regression. Grazing per rotifer was calculated by dividing the slope of the resulting regression by the rotifer concentration used in the individual flask. The corresponding food concentration for each grazing rate experiment was calculated as the geometric mean over the incubation time.

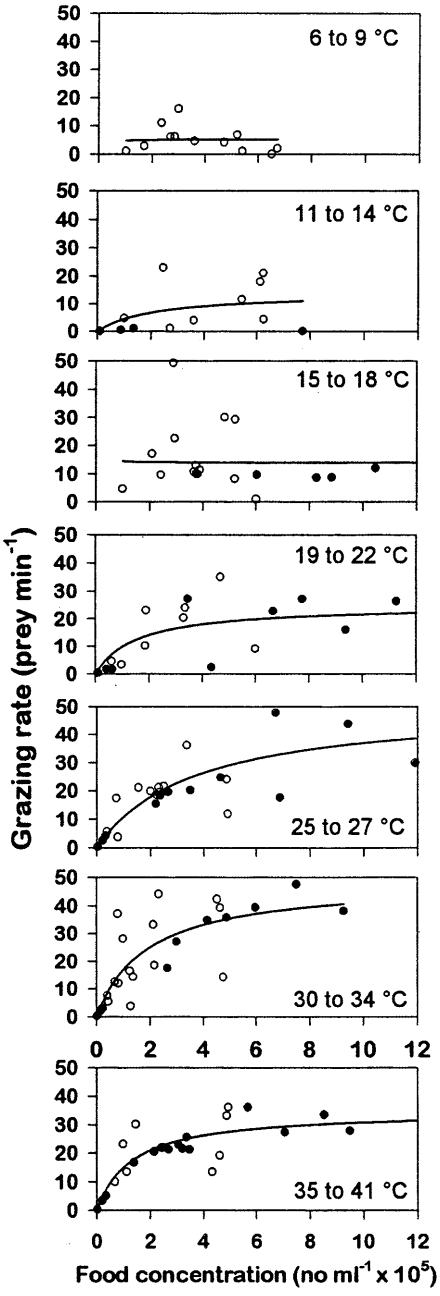

Fig. 1 *Brachionus plicatilis*. Response of grazing to prey concentration (*Isochrysis galbana*); experiments conducted in 3–6°C ranges (*blocks*) are collectively presented to indicate temperature-independent functional responses. *Points* are individual grazing rates; *solid* and *open points* represent experiments where rotifers were acclimated to treatment conditions for 4 h and ~0.5 h, respectively. The *lines* are the best fit to the data described by the functional response (for details see Results)

Modelling grazing rates

A multiple regression and an associated response plane were established from the data. Initially, several different models were

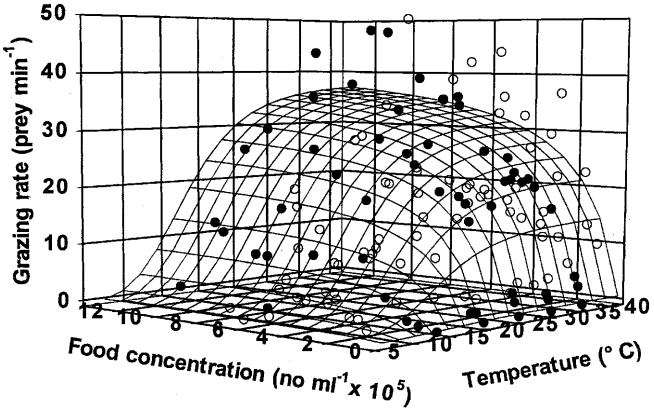

Fig. 2 *Brachionus plicatilis*. Response of grazing to prey concentration (*Isochrysis galbana*) and ambient temperature. *Points* are individual grazing rates; *solid* and *open points* represent experiments where rotifers were acclimated to treatment conditions for 4 h and ~0.5 h, respectively. The *grid* is the best fit to the data described by Eq. 2 (for details see Materials and methods and Results)

examined. The data were finally fit to Eq. 1, consisting of a Holling type 2 curve (functional response) and a sigmoidal function (Spain 1982). The Holling type 2 curve described the response of grazing rate to food concentration, and the sigmoidal function described the response of grazing rate to temperature.

$$G = (m \times F)/(k + F) \times a/(1 + bT^c) \quad (1)$$

where G is the grazing rate (flagellates rotifer^{-1} min^{-1}); F is the food concentration (flagellates ml^{-1}); T is temperature (°C); and m, k, a, b, and c are constants. The equation was fit to the data using the Marquardt–Levenberg algorithm (SigmaPlot 5.0, SPSS Inc., Ill., USA), which determines the coefficients of independent variables that give the best fit between the equation and the data.

Results

There was no apparent difference in response obtained from rotifers acclimated for 0.5 and 4 h (Figs. 1, 2). All data, available on request from the corresponding author, are presented in Fig. 1 in temperature blocks (ranging from 3°C to 6°C); these panels provide "slices" of the entire data set presented in Fig. 2. Grazing rate increased with temperature and food concentration (Figs. 1, 2). Grazing rates were more variable in blocks at lower temperatures (Fig. 1), although this partially reflects the response to temperature within blocks (cf. Fig. 2). Holling type 2 functional responses [$G = m \times F/(k + F)$; see Eq. 1 above for symbols] were fit to the data within blocks to illustrate trends. However, the variability of data within blocks and the lack of data at some low temperatures (e.g. block 15–18°C) resulted in some poor fits. Rather than treating the data in discrete sections, all data were combined (Fig. 2).

A number of predictive equations that were modifications of Eq. 1 were fit to all the data (not shown); the best fit, determined by assessing the error terms presented by the statistical analysis (standard error of estimates, adjusted R^2, analysis of variance at $\alpha = 0.05$; SigmaPlot 5.0) was obtained with Eq. 1. The data and the predictive response plane (Fig. 2) indicate that grazing rate increased with increasing temperature and food concentration; the fit has a rectangular-hyperbolic response to food concentration, regardless of temperature, and a sigmoidal relationship with temperature, regardless of food concentration (Fig. 2). That is, interactions between the effect of food concentration and temperature on grazing rate were negligible; results from models that included such terms did not provide an improved fit.

For any one temperature, between ~0 and 2×10^5 cells ml^{-1}, grazing rates rapidly increased and then gradually increased to a maximum of between 2 and 4×10^5 cells ml^{-1}. Similarly, as temperature increased from 5 to 25°C, grazing rate gradually increased from near zero to the maximum for that food concentration. Thus, a maximum grazing rate of ~30–40 cells rotifer^{-1} min^{-1} occurred above 4×10^5 cells ml^{-1} and 25°C (Figs. 1, 2). Some scatter occurred around the best fit to the data, and maximum grazing rates in some cases reached 50 cells rotifer^{-1} min^{-1} (Figs. 1, 2).

Equation 1 provided a significant relationship ($P < 0.0001$) with a reasonable predictive ability (adjusted $R^2 = 0.56$). The formula, with fitted parameters, is presented in Eq. 2:

$$G = (45 \times F)/(159000 + F) \times 0.94/(1 + 219000 \times T^{-4.35}) \quad (2)$$

where G is the grazing rate (flagellates rotifer^{-1} min^{-1}), F is the food concentration (flagellates ml^{-1}), and T is temperature (°C).

Discussion

The main aim of this study was to establish a predictive equation for the grazing response of *Brachionus plicatilis* experiencing various temperatures and offered various food concentrations of the flagellate *Isochrysis galbana*. Our data indicate a sigmoidal response of grazing rates to increasing temperature (Fig. 2); this agrees with previous work (Hirayama and Ogawa 1972). Raising temperature may influence grazing rate by increasing the rate of chemical reactions, such as enzyme kinetics, and by reducing membrane and water viscosity, thus increasing diffusion (Cossins and Bowler 1987). The combination of these effects may increase prey encounter rate and digestion. Enzyme kinetics are expected to change exponentially with temperature. However, at high temperatures, rate processes typically cease to increase, and at extremely high temperatures there is inhibition, often attributed to enzyme denaturation (Cossins and Bowler 1987). We did not observe a strong inhibitory effect at temperatures as high as 40°C, but the combination of an exponential increase and some inhibition probably produced the sigmoidal response observed in this study.

Typically, physiologists and many ecologists anticipate that, over a given temperature range, rate processes such as grazing rate will increase exponentially, following a Q_{10} relationship (Cossins and Bowler 1987). Although we observed, and modelled, a sigmoidal function, the initial portion of this function can be used to approximate a Q_{10} function. When the temperature-related portion of Eq. 2 was used to estimate rates between 5 and 10°C, the Q_{10} calculated from these values is 2.5. This value is close to a Q_{10} of 2.8, considered to be an average for zooplankton rate processes (P.J. Hansen et al. 1997) and is well within the range of 2–3 considered to be typical for many biological rates (Cossins and Bowler 1987).

Assuming grazing rate is a reasonable proxy for growth and that some inhibition occurs at high temperatures, our data seem to support the recommendation of Dhert (1996) that optimal growth would occur between 28 and 35°C. However, Hirayama and Kunsano (1972), analysing a variety of growth, survivorship, and fecundity parameters, indicated that 25°C is an optimum temperature for growth of *B. plicatilis* cultures. The grazing response becomes asymptotic near 20–25°C (Fig. 2), indirectly supporting the recommendations of Hirayama and Kunsano (1972). Thus, we support the recommendation that ~25°C is a suitable temperature for culturing *B. plicatilis*.

The data also indicate that the grazing rate for *B. plicatilis* follows a Holling type 2 (rectangular-hyperbolic; Spain 1982) response with increasing food concentration (Figs. 1, 2); this is consistent with other work for *B. plicatilis* (B. Hansen et al. 1997) and for other species of *Brachionus* fed optimal-sized prey (Rothhaupt 1990b). However, others suggest that Holling type 1 (rectilinear; Korstad et al. 1989) or Holling type 3 (sigmoidal; Navarro 1999) responses better fit the functional response for *B. plicatilis*. In practice, it is difficult to distinguish differences between these responses, and for most aquaculture purposes and ecosystem modelling there is little need to do so; nevertheless Rothhaupt (1990b) has approached this problem, recognising that prey size may alter the shape of the response. To test this hypothesis adequately would require substantially more rate measurements at low food concentrations than were collected in this study.

The point where grazing becomes asymptotic with increasing food concentration (also called the incipient limiting level, Rothhaupt 1990b) can, however, be of considerable practical and theoretical interest, as above this point an increase in prey does not raise ingestion rate. Observations from four studies on *B. plicatilis*, fed various prey species, indicated that grazing rate increases with increasing food concentration until a plateau is reached (Korstad et al. 1989; B. Hansen et al. 1997; Navarro 1999; this study). Only one of these studies can be directly compared to our work: for *B. plicatilis* feeding on *I. galbana* at 20–22°C, and a salinity of 22 ppt, Korstad et al. (1989) observed a maximum of 10–45 prey grazed min^{-1} above $\sim 4\times10^5$ prey ml^{-1} (prey number converted from carbon values, assuming 7 pg C $cell^{-1}$ for *I. galbana*; Montagnes et al. 1994); this generally agrees with our data. We suggest that feeding *B. plicatilis* $>4\times10^5$ *I. galbana* ml^{-1} will not improve its grazing rate. The point below which ingestion decreases with decreasing food concentration also indicates that under most natural conditions, where small flagellates occur at levels $<10^4$ prey ml^{-1}, rotifers are likely to be food limited. Thus, natural rotifer populations may typically be food limited, that is, controlled by bottom-up factors, rather than by predation by upper trophic levels.

Although our data appear to agree reasonably well with those in the literature, there are caveats that require recognising. First, although generalities can be made, our data should ideally only be applied to grazing rates for *B. plicatilis* feeding on *I. galbana*. Feeding rate can vary with food quality (Doohan 1973) and cell type and size (Starkweather 1980; Rothhaupt 1990b; B. Hansen et al. 1997; Navarro 1999). Second, although we acclimated the rotifers to experimental conditions for 0.5 or 4 h, this would not be sufficient to alter their physiology substantially. Several generations would be required to acclimate fully the rotifer population to each treatment combination; in practice this is exceedingly difficult. Our data, thus, represent the grazing rate of a population that has entered an environment with a new temperature and food concentration for a relatively short period. Such conditions may occur in practice, for example, in cultures due to periodic transfers and rapid experimental temperature shifts, and they may occasionally occur in nature, for example, due to blooms of phytoplankton. However, our data may not relate to long-term shifts. Now that we have recognised, and successfully modelled, the extent of the short-term response, we plan, in future studies, to examine long-term effects. Third, grazing on prey by *B. plicatilis* is size dependent (B. Hansen et al. 1997), and the size of *I. galbana* changes with long-term exposure to temperature (Montagnes and Franklin, submitted). Thus, our findings are based on the feeding of rotifers on food grown at a single temperature, and altering the prey growth temperature could alter the grazing rate. Finally, rotifer strains may respond differently (Hoff and Snell 1987; Serra and Miracle 1987), and some caution must be applied to extending our results to all strains of *B. plicatilis*.

Application of the model to the growth and culture of rotifers

Several factors are necessary for optimal growth of the rotifers, for example, a good food supply, high temperatures, and good water quality (Pillay 1990; Dhert 1996). Our data imply that grazing rate can be predicted for any combination of temperature and food concentration. This could be useful to ensure that rotifers cultured at a certain temperature are well fed.

Rotifers that are starved have a low nutritional value (Dhert 1996), fish larvae do not grow well on them (Pillay 1990), and rotifers invest limited food resources into body growth rather than egg production (Navarro 1999). Equation 2 could be used to calculate how fast a known density of rotifers will deplete a known food concentration at any given temperature, so that rotifers are not starved. However, in cultures, it is also important not to overfeed rotifers, as overfeeding leads to food particles being ingested but not digested (Doohan 1973; Galkovskaja 1987), potentially resulting in poor water quality (Dhert 1996). Good water quality is needed to reduce ciliate and bacterial contaminants and to prevent rotifers from reproducing sexually (Pillay 1990; Dhert 1996). Similarly, under natural conditions, when there is a surplus of food, rotifers may become ''sloppy feeders'', providing dissolved and particulate material that may fuel the microbial food web (Azam et al. 1983). Thus, a predictive equation that can estimate how fast a grazer is likely to deplete its food will be important to ensure that rotifers are not overfed and may be useful in determining the role of rotifers as links or sinks of material in the transfer of small phytoplankton up the food chain.

Equation 2 also indicates that grazing rates become asymptotic above 25°C (Fig. 2). Therefore, we recommend that researchers who grow rotifers avoid culturing rotifers at temperatures much above 25°C, as this would only increase heating costs without improving the grazing rate. These conclusions support recommendations to culture rotifers at an optimum temperature for reproduction of ~25°C (Hirayama and Kunsano 1972; Lubzens 1987; Dhert 1996). Finally, we suggest that grazing rate studies, using *B. plicatilis*, should be performed at temperatures between 25°C and 30°C; this provides two advantages: experiments will be rapid and temperature fluctuations of a few degrees should not bias the experiment, as grazing is relatively invariant in this range.

Acknowledgements We thank Dan Franklin, Chris Lowe, Jason Mallard, and Nick Wade for their technical assistance. We also thank the undergraduate students in the University of Liverpool's course BIOL 122, for their participation in portions of this study. We are grateful to Nick Fullerton and the Larval Rearing Centre staff for providing rotifers. Finally, we thank the anonymous reviewers for their constructive comments on the manuscript.

References

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Begon M, Harper JL, Townsend CR (1986) Ecology: individuals populations and communities. Blackwell, Oxford

Cossins AR, Bowler K (1987) Temperature biology of animals. Chapman and Hall, London

Dhert P (1996) Rotifers. In: Lavens P, Sorgeloos P (eds) Manual on the production and use of live food for aquaculture. (FAO technical paper) Food and Agriculture Organization of the United Nations, Rome, pp 61–98

Doohan M (1973) An energy budget for adult *Brachionus plicatilis* Muller (Rotatoria). Oecologia 13: 351–362

Galkovskaja GA (1987) Planktonic rotifers and temperature. Hydrobiologia 147:307–317

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum, New York, pp 29–60

Hansen B, Wernberg-Møller T, Wittrup L (1997) Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J Exp Mar Biol Ecol 215:217–233

Hansen PJ, Bjørnsen PK, Hansen BW (1997) Zooplankton grazing and growth: scaling within the 2–2,000-μm body size range. Limnol Oceanogr 42:687–704

Hernroth L (1983) Marine pelagic rotifers and tintinnids – important trophic links in the spring planktonic community of the Gullmar Fjord, Sweden. J Plankton Res 5:835–846

Hirayama K, Kunsano T (1972) Fundamental studies on physiology of rotifer for its mass culture II. Influence of water temperature on population growth of rotifer. Bull Jpn Soc Sci Fish 38:1357–1363

Hirayama K, Ogawa S (1972) Fundamental studies on physiology of rotifer for its mass culture I. Filter feeding of rotifer. Bull Jpn Soc Sci Fish 38:1207–1214

Hoff FH, Snell TW (1987) Plankton culture manual. Florida Aqua Farms, Dade City

Korstad J, Vadstein O, Olsen Y (1989) Feeding kinetics of *Brachionus plicatilis* fed *Isochrysis galbana*. Hydrobiologia 186/187:51–57

Lubzens E (1987) Raising rotifers for use in aquaculture. Hydrobiologia 147:245–255

Montagnes DJS, Berges JA, Harrison PJ, Taylor FJR (1994) Estimating carbon, nitrogen, protein, and chlorophyll *a* from cell volume in marine phytoplankton. Limnol Oceanogr 39:1044–1060

Navarro N (1999) Feeding behaviour of the rotifers *Brachionus plicatilis* and *Brachionus rotundiformis* with two types of food: live and freeze-dried microalgae. J Exp Mar Biol Ecol 237: 75–87

Pillay TVR (1990) Aquaculture: principles and practices. Fishing News Books, London

Rothhaupt KO (1990a) Differences in particle size-dependent feeding efficiencies of closely related rotifer species. Limnol Oceanogr 35:16–23

Rothhaupt KO (1990b) Change of the functional responses of rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particles. Limnol Oceanogr 35:24–32

Scott AP, Baynes SM (1978) Effect of algal diet and temperature on the biochemical composition of the rotifer, *Brachionus plicatilis*. Aquaculture 14:247–260

Serra M, Miracle MR (1987) Biometric variation in three strains of *Brachionus plicatilis* as a direct response to abiotic variables. Hydrobiologia 147:83–89

Spain JD (1982) Basic microcomputer models in biology. Addison-Wesley, London

Starkweather PL (1980) Aspects of the feeding behavior and trophic ecology of suspension-feeding rotifers. Hydrobiologia 73:63–72

Weisse T, Montagnes DJS (1998) Effect of temperature on inter- and intraspecific isolates of *Urotrichia* (Prostomatida, Ciliophora). Aquat Microb Ecol 15:285–291