Aquaculture Research

Aquaculture Research, 2013, 1–12 doi: 10.1111/are.12232

Biochemical composition and fatty acid profile in a strain of the lineage 'Nevada', belonging to the *Brachionus plicatilis* (Rotifera) complex, fed different diets

Venetia Kostopoulou[1], Helen Miliou[2] & George Verriopoulos[1]

[1]Department of Zoology-Marine Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece

[2]Department of Applied Hydrobiology, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, Athens, Greece

Correspondence: V Kostopoulou, Hellenic Centre for Marine Research, Institute of Aquaculture, PO BOX 2214, Heraklion, Crete, 71 003, Greece. E-mail: vkostop@biol.uoa.gr

Abstract

A strain of the lineage *Brachionus* 'Nevada' was batch cultured with two diets, differing in biochemical composition: baker's yeast (treatment 1), which has higher protein:lipid ratio compared with CULTURE SELCO (treatment 2). The biochemical composition (DNA, RNA, lipid, protein content) and fatty acid profile of rotifers of both treatments was analysed and related to previously published population structure data. CULTURE SELCO-fed rotifers showed higher DNA, RNA, lipid, n-3 highly unsaturated fatty acids (HUFA) average content, compared with yeast-fed rotifers, which had higher protein content. Rotifer lipid content showed significant diurnal variation in yeast-fed rotifers. Rotifer lipid and n-3 HUFA content was associated with reproductive output. DNA and RNA content was related to embryonic development while protein content, to somatic growth and mixis. The saturated and monounsaturated fatty acid rotifer content was stable irrespective of feed, in contrast to eicosapentanoic acid (EPA) and DHA. The levels of AA were similar in both rotifer populations, but those of EPA and docosahexaenoic acid (DHA) were about half in yeast-fed compared with CULTURE SELCO-fed rotifers. CULTURE SELCO resulted in a temporally stable rotifer lipid profile and a better enriched parthenogenetic population.

Keywords: rotifer, mass culture, hatcheries, HUFA, enrichment, dry feed

Introduction

The rotifer *Brachionus plicatilis* (Müller 1786) is commonly used in aquaculture as first feed for fish larvae (Lubzens, Zmora & Barr 2001). Many aspects relating to its mass culture have been studied, such as population dynamics (James, Bou-Abbas, Al-Khars, Al-Hinty & Salman 1983; Yúfera & Navarro 1995; Alver, Alfredsen & Olsen 2006; Kostopoulou, Miliou, Katis & Verriopoulos 2006), morphometry (Yúfera 1982; Snell & Carrillo 1984; Fu, Hirayama & Natsukari 1991; Kostopoulou, Miliou & Verriopoulos 2009), biochemical and fatty acid composition (Lubzens, Marko & Tietz 1985; Frolov, Pankov, Geradze, Pankova & Spektorova 1991; Carić, Sanko-Njire & Skaramuca 1993; Fernández-Reiriz, Labarta & Ferreiro 1993) among others. However, success in rotifer mass culture has still room for improvement. Population crashes, microbial aspects and nutritional quality of rotifers constitute active areas of research (Dhert, Rombaut, Suantika & Sorgeloos 2001; Lubzens *et al.* 2001; Conceição, Yúfera, Makridis, Morais & Dinis 2010).

Rotifer biochemical and fatty acid composition provide an indication of the nutritional value of rotifers for fish larvae (Carić *et al.* 1993; Whyte, Clarke, Ginther, Jensen & Townsend 1994) and for this reason they have been studied mostly in relation to enrichment. Most studies have focused on lipids and, in particular, highly unsaturated fatty acids (HUFA), due to their pivotal role in the early

stages of fish life. HUFA are required for the normal growth and survival of fish larvae (Watanabe & Kiron 1994) and their deficiency leads to delays in fish growth, higher mortality and reduced resistance to stress (Izquierdo 1996). HUFA have to be transferred to fish larvae via the live food chain. However, rotifers are incapable of synthesizing sufficient amounts of HUFA (Lubzens *et al.* 1985) and have to be enriched before being offered as prey.

Biochemical composition also provides an indirect measure of growth, owing to its fundamental role in processes such as cellular growth and multiplication. The RNA:DNA ratio has been related to population growth and nutritional status in cladocerans (Vrede, Persson & Aronsen 2002) and fish larvae (Buckley, Caldarone & Ong 1999). RNA has been positively correlated with growth rate (Båmstedt & Skjoldal 1980; Acharya, Kyle & Elser 2004), whereas protein levels have been linked to changes in somatic growth (Guisande & Serrano 1989). Biochemical composition could therefore represent a 'qualitative' measure of growth, reflecting changes in the physiological state and developmental stages of an animal (Riccardi & Mangoni 1999; Gorokhova & Kyle 2002).

In this study, the biochemical composition and fatty acid content of a strain of *B.* 'Nevada', one of the lineages grouped with *B. plicatilis* s.s. (Gómez, Serra, Carvalho & Lunt 2002), is presented. Owing to the role of rotifers in aquaculture, the experimental design was based on mass culture conditions characteristic of Mediterranean hatcheries, using two commonly applied diets. The results are linked to previous studies on population structure (Kostopoulou *et al.* 2006), morphometry (Kostopoulou *et al.* 2009) and mixis patterns (Kostopoulou, Miliou, Krontira & Verriopoulos 2007).

Materials and methods

Experimental stock population

The strain (isolate K) used in this study has been cultured for at least 7 years in an aquaculture farm in Greece (OCTAPUS S.A.). It was identified on the basis of mitochondrial gene COI sequencing and named according to Gómez *et al.* (2002). It belongs to *Brachionus* 'Nevada' (GenBank accession no. AM180752), a newly described lineage of the *B. plicatilis* species complex. *B.* 'Nevada' has been grouped with *B. plicatilis* s.s., which represents the large morphotype (Gómez *et al.* 2002).

The feeding regime included the microalga *Tetraselmis suecica* (LB 2286), and baker's yeast *Saccharomyces cerevisiae* or the microparticulate compound diet CULTURE SELCO® (INVE N.V., Ghent, Belgium). *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 g L^{-1} salinity under constant illumination and aeration (Moretti, Pedini Fernandez-Criado, Cittolin & Guidastri 1999). The culture medium consisted of diluted seawater that was UV treated.

A pre-experimental low-density (~10 individual mL^{-1}) culture of a parthenogenetic population of *B.* 'Nevada' – referred to hereafter as 'initial population' - was maintained under the abovementioned experimental conditions for several generations, using *T. suecica* as food (mean ± SE: 1.02×10^5 ± 0.9 cells ml^{-1}).

Experimental procedure

Rotifers were batch cultured in tanks (2.5 m^3 capacity) for 4 days. Two feeding regimes used in Mediterranean hatcheries were chosen: baker's yeast *Saccharomyces cerevisiae* and *Tetraselmis suecica* (treatment 1, three replicates), CULTURE SELCO and *T. suecica* (treatment 2, three replicates). The 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 (except day 0), 15:00 (except day 0), 17:00, 22:00, 2:00 and 5:00. The phytoplankton levels used were near (1.5×10^4 cells mL^{-1} in treatment 1) or below (0.2×10^4 cells mL^{-1} in treatment 2) 'green water' levels (Reitan, Rainuzzo, Øie & Olsen 1993; Dhert *et al.* 2001) and not high enough to satiate rotifers (Hirayama & Ogawa 1972). The levels of yeast and CULTURE SELCO corresponded to recommended levels for mass culture production (Moretti *et al.* 1999; Suantika, Dhert, Nurhudah & Sorgeloos 2000).

The biochemical composition of both dry feeds is listed in Table 1. Protein and lipid content (% dry weight) as measured in this study falls within reported values. Based on the values found in this study, the protein:lipid ratio for yeast and CULTURE SELCO is calculated to be 29.85 and 2.19 respectively.

Rotifer samples were collected from all tanks for biochemical assays and fatty acid analyses (500 mL). Sampling started on day 0 at 16:00 ('0 h'), proceeded with day 1 morning ('16 h'), day 1 afternoon ('24 h'), day 2 morning ('40 h'),

Table 1 Protein and lipid content (% dry weight) of the two feeds used in this study and of rotifers cultured therein. Values appear as range or mean (±[a:] SE or [b:]SD)

	Proteins	Lipids	Source
Saccharomyces cerevisiae	53.2	1.0	Ben-Amotz *et al.* (1987)
	30.65	3.4	Dendrinos and Thorpe (1987)
	30.5 ± 2.6[a]	7.7 ± 2.1[a]	Frolov *et al.* (1991)
	48.95 ± 0.49[a]	1.64 ± 0.39[a]	This study
S. cerevisiae-fed rotifer	65–69	15–22	Watanabe *et al.* (1983)
	55.4	4.5	Ben-Amotz *et al.* (1987)
	41.60	14.0	Dendrinos and Thorpe (1987)
		9.8 ± 0.2[a]	Whyte and Nagata (1990)
	46.2 ± 4.1[a]	11.8 ± 1.4[a]	Frolov *et al.* (1991)
	49.00 ± 1.17[a]	9.77	Nagata and Whyte (1992)
	34–35.1	13.5–15.4	Carić *et al.* (1993)
	36.06 ± 0.52[b]	10.48 ± 0.97[b]	Fernández-Reiriz *et al.* (1993)
	41.98 ± 2.72[b]		Tamaru *et al.* (1993)
CULTURE SELCO	35	15	Suantika *et al.* (2000)
		18	Dhert *et al.* (2001)
	36.8	16.8	www.inve.com
	45	15	www.aquaculture.ugent.be
	35.3 ± 0.45[a]	16.1 ± 0.56[a]	This study
CULTURE SELCO-fed rotifer	No data available		

day 2 afternoon ('48 h'), day 3 morning ('64 h'), day 3 afternoon ('72 h') and ended on day 4 at 8:00 ('88 h'). The samples of day 0 were used solely for the analyses of the initial population (fed on *Tetraselmis suecica*). The abundance (%) of the population structure of the sampled rotifer populations – comprising of immature individuals, non-ovigerous females, females with one egg, females with multiple eggs, females with sac, post-reproductive females – has been calculated in a previous study (Kostopoulou *et al.* 2006), where a more analytical description of the experimental design can be found (Experiment 1). Only parthenogenetic individuals were present in the population.

Chemical composition analyses

For the biochemical assays, samples were lyophilized, weighed and kept at −80°C, prior to analysis. DNA, RNA and total lipid analyses were assayed according to the method of Holland and Gabbott (1971), modified by Holland and Hannant (1973). Standard curves were run with known concentrations of calf thymus DNA (SERVA, 18560), calf liver RNA (SIGMA, R-7250) and tripalmitin (C 16:0) (SIGMA, T-5888) in the appropriate range of values. Proteins were determined according to Hach, Brayton and Kopelove (1985) and were estimated as $N \times 6.25$.

Lipids were extracted in chloroform:methanol (2:1, v:v) using the method of Folch, Lees and Sloane-Stanley (1957). Samples were stored under nitrogen at −80°C prior to analysis. Fatty acid methyl esters (FAMEs) were prepared by transesterification with anhydrous methanol containing 2% sulphuric acid and 0.01% (w/v) butylated hydroxytoluene for 16 h at 50°C (Christie 1982). FAMEs were separated using gas liquid chromatography on a Perkin Elmer Gas Chromatograph (Autosystem XL) equipped with a flame ionization detector and a split/splitless injector fitted to a capillary column of fused silica (FameWax, Restek, length: 30 m, i.d.: 0.32 mm, film thickness: 0.25 μm). Helium was used as the carrier gas. Injector and detector temperatures were set at 225°C and 250°C respectively. The oven temperature was programmed to rise from 130°C to 175°C at a rate of 5°C min^{-1} and then to 225°C at a rate of 20°C min^{-1}, where it was held for 10 min. Peak areas were integrated using Turbochrom Navigator Software (Version 4.1, Perkin Elmer, San Jose, CA, USA). FAMEs were identified by reference to a well-characterized fish oil and to authentic standards (ALLTECH, SIGMA and LARODAN).

Data analysis

The data were subjected to statistical analysis (Statgraphics Plus v.5.0, Statistical Graphics Corporation,

Warrenton, Virginia, USA). Normal distribution (Shapiro–Wilks test) and homogeneity of variance (Levene's test) were assessed prior to analysis. The data of the fatty acid content of the two diets had to be square root transformed. Regarding data on fatty acid composition, a two-way ANOVA was conducted to estimate time effects as well as interaction effects between diet and time. As these effects were not significant ($P > 0.05$), all samples for each tested feed ($n = 21$) were taken into consideration for the comparison with the initial population (rotifers fed only on *Tetraselmis suecica;* $n = 4$). With respect to biochemical, fatty acid and population structure analyses, comparisons between and within treatments and significance of regressions were tested using ANOVA, followed by LSD multiple range test when significant differences were found at 0.05 level. Untransformed mean values ± standard error (SE) are presented. The measurements taken at the beginning of the batch cultures (day 0) were not included in the statistical tests.

Results

DNA, RNA and lipids (Fig. 1) were significantly higher in treatment 2 (0.20 ± 0.02, 3.16 ± 0.16 and 11.78 ± 0.52% of dry weight respectively) compared with treatment 1 (0.15 ± 0.01, 2.62 ± 0.13 and 9.25 ± 0.48% of dry weight respectively). No significant difference was found in the RNA:DNA ratio. Protein content was significantly higher in treatment 1 than in treatment 2 (55.65 ± 0.99% and 52.23 ± 0.76% of dry weight respectively).

Regarding the temporal variation in the studied biochemical parameters (Fig. 2), DNA and RNA showed similar responses. In treatment 1, a significant diurnal variation was evident, whereas in treatment 2, significantly higher values were observed within the first 40 h of sampling, decreasing thereafter to reach higher values again towards the end of the sampling period (88 h). DNA levels were significantly different between treatments at 24 and 64 h, whereas RNA levels differed at 24 and 40 h. Lipids showed the opposite picture: in treatment 1, the reverse significant diurnal variation was apparent, while in treatment 2, slightly higher values were monitored after 48 h. Between treatments, significant differences were found at 16 and 64 h. Proteins showed no significant temporal variation, but tended to follow the fluctuations of RNA.

Mean values of both DNA and RNA were positively related ($P < 0.001$) to the abundance (%) of ovigerous females – comprising of females with single and multiple eggs as presented in Kostopoulou *et al.* (2006) – in both treatments (Fig. 3). Lipids were positively related to the abundance of immature individuals in treatment 1 (Fig. 4).

The fatty acid profile of feeds is listed in Table 2. *Tetraselmis suecica* showed the highest content in 20:4n-6 (arachidonic acid, AA). Yeast was rich in 18:2n-6, while CULTURE SELCO in 20:5n-3 (eicosapentanoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA). Specifically, there was a 100-fold and a tenfold difference in EPA and DHA levels, respectively, between the two dry feeds. The total content (% total fatty acids) in n-3 PUFA (polyunsaturated fatty acids) was significantly higher ($P < 0.001$) in

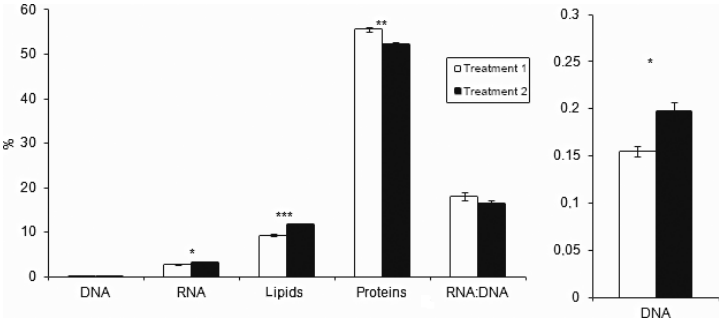

Figure 1 Mean values (± SE; $n = 3$) of the DNA, RNA, lipid, protein content and RNA:DNA ratio in the rotifer populations of treatments 1 and 2, after 16, 24, 40, 48, 69, 72 and 88 h of rearing. Asterisks indicate significant differences between treatments (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$). Inlet on the right shows detail for low (%) values.

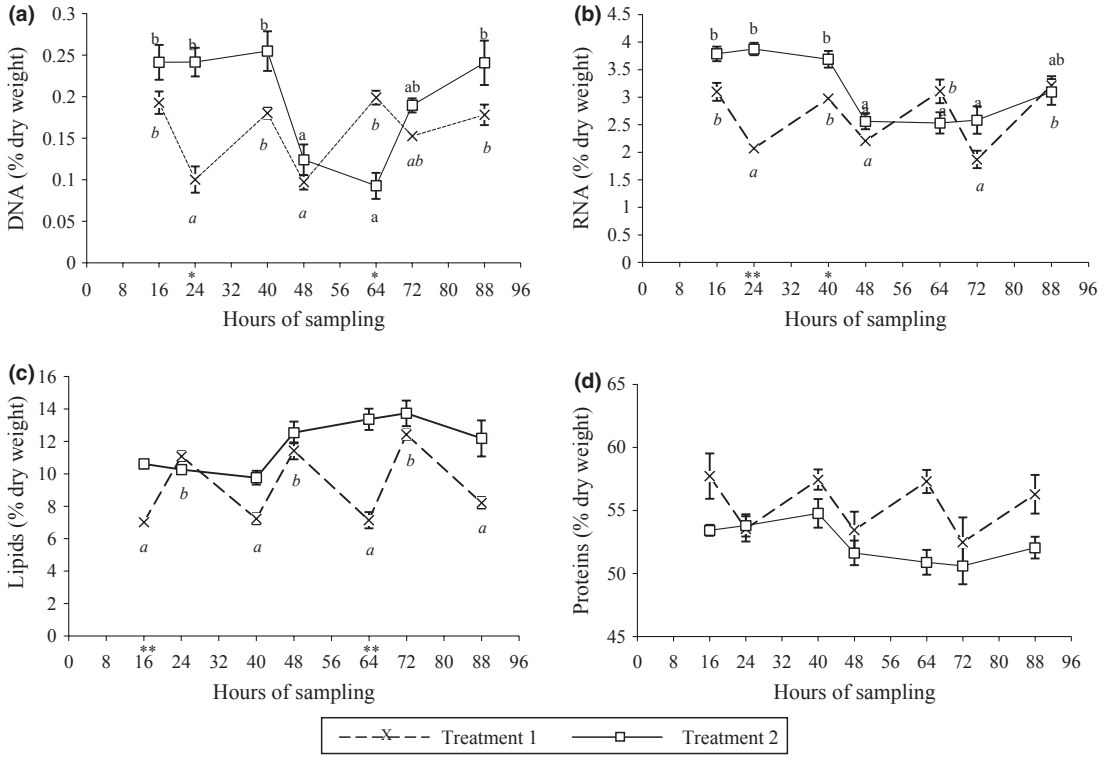

Figure 2 Mean values (± SE; $n = 3$) of (a) DNA, (b) RNA, (c) lipid and (d) protein content *versus* time period of sampling (16–88) in the rotifer populations of treatments 1 and 2. Letters indicate significant differences within each treatment ($P < 0.05$; characters in italics: treatment 1, normal characters: treatment 2). Asterisks indicate significant differences between treatments at designated hours of sampling (*, $P < 0.05$; **, $P < 0.01$).

CULTURE SELCO compared with yeast, whereas the opposite was observed for n-6 PUFA. Consequently, the ratio of n-3–n-6 PUFA was significantly higher ($P < 0.001$) in CULTURE SELCO than yeast. The ratio DHA to EPA was significantly higher ($P < 0.001$) in yeast compared with CULTURE SELCO. The levels (% total fatty acids) of n-3 and n-6 HUFA were significantly higher in CULTURE SELCO than in yeast.

Rotifer fatty acid composition (% total fatty acid) was influenced by the diet in the case of the following fatty acids: 16:4n-1, 20:5n-3 (EPA), 22:2n-6, 22:5n-3, 22:6n-3 (DHA) and 18:2n-6 (Table 3). These were all statistically higher in CULTURE SELCO and CULTURE SELCO-fed rotifers, except for the last one, which was detected in greater levels (% total fatty acids) in yeast and yeast-fed rotifers. There were other fatty acids whose difference in feed did not influence rotifer composition, which was similar in both treatments (14:0, 16:0, 18:0, 18:4n-3, 20:1n-9, 20:4n-6 (arachidonic acid, AA), 22:1n-11, 21:5n-3 and 24:1n-9). Rotifers had a higher fatty acid content compared with the feed in two cases (14:0, 20:1n-9). In the rest, the feed had a higher level of the particular fatty acid than the rotifer (16:0, 18:0, 18:4n-3, 20:4n-6, 22:1n-11, 21:5n-3, 24:1n-9). Another category was that of fatty acids that were either absent or present in trace amounts in feeds, but present in higher levels in rotifers (14:1, 15:0, 15:1, 16:1n7, 16:2n-4, 17:0, 18:1n9, 18:1n7, 18:1n5, 18:3n-6, 18:4n-1, 20:0, 20:2n-6, 20:3n-6, 20:3n-3, 20:4n-3, 22:1n-9, 22:4n-6, 22:5n-6). Finally, a specific fatty acid (18:3n-3) was significantly higher in yeast-fed than CULTURE SELCO-fed rotifers, although the profile of the corresponding feeds showed the opposite significant difference. This resulted in rotifers of similar n-3 PUFA content, but not n-3 HUFA. Namely, n-3 HUFA content was significantly higher in CULTURE SELCO-fed rotifers, compared with yeast-fed ones. On the other hand, the n-6 PUFA and HUFA levels were similar. No significant temporal difference was observed in the levels of all fatty acids identified.

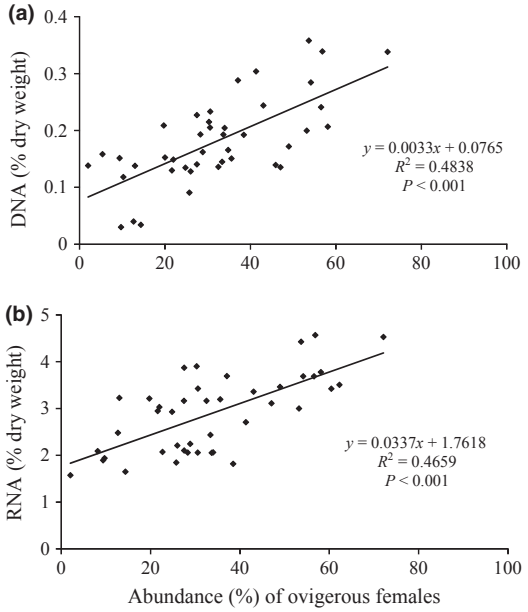

Figure 3 Linear regression of (a) DNA and (b) RNA with ovigerous females. The data for the population structure (ovigerous females) are derived from Kostopoulou *et al.* (2006).

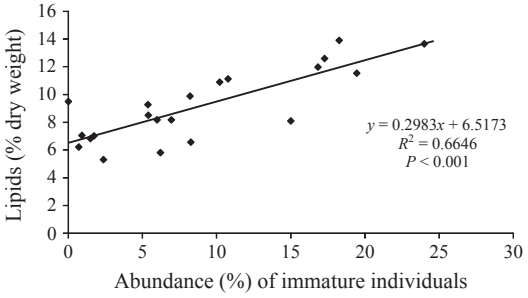

Figure 4 Linear regression of lipids with immature individuals of treatment 1. The data for the population structure (immature individuals) are derived from Kostopoulou *et al.* (2006).

Discussion

Lipid values reported here are in agreement with previous studies (Table 1). The lipid level of rotifers has been reported to be positively correlated with that of their diet (Watanabe, Kitajima & Fujita 1983; Ben-Amotz, Fishler & Schneller 1987; Frolov *et al.* 1991; Øie, Reitan & Olsen 1994; Fernández-Reiriz & Labarta 1996). The increased lipid levels in rotifers fed mainly on CULTURE SELCO (treatment 2) can thus be attributed to the increased lipid level of this feed (Table 1). The higher lipid levels in rotifers fed on CULTURE SELCO were almost constant throughout the experimental period and did not appear to be influenced by the structure of the population. In contrast, the 'lipid-poor' population of rotifers fed on yeast showed distinct diurnal variation in its lipid content, in synchrony with the newly hatched portion of the population, as shown in Fig. 3b of Kostopoulou *et al.* (2006). This is reinforced by the significant positive relationship of lipids with immature individuals of the latter population (Fig. 4 of this study). It is argued that the abovementioned temporal decrease in rotifer lipid content is due to the lower lipid input of the feed (yeast). Rotifers supply their offspring with the necessary energy, which decreases thereafter as it is not compensated by the food.

Proteins also agree with previously reported values (Table 1). Proteins are considered to be the most conservative of natural substances (Frolov *et al.* 1991). However, they also change with diet (Carić *et al.* 1993; Whyte *et al.* 1994; Lubzens *et al.* 2001). In this study, protein (% dry weight) was higher in the rotifers fed on yeast compared with CULTURE SELCO. According to Nagata and Whyte (1992), yeast-fed rotifers have a high protein content compared with algal-fed ones, whereas Frolov *et al.* (1991) have documented a higher protein content of yeast-fed rotifers compared with the corresponding content of yeast itself. On the other hand, enrichment with lipid emulsions results in a decrease in the protein content of rotifers (Fernández-Reiriz *et al.* 1993; Makridis & Olsen 1999). The oil emulsions, which aim to enrich and manipulate the lipid fraction, also appear to reduce the free amino acid (FAA) content, leading to protein consumption (Aragão, Conceição, Dinis & Fyhn 2004). A previous study (Kostopoulou *et al.* 2009) has shown that the rotifers of treatment 1 were also larger in size, creating a link between somatic growth (i.e. structural tissues) and protein content, in agreement with previous studies (Guisande & Serrano 1989; Øie & Olsen 1997). In another study, Kostopoulou *et al.* (2007) demonstrated that yeast influenced the fecundity of male-producing mictic females, resulting in a higher abundance of males, compared with CULTURE SELCO. It is argued that a possible relationship exists between protein content and production of males in rotifers.

The significantly higher levels of nucleic acids in rotifers fed on CULTURE SELCO could be attributed

Table 2 Selected† fatty acid composition (% of total fatty acids) of the feeds used in rotifer culture. Mean ± SE are presented. *Tetraselmis suecica* composition is indicative of the exponential phase of growth and not compared with that of the other two dry feeds

Fatty acids	*Tetraselmis suecica* *n* = 3	Yeast *n* = 3	CULTURE SELCO *n* = 3	*P*-value
14:0	1.12 ± 0.08	0.23 ± 0.02	1.47 ± 0.31	**
16:0 + 16:1‡	27.49 ± 0.45	34.09 ± 0.94	19.02 ± 2.95	*
16:4n-1	ND	ND	0.33 ± 0.12	**
18:0 + 18:1§	26.83 ± 1.21	39.53 ± 0.65	28.69 ± 2.04	*
18:2n-6	1.48 ± 0.38	23.17 ± 0.85	6.62 ± 0.91	***
18:3n-3	1.98 ± 0.18	ND	1.20 ± 0.17	***
18:4n-3	1.11 ± 0.07	ND	1.48 ± 0.14	***
20:1n-9	ND	0.35 ± 0.02	1.82 ± 0.38	**
20:2n-6	1.38 ± 0.09	ND	0.04 ± 0.04	ns
20:4n-6 (AA)	2.01 ± 0.23	ND	0.97 ± 0.27	***
20:4n-3	0.76 ± 0.17	ND	0.13 ± 0.13	ns
20:5n-3 (EPA)	1.12 ± 0.08	0.12 ± 0.04	11.21 ± 0.91	***
22:1n-11	0.33 ± 0.18	0.58 ± 0.07	3.15 ± 1.12	*
22:2n-6	ND	0.21 ± 0.03	0.95 ± 0.13	**
21:5n-3	ND	0.01 ± 0.01	1.42 ± 0.51	**
22:5n-3	0.14 ± 0.15	0.17 ± 0.04	3.08 ± 0.92	**
22:6n-3 (DHA)	0.83 ± 0.10	0.94 ± 0.23	13.42 ± 0.34	***
24:1n-9	0.05 ± 0.05	0.27 ± 0.05	2.92 ± 1.26	*
Sum n-3 PUFA	5.94 ± 0.12	1.24 ± 0.31	31.94 ± 1.51	***
Sum n-6 PUFA	4.87 ± 0.23	23.38 ± 0.88	9.18 ± 0.57	***
n-3/n-6 PUFA	1.22 ± 0.08	0.05 ± 0.01	3.50 ± 0.26	***
Sum n-3 HUFA	2.85 ± 0.12	1.24 ± 0.30	29.26 ± 1.53	***
Sum n-6 HUFA	3.39 ± 0.10	0.21 ± 0.03	2.29 ± 0.18	***
n-3/n-6 HUFA	0.84 ± 0.03	6.01 ± 1.54	12.86 ± 0.72	*
DHA/EPA	0.74 ± 0.10	7.68 ± 0.60	1.21 ± 0.08	***

†Fatty acids of less than 0.8 (% of total fatty acids), showing no significant differences between treatments, are not listed.
‡Including 16:1n-7.
§Including 18:1n-9, 18:1n-7, 18:1n-5.
ND, not detectable; AA, arachidonic acid; EPA, eicosapentanoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

to the increased proportion of ovigerous females present in these populations (Kostopoulou *et al.* 2006), a relationship also demonstrated in this study (Figure 3). DNA and RNA appear elevated during embryonic development in *Daphnia* (Gorokhova & Kyle 2002), as well as in ovigerous copepods (Miliou, Moraitou-Apostolopoulou & Argyridou 1992). The correlation of nucleic acids with ovigerous females could reflect active embryonic development during that phase of the life cycle. It is suggested that DNA and RNA content, through its relationship with ovigerous females, could be ultimately used as an alternative index to measure growth rate and/or egg–female ratio, although additional data are needed.

The fatty acid profile of rotifers was influenced by the dry feeds in a varied way. The saturated and monounsaturated fatty acids were similar in the rotifers of the two treatments, although the respective feeds showed significant differences. In particular, the saturated fatty acids of the C16 and C18 series were significantly higher in yeast, compared with CULTURE SELCO, but similar in the corresponding rotifers. On the other hand, the saturated fatty acids of the C14 series and the monounsaturated fatty acids of the C20, C22 and C24 series were elevated in CULTURE SELCO, but did not yield a significant difference in the rotifers. It has been suggested that rotifers are able to regulate the incorporation of saturates and monoenes into their body tissues by either inhibiting the absorption of these fatty acids or enhancing their catabolism (Rodríguez, Pérez, Díaz, Izquierdo, Fernández-Palacios & Lorenzo 1997; Rodríguez, Pérez, Badía, Izquierdo, Fernández-Palacios & Lorenzo Hernández 1998). In the cases where the fatty

Table 3 Fatty acid composition of rotifers (% of total fatty acids) fed on yeast (Treatment 1) or CULTURE SELCO (Treatment 2). Initial refers to the rotifer population of day 0 fed on *Tetraselmis suecica* (beginning of culture). Mean ± SE are presented. For abbreviations, see Table 2

	Rotifers			
Fatty acids	Initial *n* = 4	Treatment 1 *n* = 21	Treatment 2 *n* = 21	*P*-value
14:0	1.49 ± 0.15	1.55 ± 0.08	1.84 ± 0.11	ns
14:1	0.66 ± 0.13	0.81 ± 0.07	0.68 ± 0.10	ns
15:0	0.49 ± 0.03	0.54 ± 0.03	0.54 ± 0.02	ns
15:1	0.44 ± 0.05	0.33 ± 0.03	0.37 ± 0.03	ns
16:0	12.70 ± 0.44	15.15 ± 0.84	16.08 ± 0.93	ns
16:1n7	8.62 ± 1.37	8.53 ± 0.49	10.30 ± 0.51	ns
16:2n-4	1.17 ± 0.37	0.83 ± 0.15	0.75 ± 0.11	ns
17:0	0.75 ± 0.10	0.66 ± 0.09	0.79 ± 0.08	ns
16:4n-1	0.09 ± 0.04^{a}	0.08 ± 0.02^{a}	0.23 ± 0.02^{b}	***
18:0	1.58 ± 0.05	1.60 ± 0.06	1.32 ± 0.1	ns
18:1n9	14.22 ± 1.27	12.64 ± 0.81	13.00 ± 1.31	ns
18:1n7	11.26 ± 0.68	10.84 ± 0.39	9.94 ± 0.62	ns
18:1n5	0.53 ± 0.14	0.59 ± 0.07	0.55 ± 0.08	ns
18:2n-6	12.63 ± 0.74^{b}	11.65 ± 0.67^{b}	9.91 ± 0.51^{a}	*
18:3n-6	0.28 ± 0.03	0.25 ± 0.03	0.28 ± 0.04	ns
18:3n-3	14.36 ± 1.96^{b}	14.40 ± 0.96^{b}	9.43 ± 0.58^{a}	***
18:4n-3	0.45 ± 0.26	0.40 ± 0.10	0.33 ± 0.06	ns
18:4n-1	0.05 ± 0.03	0.15 ± 0.02	0.18 ± 0.04	ns
20:0	0.08 ± 0.03	0.11 ± 0.02	0.15 ± 0.02	ns
20:1n-9	2.03 ± 0.24	2.99 ± 0.13	2.78 ± 0.26	ns
20:2n-6	0.29 ± 0.03^{ab}	0.39 ± 0.03^{b}	0.24 ± 0.04^{a}	*
20:3n-6	0.33 ± 0.01^{ab}	0.38 ± 0.02^{b}	0.27 ± 0.03^{a}	**
20:4n-6 (AA)	0.78 ± 0.08	0.88 ± 0.05	0.84 ± 0.09	ns
20:3n-3	0.17 ± 0.04^{ab}	0.27 ± 0.03^{b}	0.07 ± 0.03^{a}	***
20:4n-3	1.69 ± 0.19^{ab}	2.16 ± 0.10^{b}	1.57 ± 0.13^{a}	**
20:5n-3 (EPA)	3.58 ± 0.33^{a}	2.90 ± 0.28^{a}	5.83 ± 0.26^{b}	***
22:1n-11	0.74 ± 0.18	1.01 ± 0.09	1.22 ± 0.12	ns
22:1n-9	0.21 ± 0.03	0.20 ± 0.03	0.25 ± 0.06	ns
22:2n-6	0.59 ± 0.08^{a}	0.85 ± 0.08^{a}	1.08 ± 0.09^{b}	*
21:5n-3	0.21 ± 0.03	0.35 ± 0.04	0.31 ± 0.05	ns
22:4n-6	0.11 ± 0.06	0.20 ± 0.04	0.14 ± 0.03	ns
22:5n-6	0.06 ± 0.05	0.16 ± 0.03	0.10 ± 0.03	ns
22:5n-3	0.72 ± 0.06^{b}	0.44 ± 0.05^{a}	1.15 ± 0.03^{c}	***
22:6n-3 (DHA)	2.25 ± 0.33^{a}	2.10 ± 0.29^{a}	4.52 ± 0.46^{b}	***
24:1n-9	1.14 ± 0.14	1.34 ± 0.20	1.65 ± 0.20	ns
Sum	96.78 ± 0.45	97.78 ± 0.72	98.70 ± 0.68	ns
Sum n-3 PUFA	23.42 ± 1.97	23.03 ± 0.97	23.20 ± 1.21	ns
Sum n-6 PUFA	15.08 ± 0.76	14.77 ± 0.72	12.88 ± 0.45	ns
n-3/n-6 PUFA	1.55 ± 0.08	1.62 ± 0.13	1.84 ± 0.14	ns
Sum n-3 HUFA	8.61 ± 0.50^{a}	8.22 ± 0.44^{a}	13.44 ± 0.76^{b}	***
Sum n-6 HUFA	1.88 ± 0.06	2.47 ± 0.14	2.44 ± 0.14	ns
n-3/n-6 HUFA	4.58 ± 0.21^{b}	3.37 ± 0.15^{a}	5.57 ± 0.20^{c}	***
DHA/EPA	0.63 ± 0.08	0.85 ± 0.18	0.76 ± 0.06	ns

Means in the same row having a different letter as superscript are significantly different (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant).

acids reached a higher percentage in rotifers than in corresponding feed, endogenous synthesis is hypothesized (Whyte & Nagata 1990; Lie, Haaland, Hemre, Maage, Lied, Rosenlund, Sandnes & Olsen 1997; Navarro, Yúfera & García-Gallego 2001). Such stable rotifer composition, regardless of feed, can be linked to a need for these acids during growth and development (Whyte & Nagata 1990).

Rotifer fatty acid composition was influenced by the dry feeds in the case of the following fatty acids: 16:4n-1, 18:2n-6, 20:5n-3, 22:2n-6, 22:5n-3 and 22:6n-3. Such correspondence between feed and rotifer composition is in agreement with previous studies (Walford & Lam 1987; Frolov *et al.* 1991; Fernández-Reiriz *et al.* 1993; Tamaru, Murashige, Lee, Ako & Sato 1993; Reitan, Rainuzzo & Olsen 1994; Whyte *et al.* 1994; Fernández-Reiriz & Labarta 1996; Lie *et al.* 1997; Copeman, Parrish, Brown & Harel 2002; Castell, Blair, Neil, Howes, Mercer, Reid, Young-Lai, Gullison, Dhert & Sorgeloos 2003). The fatty acids that have received most attention are EPA (20:5n3) and DHA (22:6n3), because of their important role in fish larval growth and development (Watanabe & Kiron 1994). In this study, rotifers were enriched in those two fatty acids when cultured with CULTURE SELCO, in contrast to yeast. The latter is considered nutritionally deficient in HUFA for rotifers (Lubzens *et al.* 1985; Walford & Lam 1987; Whyte & Nagata 1990; Frolov *et al.* 1991; Fernández-Reiriz *et al.* 1993; Tamaru *et al.* 1993; Fernández-Reiriz & Labarta 1996; Nichols, Hart, Nichols & McMeekin 1996), in accordance with this study. On the other hand, although CULTURE SELCO is more enriched than yeast, it is nevertheless on the lower range of most artificially prepared enrichment emulsions (Reitan *et al.* 1994; Copeman *et al.* 2002; Castell *et al.* 2003; Park, Puvanendran, Kellett, Parrish & Brown 2006). Consequently, the levels of EPA and DHA obtained in CULTURE SELCO-fed rotifers were accordingly lower than most emulsion-fed rotifers. Based on the bibliography (Reitan *et al.* 1994; Hache & Plante 2011), the highest levels in the abovementioned fatty acids were attained in rotifers (18% EPA and 34.4% DHA), when they were cultured using emulsions with a composition of 27.3% and 33.8% of EPA and DHA respectively. Studies (Reitan *et al.* 1994; Copeman *et al.* 2002; Park *et al.* 2006) where a higher percentage of either EPA or DHA was provided did not yield better results. It is therefore suggested that there must be a threshold above which no further enrichment is possible.

The higher content of n-3 HUFA in CULTURE SELCO-fed rotifers, together with their higher lipid content can be associated with their population structure. Based on a previous study (Kostopoulou *et al.* 2006), CULTURE SELCO-fed rotifers were characterized by a higher proportion of ovigerous females. A relationship therefore appears to exist between lipid content and reproductive output (Schmid-Araya 1992; Olsen, Reitan & Vadstein 1993; Gilbert 2004). HUFA act as precursors to eicosanoids, which have a regulatory function in egg production, egg laying and hatching (Brett & Müller-Navarra 1997). Furthermore, n-3 HUFA have been associated with an increased proportion of gravid females in cladocerans (Sundbom & Vrede 1997).

Certain fatty acids (18:3n-3, 18:3n-6, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:6n-3, 20:2n-6, 20:3n-6, 20:4n-6, 21:5n-3, 22:5n-3, 22:2n-6, 22:4n-6 and 22:5n-6) were absent or were detected in lower levels in yeast, compared with the corresponding rotifers. A number of these fatty acids even exceeded the levels of CULTURE SELCO-fed rotifers, whereas most of them were similar to the initial population (fed on *Tetraselmis suecica*). It is therefore postulated that they may have originated from lipids in the starting rotifer population or from selective feeding on *T. suecica*, at least in some of the cases, such as 18:4n-3, 20:2n-6 and 20:4n-6. However, certain polyunsaturated fatty acids showed a relatively low content in *T. suecica* (e.g. 18:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3) or were not detected at all (e.g. 20:3n-6, 20:3n-3, 22:2n6, 21:5n-3). Thus, an activation of n-3 and n-6 conversion pathways in both yeast- and phytoplankton-fed rotifer populations cannot be ruled out. It is well known that the fatty acids 18:3n-3, 18:4n-3, 20:3n-3 and 20:4n-3 may act as precursors in *de novo* synthesis of 20:5n-3 and 22:6n-3 (n-3 conversion pathway). In addition, the increased levels of 20:2n-6 and 20:3n-6 may indicate formation of 20:4n-6 and thereafter of 22:2n-6, 22:4n-6 and 22:5n-6 (n-6 conversion pathway). It has been suggested that when the feed is deficient in HUFA, as is the case in yeast, rotifers can activate specific pathways that lead to the production of those HUFA (Lubzens *et al.* 1985; Frolov *et al.* 1991; Robin 1995; Nichols *et al.* 1996; Navarro *et al.* 2001). Finally, the level of 20:4n-6 was similar in yeast-fed and CULTURE SELCO-fed rotifers, although the corresponding dry feeds showed significant differences. However, this was not the case for EPA and DHA, which were half in yeast-fed, compared with CULTURE SELCO-fed rotifers.

Overall, CULTURE SELCO gave the best fatty acid profile in terms of n-3 HUFA, followed by phytoplankton (reflecting the initial rotifer population) and then yeast. The levels of 20:5n-3 and 22:6n-3 were slightly lower, while those of 22:5n-3 significantly lower, in yeast-fed rotifers compared

with phytoplankton-fed rotifers. The deficiency of yeast compared with phytoplankton has also been noted in previous studies (Dendrinos & Thorpe 1987; Whyte & Nagata 1990). Rotifer populations fed on CULTURE SELCO have also been shown to attain higher growth rates (Kostopoulou *et al.* 2006) through faster deposition of eggs (Kostopoulou *et al.* 2009). On the other hand, when rotifers were fed on yeast, they reached higher mixis (i.e. sexual reproduction) levels, compared with CULTURE SELCO (Kostopoulou *et al.* 2007). It is therefore suggested that CULTURE SELCO results in more productive and better enriched parthenogenetic rotifer populations in comparison with yeast.

In conclusion, this study compared rotifer populations that were mass cultured using two diets differing in biochemical composition. The lipid-rich diet (CULTURE SELCO) yielded populations of higher DNA, RNA, lipid and n-3 HUFA content, in contrast to the temporally unstable lipid-poor diet (yeast), which resulted in rotifers of higher protein content. CULTURE SELCO appears to offer a more temporally stable lipid content, compared with yeast. It is therefore suggested that the two populations require different enrichment protocols to reach appropriate biochemical compositions, before being fed to the fish larvae.

Acknowledgments

The authors thank the personnel of OCTAPUS hatchery and in particular Mr. Peter Tarrant, Mrs. Maria Simpsiridou and Mr. Grigoris Giannoulis. The identification of the strain was carried out at the Institute Cavanilles of Biodiversity and Evolutionary Biology (University of Valencia, Spain) by Dr. Sergi Campillo. This study was supported by the National Foundation of Scholarships and the Internal Research Programme of the National and Kapodistrian University of Athens.

References

Acharya K., Kyle M. & Elser J.J. (2004) Biological stoichiometry of *Daphnia* growth: an ecophysiological test of the growth rate hypothesis. *Limnology and Oceanography* **49**, 656–665.

Alver M.O., Alfredsen J.A. & Olsen Y. (2006) An individual-based population model for rotifer (*Brachionus plicatilis*) cultures. *Hydrobiologia* **560**, 93–108.

Aragão C., Conceição L.E.C., Dinis M.T. & Fyhn H.-J. (2004) Amino acid pools of rotifers and *Artemia* under different conditions: nutritional implications for fish larvae. *Aquaculture* **234**, 429–445.

Båmstedt U. & Skjoldal H.R. (1980) RNA concentration of zooplankton: relationship with size and growth. *Limnology and Oceanography* **25**, 304–316.

Ben-Amotz A., Fishler R. & Schneller A. (1987) Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. *Marine Biology* **95**, 31–36.

Brett M.T. & Müller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology* **38**, 483–499.

Buckley L., Caldarone E. & Ong T.-L. (1999) RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* **401**, 265–277.

Carić 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.

Castell J., Blair T., Neil S., Howes K., Mercer S., Reid J., Young-Lai W., Gullison B., Dhert P. & Sorgeloos P. (2003) The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (*Brachionus plicatilis*) fed to larval haddock (*Melanogrammus aeglefinus*). *Aquaculture International* **11**, 109–117.

Christie W.W. (1982) *Lipid Analysis*. Pergamon, Oxford 207 pp.

Conceição L.E., Yúfera M., Makridis P., Morais S. & Dinis M.T. (2010) Live feeds for early stages of fish rearing. *Aquaculture Research* **41**, 613–640.

Copeman L.A., Parrish C.C., Brown J.A. & Harel M. (2002) Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture* **210**, 285–304.

Dendrinos P. & Thorpe J.P. (1987) Experiments on the artificial regulation of the amino acid and fatty acid contents of food organisms to meet the assessed nutritional requirements of larval, post-larval and juvenile dover sole [*Solea solea* (L.)]. *Aquaculture* **61**, 121–154.

Dhert P., Rombaut G., Suantika G. & Sorgeloos P. (2001) Advancement of rotifer culture and manipulation techniques in Europe. *Aquaculture* **200**, 129–146.

Fernández-Reiriz M.J. & Labarta U. (1996) Lipid classes and fatty acid composition of rotifer (*Brachionus plicatilis*) fed two algal diets. *Hydrobiologia* **330**, 73–79.

Fernández-Reiriz M.J., Labarta U. & Ferreiro M.J. (1993) Effects of commercial enrichment diets on the nutritional value of the rotifer (*Brachionus plicatilis*). *Aquaculture* **112**, 195–206.

Folch J., Lees N. & Sloane-Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Frolov A.V., Pankov S.L., Geradze K.N., Pankova S.A. & Spektorova L.V. (1991) Influence of the biochemical composition of food on the biochemical composition of the rotifer *Brachionus plicatilis*. *Aquaculture* **97**, 181–202.

Fu Y., Hirayama K. & Natsukari Y. (1991) Morphological differences between two types of the rotifer *Brachionus plicatilis* O.F. Müller. *Journal of Experimental Marine Biology and Ecology* **151**, 29–41.

Gilbert J.J. (2004) Females from resting eggs and parthenogenetic eggs in the rotifer *Brachionus calyciflorus*: lipid droplets, starvation resistance and reproduction. *Freshwater Biology* **49**, 1505–1515.

Gómez A., Serra M., Carvalho G.R. & Lunt D.H. (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution* **56**, 1431–1444.

Gorokhova E. & Kyle M. (2002) Analysis of nucleic acids in *Daphnia*: development of methods and ontogenetic variations in RNA-DNA content. *Journal of Plankton Research* **24**, 511–522.

Guisande C. & Serrano L. (1989) Analysis of protein, carbohydrate and lipid in rotifers. *Hydrobiologia* **186**/187, 339–346.

Hach C.C., Brayton S.V. & Kopelove A.B. (1985) A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. *Journal of Agricultural and Food Chemistry* **33**, 1117–1123.

Hache R. & Plante S. (2011) The relationship between enrichment, fatty acid profiles and bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and *Artemia* (*Artemia salina* strain *fransiscana*). *Aquaculture* **311**, 201–208.

Hirayama K. & Ogawa S. (1972) Fundamental studies on physiology of rotifer for its mass culture – I. Filter feeding of rotifer. *Bulletin of the Japanese Society of Scientific Fisheries* **38**, 1207–1214.

Holland D.L. & Gabbott P.A. (1971) A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. *Journal of the Marine Biological Association of the United Kingdom* **51**, 659–668.

Holland D.L. & Hannant P.J. (1973) Addendum to a micro-analytical scheme for the biochemical analysis of marine invertebrate larvae. *Journal of the Marine Biological Association of the United Kingdom* **53**, 833–836.

Izquierdo M.S. (1996) Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutrition* **2**, 183–191.

James C.M., Bou-Abbas M., Al-Khars A.M., Al-Hinty S. & Salman A.E. (1983) Production of the rotifer *Brachionus plicatilis* for aquaculture in Kuwait. *Hydrobiologia* **104**, 77–84.

Kostopoulou V., Miliou H., Katis G. & Verriopoulos G. (2006) Changes in the population structure of the lineage 'Nevada' belonging to the *Brachionus plicatilis* species complex, batch-cultured under different feeding regimes. *Aquaculture International* **14**, 451–466.

Kostopoulou V., Miliou H., Krontira Y. & Verriopoulos G. (2007) Mixis in rotifers of the lineage 'Nevada', belonging to the *Brachionus plicatilis* species complex, under different feeding regimes. *Aquaculture Research* **38**, 1093–1105.

Kostopoulou V., Miliou H. & Verriopoulos G. (2009) Morphometric changes in a strain of the lineage 'Nevada', belonging to the *Brachionus plicatilis* (Rotifera) complex. *Aquaculture Research* **40**, 938–949.

Laing I. (1991) *Cultivation of Marine Unicellular Algae. Ministry of Agriculture Fisheries and Food Laboratory Leaflet 67*, 31pp. Directorate of Fisheries Research, Lowestoft, UK.

Lie Ø., Haaland H., Hemre G.-I., Maage A., Lied E., Rosenlund G., Sandnes K. & Olsen Y. (1997) Nutritional composition of rotifers following a change in diet from yeast and emulsified oil to microalgae. *Aquaculture International* **5**, 427–438.

Lubzens E., Marko A. & Tietz A. (1985) De novo synthesis of fatty acids in the rotifer, *Brachionus plicatilis*. *Aquaculture* **47**, 27–37.

Lubzens E., Zmora O. & Barr Y. (2001) Biotechnology and aquaculture of rotifers. *Hydrobiologia* **446**/447, 337–353.

Makridis P. & Olsen Y. (1999) Protein depletion of the rotifer *Brachionus plicatilis* during starvation. *Aquaculture* **174**, 343–353.

Miliou H., Moraitou-Apostolopoulou M. & Argyridou M. (1992) Biochemical composition of *Tisbe holothuriae* (Copepoda: Harpacticoida) and its differentiation in relation to developmental stages. *Ophelia* **36**, 159–166.

Moretti A., Pedini Fernandez-Criado M., Cittolin G. & Guidastri R. (eds.) (1999) *Manual on Hatchery Production of Seabass and Gilthead Seabream*, Vol. **1**. FAO, Rome.

Nagata W.D. & Whyte J.N.C. (1992) Effects of yeast and algal diets on the growth and biochemical composition of the rotifer *Brachionus plicatilis* (Müller) in culture. *Aquaculture and Fisheries Management* **23**, 13–21.

Navarro N., Yúfera M. & García-Gallego M. (2001) Use of freeze-dried microalgae for rearing gilthead seabream, *Sparus aurata* L., larvae. II. Biochemical composition. *Hydrobiologia* **452**, 69–77.

Nichols D.S., Hart P., Nichols P.D. & McMeekin T.A. (1996) Enrichment of the rotifer *Brachionus plicatilis* fed an Antarctic bacterium containing polyunsaturated fatty acids. *Aquaculture* **147**, 115–125.

Øie G. & Olsen Y. (1997) Protein and lipid content of the rotifer *Brachionus plicatilis* during variable growth and feeding condition. *Hydrobiologia* **358**, 251–258.

Øie G., Reitan K.I. & Olsen Y. (1994) Comparison of rotifer culture quality with yeast plus oil and algal-based cultivation diets. *Aquaculture International* **2**, 225–238.

Olsen Y., Reitan K.I. & Vadstein O. (1993) Dependence of temperature on loss rates of rotifers, lipids and ω3 fatty acids in starved *Brachionus plicatilis* cultures. *Hydrobiologia* **255**, 13–20.

Park H.G., Puvanendran V., Kellett A., Parrish C.C. & Brown J.A. (2006) Effect of enriched rotifers on growth, survival, and composition of larval Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Sciences* **63**, 285–295.

Reitan K.I., Rainuzzo J.R., Øie G. & Olsen Y. (1993) Nutritional effects of algal addition in first-feeding of turbot (*Scophthalmus maximus* L.). *Aquaculture* **118**, 257–275.

Reitan K.I., Rainuzzo J.R. & Olsen Y. (1994) Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. *Aquaculture International* **2**, 33–48.

Riccardi N. & Mangoni M. (1999) Considerations on the biochemical composition of some freshwater zooplankton species. *Journal of Limnology* **58**, 58–65.

Robin J.H. (1995) Effect of diets containing γ-linoleic acid on n-6 highly unsaturated fatty acid content of rotifer (*Brachionus plicatilis*). *Hydrobiologia* **313**, 185–190.

Rodríguez C., Pérez J.A., Díaz M., Izquierdo M.S., Fernández-Palacios H. & Lorenzo A. (1997) Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. *Aquaculture* **150**, 77–89.

Rodríguez C., Pérez J.A., Badía P., Izquierdo M.S., Fernández-Palacios H. & Lorenzo Hernández A. (1998) The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. *Aquaculture* **169**, 9–23.

Schmid-Araya J.M. (1992) The biochemical composition and calorific content of a rotifer and its algal food: comparison of a two-stage chemostat and batch culture. *Oecologia* **92**, 327–338.

Snell T.W. & Carrillo K. (1984) Body size variation among strains of the rotifer *Brachionus plicatilis*. *Aquaculture* **37**, 359–367.

Suantika G., Dhert P., Nurhudah M. & Sorgeloos P. (2000) High-density production of the rotifer *Brachionus plicatilis* in a recirculation system: consideration of water quality, zootechnical and nutritional aspects. *Aquacultural Engineering* **21**, 201–214.

Sundbom M. & Vrede T. (1997) Effects of fatty acid and phosphorus content of food on the growth, survival and reproduction of *Daphnia*. *Freshwater Biology* **38**, 665–674.

Tamaru C.S., Murashige R., Lee C.-S., Ako H. & Sato V. (1993) Rotifers fed various diets of baker's yeast and/or *Nannochloropsis oculata* and their effect on the growth and survival of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos)* larvae. *Aquaculture* **110**, 361–372.

Vrede T., Persson J. & Aronsen G. (2002) The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*. *Limnology and Oceanography* **47**, 487–494.

Walford J. & Lam T.J. (1987) Effect of feeding with microcapsules on the content of essential fatty acids in live foods for the larvae of marine fishes. *Aquaculture* **61**, 219–229.

Walne P.R. (1966) *Experiments in the large-scale culture of Ostrea edulis L.* Ministry of Agriculture Fisheries and Food, Fisheries Investigations 2, Series 2. *London* **25**, 1–53.

Watanabe T. & Kiron V. (1994) Prospects in larval fish dietetics. *Aquaculture* **124**, 223–251.

Watanabe T., Kitajima C. & Fujita S. (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture* **34**, 115–143.

Whyte J.N.C. & Nagata W.D. (1990) Carbohydrate and fatty acid composition of the rotifer *Brachionus plicatilis*, fed monospecific diets of yeast or phytoplankton. *Aquaculture* **89**, 263–272.

Whyte J.N.C., Clarke W.C., Ginther N.G., Jensen J.O.T. & Townsend L.D. (1994) Influence of composition of *Brachionus plicatilis* and *Artemia* on growth of larval sablefish (*Anoplopoma fimbria* Pallas). *Aquaculture* **119**, 47–61.

Yúfera M. (1982) Morphometric characterization of a small-sized strain of *Brachionus plicatilis* in culture. *Aquaculture* **27**, 55–61.

Yúfera M. & Navarro N. (1995) Population growth dynamics of the rotifer *Brachionus plicatilis* cultured in non-limiting food condition. *Hydrobiologia* **313**, 399–405.

