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Determination of the Optimal Conditions for the Mass Culture of Large-Type Rotifers (*Brachionus plicatilis*) at Low Temperatures

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Abstract: We aimed to determine the optimal conditions for the mass culture of rotifers, which can be used as feed for cold-water fish species at low temperatures. The growth and specific growth rates (SGRs) of rotifers were assessed considering water temperature, salinity, density, dissolved oxygen (DO) levels, and the amount of *Chlorella* supplied as feed. The growth of rotifers was higher at 15 °C than at 10 °C and at salinities of ~11–17 psu. Initial inoculation densities of 500 and 700 individuals/mL resulted in the highest rotifer density, and SGR was highest at 100 individuals/mL. DO concentration did not significantly affect the growth and SGRs of rotifers. Enrichment with fatty acids is important to supplement the diet of cold-water fish species. Highly unsaturated fatty acid content increased with enrichment time to 14.04 ± 0.86% at 12 h and 15.58 ± 2.20% at 24 h. Thus, the optimal conditions for rotifer mass culture are a water temperature of 15 °C, salinity of 11–17 psu, initial inoculation density of 300–500 individuals/mL, DO concentration of 8 mg/L or more, and *Chlorella* supply at 7.5 × 10^{12} cells/mL. Therefore, the present study suggests optimal culture conditions of rotifers at low temperatures for breeding cold-water fish species.

Keywords: *Brachionus plicatilis*; culture condition; low temperature; mass culture; rotifer

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1. Introduction

Rotifers of the genus *Brachionus* are the most widely used live feed for marine fish. Rotifers are important to feed zooplankton because their size and slow motility make them suitable starter feed for larval fish during early exogenous feeding [1]. Furthermore, rotifers are easily digested and absorbed by fish larvae and can be easily enriched and cultured en masse; therefore, they are most commonly used as live feed for larval fish and crustaceans during the early stages of life [2–10]. Rotifers can be generally categorized into three types based on their size: *Brachionus plicatilis* (250–300 μm), *B. rotundiformis* (160–220 μm), and *B. rotundiformis* ssp. (100–140 μm) constitute the large, small, and super small types, respectively. Rotifers differ in size, optimal growth, nutritional value, and reproduction depending on the specific strain [11,12] and environmental factors, including water temperature, salinity, and light [13,14]. Their size is significantly affected by enrichment [15–17] during culture. Considering these factors, it is necessary to utilize suitable rotifer strains depending on the mouth size of the fish larvae.

Various studies have been conducted on the nutritional aspects and culture methods of rotifers [7,18–20] and on the increase in the growth rate of rotifers owing to environmental changes in water temperature, salinity, density, and dissolved oxygen (DO) levels [9,21–26].

The production of cold-water fish species inhabiting waters below 10 °C, such as walleye pollock (*Gadus chalcogrammus*) and Pacific cod (*Gadus macrocephalus*), has been recently established [27,28]. Embryos hatched after about 14 days at 5 °C. The egg yolk was absorbed for 3~4 days, and then the larvae were fed four times daily with enriched rotifers.

When rotifers cultured at high temperatures and low salinity are introduced to the rearing tanks of cold-water fish larvae in a seawater environment, they weaken and die due to water temperature and salinity differences [3,29]. The larvae are distributed in the surface layer because of their lower specific gravity relative to that of seawater and their weak swimming capability. Rotifers with reduced activity sink to the bottom of the tank. Therefore, they are less effective as feed sources for these larvae. In addition, rotifers that accumulate at the bottom can cause bacterial infection that may lead to larval death [30]. To avoid this, rotifer culture water temperature and salinity should be similar to the rearing conditions of the artificial seed production tank [31–33]. However, rotifer culture technology and nutritional enhancement methods suitable for cold-water fish species inhabiting waters with temperatures below 10 °C have not yet been established.

To supplement the diet of cold-water fish species, rotifer enrichment with fatty acids, including n-3 highly unsaturated fatty acids (n-3 HUFA), has been attempted at temperatures lower than the rotifer culture temperature [34–36]. However, the mass culture of rotifers at low temperatures under these conditions presents challenges that remain unresolved [21,31].

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n-3 fatty acids with bioregulatory functions that play important roles in the early growth and survival of larvae. HUFAs play an important role in biological membrane fluidity, alter enzymatic activity, and act as prostaglandin precursors [37].

In fish, the qualitative and quantitative requirements for essential fatty acids may vary depending on the species, temperature, and salinity [38]. Freshwater fish species use linoleic acid (C18:2n-6) and alpha-linoleic acid (C18:3n-3) as precursor fatty acids, which can be used to synthesize EPA and DHA in the body. However, as the enzymes required to synthesize n-3 HUFAs, such as EPA and DHA, in seawater fish species are extremely limited or absent, the diet must be exogenously supplemented with EPA and DHA [39].

In this study, we aimed to investigate the mass culture conditions for rotifers at low temperatures considering salinity, density, DO concentration, and *Chlorella* supply. To determine the optimal enrichment time of rotifers at low water temperatures, the effects of time-dependent rotifer enrichment were also assessed through fatty acid analysis.

2. Materials and Methods

2.1. Culturing Rotifers at Low Temperatures

Large-type rotifers (*B. plicatilis*, Uljin strain), obtained from Gangneung-Wonju National University, Gangwon-do, Republic of Korea, in 2015, were used in this study and subcultured in a 1-ton culture tank at a water temperature of ~22–23 °C and salinity of ~18–20 practical salinity units (psu). Freshwater-concentrated *Chlorella* (*Chlorella vulgaris*, Daesang, Seoul, Republic of Korea) was supplied at 1.0×10^{13}/mL daily. Maintaining low temperatures in the culture system (tank) was crucial for this study. Therefore, the culture system comprised internal and external tanks with a dual structure that could control and maintain the water temperature of the internal tank by circulating water at an adjusted temperature to the external tank. Rotifers were cultured at 20 °C using this tank, and the water temperature was lowered by 1 °C per month to select rotifers with good vitality, which were then cultured at 15 °C for 3 months. Subsequently, the same process of reducing the water temperature by 1 °C was performed every month, and the rotifers were cultured at 10 °C for 3 months or more. For the study, rotifers cultured at 10 °C for more than 3 months were used; 10% of the water in the tank was removed by draining the rearing tank daily, and then clean seawater and freshwater were supplemented in equal parts. All experiments were repeated thrice. Water environmental parameters such as temperature, salinity, DO concentration, and pH were recorded daily using a ProDSS Multiparameter Digital Water Quality Meter (YSI Inc., Yellow Springs, OH, USA).

2.2. Culture Conditions According to Temperature, Salinity, Density, DO Concentration, and Chlorella Abundance

2.2.1. Temperature

In a circular culture tank (Ø100 × 1000 cm, approximately 1000 L) set at 10 and 15 °C, filtered seawater using a cartridge with a 5 μm pore and freshwater were supplied in equal parts to maintain a culture water salinity of approximately 15–18 psu. The rotifers were inoculated at a density of about 300 individuals/mL, 10% of the water was exchanged daily, approximately 1.0×10^{13} *Chlorella* was counted using a hemocytometer and supplied daily [40], and the rotifers were cultured for 14 days. Subsequently, the rotifers were harvested using an 80-μm sieve, rinsed with seawater, and then observed using stereomicroscopy (SteREO Discovery V8; Carl Zeiss GmbH, Oberkochen, Germany) for counting. All conditions, except water temperature, were maintained at constant levels.

2.2.2. Salinity

Culture water with a salinity of 11, 14, 17, 20, and 23 psu was prepared at 15 °C. The rotifers were inoculated at a density of 300 individuals/mL, 10% of the water was exchanged daily, and 1.0×10^{13} *Chlorella* was supplied daily. All conditions, except salinity, were maintained at constant levels.

2.2.3. Density

Culture water with a salinity of 17 psu was prepared at 15 °C. Rotifers at densities of 100, 300, 500, and 700 individuals/mL were inoculated in each tank, 10% of the water was exchanged daily, and 1.0×10^{13} *Chlorella* was supplied daily. All conditions, except inoculation density, were maintained at constant levels.

2.2.4. DO

Culture water with a salinity of 17 psu was prepared at 15 °C, and rotifers were inoculated at a density of 300 individuals/mL. The growth rate of rotifers was investigated at approximately 8, 11, 14, 17, 20, and 23 mg/L of DO. Errors occurred within the range of 0.5 mg/L in each experimental DO. The DO concentration was controlled using liquid oxygen, and 1.0×10^{13} *Chlorella* was supplied daily. All conditions, except DO, were maintained at constant levels.

2.2.5. *Chlorella* Supply

Culture water with a salinity of 17 psu was prepared at 15 °C, and rotifers were inoculated at a density of 300 individuals/mL with 8 ppm of DO. Ten percent of water was exchanged daily, and *Chlorella* was supplied daily at concentrations of 6.25×10^{12}, 7.5×10^{12}, and 8.75×10^{12} to investigate the *Chlorella* growth rate depending on supply. All conditions, except the amount of *Chlorella*, were maintained at constant levels.

2.2.6. Daily Growth Rate

Rotifer density was measured daily using a stereomicroscope (SteREO Discovery V8; Carl Zeiss GmbH, Oberkochen, Germany) at 40×, and the intrinsic rate of reproduction [specific growth rate (SGR)] was calculated according to the method described by Rico-Martínez and Dodson [41] as follows:

$$SGR = (1/T)\ \mathrm{In}(N_T/N_0) \quad (1)$$

where T is the number of incubation days from inoculation to *B. plicatilis* peak density, N_T is the population density of *B. plicatilis* at T days, and N_0 is the inoculation of *B. plicatilis* density.

2.3. Analysis of Ammonium, Nitrite, and Nitrate

Dissolved ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) were analyzed using nutrient autoanalyzers (QuAAtro, 4 Channel; Seal Analytical, Hampshire, UK; SBE 9plus, UNDERWATER UNIT; Sea-Bird Electronics, Bellevue, WA, USA; AS-C3 Dissolved Inorganic Carbon Analyzer; Apollo SciTech, Newark, DE, USA) to investigate changes in ammonium during the growth of rotifers. Water samples from rotifer culture were collected daily for 12 days and then filtered using grade GF/F glass microfiber filters (Whatman; Merck KGaA, Darmstadt, Germany). All the samples were frozen until further analysis.

2.4. Rotifer Enrichment

To investigate the effects of enrichment on rotifers at low temperatures, rotifers, at a density of 1000 individuals/mL and 15 °C, were fed 70 mg/L super capsule powder (SCP; Chlorella Industry Co., Ltd., Kyoto, Japan) and 150 mg/L S.presso (INVE aquaculture, Dendermonde, Belgium) for 6, 12, and 24 h. Fatty acids were analyzed after rotifer enrichment by first extracting total lipids using a mixture of chloroform and methanol (2:1), as described by Folch et al. [42]. Thereafter, the extracted methyl ester lipids were used with 14% Bf3-methanol (Sigma-Aldrich, St. Louis, MO, USA) solution, and the fatty acids were analyzed using a gas chromatograph (Clarus 600 Gas Chromatograph; PerkinElmer, Waltham, MA, USA) with an SPTM-2560 column (Sigma-Aldrich; 100 m × 0.25 mm i.d., film thickness = 0.20 μm).

2.5. Data Analysis

Each set of experiments was performed in triplicate, and the mean values were calculated. Two-way analysis of variance was used to compare the reproductive potential in relation to salinity and feed concentration against the three temperatures. All analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Effects of Culture Environment on Rotifer Growth

3.1.1. Water Temperature

Figure 1 shows the growth of large-type rotifers at 10 and 15 °C. Rotifers cultured at 15 °C showed the highest density at 1331 individuals/mL on day 11, after which growth decreased (Figure 1a). However, the growth of rotifers cultured at 10 °C steadily increased until day 14, and the density was highest at 661.3 individuals/mL on day 14. Rotifers cultured at 10 °C showed a lower growth density than those cultured at 15 °C. Figure 1b shows the SGR according to culture temperature. Rotifers cultured at 15 °C had a higher SGR of 0.15 ± 0.01 than those cultured at 10 °C with an SGR of 0.36 ± 0.01.

3.1.2. Salinity

Analysis of the growth of low-temperature cultured rotifers according to salinity revealed that the highest growth occurred at 17 psu with 1290 individuals/mL on day 12. Growth at 14 psu was slightly higher than that at 11 psu. Rotifers cultured at 20 and 23 psu showed low growth (Figure 2a). SGR was highest at 17 psu with a value of 0.12 ± 0.016, followed by 0.11 ± 0.005 at 14 psu, 0.10 ± 0.012 at 11 psu, 0.07 ± 0.03 at 20 psu, and 0.07 ± 0.03 at 23 psu, in that order (Figure 2b).

3.1.3. Inoculation Density

For inoculation density, the highest growth was observed at an initial inoculation density of 500 and 700 individuals/mL, resulting in 1654–1668 individuals/mL on day 12. When 300 individuals/mL were inoculated, 1521 individuals/mL were observed on day 12, and no decrease in growth was observed. Inoculation with 100 individuals/mL resulted in 791 individuals/mL on day 12, showing the lowest growth (Figure 3a). The SGR was highest at 0.64 ± 0.01 following inoculation with 500 individuals/mL and lowest

at 0.54 ± 0.02 after inoculation with 100 individuals/mL (Figure 3b). The fastest time to reach 1000 individuals/mL was 4 days after inoculation with 700 individuals/mL. Thus, the lower the inoculation density, the larger the number of days required to reach 1000 individuals/mL (Figure 3c).

The pH of the rotifer culture water changed slightly depending on the inoculation density, i.e., 100, 300, 500, and 700 individuals/mL, ranging from 7.29 to 7.80, with no significant differences between inoculation densities. However, pH decreased with increasing inoculation density (Figure 3d).

3.1.4. DO

High rotifer growth was observed at DO concentrations of 14 and 17 mg/L on day 11 (Figure 4a). The growth of rotifers stood at 1212 individuals/mL at a DO concentration of 17 mg/L on day 11, the highest among all the experimental groups. The highest SGR, 0.13, occurred at a DO concentration of 17 mg/L (Figure 4b).

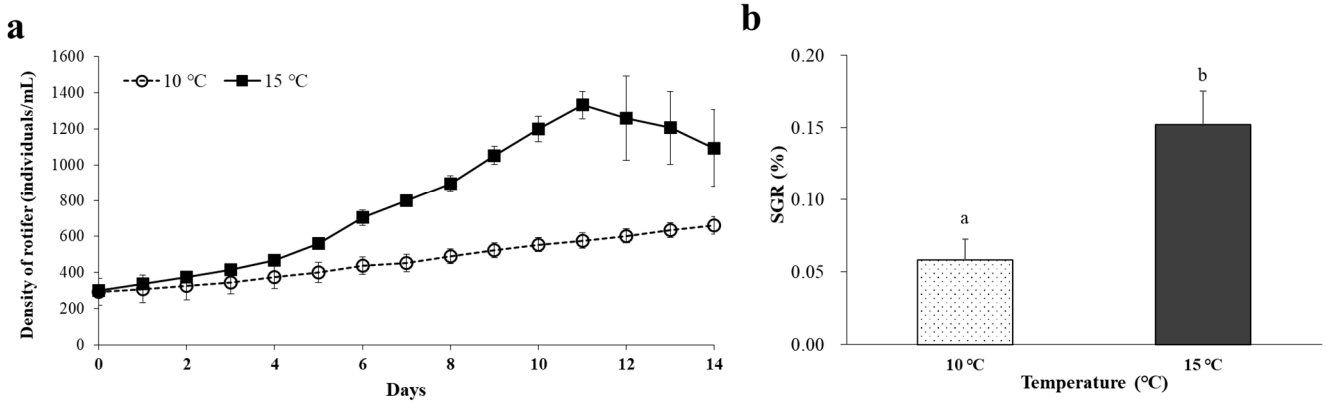

Figure 1. Effects of water temperature on growth of rotifer. (**a**) Growth density of rotifers at different water temperatures. (**b**) Specific growth rate (SGR) of rotifers at different water temperatures. Values are presented as the mean ± standard deviation (n = 3). Each experiment was performed in triplicate. Different letters indicate significant differences ($p < 0.05$).

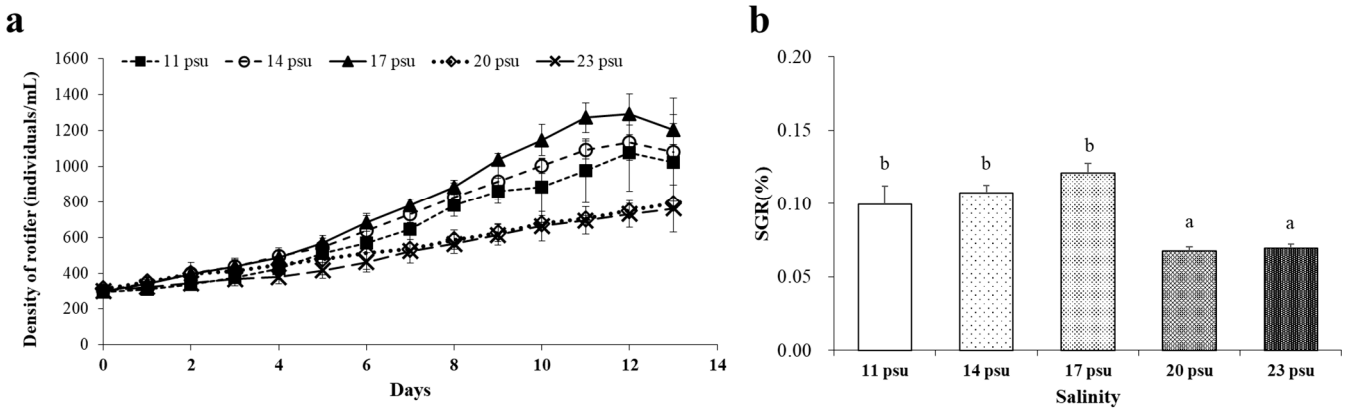

Figure 2. Effects of salinity on rotifer growth at 15 °C. (**a**) Growth density of rotifers at different salinities. (**b**) Specific growth rate (SGR) of rotifers at different salinities. Values are presented as the mean ± standard deviation (n = 3). Each experiment was performed in triplicate. Different letters indicate significant differences ($p < 0.05$).

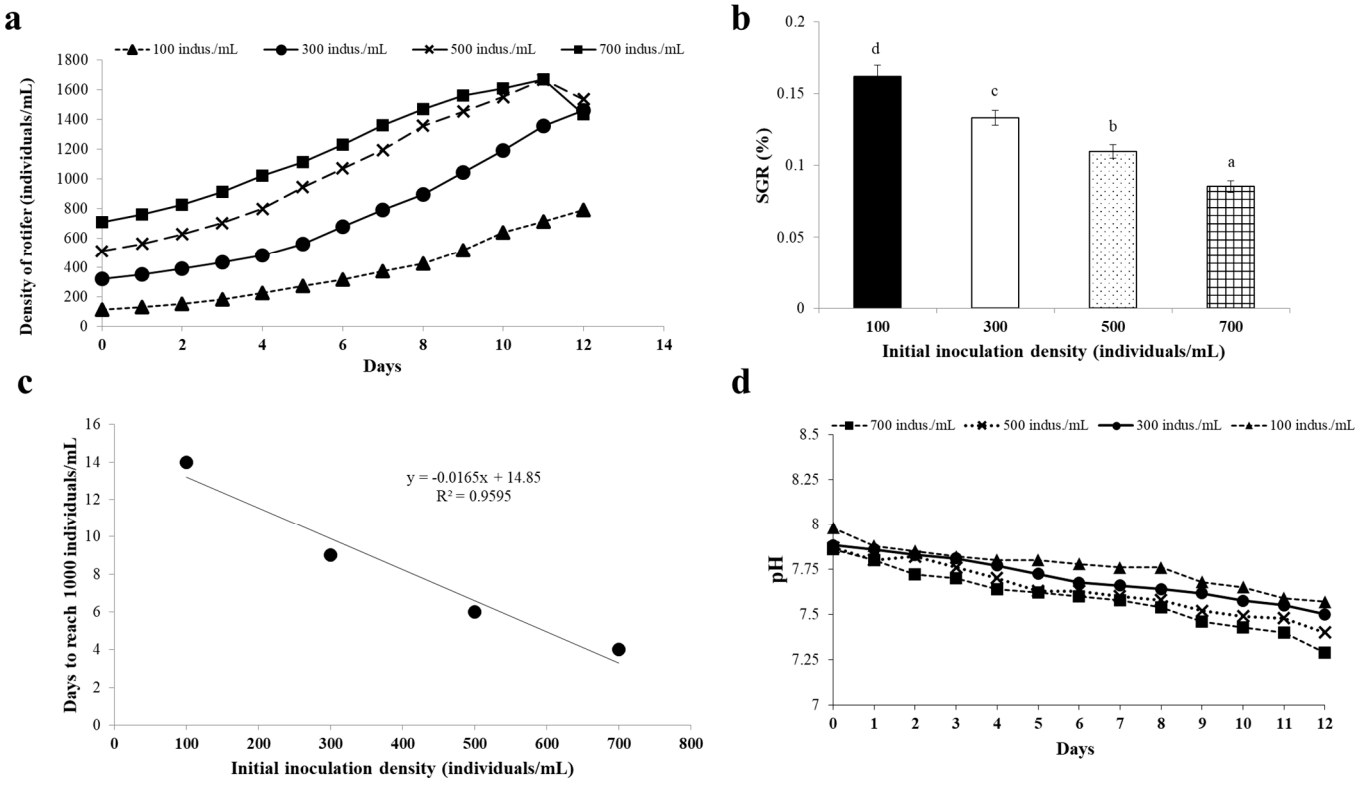

Figure 3. Effects of inoculation density (100, 300, 500, and 700 individuals/mL) on growth of rotifer. (**a**) Growth density of rotifers at different initial inoculation densities. (**b**) Specific growth rate (SGR) at different initial inoculation densities. (**c**) Days to reach 1000 individuals/mL at different initial inoculation densities. (**d**) Changes in pH at different initial inoculation densities. Values are presented as the mean ± standard deviation (n = 3). Each experiment was performed in triplicate. Different letters indicate significant differences ($p < 0.05$).

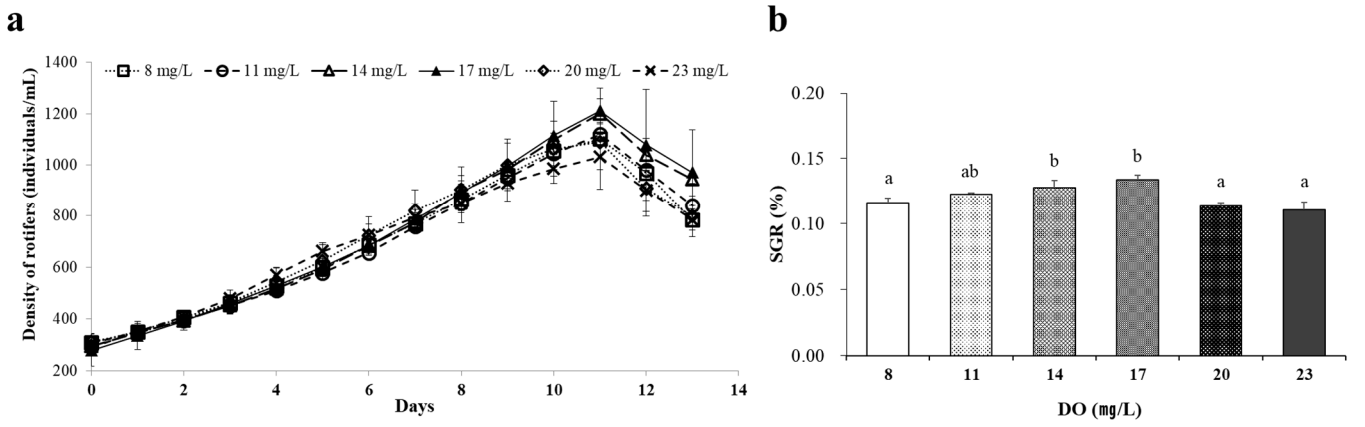

Figure 4. Effects of dissolved oxygen (DO) concentration on growth of rotifer. (**a**) Growth density of rotifers and different DO concentrations. (**b**) Specific growth rate (SGR) of rotifers at different DO concentrations. Values are presented as the mean ± standard deviation (n = 3). Each experiment was performed in triplicate. Different letters indicate significant differences ($p < 0.05$).

3.1.5. *Chlorella* Supply

The growth of rotifers depending on *Chlorella* supply was highest at 1267 individuals/mL on day 12 when 7.5×10^{12} cells/mL of *Chlorella* was supplied. When 8.75×10^{12} cells/mL of *Chlorella* was supplied, growth was slightly higher than when 7.5×10^{12} cells/mL was supplied until day 6; however, from day 7, rotifer growth decreased and was lower than when 7.5×10^{12} cells/mL of *Chlorella* was supplied. After supplying 6×10^{12} cells/mL

of *Chlorella*, the maximum level of growth was 789 individuals/mL on day 13, with all experimental groups recording the lowest growth (Figure 5a). SGR was highest at 0.11 when 7.5×10^{12} cells/mL *Chlorella* was supplied (Figure 5b).

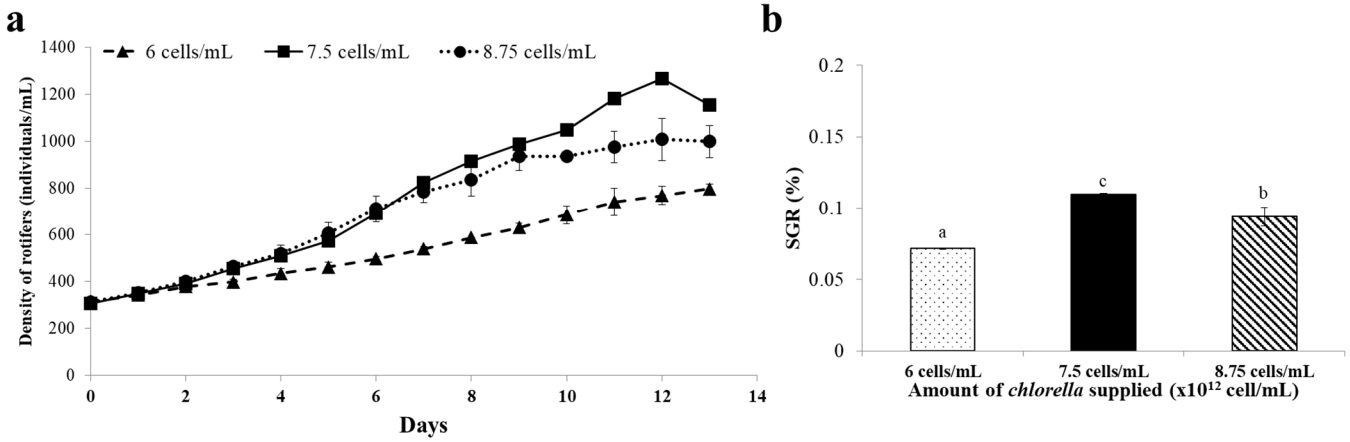

Figure 5. Effects of *Chlorella* supply on growth of rotifer. (**a**) Growth density of rotifers at different concentrations of *Chlorella* supplied. (**b**) Specific growth rate (SGR) of rotifers at different *Chlorella* concentrations. Values are presented as the mean ± standard deviation ($n = 3$). Each experiment was performed in triplicate. Different letters indicate significant differences ($p < 0.05$).

3.2. Ammonium, Nitrite, and Nitrate

The ammonium concentration in the culture water ranged from 33.8 to 98.8 µmol/L and tended to increase with culture duration. The nitrite concentration ranged from 9.7 to 117.4 µmol/L, and NO_3^- concentration ranged from 179.5 to 970.0 µmol/L, also increasing with culture duration. The highest concentrations of ammonium, nitrite, and nitrate were observed on day 11 at the maximum rotifer density (Figure 6).

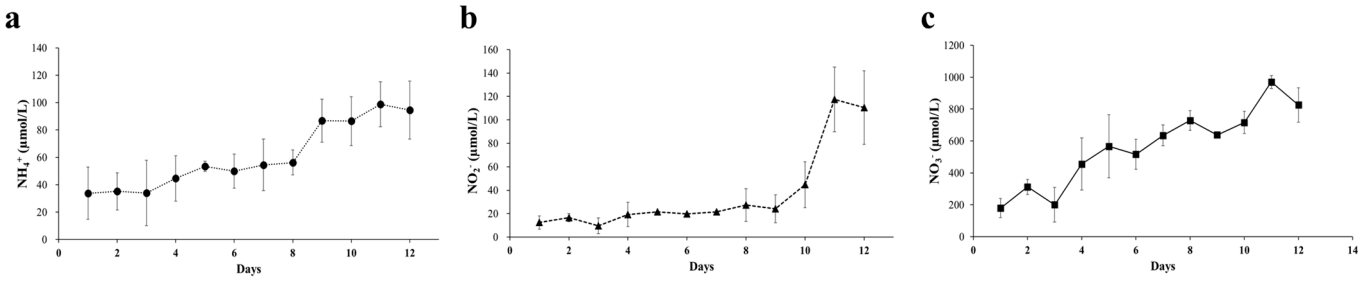

Figure 6. Changes in ammonium (NH_4^+) (**a**), nitrite (NO_2^-) (**b**), and nitrate (NO_3^-) (**c**) concentrations during culture. Values are presented as the mean ± standard deviation ($n = 3$).

3.3. Enrichment of Rotifers

The effect of enrichment on low-temperature cultured rotifers is shown in Table 1. Rotifers without enrichment had a high saturated fatty acid content at 26.68 ± 1.63%. Moreover, although the monosaturated fatty acid (MUFA) content was high in the enriched rotifers at 6 h, enrichment time did not have a significant effect. Enrichment time significantly influenced HUFA content; unenriched rotifers had the lowest HUFA content at 3.89 ± 0.08%. At 6, 12, and 24 h of enrichment, HUFA content was 11.75 ± 0.32%, 14.04 ± 0.86%, and 15.58 ± 2.20%, respectively; however, no significant difference was observed between the 12- and 24-h time points.

Table 1. Major fatty-acid composition (area % of total fatty acids) of the enriched rotifers at different time points.

FFA (%)	Before Enrichment	Enrichment Time		
		6 h	12 h	24 h
12:0	0.31 ± 0.03 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
14:0	1.86 ± 0.04 ^{b}	1.42 ± 0.06 ^{a}	1.41 ± 0.08 ^{a}	1.54 ± 0.07 ^{a}
15:0	0.49 ± 0.04 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
16:0	16.87 ± 0.54 ^{c}	15.44 ± 0.02 ^{b}	14.23 ± 0.13 ^{a}	14.89 ± 0.49 ^{a}
16:1	1.25 ± 0.10 ^{c}	1.09 ± 0.00 ^{b}	0.77 ± 0.06 ^{a}	0.80 ± 0.01 ^{a}
18:0	4.43 ± 0.13 ^{c}	3.07 ± 0.03 ^{a}	3.92 ± 0.24 ^{b}	4.08 ± 0.18 ^{b}
18:1n-9 trans	2.02 ± 0.07 ^{c}	1.92 ± 0.05 ^{b}	1.73 ± 0.15 ^{a}	1.73 ± 0.13 ^{a}
18:1n-9 cis	5.90 ± 0.40 ^{a}	7.97 ± 0.32 ^{b}	8.49 ± 0.19 ^{c}	8.42 ± 0.45 ^{bc}
18:2n-6 trans	0.28 ± 0.15 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
18:2n-6 cis	46.63 ± 1.74 ^{c}	41.56 ± 0.42 ^{b}	38.77 ± 0.23 ^{a}	36.60 ± 3.07 ^{a}
20:0	0.31 ± 0.02 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
20:1	1.40 ± 0.04 ^{a}	1.69 ± 0.00 ^{c}	1.46 ± 0.02 ^{b}	1.51 ± 0.05 ^{bc}
18:3n-3	3.35 ± 0.24 ^{a}	4.58 ± 0.02 ^{b}	6.42 ± 0.20 ^{c}	6.15 ± 0.12 ^{c}
20:2	9.00 ± 0.40 ^{c}	8.11 ± 0.09 ^{b}	6.56 ± 0.24 ^{a}	6.41 ± 0.44 ^{a}
22:0	0.39 ± 0.09 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
20:3n-6	1.63 ± 0.01 ^{b}	1.60 ± 0.01 ^{b}	1.11 ± 0.14 ^{a}	1.01 ± 0.22 ^{a}
22:1n-9	0.49 ± 0.03 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
20:3n-3	0.76 ± 0.02 ^{a}	0.81 ± 0.02 ^{b}	1.40 ± 0.05 ^{c}	1.35 ± 0.05 ^{c}
20:4n-6	0.34 ± 0.06 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
23:0	1.21 ± 0.09 ^{c}	0.56 ± 0.03 ^{a}	0.66 ± 0.04 ^{b}	0.72 ± 0.08 ^{b}
22:2	0.80 ± 0.00 ^{c}	0.62 ± 0.08 ^{b}	0.24 ± 0.00 ^{a}	0.20 ± 0.35 ^{a}
24:0	0.81 ± 0.02 ^{c}	0.50 ± 0.01 ^{a}	0.61 ± 0.05 ^{b}	0.67 ± 0.07 ^{b}
20:5n-3	0.70 ± 0.14 ^{a}	2.52 ± 0.16 ^{b}	3.73 ± 0.17 ^{c}	4.50 ± 0.89 ^{d}
24:1	0.76 ± 0.09 ^{a}	0.72 ± 0.01 ^{a}	0.70 ± 0.20 ^{a}	0.70 ± 0.05 ^{a}
22:6n-3	0.45 ± 0.08 ^{a}	5.83 ± 0.27 ^{a}	7.80 ± 0.45 ^{a}	8.71 ± 1.51 ^{a}
SFA	26.68 ± 1.63 ^{b}	20.98 ± 0.03 ^{a}	20.84 ± 0.48 ^{a}	21.91 ± 0.58 ^{a}
MUFA	11.83 ± 0.43 ^{a}	13.39 ± 0.28 ^{b}	13.15 ± 0.25 ^{b}	13.16 ± 0.50 ^{b}
HUFA	3.89 ± 0.08 ^{a}	11.75 ± 0.32 ^{b}	14.04 ± 0.86 ^{c}	15.58 ± 2.20 ^{c}

Values are presented as the mean ± standard deviation (n = 3). Each experiment was performed in triplicate. Different superscript letters indicate significant differences ($p < 0.05$). FFA, free fatty acid; HUFA, highly unsaturated fatty acid; MUFA, monosaturated fatty acid; SFA, saturated fatty acid.

4. Discussion

This study investigated the effects of water temperature, salinity, density, DO concentration, and the amount of *Chlorella* supplied on the mass culture of large-type rotifers at low temperatures to determine their better rearing water quality conditions.

Rotifers generally proliferate well at high water temperatures of ~20–30 °C [43]. If rotifers active at high temperatures are supplied as an initial feed for cold-water fish species, the water temperature difference decreases their activity, making them sink and pollute the water [3,29].

The increase in water temperatures due to global warming has led to a decrease in the habitats of cold-water fish species [27]. In a previous study, the walleye pollock larvae survival rate was the highest, with 50% at 5 °C. The larvae survival rate also declined as water temperatures increased and was lowest, with 0.3% at 14 °C [27]. To preserve these species and replace fishery with aquaculture production, the development of cold-water fish breeding technology, mass cultures of low-temperature rotifers, and optimal environments to maintain these fish and rotifers are required.

In the present study, rotifers cultured at 15 °C showed faster growth compared with those cultured at 10 °C. Previous studies have demonstrated that a lower water temperature results in a lower rotifer growth rate and a longer growth period [43,44]. Generally, when rotifers are cultured at water temperatures of approximately 25 °C, maximum density is

reached within 5–8 days [13,45,46]. However, in the present study, it took approximately 11 days for rotifers cultured at 15 °C to reach the highest density. In addition, the density of rotifers cultured at 10 °C was at half their highest density at 15 °C on day 14, suggesting that 15 °C is a more appropriate temperature for mass culture at low temperature when considering the culture period. Also, since rotifers can be cultured at low temperatures of 10 °C and 15 °C they can be suitable as feed for cold-water fish larvae reared at those cold temperatures.

The lifespan of rotifers was extended, but reproduction decreased owing to low temperatures. This phenomenon is referred to as the cost of reproduction and has been verified in various organisms [47–51]. These results indicate that culture temperature plays an important role in the growth of rotifers, and culturing rotifers at low temperatures requires more time, as observed in previous studies [43,44].

The effects of salinity on rotifer growth have been previously reported. *Colurella dicentra*, a small rotifer, showed the highest density at low salinity [52], whereas *Synchaeta cecilia valentina* and *Synchaeta littoralis* showed the highest growth rates at 20 and 25 psu, respectively. In accordance with the salinity range reported by Yin and Zhao [53] reported that the growth and egg-laying rate for *B. plicatilis* and *Brachionus rotundiformis* were high at ~10–20 psu, which is an optimal salinity range for the mass culture of rotifers [54,55]. Moreover, when *Brachionus koreanus* was exposed to different salinities, the fastest growth rate was observed at a low salinity of 15 psu, while its lifespan was extended at a high salinity (35 psu) [56]. Salinity combined with water temperature is a factor that has an important influence on rotifers, and studies on the optimal salinity of various rotifers have been conducted. In the present study, rotifer growth was high at 17 psu, with the rotifers exhibiting superior growth at 11–14 psu than at 20–23 psu. These results indicate that the growth rate was higher at salinities less than 20 psu, and the optimal salinity for *B. plicatilis* at low temperatures was 17 psu.

In the growing aquaculture industry, the production of high-density rotifers as feed for fish larvae is essential for a stable supply of rotifers. Factors limiting the high-density culture of rotifers include lack of feed, low DO concentrations, and the accumulation of toxic undissociated ammonia in the culture water [57]. In addition to these, in the present study, whether the initial inoculation density affects rotifer propagation was investigated. The growth of rotifers in response to the initial inoculation density peaked on day 11 when 500 and 700 individuals/mL were inoculated. When 300 individuals/mL were inoculated, the density increased until day 12, and when 100 individuals/mL were inoculated, the lowest growth density was observed at 791 individuals/mL. Suantika et al. (2000) reported a maximum rotifer *Brachionus* biomass concentration of 500–1000 individuals/mL [58]. The time to reach 1000 individuals/mL was the fastest at 4 days when 700 individuals/mL were inoculated; however, the growth rate gradually decreased thereafter. Although the initial inoculation density of rotifers dictates when the culture reaches its highest density, it appears that the highest density reached becomes the same over time [59].

Seasonal water temperature and DO concentrations in the wild affect zooplankton growth [60]. Rotifer growth is known to be affected by DO concentration [26]. Yoshimura et al. [57] reported that DO concentrations of at least 2 mg/L or more are required for rotifer growth. Czarnoleski et al. (2015) studied the body size of rotifers (*Keratella cochlearis*) at different depths in 20 European lakes and found that rotifers were larger not only in cold water but also in oxygen-rich environments. In hypoxic waters, rotifers remained small regardless of temperature [61]. This means that oxygen has an important effect on the growth of rotifers. In the present study, the highest rotifer density and SGR did not differ substantially at DO concentrations of 8–23 mg/L. However, on the one hand, when DO concentrations were 11, 14, and 17 mg/L, the highest growth density was observed on day 11. On the other hand, the maximum growth density was observed on day 10 when the DO concentrations were 8, 20, and 23 mg/L. Nevertheless, the maximum growth density at DO concentrations of 8 and 23 mg/L differed from that of the other experimental groups. Based on these results, there was no significant difference in the growth of rotifers

depending on the DO concentration, and considering the cost of using liquid oxygen to regulate supersaturation and the difficulty of controlling the concentration, the DO concentration of about 8 mg/L appears to be sufficient for the culturing of rotifers at low temperatures.

Nitrogen is an essential element for all living organisms. In the environment, N actively cycles between water, air, and soil in various concentrations and forms, such as dinitrogen gas (N_2), ammonium, nitrite, nitrate, and organic matter [62]. The available sources of inorganic N in the environment are ammonium, nitrite, and nitrate, which present variable concentrations in different habitats [63]. In seawaters, the assumed concentration of nitrate is between 7 and 31 μM; of ammonium, 0.001–0.3 μM; and of nitrite, about 0.006–0.1 μM [63]. In an aquatic environment, ammonia presents two main forms: conjugated unionized ammonia and ionized ammonium [64]. The concentration of undissociated ammonia is a major factor inhibiting rotifer growth [57]. Han et al. (2022) reported that survival, life history parameters, and reproductive patterns of rotifers treated with high concentrations of NH3-N (≥8.5 mg/L) were suppressed [65]. The present study showed an increase in ammonium with propagation density. In particular, nitrite increased rapidly when rotifers reached their maximum density. It seems difficult to control the concentration of nitrite and nitrate with a 10% water change as the density of rotifers increases.

Determining the optimal amount of feed is important because feed negatively affects the growth, egg-laying rate, and survival rate of rotifers [41,66]. Furthermore, the amount of available feed is correlated with the reproductive rate of rotifers and directly affects digestibility, anabolism, and growth [67,68]. The algae that are widely used in mass culturing rotifers *B. plicatilis* and *B. rotundiformis* include seawater *C. ellipsoidea*, *Nannochloropsis oculata*, *Isochrysis galbana*, and *Tetraselmis* sp. and freshwater *C. vulgaris* [68,69]. In particular, freshwater *C. vulgaris* is generally the most widely used because of its small size, good growth, and economical use [36]. According to the results of the present study, the amount of *Chlorella* (*C. vulgaris*) supplied affected the growth density of rotifers. The highest growth density was obtained when *Chlorella* supplied was 7.5×10^{12} cells/mL; when *Chlorella* supplied was 6×10^{12} cells/mL, the amount of *Chlorella* was insufficient. Therefore, 7.5×10^{12} cells/mL of *Chlorella* may be appropriate for rotifers cultured at low temperatures.

Unsaturated fatty acids are the major components of cell membranes and precursors of bioactive molecules, which have hormone-like activity [70]. Some studies have shown a lack of essential nutrients in rotifers; therefore, they should be enriched before feeding to fish larvae [71,72]. Commercial enhancement products are more widely used because they are easier to handle [73]. Studies revealed that DHA is associated with bone and cartilage development in fish and contributes to the prevention of bone deformities [74,75]. Previous studies have also demonstrated that the metabolic rate of organisms is inhibited at low temperatures [76–78]. Lee et al. [79] reported that the amount of total fatty acids is reduced significantly at low temperatures (20 and 15 °C) compared with that at 25 °C in *B. koreanus*. In general, rotifers were enriched within 12 h at ~25–27 °C [80–82]. However, studies on the optimal enrichment time for rotifers cultured at low temperatures are limited. In the present study, fatty acids were analyzed after enrichment for 6, 12, and 24 h. The results showed that enriched rotifers had higher MUFA and HUFA content than those of unenriched rotifers. Although there was no significant difference in the content of MUFAs and HUFAs at 12 and 24 h, they were slightly higher at 24 h. In addition, although the present study did not compare the effect of enrichment between high and low temperatures, it was confirmed that HUFA content increased more at 12 h relative to levels before enrichment at low temperatures. Nevertheless, rotifer enrichment at low temperatures is prolonged compared with that at ~25–27 °C. Therefore, the optimal rotifer enrichment time at low temperatures was determined to be between 12 and 24 h.

5. Conclusions

Considering the growth period and density of rotifers cultured at low temperatures, the optimal water temperature was 15 °C. The optimal salinity was approximately 17 psu; a salinity of more than 20 psu inhibited the growth of rotifers. The growth density can be increased when the initial inoculation density is 300 cells/mL or more, and no significant difference in rotifer growth was observed at a DO concentration of 8 mg/L or more. When the initial inoculation density was 300 cells/mL, the optimal amount of *Chlorella* was 7.5 cells/mL, and the ammonium concentration increased depending on the growth of the rotifer. The optimal enrichment time of rotifers at low temperatures is within 12 to 24 h, as the HUFA content. Therefore, for rotifer mass culture at low temperatures, optimal conditions should include a water temperature of 15 °C considering culture period, salinity of approximately 17 psu, initial inoculation density of 300 individuals/mL or more, a DO concentration of approximately 8 mg/L, *Chlorella* cells at the concertation of 7.5 cells/mL, and an enrichment time of 12–24 h.

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References

1. Battaglene, S.C.; Purser, G.; Hart, P.R.; Morehead, D.T. Priorities for live feed production and research in Tasmania. In *The Second Hatchery Feeds and Technology Workshop*; Kolkovski, S.J., Heine, S.C., Eds.; University of Tasmania: Tasmania, Australia, 2004; pp. 104–112.
2. Hirayama, K.; Kusano, T. Fundamental studies on physiology of rotifer for its mass cultrure-II. *Nippon. Suissan Gakkaishi* **1972**, *38*, 1357–1363. [CrossRef]
3. Lubzens, E. Raising rotifers for use in aquaculture. In *Rotifer Symposium IV*; Springer: Dordrecht, The Netherlands, 1987; Volume 147, pp. 245–255, ISBN 9401083029.
4. Yukino, T.; Hayashi, M.; Yoshimatsu, T.; Maruyama, I.; Murata, H. Nutritional enrichment and cultivation of rotifers by feeding of docosahexaenoic acid-enriched *Chlorella vulgaris* K-22. *Aquac. Sci.* **2004**, *52*, 381–386. [CrossRef]
5. Hayashi, M.; Yukino, T.; Watanabe, F.; Miyamoto, E.; Nakano, Y. Effect of vitamin B12-enriched thraustochytrids on the population growth of rotifers. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 222–225. [CrossRef]
6. Hirayama, K.; Ogawa, S. Fundamental studies on physiology of rotifer for its mass culture-I. *Nippon Suissan Gakkaishi* **1972**, *38*, 1207–1214. [CrossRef]
7. Fu, Y.; Hada, A.; Yamashita, T.; Yoshida, Y.; Hino, A. Development of a continuous culture system for stable mass production of the marine rotifer Brachionus. *Hydrobiologia* **1997**, *358*, 145–151. [CrossRef]
8. Fielder, D.S.; Purser, G.J.; Battaglene, S.C. Effect of rapid changes in temperature and salinity on availability of the rotifers *Brachionus rotundiformis* and *Brachionus plicatilis*. *Aquaculture* **2000**, *189*, 85–99. [CrossRef]
9. Altaff, K.; Janakiraman, A. Effect of temperature on mass culture of three species of zooplankton, *Brachionus plicatilis*, *Ceriodaphnia reticulata* and *Apocyclops dengizicus*. *Int. J. Fish. Aquat. Stud.* **2015**, *2*, 49–53.
10. Önal, U.; Topaloğlu, G.; Sepil, A. The performance of continuous rotifer (*Brachionus plicatilis*) culture system for ornamental fish production. *J. Life Sci.* **2015**, *10*, 207–213. [CrossRef]
11. Minkoff, G.; Lubzens, E.; Kahan, D. Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. *Hydrobiologia* **1983**, *104*, 61–69. [CrossRef]
12. Yoseda, K.; Asami, K.; Fukumoto, M.; Takaira, S.; Kurokawa, Y.; Kawai, S. Effects of two types of rotifer on first-feeding success and early survival in coral trout *Plectropomus leopardus* larvae. *Aquac. Sci.* **2003**, *51*, 101–108. [CrossRef]
13. Lubzens, E.; Minkoff, G. Influence of the age of algae fed to rotifers (*Brachionus plicatilis* O.F. Müller) on the expression of mixis in their progenies. *Oecologia* **1988**, *75*, 430–435. [CrossRef] [PubMed]

14. Stelzer, C.-P. Phenotypic plasticity of body size at different temperatures in a planktonic rotifer: Mechanisms and adaptive significance. In *Functional Ecology*; British Ecological Society: London, UK, 2002; Volume 16, pp. 835–841.
15. Snell, T.W.; Bieberich, C.J.; Fuerst, R. The effects of green and blue-green algal diets on the reproductive rate of the rotifer *Brachionus plicatilis*. *Aquaculture* **1983**, *31*, 21–30. [CrossRef]
16. Park, H.-G.; Hur, S.-B. Production and hatching rate of resing egg of Korean rotifer, *Brachionus plicatilis* (S-type) with different diets. *J. Aquac.* **1996**, *9*, 329–337.
17. Yoshimatsu, T.; Higuchi, T.; Zhang, D.; Fortes, N.R.; Tanaka, K.; Yoshimura, K. Effect of dietary cobalt supplementation on the population growth of rotifer *Brachionus rotundiformis*. *Fish. Sci.* **2006**, *72*, 214–216. [CrossRef]
18. Theilacker, G.H.; McMaster, M.F. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. *Mar. Biol.* **1971**, *10*, 183–188. [CrossRef]
19. Takeuchi, T. A review of feed development for early life stages of marine finfish in Japan. *Aquaculture* **2001**, *200*, 203–222. [CrossRef]
20. Tomoda, T.; Koiso, M.; Shima, Y. Dietary value of marine rotifer *Brachionus plicatilis* after enrichment produced by batch culture and extensive continuous culture methods. *Nippon. Suissan Gakkaishi* **2007**, *73*, 505–507. [CrossRef]
21. Ito, S.; Sakamoto, H.; Hori, M.; Hirayama, K. Morphological characteristics and suitable temperature for the growth of several strains of the rotifer, *Brachionus plicatilis*. *Bull. Fac. Fish. Nagasaki Univ.* **1981**, *51*, 9–16.
22. Miracle, M.R.; Serra, M. Salinity and temperature influence in rotifer life history characteristics. In *Rotifer Symposium V*; Springer: Dordrecht, The Netherlands, 1989; pp. 81–102, ISBN 9401066949.
23. Hagiwara, A.; Kotani, T.; Snell, T.W.; Assava-Aree, M.; Hirayama, K. Morphology, reproduction, genetics, and mating behavior of small, tropical marine *Brachionus* strains (Rotifera). *J. Exp. Mar. Bio. Ecol.* **1995**, *194*, 25–37. [CrossRef]
24. Snell, T.W.; Moffat, B.D. A 2-d Life cycle test with the rotifer *Brachionus calyciflorus*. *Environ. Toxicol. Chem.* **1992**, *11*, 1249–1257. [CrossRef]
25. Kuefler, D.; Avgar, T.; Fryxell, J.M. Density- and resource-dependent movement characteristics in a rotifer. *Funct. Ecol.* **2013**, *27*, 323–328. [CrossRef]
26. Lingampally, V.; Solanki, V.R.; Anuradha, D.L.; Raja, S.S. Investigating the Effect of Dissolved Oxygen on Rotifers of Chakki Talab, Bodhan, Telangana, India. *New Vis. Biol. Sci.* **2021**, *3*, 142–147.
27. Kim, S.-S.; Lee, C.-J.; Yoo, H.-K.; Choi, J.; Byun, S.-G.; Kim, W.-J.; Lim, H.-J.; Park, J.-S. Effect of water temperature on walleye pollock (*Gadus chalcogrammus*) embryos, larvae and juveniles: Survival, HSP70 expression, and physiological responses. *Aquaculture* **2022**, *554*, 738136. [CrossRef]
28. Choi, J.; Han, G.S.; Lee, K.W.; Byun, S.-G.; Lim, H.J.; Lee, C.-H.; Lee, D.-Y.; Kim, H.S. Effects of feeding differentially enriched Artemia nauplii on the survival, growth, fatty acid composition, and air exposure stress response of Pacific cod (*Gadus macrocephalus*) larvae. *Aquac. Rep.* **2021**, *21*, 100829. [CrossRef]
29. Øie, G.; Olsen, Y. Influence of rapid changes in salinity and temperature on the mobility of the rotifer *Brachionus plicatilis*. In *Rotifer Symposium VI*; Springer: Dordrecht, The Netherlands, 1993; pp. 81–86, ISBN 9401047006.
30. Makridis, P.; Fjellheim, A.J.; Skjermo, J.; Vadstein, O. Control of the bacterial flora of *Brachionus plicatilis* and *Artemia franciscana* by incubation in bacterial suspensions. *Aquaculture* **2000**, *185*, 207–218. [CrossRef]
31. Tomoda, T.; Dan, S. Stagnant water larviculture using the rotifer *Brachionus plicatilis* acclimated at low temperature in Pacific cod *Gadus macrocephalus*. *Aquac. Sci.* **2014**, *62*, 307–318. [CrossRef]
32. Tomoda, T. Temperature effects of rotifer *Brachionus plicatilis* mass culture on rearing efficiency of larval Japanese flounder *Paralichthys olivaceus*. *Nippon Suisan Gakkaishi* **2008**, *74*, 625–635. [CrossRef]
33. Hagiwara, A.; Suga, K.; Akazawa, A.; Kotani, T.; Sakakura, Y. Development of rotifer strains with useful traits for rearing fish larvae. *Aquaculture* **2007**, *268*, 44–52. [CrossRef]
34. Reitan, K.I.; Rainuzzo, J.R.; Øie, G.; Olsen, Y. A review of the nutritional effects of algae in marine fish larvae. *Aquaculture* **1997**, *155*, 207–221. [CrossRef]
35. Cahu, C.L.; Infante, J.L.Z.; Peres, A.; Quazuguel, P.; Le Gall, M.M. Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing: Effect on digestive enzymes. *Aquaculture* **1998**, *161*, 479–489. [CrossRef]
36. Assavaaree, M.; Hagiwara, A.; Ide, K.; Maruyama, K.; Lubzens, E. Low-temperature preservation (at 4 °C) of marine rotifer *Brachionus*. *Aquac. Res.* **2001**, *32*, 29–39. [CrossRef]
37. Sargent, J.; Bell, G.; McEvoy, L.; Tocher, D.; Estevez, A. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* **1999**, *177*, 191–199. [CrossRef]
38. NRC. *National Research Council, Nutrient Requirements of Fish*; National Academies Press: Washington, DC, USA, 1993; ISBN 0309048915.
39. Sargent, J.; McEvoy, L.; Estevez, A.; Bell, G.; Bell, M.; Henderson, J.; Tocher, D. Lipid nutrition of marine fish during early development: Current status and future directions. *Aquaculture* **1999**, *179*, 217–229. [CrossRef]
40. Abd Rahman, A.R.; Che Cob, Z.; Jamari, Z.; Mohamed, A.M.; Toda, T.; Haji Ross, O. The Effects of Microalgae as Live Food for *Brachionus plicatilis* (Rotifer) in Intensive Culture System. *Trop. Life Sci. Res.* **2018**, *29*, 127–138. [CrossRef] [PubMed]
41. Rico-Martínez, R.; Dodson, S.I. Culture of the rotifer *Brachionus calyciflorus* Pallas. *Aquaculture* **1992**, *105*, 191–199. [CrossRef]
42. Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]

43. Sarma, S.S.S. Effect of Chlorella density and temperature on somatic growth and age at maturity of the rotifer *Brachionus patulus* (Muller)(Rotifera). *Curr. Sci.* **1989**, *58*, 788–791.
44. Yona, G.K. Effect of culture temperature on rotifers (*Brachionus plicatilis sensu strictu*) size and reproduction activities. *Tanzan. J. Agric. Sci.* **2018**, *17*, 23–30.
45. Tomas, C.; Bum, H.U.R.S.; Jun, K.I.M.H. Lifespan and fecundity of three types of rotifer, *Brachionus plicatilis* by an individual culture. *Korean J. Fish. Aquat. Sci.* **1993**, *26*, 511–518.
46. Kobayashi, T.; Nagase, T.; Hino, A.; Takeuchi, T. Influence of photoperiod on the eicosapentaenoic acid concentration of *Nannochloropsis*, and fatty acid composition of S-type rotifer *Brachionus* in a continuous culture system. *Aquac. Sci.* **2009**, *57*, 133–139. [CrossRef]
47. Kuhla, A.; Hahn, S.; Butschkau, A.; Lange, S.; Wree, A.; Vollmar, B. Lifelong caloric restriction reprograms hepatic fat metabolism in mice. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2014**, *69*, 915–922. [CrossRef] [PubMed]
48. Heck, M.J.; Pehlivanovic, M.; Purcell, J.U.; Hahn, D.A.; Hatle, J.D. Life-extending dietary restriction reduces oxidative damage of proteins in grasshoppers but does not alter allocation of ingested nitrogen to somatic tissues. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2017**, *72*, 616–623. [CrossRef] [PubMed]
49. Pierce, G.J.; Valavanis, V.D.; Guerra, A.; Jereb, P.; Orsi-Relini, L.; Bellido, J.M.; Katara, I.; Piatkowski, U.; Pereira, J.; Balguerias, E.; et al. A review of cephalopod-environment interactions in European Seas. *Hydrobiologia* **2008**, *612*, 49–70. [CrossRef]
50. Sarma, S.S.S.; Nandini, S.; Gulati, R.D. Cost of reproduction in selected species of zooplankton (rotifers and cladocerans). *Hydrobiologia* **2002**, *481*, 89–99. [CrossRef]
51. Stelzer, C.-P. Evolution of rotifer life histories. *Hydrobiologia* **2005**, *546*, 335–346. [CrossRef]
52. Chigbu, P.; Suchar, V.A. Isolation and culture of the marine rotifer, *Colurella dicentra* (Gosse, 1887), from a Mississippi Gulf Coast estuary. *Aquac. Res.* **2006**, *37*, 1400–1405. [CrossRef]
53. Yin, X.W.; Zhao, W. Studies on life history characteristics of *Brachionus plicatilis* O. F. Müller (Rotifera) in relation to temperature, salinity and food algae. *Aquat. Ecol.* **2008**, *42*, 165–176. [CrossRef]
54. Oltra, R.; Todolf, R. Effects of temperature, salinity and food level on the life history traits of the marine rotifer *Synchaera cecilia valentina*, n. subsp. *J. Plankton Res.* **1997**, *19*, 693–702. [CrossRef]
55. Bosque, T.; Hernandez, R.; Pérez, R.; Todolí, R.; Oltra, R. Effects of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. *J. Exp. Mar. Bio. Ecol.* **2001**, *258*, 55–64. [CrossRef]
56. Lee, M.-C.; Park, J.C.; Kim, D.-H.; Kang, S.; Shin, K.-H.; Park, H.G.; Han, J.; Lee, J.-S. Interrelationship of salinity shift with oxidative stress and lipid metabolism in the monogonont rotifer *Brachionus koreanus*. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2017**, *214*, 79–84. [CrossRef]
57. Yoshimura, K.; Kitajima, C.; Miyamoto, Y.; Kishimoto, G. Factors inhibiting growth of the rotifer *Brachionus plicatilis* in high density cultivation by feeding condensed Chlorella. *Nippon. Suissan Gakkaishi* **1994**, *60*, 207–213. [CrossRef]
58. Suantika, G.; Dhert, P.; Nurhudah, M.; Sorgeloos, P. High-density production of the rotifer *Brachionus plicatilis* in a recirculation system: Consideration of water quality, zootechnical and nutritional aspects. *Aquac. Eng.* **2000**, *21*, 201–213. [CrossRef]
59. Zhang, K.; Wan, Q.; Xi, Y.-L. Competition between *Brachionus calyciflorus* and *Brachionus angularis* (Rotifera) in relation to algal food level and initial population density. *Ann. Limnol. Int. J. Limnol.* **2019**, *55*, 2. [CrossRef]
60. Karpowicz, M.; Ejsmont-Karabin, J.; Kozłowska, J.; Feniova, I.; Dzialowski, A.R. Zooplankton community responses to oxygen stress. *Water* **2020**, *12*, 706. [CrossRef]
61. Czarnoleski, M.; Ejsmont-Karabin, J.; Angilletta, M.J., Jr.; Kozlowski, J. Colder rotifers grow larger but only in oxygenated waters. *Ecosphere* **2015**, *6*, 1–5. [CrossRef]
62. Mandal, S.; Shurin, J.B.; Efroymson, R.A.; Mathews, T.J. Functional divergence in nitrogen uptake rates explains diversity–productivity relationship in microalgal communities. *Ecosphere* **2018**, *9*, e02228. [CrossRef]
63. Sanz-Luque, E.; Chamizo-Ampudia, A.; Llamas, A.; Galvan, A.; Fernandez, E. Understanding nitrate assimilation and its regulation in microalgae. *Front. Plant Sci.* **2015**, *6*, 899. [CrossRef]
64. Randall, D.J.; Tsui, T.K.N. Ammonia toxicity in fish. *Mar. Pollut. Bull.* **2002**, *45*, 17–23. [CrossRef]
65. Han, C.; Kim, H.-J.; Sakakura, Y.; Hagiwara, A. Species-specific ammonia tolerance in the marine rotifers *Brachionus plicatilis* and *Brachionus rotundiformis*: Reproductive characteristics and its mechanisms. *Aquaculture* **2022**, *550*, 737837. [CrossRef]
66. Stemberger, R.S.; Gilbert, J.J. Body size, food concentration, and population growth in planktonic rotifers. *Ecology* **1985**, *66*, 1151–1159. [CrossRef]
67. Starkweather, P.L. Aspects of the feeding behavior and trophic ecology of suspension-feeding rotifers. In *Rotatoria*; Springer: Dordrecht, The Netherlands, 1980; pp. 63–72, ISBN 9400992114.
68. Korstad, J.; Vadstein, O.; Olsen, Y. Feeding kinetics of *Brachionus plicatilis* fed Isochrysis galbana. *Hydrobiologia* **1989**, *186*, 51–57. [CrossRef]
69. PARK, H.G.; KIM, S.K.; PARK, K.Y.; PARK, Y.J. High density cultivation of rotifer, *Brachionus rotundiformis* in the different diets. *Korean J. Fish. Aquat. Sci.* **1999**, *32*, 280–283.
70. Tocher, D.R. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac. Res.* **2010**, *41*, 717–732. [CrossRef]
71. Hamre, K. Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries. *Aquaculture* **2016**, *450*, 136–142. [CrossRef]

72. Kotani, T. The current status of the morphological classification of rotifer strains used in aquaculture. In *Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology*; Hagiwara, A., Yoshinaga, T., Eds.; Springer: Singapore, 2017; pp. 3–13.
73. Eryalcin, K. Effects of different commercial feeds and enrichments on biochemical composition and fatty acid profile of rotifer (*Brachionus Plicatilis*, Muller 1786) and *Artemia franciscana*. *Turk. J. Fish. Aquat. Sci.* **2018**, *18*, 81–90. [CrossRef] [PubMed]
74. Roo, F.J.; Hernández-Cruz, C.M.; Socorro, J.A.; Fernández-Palacios, H.; Montero, D.; Izquierdo, M.S. Effect of DHA content in rotifers on the occurrence of skeletal deformities in red porgy *Pagrus pagrus* (Linnaeus, 1758). *Aquaculture* **2009**, *287*, 84–93. [CrossRef]
75. Izquierdo, M.S.; Scolamacchia, M.; Betancor, M.; Roo, J.; Caballero, M.J.; Terova, G.; Witten, P.E. Effects of dietary DHA and α-tocopherol on bone development, early mineralisation and oxidative stress in *Sparus aurata* (Linnaeus, 1758) larvae. *Br. J. Nutr.* **2013**, *109*, 1796–1805. [CrossRef]
76. Conti, B. Considerations on temperature, longevity and aging. *Cell. Mol. Life Sci.* **2008**, *65*, 1626–1630. [CrossRef]
77. Gillooly, J.F.; Brown, J.H.; West, G.B.; Savage, V.M.; Charnov, E.L. Effects of size and temperature on metabolic rate. *Science* **2001**, *293*, 2248–2251. [CrossRef]
78. Waalen, J.; Buxbaum, J.N. Is older colder or colder older? The association of age with body temperature in 18,630 individuals. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2011**, *66*, 487–492. [CrossRef]
79. Lee, M.-C.; Yoon, D.-S.; Lee, Y.; Choi, H.; Shin, K.-H.; Park, H.G.; Lee, J.-S. Effects of low temperature on longevity and lipid metabolism in the marine rotifer *Brachionus koreanus*. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2020**, *250*, 110803. [CrossRef] [PubMed]
80. Fu, Z.; Yang, R.; Zhou, S.; Ma, Z.; Zhang, T. Effects of Rotifers Enriched With Different Enhancement Products on Larval Performance and Jaw Deformity of Golden Pompano Larvae *Trachinotus ovatus* (Linnaeus, 1758). *Front. Mar. Sci.* **2021**, *7*, 626071. [CrossRef]
81. Ozdogan, H.B.E.; Savas, S. The effect on fatty acid contents of Rotifer (*Brachionus plicatilis*) of Algamac 3050 and Olio ω-3 supplemented with or without L-Carnitine. *Iran. J. Fish. Sci.* **2022**, *21*, 1097–1107. [CrossRef]
82. Rainuzzo, J.R.; Reitan, K.I.; Olsen, Y. Effect of short- and long-term lipid enrichment on total lipids, lipid class and fatty acid composition in rotifers. *Aquac. Int.* **1994**, *2*, 19–32. [CrossRef]