Aquaculture 546 (2022) 737282

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effects of salinity and temperature on reproductivity and fatty acid synthesis in the marine rotifer *Brachionus rotundiformis*

Min-Chul Lee [a,1], Deok-Seo Yoon [a,1], Jun Chul Park [a], Hyuntae Choi [b], Kyung-Hoon Shin [b], Atsushi Hagiwara [c,d], Jae-Seong Lee [a,*], Heum Gi Park [e,*]

[a] Department of Biological Sciences, College of Science, Sungkyunkwan University, Suwon 16419, South Korea
[b] Department of Marine Science and Convergence Engineering, Hanyang University, Ansan 15588, South Korea
[c] Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan
[d] Organization for Marine Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan
[e] Department of Marine Resource Development, College of Life Sciences, Gangneung-Wonju National University, Gangneung 25457, South Korea

ARTICLE INFO

Keywords:
Life cycle
Culture
Elovl
Fad
PUFA

ABSTRACT

To examine the effect of salinity and temperature in the marine tropical rotifer *Brachionus rotundiformis*, we analyzed various *in vivo* life cycle parameters, fatty acid (FA) composition, and transcriptional levels of elongation of very long-chain fatty acid (*Elovl*) and fatty acid desaturase (*Fad*) genes. In the case of life cycle parameters, the reproduction of *B. rotundiformis* showed the highest fecundity under 5 practical saline unit (PSU) and 30 °C conditions, while the concentrations of total FAs were the highest in 15 PSU and 25 °C-exposed groups, respectively. Besides, changes in culture condition (salinity and temperature) resulted in a decrease in n-3 FAs. Therefore, the correlation between nutrient components and the total yield should be considered to determine the optimal culture conditions for *B. rotundiformis*. The changes in the transcriptional level of *Elovl* and *Fad* genes appear to be involved in the compositional modulations of FA, and in particular, *Elovl9b* could be suggested as useful biomarker due to its temperature-dependent modulation.

1. Introduction

Rotifers (phylum Rotifera), which are microzooplankton, are widely distributed and play a bridge as a food source for higher-level throughout aquatic ecosystems (Hutchinson, 1957). Also, rotifers have characteristics such as small size and slow motility and can be cultured at a relatively high density. In addition to their advantageous physiological features, their nutritional status could be enhanced by fatty acids (Lubzens et al., 1989). Based on these characteristics, rotifers are ideal species for use as food for fish larva in aquaculture (Kafuku and Ikenoue, 1983; Lubzens, 1987). Therefore, it is possible to increase the production of aquaculture by supplying food tailored to the development stage of fish using rotifers (Kuronuma and Fukusho, 1987; Snell and Carrillo, 1984). In particular, monogonont *Brachionus* rotifers (e.g., *Brachionus plicatilis*, *B. rotundiformis*, *B. manjavacas*, and *B. koreanus*) have been known as suitable model species in aquaculture, ecology, gerontology, and ecotoxicology research (Dahms et al., 2011; Won et al., 2017; Gribble and Mark Welch, 2017). Recent studies have been reported with emphasis on the effects of antioxidants (Snell et al., 2012), caloric restriction (Ozaki et al., 2010; Kailasam et al., 2011), and temperature changes (Johnston and Snell, 2016). The marine rotifer *B. rotundiformis* used in this study has advantages in conducting molecular researches as genome-wide analysis has been completed (http://rotifer.skku.edu:8080/Br) (Kang et al., 2020) therefore, can contribute to the aquaculture in the aspect of molecular-based researches.

To date, numerous studies have contributed to aquaculture by utilizing rotifers. Among them, studies to determine the optimal culture conditions were highlighted by controlling the physiological activity of rotifers through changes in temperature or salinity. For example, *B. rotundiformis* Japanese strain showed the most productivity under high temperatures (>30 °C), while *B. plicatilis* had the most productivity at lower temperatures (<25 °C) (Fukusho, 1983; Fukusho and Iwamoto, 1980). Different optimal culture conditions have also been reported from strains originating outside of Japan (Lubzens et al., 1989). Moreover, salinity-induced effects on biological processes have led to changes in the life cycle parameters (e.g., growth, reproduction, lifespan, and

* Corresponding authors.
E-mail addresses: jslee2@skku.edu (J.-S. Lee), hgpark@gwnu.ac.kr (H.G. Park).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.aquaculture.2021.737282
Received 12 January 2021; Received in revised form 23 July 2021; Accepted 2 August 2021
Available online 6 August 2021

population) in rotifers (Cervetto et al., 1999; Yin and Zhao, 2008). For example, when *B. koreanus* was exposed to 15, 25, and 35 practical saline unit (PSU), the fastest reproduction rate was observed at 15 PSU, while the lifespan was prolonged at 35 PSU (Lee et al., 2017a). In addition to reproduction and lifespan, the effects of changes in temperature and salinity on various end-points of rotifers have been studied. Among them, changes in fatty acid (FA) and lipid metabolism are drawing attention. Lipid metabolism is one of the most representative metabolic processes of living organisms, as lipid metabolic pathway is well conserved across various species. Also, changes in FA compositions contribute to reduction in the stress caused by external factors (e.g., xenobiotics, salinity, temperature, and food). In aquaculture, it is important to improve the quantity and quality of FA in fish by supplying appropriate food sources. However, traditional approaches analyzed FA and lipid content in fishes, so additional studies to control their FAs and lipid are needed.

In this study, the tropical marine rotifer *B. rotundiformis* was cultured under different salinity and temperature conditions to observe alteration in their reproduction, fatty acid content, and lipid metabolism. This study aims to establish basic information about the optimal culture conditions for *B. rotundiformis* by analyzing fecundity and lipid metabolism according to changes in temperature and salinity.

2. Materials and methods

2.1. Rotifer stock culture

The monogonont rotifer *B. rotundiformis* was originally collected in Java Island, Indonesia in 1986 by Prof. Kazutsugu Hirayama (Nagasaki University, Nagasaki, Japan) and kindly provided by Prof. Atsushi Hagiwara (Nagasaki University) on January 7th, 2016 (Kim et al., 2017). A single rotifer *B. rotundiformis* was isolated and maintained in filtered 15 PSU artificial seawater (ASW; TetraMarine Salt Pro, Tetra, Cincinnati, OH, USA). Cultures were maintained in 3 L tank under a light:dark 12:12 h photoperiod at 25 °C. The green microalga *Tetraselmis suecica* (6×10^4 cells/mL) was used as a live diet, and provided it every 24 h. Species identification was conducted by analysis of mitochondrial DNA cytochrome oxidase I and morphologic characteristics (Kim et al., 2017).

2.2. Measurement of fecundity and reproductive day

To examine fecundity and reproductive day of *B. rotundiformis* at different salinities (5, 15 [control], 25, and 35 PSU at 25 °C) and temperatures (15, 20, 25 [control], and 30 °C at 15 PSU), cumulative offspring and reproductive day were recorded. Briefly, we used a total of 36 newborn *B. rotundiformis* offsprings (< 2 h after hatching) for each temperature and salinity condition. Individual rotifer was transferred into a new 24-well culture plate (SPL Life Science Co. Ltd., Seoul, South Korea) containing 1 mL ASW, with each containing microalga *T. suecica* (6×10^4 cells/mL) every 24 h. Stereomicroscopy (M205-A, Leica Microsystems, Wetzlar, Germany) was used to observe *B. rotundiformis*. The number of newborn rotifers was counted every 8 h to examine offspring production and reproductive days were counted until rotifers were died. All experiments were performed in biological triplicate.

2.3. Analysis of fatty acid profiles

To analyze FA composition under different salinities (5, 15 [control], 25, and 35 PSU) and temperatures (15, 20, 25 [control], and 30 °C), we collected approximately 6000 adult individuals and extracted total lipids following the protocol from Folch et al. (1957) with minor modifications. Briefly, the lipids were extracted with dichloromethane/methanol 2:1 (*v*/v). Nonadecanoic acid (C19:0) was added to the extracts as an internal standard. Extraction procedures were repeated three times with sonication. The lipid fraction was separated from the water-methanol phase and converted into FA methyl esters (FAMEs) by saponification using 0.5 M KOH-methanol, followed by methylation with borontrifluoride-methanol. Concentrations and compositions of FAMEs were analyzed in a gas chromatograph (HP 7890A, Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector using a fused silica capillary column (HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as a carrier gas. Samples were injected in splitless mode at an initial oven temperature of 60 °C, which was increased to 320 °C at 5 °C/min and then maintained for 10 min. The FAs were identified from the retention times of standards and mass spectra from gas chromatograph-mass spectrometry (HP-7820A; Agilent Technologies) equipped with a fused silica capillary column (DB-5, 60 m × 0.25 mm i.d., 0.25 μm film thickness). All experiments were performed in biological triplicate, with 4,4-dimethyloxazoline (DMOX) derivatization (Garrido and Medina, 1994; Spitzer, 1996) used to check the positions and numbers of double bonds within unsaturated FAs. Briefly, FAMEs were converted into DMOX derivatives with the addition of 2-amino-2-methylpropanol. DMOX derivatives were analyzed and identified by their mass spectra from gas chromatography–mass spectrometry.

2.4. Expression of lipid metabolism-related genes

The elongation of very long-chain fatty acid (*Elovl*) and fatty acid desaturase (*Fad*) genes were identifed by RNA-seq data and the *B. rotundiformis* Jbrowse (http://rotifer.skku.edu:8080/Br) (Kang et al., 2020). Genes were subjected to BLAST analysis in the GenBank non-redundant (NR; including all GenBank, EMBL, DDBJ, and PDB sequences except EST, STS, GSS, and HTGS) amino acid sequence database (http://blast.ncbi.nlm.nih.gov). To investigate expression of *Elovl* and *Fad* genes, we measured mRNA expression levels for 24 h under different salinities (5, 15 [control], 25, and 35 PSU at 25 °C) and temperatures (15, 20, 25 [control], and 30 °C at 15 PSU). Total RNAs were extracted with TRIZOL reagent (Invitrogen, Paisley, Scotland, UK) according to the manufacturer's instructions. Quantity and purity were analyzed spectrometrically at 230, 260, and 280 nm (QIAxpert, Qiagen, Hilden, Germany). To synthesize cDNA for quantitative real-time reverse transcription PCR (qRT-PCR), we used 1 μg of total RNA and oligo(dT)$_{20}$ primer for reverse transcription (SuperScript II RT kit, Invitrogen, Carlsbad, CA, USA). All qRT-PCR amplification was conducted at 95 °C/4 min; 40 cycles of 95 °C/30 s, 58 °C/30 s, 72 °C/30 s, and 72 °C/10 min using SYBR Green as a probe (Molecular Probes Inc., Eugene, OR, USA) in a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA). To confirm amplification of specific products, melting curve cycles were run at the following conditions: 95 °C/1 min; 55 °C/1 min; and 80 cycles of 55 °C/10 s with a 0.5 °C increase per cycle using qRT-PCR F or R primers (Suppl. Table 1). The *B. rotundiformis* elongation factor 1-alpha gene, which exhibited stable expression throughout the experiments, was used as an internal control to normalize expression levels between samples. All experiments were performed in technical triplicate. The relative fold-change in gene expression compared with the control was calculated by the $2^{-\Delta\Delta C}{}_T$ comparative method (Livak and Schmittgen, 2001).

2.5. Statistical analysis

SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are expressed as mean ± S.D. Significant differences

between the control and exposed groups were analyzed using one-way analysis of variance ANOVA followed by Tukey's tests. Overall, differences with $P < 0.05$ were considered significant.

3. Results

3.1. Effects of salinity and temperature on in vivo life parameters

Since stock culture was maintained at 25 °C at 15 PSU, we considered 15 PSU and 25 °C as the control for salinity and temperature experiment, respectively. Rotifer fecundity was significantly decreased ($P < 0.05$) in 25 and 35 PSU compared to the control (15 PSU) in a salinity-dependent manner, while 5 PSU showed the significantly highest reproduction ($P < 0.05$). Under different temperatures, cumulative offspring production was significantly increased ($P < 0.05$) in a temperature-dependent manner, and every group showed significant differences ($P < 0.05$) (Fig. 1A). During reproductive period, 5 and 15 PSU groups showed the longest reproductive period compared to 25 and 35 PSU at significant level ($P < 0.05$). In the temperature experiment, 25 °C groups were the longest compared to 15 and 20 °C, and 15 °C did not produce any offspring during their lifetime. Besides, a lifespan of *B. rotundiformis* showed a negative relationship with an increase in salinity, but the effects of temperature was not related to the lifespan in *B. rotundiformis* (Fig. 1B).

3.2. Effects of salinity and temperature on the content of total, saturated, monounsaturated, and polyunsaturated fatty acids

The composition of FA was measured in response to different salinities (5, 15 [control], and 25 PSU) and temperatures (20, 25 [control], and 30 °C). The fatty acid composition of 35 PSU and 15 °C were not measured, as the reproductivity under 35 PSU and 15 °C was not observed. In the salinity exposure experiment (Fig. 2A), 15 PSU (control) was the highest in all groups, particularly on total fatty acid (TFA), n-3, and n-6 polyunsaturated fatty acid (PUFA). Although the reproduction was the highest in 5 PSU compared to the other groups, the TFA, n-3, and n-6 PUFA in 5 PSU groups were the lowest compared to 15 and 25 PSU groups. In the temperature exposure experiment (Fig. 2B), 25 °C (control) represented the highest in TFA, n-3, and n-6 PUFA.

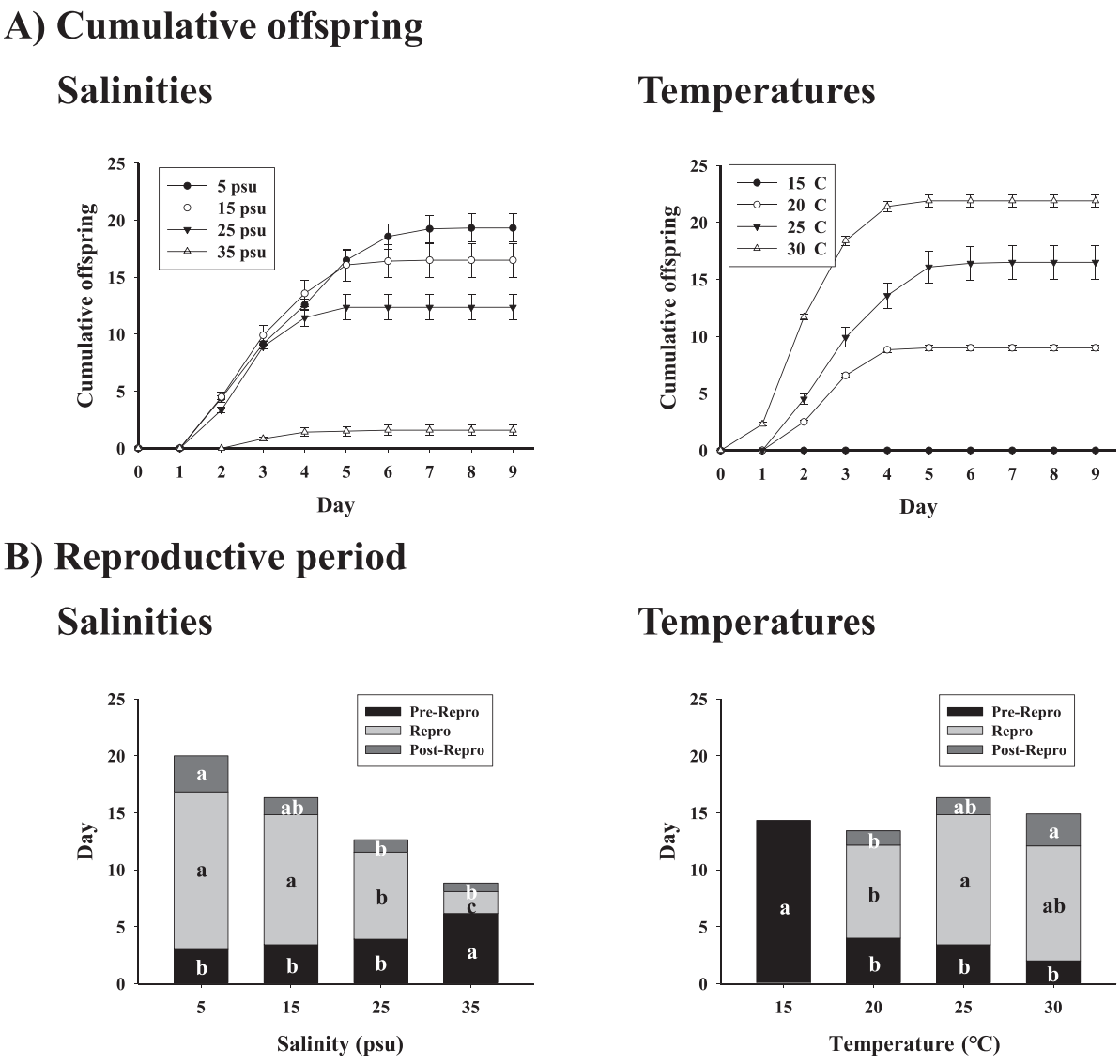

Fig. 1. Effects of different salinities (5, 15 [control], 25, and 35 PSU) and temperatures (15, 20, 25 [control], and 30 °C) on A) cumulative offspring and B) reproductive day. Data are the mean ± standard deviation of three replicates. Significant differences from control values are indicated by different letters ($P < 0.05$) analyzed by analysis of variance. Data are the mean ± standard deviation of triplicates.

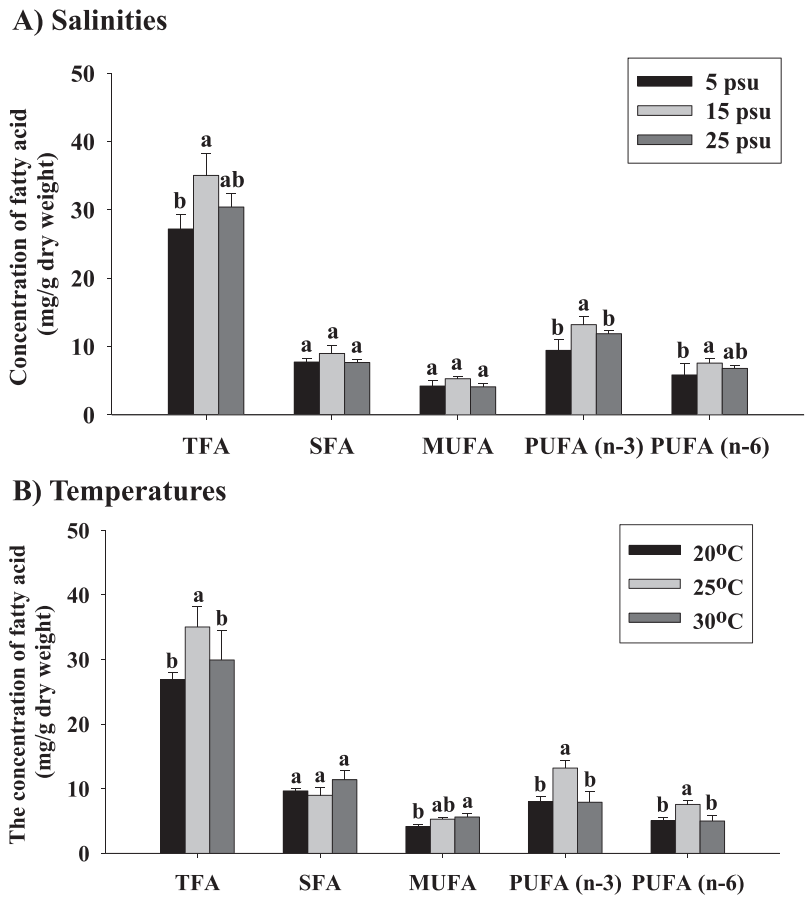

Fig. 2. The content of total fatty acid (TFA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), n-3 polyunsaturated fatty acid (PUFA), and n-6 PUFA under different A) salinities (5, 15 [control], 25, and 35 practical salinity unit) and B) temperatures (15, 20, 25 [control], and 30 °C) in *Brachionus rotundiformis*. Significant differences from control values are indicated by different letters ($P < 0.05$) analyzed by analysis of variance. Data are the mean ± standard deviation of three replicates.

3.3. Effects of salinity and temperature on the content single fatty acids

The composition of single FA was analyzed following FA metabolic pathway. In the salinity exposure experiment, most of the single FA were down-regulated compared to the control (15 PSU), except for 22:0, 24:0, and 20:3n-6 in 5 PSU (Fig. 3A). However, in the temperature exposure experiment, most SFA was significantly increased ($P < 0.05$) compared to the control (25 °C) in 30 °C (Fig. 3B), and the longer the SFA, the higher the composition of SFA compared to the control. Also, the composition of 20:1n-9, 24:1n-9, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3 were higher than the control in 30 °C, with significant up-regulation ($P < 0.05$) particularly observed in 24:1n-9 and 22:5n-3.

3.4. Modulation of lipid metabolism-related genes under salinity and temperature exposure

In the salinity exposure experiment, most of the genes were down-regulated except for *Fad5/6–3* (Fig. 4A). However, in the temperature exposure experiment, the transcript levels of *Fad* genes were up-regulated at 20 and 30 °C compared to the control (Fig. 4B), but *Elovl* genes were not significantly modulated compared to the control, except for *Elovl9b*, which showed temperature-dependent up-regulation pattern.

4. Discussion

In the life cycle parameter results, we found that the abiotic factors (salinity and temperature) induced negative effects on the reproduction in the tropical rotifer *B. rotundiformis*. In particular, it was found that the total fecundity of *B. rotundiformis* was extremely reduced at 35 PSU and 15 °C, indicating high salinity and the low temperature had a significant adverse effect on the reproduction in *B. rotundiformis*. On the other hand, the reproduction was best shown at 5 PSU and 30 °C, so the corresponding salinity and temperature could be regarded as the optimal culture conditions for *B. rotundiformis* in terms of reproduction. A previous study has revealed that the total fecundity of the temperate rotifer *B. koreanus*, was not significantly affected under the same salinity exposure (15, 25, and 35 PSU) (Lee et al., 2017b). Thus, compared to the temperate rotifer, tropical rotifer *B. rotundiformis* responds at a higher sensitivity to salinity changes. On the other hand, at 15 °C, both *B. koreanus* and *B. rotundiformis* showed a significant decrease in reproduction (Lee et al., 2020), indicating that both temperate and tropical *Brachionus* species were significantly affected by low temperature. When exposed to specific environmental conditions (temperature, salinity, pH, and fasting), the reproduction is lowered while the lifespan is extended (Kauler and Enesco, 2011; Yin and Niu, 2011; Yoshinaga et al., 2003). This phenomenon known as the cost of reproduction,has been reported in various rotifers (Snell and King, 1977; Sarma et al., 2002; Stelzer, 2005). However, in this study, prolonged life span was demonstrated in salinity and temperature groups which showed the most reproduction ponential (Suppl. Table 2), which contrasts to the results drawn from previous studies. When environmental stressors induce extremely negative effects on organisms, energy reallocation does not appear since the both lifespan and reproduction of organisms are extremely reduced. Therefore, the cost of reproduction can be observed at a specific condition that does not seriously affect the reproduction of organisms. In this study, the cost of reproduction was not observed even under conditions where the reproduction did not decrease extremely, but the exact cause of this could not be examined. Therefore, further research will be conducted to examine whether this was a species-specific phenomenon.

Fatty acids of the rotifer *B. rotundiformis* were measured after

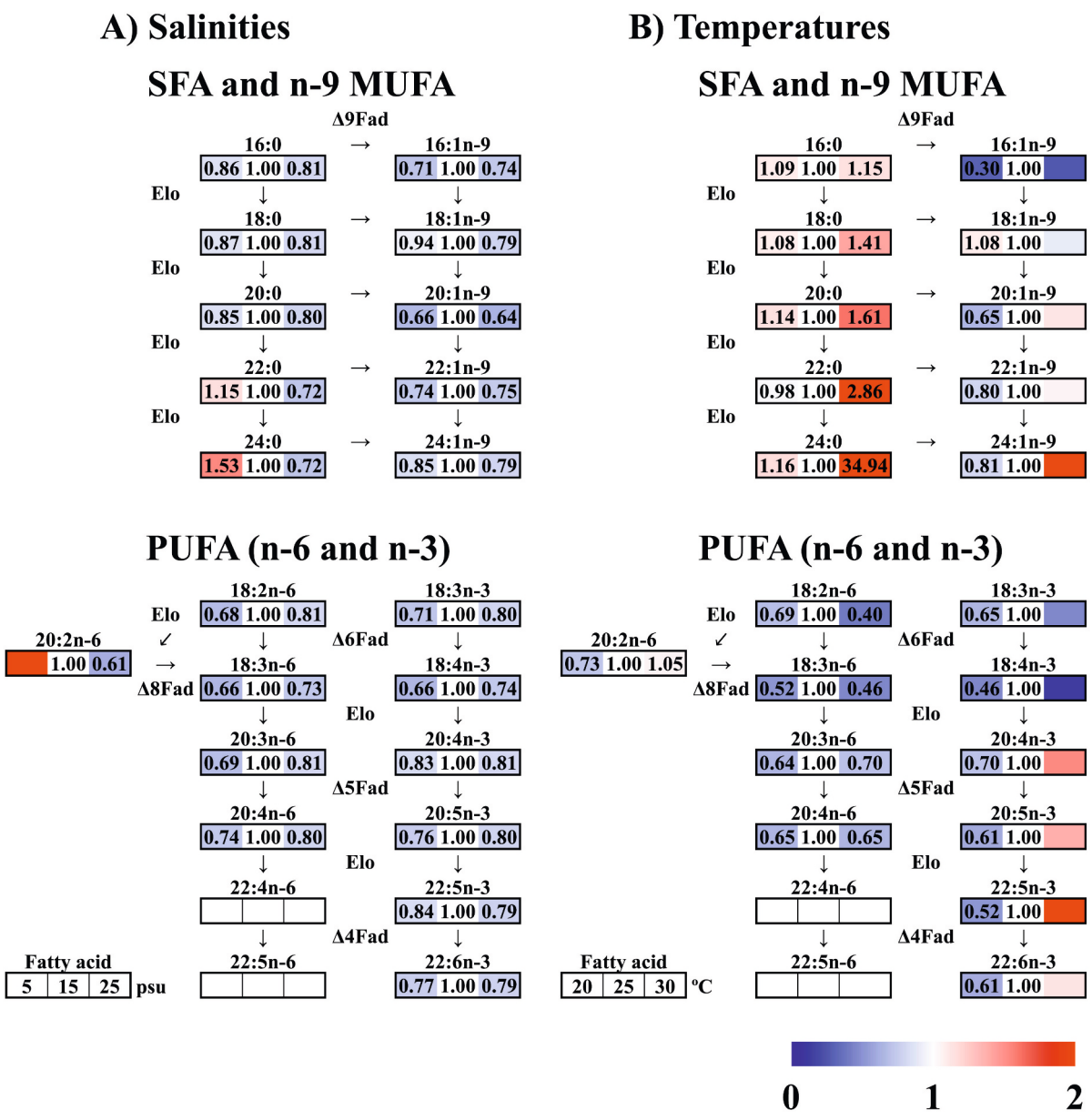

Fig. 3. The content single fatty acids under different A) salinities (5, 15 [control], 25, and 35 practical salinity unit) and B) temperatures (15, 20, 25 [control], and 30 °C) in *Brachionus rotundiformis* represented by a heat map. Elo, elongase; Fad, fatty acid desaturase; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

exposure to salinity (5, 15, and 25 PSU) and temperature (20, 25, and 30 °C) for 24 h, except for 35 PSU and 15 °C, which showed little reproduction. TFAs were the highest in the control (15 PSU and 25 °C) and the lowest at 5 PSU and 20 °C. SFA contents were not different among all all groups, but in the case of PUFA, both n-3 and n-6 PUFA were lower than that of the control. The reduction of TFAs due to high salinity exposure and low temperature have also been reported in the temperate rotifer *B. koreanus* (Lee et al., 2017a; Lee et al., 2020). Besides, a previous study has reported that the copepod *Paracyclopina nana* showed lower FAs in high salinity exposures (25 and 30 PSU) compared to control (15 PSU) (Lee et al., 2017b). On the other hand, changes in the salinity from 8 to 23 PSU, no changes in the TFA and MUFA contents were observed, while SFA and PUFA were decreased and increased, respectively, in the razor clam *Sinonovacula constricta* (Ran et al., 2017). Taken together, *B. rotundiformis* did not appear to have a positive effect by the synthesis or storage of fatty acids by changes under salinity and temperature changes. However, since the highest reproduction was shown at 5 PSU and 30 °C, it would be appropriate to change the salinity and temperature according to the purpose of cultivation by deciding whether to cultivate rotifers with optimal FAs or to cultivate as many rotifers as possible within a short time.

Next, the composition of a single FA was summarized by referring to a previous study (Monroig et al., 2013). As one of the main energy sources, FAs play important roles such as cell structure, homeostasis, and immunity. Besides, since FAs are dependent on environmental changes, the composition of single FAs can be affected by temperature, salinity, and the type of food source (Arts et al., 2009). In general, FAs can also be synthesized from glucose under the positive nutritional

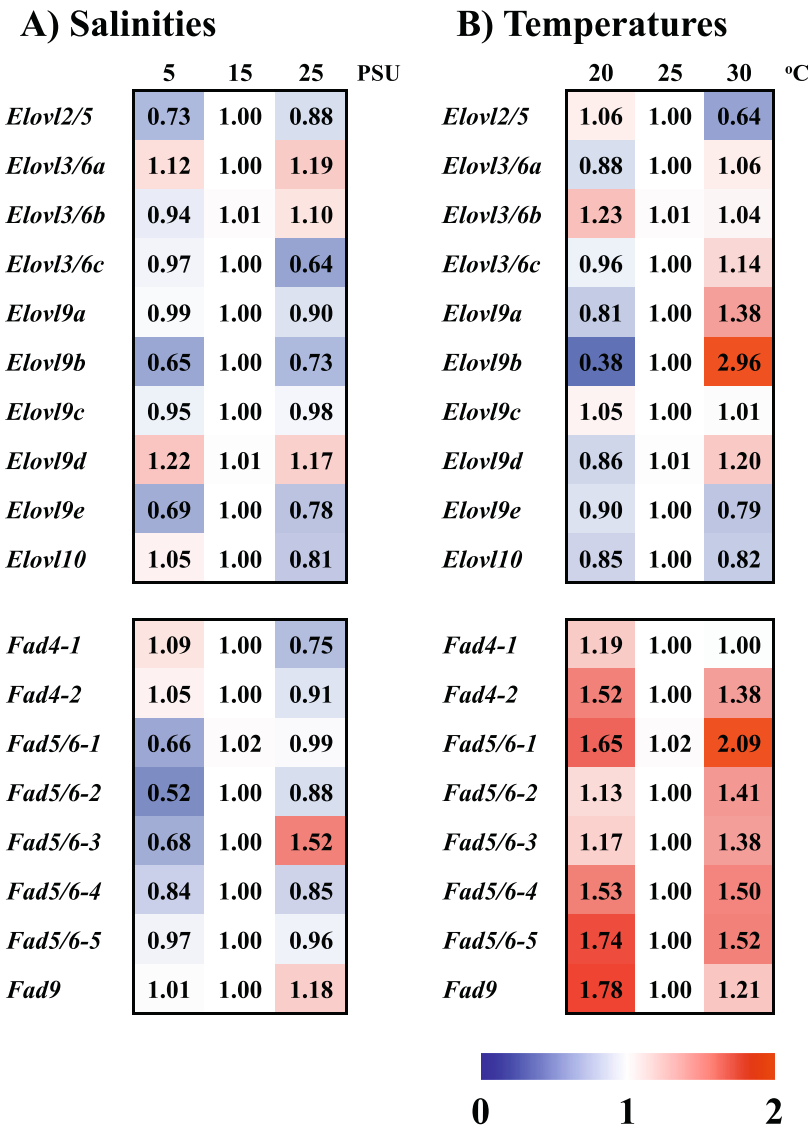

Fig. 4. Transcriptional levels of elongation of very long-chain fatty acid (*Elovl*) and fatty acid desaturase (*Fad*) genes to different A) salinities (5, 15 [control], 25, and 35 practical salinity unit) and B) temperatures (15, 20, 25 [control], and 30 °C) represented by a heap map.

status, and not all FAs can be synthesized in organisms. In synthesizing FAs, enzymes that can increase the length of FAs and change the degree of unsaturation of FAs are required. In this process, *Elovl* and *Fad* genes are responsible for synthesizing various types of FAs. Although, various types of Elovl and Fad genes have been identified, most animals lack Δ12 and 15 desaturases, so it impossible to synthesize long chain PUFAs and thus,most animals require 18:2n-6 and 18:3n-3 FAs for synthesizing PUFAs, thus, the two FAs are referred to as an essential FAs (Kelly and Scheibling, 2011; Dalsgaard et al., 2003). Therefore, in a normal feeding condition, the composition of 18:2n-6 and 18:3n-3 FAs occupies a very important part in the PUFA composition in rotifers. *Brahionus* species is known to synthesize n-6 and n-3 FAs from 18:2n-6 and 18:3n-3 (Lee et al., 2019). Since both 18:2n-6 and 18:3n-3 FAs were reduced due to changes in salinity or temperature, we suggested that a negative effect on the lipid metabolism might occur in the tropical rotifer *B. rotundiformis*. On the other hand, among the n-3 PUFAs, 22:5n-3 increased at 30 °C, and the average values of 20:4n-3, 20:5n-3, and 22:6n-3 showed a tendency to increase. n-3 PUFAs not only play important roles in the immune response, but also play pivotal roles in growth, reproduction, and neurodevelopment (Brett and Müller-Navarra, 1997; Müller-Navarra et al., 2000; Goedkoop et al., 2007; Stanley-Samuelson et al., 1988). In this study, the green microalgae *T. suecica* was administered as a food source. A previous study has revealed that 22:5n-3 and 22:6n-3 were not detected in *T. suecica* (Lee et al., 2019). Nevertheless, in 30 °C group, these fatty acids were detected at higher levels than the control suggested that these fatty acids play a specific role. It is not clear what role it plays, however, compared with the in vivo analysis results, it can be estimated that those FAs might play an important role in the reproduction of *B. rotundiformis* but further analysis is required.

Transcriptional level was measured according to changes in salinity and temperature using *B. rotundiformis Elovl* (Lee et al., 2019b) and *Fad* (Lee et al., 2019a) genes. In salinity exposure groups, the expression of most genes was decreased or showed no differences compared to the control. A similar result has been reported in the temperate rotifer *B. koreanus* (Lee et al., 2017a), indicating the change in salinity could induce a negative effect on fatty acid production in *Brachionus* species. On the other hand, in temperature experiment groups, *Elovl9b* might be used as a biomarker according to temperature changes, as the transcriptional level of *Elovl9b* showed a temperature-dependent manner.

Besides, most of the *Fad* genes showed a tendency to increase, of which the *Fad5/6–1* gene significantly increased at 30 °C. Since *Elovl* and *Fad* genes are responsible for changing the structure of FAs (Castro et al., 2016), changes in the transcriptional level of the gene according to salinity and temperature fluctuation can affect fatty acid composition in rotifers. For example, in *B. koreanus* exposed to chronic caloric restriction, the transcriptional levels of *Elovl* and *Δ4 Fad* genes increased, leading to an increase in the composition of docosahexaenoic acid (22:6n-3, DHA) (Lee et al., 2018). Under the temperature changes, although the transcription of most of the *Fad* genes were up-regulated, the total concentration of n-3 and n-6 PUFAs decreased, so that the increase in the transcription of the *Fad* gene might be related to the increase of specific FAs (e.g., 22:5n-3 at 30 °C), or the consumption of FAs was higher than the synthesis of FAs. In particular, in a previous study (Lee et al., 2020), although the metabolic rate of *B. koreanus* was lowered, the pathway to synthesize glycerol was activated to secure the fluidity of body fluids. Taken together, we assumed that an increase in the *Fad* genes, which increases the degree of unsaturation of FAs, had a similar effect at low temperature, but further studies are needed.

In this study, the effects of salinity and temperature on the tropical rotifer *B. rotundiformis* were investigated by analyzing fecundity, fatty acid composition, and expression levels of related genes. *B. rotundiformis* showed the best reproduction at 5 PSU and 30 °C, but the FA composition was highest at 15 PSU and 25 °C. In particular, n-3 FAs, which are generally preferred FAs in aquaculture, were significantly lowered by changes in salinity and temperature. Therefore, it would be appropriate to change the culture condition according to the purpose of culture, rather than to determine the optimal culture conditions for rotifers. The transcriptional levels of *Elovl* and *Fad* genes were mostly reduced in the salinity experiment, but their transcription levels were modulated in no distinctive manner under different temperature groups. However, the transcriptional level of the *Elovl9b* gene showed a temperature-dependent manner, so this gene could be utilized as a biomarker. Through this study, the correlation between the life cycle parameters and lipid metabolism of tropical rotifer *B. rotundiformis*, and our data couldbe used as fundamental data for the utilization of *B. rotundiformis* in aquaculture.

Author statement

Name of the author and e-mail ID	Types of contribution
Min-Chul Lee, mclee@skku.edu	Investigation, Writing - original draft
Deok-Seo Yoon, dsyoon108@skku.edu	Investigation, Writing - original draft
Jun Chul Park, parkjc@skku.edu	Data curation, Writing - original draft
Hyuntae Choi, dmb122@hanyang.ac.kr	Methodology
Kyung-Hoon Shin, shinkh@hanyang.ac.kr	Methodology
Atsushi Hagiwara, hagiwara@nagasaki-u.ac.jp	Methodology, Resources
Jae-Seong Lee, jslee2@skku.edu	Conceptualization, Supervision, Writing - review & editing
Heum Gi Park, hgpark@gwnu.ac.kr	Conceptualization, Supervision, Funding acquisition, Writing - review & editing

Declaration of Competing Interest

The authors have no conflict of interest in this paper.

Acknowledgments

This research was supported by a grant from the National Research Foundation (2018R1D1A1B07050654) funded to Heum Gi Park.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.737282.

References

Arts, M.T., Brett, M.T., Kainz, M.J., 2009. Lipids in Aquatic Ecosystems. Springer, New York.
Brett, M., Müller-Navarra, D., 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw. Biol. 38, 483–499.
Castro, L.F.C., Tocher, D.R., Monroig, Ó., 2016. Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of *Fads* and *Elovl* gene repertoire. Prog. Lipid Res. 62, 25–40.
Cervetto, G., Gaudy, R., Pagano, M., 1999. Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). J. Exp. Mar. Biol. Ecol. 239, 33–45.
Dahms, H.-U., Hagiwara, A., Lee, J.-S., 2011. Ecotoxicology, ecophysiology, and mechanistic studies with rotifers. Aquat. Toxicol. 101, 1–12.
Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. In: Southward, A., Tyler, P., Young, C., Fuiman, L. (Eds.), Adv. Mar. Biol, Vol. 46. Academic Press LTD, London, England, pp. 225–340.
Folch, J., Lees, M., Sloane Stanley, G., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Fukusho, K., 1983. Present status and problems in the culture of the rotifer for *Brachionus plicatilis* for fry production of marine fishes in Japan. In: Hector, R. (Ed.), Symposium Internacional de Aquacultura. Coquimbo, Chile, pp. 361–374.
Fukusho, K., Iwamoto, H., 1980. Cyclomorphosis in size of the cultured rotifer, *Brachionus plicatilis*. Bull. Natl. Res. Inst. Aquaculture 1, 29–37.
Garrido, J.L., Medina, I., 1994. One-step conversion of fatty acids into their 2-alkenyl-4,4-dimethyloxazoline derivatives directly from total lipids. J. Chromatogr. 673, 101–105.
Goedkoop, W., Demandt, M., Ahlgren, G., 2007. Interactions between food quantity and quality (long-chain polyunsaturated fatty acid concentrations) effects on growth and development of *Chironomus riparius*. Can. J. Fish. Aquat. Sci. 64, 425–436.
Gribble, K.E., Mark Welch, D.B., 2017. Genome-wide transcriptomics of aging in the rotifer *Brachionus manjavacas*, an emerging model system. BMC Genomics 18, 217.
Hutchinson, G.E., 1957. A Treatise on Limnology. Wiley, New York.
Johnston, R.K., Snell, T.W., 2016. Moderately lower temperatures greatly extend the lifespan of *Brachionus manjavacas* (Rotifera): thermodynamics or gene regulation? Exp. Gerontol. 78, 12–22.
Kafuku, T., Ikenoue, H., 1983. Modern methods of aquaculture in Japan. In: Developments in Aquaculture and Fisheries Science, Vol. 11. Kodansha Ltd., Tokyo, and Elsevier, Amsterdam, 216 pp.
Kailasam, M., Kaneko, G., Oo, A.K.S., Ozaki, Y., Thirunavukkarasu, A.R., Watabe, S., 2011. Effects of calorie restriction on the expression of manganese superoxide dismutase and catalase under oxidative stress conditions in the rotifer *Brachionus plicatilis*. Fish. Sci. 77, 403–409.
Kang, H.-M., Kim, M.-S., Choi, B.-S., Kim, D.-H., Kim, H.-J., Hwang, U.-K., Hagiwara, A., Lee, J.-S., 2020. The genome of the marine monogonont rotifer *Brachionus rotundiformis* and insight into species-specific detoxification components in *Brachionus* spp. Comp. Biochem. Physiol. D 36, 100714.
Kauler, P., Enesco, H.E., 2011. The effect of temperature on life history parameters and cost of reproduction in the rotifer *Brachionus calyciflorus*. J. Freshw. Ecol. 26, 399–408.
Kelly, J.R., Scheibling, R.E., 2011. Fatty acids as dietary tracers in benthic food webs. Mar. Ecol. Prog. Ser. 446, 1–22.
Kim, H.-S., Hwang, D.-S., Kim, H.-J., Sakakura, Y., Hagiwara, A., Lee, J.-S., 2017. Complete mitochondrial genome of the monogonont rotifer *Brachionus rotundiformis* (Rotifera, Brachionidae). Mito. DNA B 2, 39–40.
Kuronuma, K., Fukusho, K., 1987. Rearing of Marine Finfish Larvae in Japan. IDRC TS 47e. International Development Research Centre, Ottawa, 109 pp.
Lee, M.-C., Park, J.C., Kim, D.-H., Kang, S., Shin, K.-H., Park, H.G., Lee, J.-S., 2017a. Interrelationship of salinity shift with oxidative stress and lipid metabolism in the monogonont rotifer *Brachionus koreanus*. Comp. Biochem. Physiol. A 214, 79–84.
Lee, S.-H., Lee, M.-C., Puthumana, J., Park, J.C., Kang, S., Hwang, D.-S., Han, J., 2017b. Effects of salinity on growth, fatty acid synthesis, and expression of stress response genes in the cyclopoid copepod *Paracyclopina nana*. Aquaculture 470, 182–189.
Lee, M.-C., Park, J.C., Yoon, D.-S., Han, J., Kang, S., Kamizono, S., Lee, J.-S., 2018. Aging extension and modifications of lipid metabolism in the monogonont rotifer *Brachionus koreanus* under chronic caloric restriction. Sci. Rep. 8, 1741.
Lee, M.-C., Park, J.C., Yoon, D.-S., Choi, H., Shin, K.-H., Kim, H.-J., Lee, J.-S., 2019. Lipid metabolism modulation by five different food types in the monogonont marine rotifer *Brachionus koreanus*. Aquaculture 503, 596–601.
Lee, M.-C., Hagiwara, A., Park, H.G., Lee, J.-S., 2019a. Genome-wide identification and expression of eight fatty acid desaturase genes, and the fatty acid profile, in the marine rotifer *Brachionus koreanus* fed the alga *Tetraselmis suecica*. Fish. Sci. 85, 397–406.
Lee, M.-C., Park, J.C., Yoon, D.-S., Choi, H., Kim, H.-J., Shin, K.-H., Lee, J.-S., 2019b. Genome-wide characterization and expression of the elongation of very long chain fatty acid genes (*Elovl*) and fatty acid profiles in the alga (*Tetraselmis suecica*) fed marine rotifer *Brachionus koreanus*. Comp. Biochem. Physiol. D 30, 179–185.

Lee, M.-C., Yoon, D.-S., Lee, Y., Choi, H., Shin, K.-H., Park, H.G., Lee, J.-S., 2020. Effects of low temperature on longevity and lipid metabolism in the marine rotifer *Brachionus koreanus*. Comp. Biochem. Physiol. A 250, 110803.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_T$ method. Methods 25, 402–408.

Lubzens, E., 1987. Raising rotifers for use in aquaculture. Hydrobiologia 147, 245–255.

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

Monroig, Ó., Tocher, D.R., Navarro, J.C., 2013. Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Mar. Drugs 11, 3998–4018.

Müller-Navarra, D.C., Brett, M.T., Liston, A.M., Goldman, C.R., 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403, 74–77.

Ozaki, Y., Kaneko, G., Yanagawa, Y., Watabe, S., 2010. Calorie restriction in the rotifer *Brachionus plicatilis* enhances hypoxia tolerance in association with the increased mRNA levels of glycolytic enzymes. Hydrobiologia 649, 267–277.

Ran, Z., Chen, H., Ran, Y., Yu, S., Li, S., Xu, J., Yan, X., 2017. Fatty acid and sterol changes in razor clam *Sinonovacula constricta* (Lamarck 1818) reared at different salinities. Aquaculture 473, 493–500.

Sarma, S.S.S., Nandini, S., Gulati, R.D., 2002. Cost of reproduction in selected species of zooplankton (rotifers and cladocerans). Hydrobiologia 481, 89–99.

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

Snell, T.W., King, C.E., 1977. Lifespan and fecundity patterns in rotifers: the cost of reproduction. Evolution 31, 882–890.

Snell, T.W., Fields, A.M., Johnston, R.K., 2012. Antioxidants can extend lifespan of *Brachionus manjavacas* (Rotifera), but only in a few combinations. Biogerontology 13, 261–275.

Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, G.J., de Renobales, M., 1988. Fatty acids in insects: composition, metabolism, and biological significance. Arch. Insect Biochem. Physiol. 9, 1–33.

Stelzer, C.-P., 2005. Evolution of rotifer life histories. Hydrobiologia 546, 335–346.

Won, E.-J., Han, J., Kim, D.-H., Dahms, H.-U., Lee, J.-S., 2017. Rotifers in ecotoxicology. In: Hagiwara, A., Yoshinaga, T. (Eds.), Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology. Springer, Tokyo, pp. 149–176.

Yin, X.W., Niu, C.J., 2011. Testing the cost of reproduction with the rotifer *Brachionus patulus* at different pH levels. Limnology 12, 145–149.

Yin, X.W., Zhao, W., 2008. Studies on life history characteristics of *Brachionus plicatilis* O. F. Müller (Rotifera) in relation to temperature, salinity and food algae. Aquat. Ecol. 42, 165–176.

Yoshinaga, T., Hagiwara, A., Tsukamoto, K., 2003. Life history response and age-specific tolerance to starvation in *Brachionus plicatilis* O.F. Müller (Rotifera). J. Exp. Mar. Biol. Ecol. 287, 261–271.