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Research Article

Live Microalgae-Based Diets as Enrichment to Improve the Nutritional Profile of the Calanoid Copepod *Acartia tonsa* (Dana, 1849) Nauplii

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Most emerging species in marine aquaculture and the aquarium industry have a sensitive and small-mouthed larval stage, and their culture is very challenging due to a lack of appropriate early feeding protocols. The use of copepods has been widely recognized as a good alternative to traditional fish live feeds (rotifers and *Artemia* spp.), especially for new species targeted for the multibillion-dollar marine aquarium trade. However, most copepod culture trials are focused on biomass production, while their nutritional requirements and enrichment protocols remain scarcely known. The main objective of this study was to determine the dietary contribution of three microalgae-based diets, provided as a mono R (*Rhodomonas salina*) or binary I–R (*Isochrysis galbana* + *R. salina*) and T–R (*Tetraselmis suecica* + *R. salina*), on the biochemical composition (proximate and fatty acids (FAs)) and survival of the calanoid copepod *Acartia tonsa* (nauplii) versus the conventional rotifer *Brachionus plicatilis* throughout 96 hr exposure. Both live preys were characterized by the highest protein and total n-3 long-chain polyunsaturated FAs (LC-PUFAs) content when fed with the binary diet I–R compared to other treatments, although the nutritional value of *A. tonsa* nauplii was always much higher compared to *B. plicatilis*. In general, there was a wide difference in proximate and FAs composition between the two preys, denoting *A. tonsa* as better live feed in terms of capacity to retain n-3 LC-PUFAs, especially eicosapentanoic acid (20 : 5n-3) and docosahexanoic acid (22 : 6n-3). The present study highlights the nutritional profile of enriched *A. tonsa* nauplii and contributes to the production of high-quality live feeds, which may be able to reach the nutritional needs of new marine fish species during their larval development, as well as contributing to the improvement of *A. tonsa* culture.

1. Introduction

1.1. Marine Fish Larvae Production and Conventional Live Feeds. In the last decades, the production of high-quality marine fish species and its diversification were constrained by a bottleneck of nonoptimized nutritional food sources for larval stages, inducing low growth and survival [1, 2]. The success in the larval breeding of new marine species is determined mainly during the early life stages of development, during the transition from endogenous to exogenous nutrition [3, 4]. At this stage, the live feed should be adapted to the morphological characteristics of the larvae and their needs in terms of nutritional requirements. In contrast to their natural environment, where larvae have access to a large variety

of feeding regimes, the main live preys employed in hatcheries consists of rotifers (*Brachionus* spp.) and brine shrimps (*Artemia* spp.). Rotifers have small sizes, suitable as first feeding for the earliest stages of several fish and shrimp larvae species [5]. Meanwhile, *Artemia* shows larger size variability compared to rotifers and can be hatched from dormant cysts, which can be easily distributed and stored for prolonged periods of time [5]. However, both preys have a limited ability to synthesize important long-chain polyunsaturated fatty acids (LC-PUFAs) from shorter FAs [6–8]. The rate of synthesis of these FAs is rather low, especially for docosahexaenoic acid (DHA: 22 : 6 n-3), and to supply large amounts of PUFAs to marine fish larvae, live preys must be enriched with PUFA-rich food [7]. Several techniques have been developed to improve the biochemical composition of rotifers and *Artemia* (e.g., oil emulsions, phytoplankton, etc.) or even to seek new types of live preys whose composition may fulfill the nutritional requirements of certain fish species [9].

1.2. Copepods as Live Feed. The use of copepods has been widely recognized as a good alternative to traditional live feeds [10–12]. Copepods can improve the survival and growth for a variety of fish larvae due to their small naupliar stages, swimming behavior, and FAs profile, which includes a high content of LC-PUFAs [9, 12]. Therefore, there is a considerable interest in studying the mechanisms responsible for its production [13, 14]. New techniques allow producing large quantities of calanoid copepods using cold-stored subitaneous eggs [14, 15–20]. However, cold-stored copepod embryos still retain a physiological activity during this period, and their nutritional quality may change over this time [14–17]. In particular, the major biochemical differences that occur during the storage period are related to the embryo FAs content [17]. Previous studies demonstrated that after 6 months of cold storage, the quality of copepods obtained from those eggs was suboptimal for the Saddleback clownfish (*Amphiprion polymnus*) larval rearing [21]. Therefore, unless proper enrichment can be assured, these changes in FAs may affect larval survival and development. Most calanoid copepods, which constitute the natural food source for many marine fish larvae [22], are unable to elongate and desaturate the 18 : 3n-3 FA to produce significant amounts of the LC-PUFAs, eicosapentanoic acid (EPA: 20 : 5n-3) and DHA FAs [23]. Their high content in n-3 LC-PUFAs is incorporated directly through the phytoplankton, exhibiting reduced growth and egg production when, in opposition, fed diets deficient in EPA and DHA [24, 25]. Moreover, food quantity and quality affect copepod ingestion, survival, growth, and fecundity [9, 14, 26–28]. The use of a mixed diet has been proven to sustain large copepod cultures for many generations [29]. Otherwise, monospecific diets may cause nutritional deficiencies because of the inadequate content of one or more essential nutrients [30].

1.3. Acartia tonsa Enrichment Study. Since most copepod culture trials are focussed on the quantity of mass production, the nutritional requirement and enrichment potential of copepod remain scarcely known [9, 14]. The nutritional value of copepods is less predictable due to the variety of algae species available as feed [9, 31]. Little information to date is available on adequate protocols to enrich copepods with nutritional and trace elements required for marine fish larvae [32, 33]. The copepod *Acartia tonsa* (Dana, 1848) is a ubiquitous cosmopolitan estuarine species that is often used in biology and ecotoxicology studies, among others [34], but also as live prey for aquaculture [14, 22, 35]. Indeed, many studies have already highlighted the relevance of *A. tonsa* as live feed, favoring fish larval survival and growth in comparison to rotifers and *Artemia* nauplii [13, 31]. In this study, we examined the dietary contribution of three microalgae species (*Rhodomonas salina*, *Isochrysis galbana*, and *Tetraselmis suecica*), provided as a mono- or binary-diets, on the biochemical composition (proximate and FAs) and survival of *A. tonsa* nauplii versus the conventional live feed *Brachionus plicatilis*. Specifically, we focus on the relationship between the changes of FAs compositions depending on food source, in order to produce high-quality live feeds [2], able to reach the nutritional needs of new marine fish larvae species. Therefore, the identification of an adequate microalgal enrichment for *A. tonsa* may enhance the production of high-quality marine fish as also contribute to the culture of this copepod species.

Table 1: Concentration of microalgae cultures expressed as mean value with standard deviation ($n \times 10^6$ cells/mL $\pm$ SD) during the 4-day experiment.

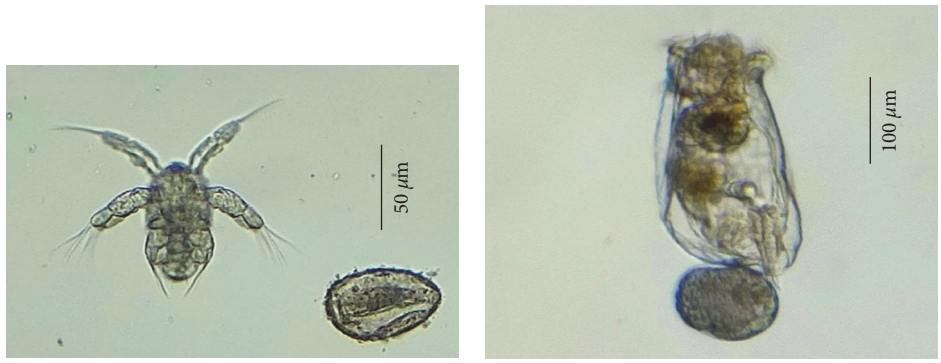
Microalgae	Day 1	Day 2	Day 3	Day 4
Tetraselmis suecica	2.1 ± 0.1	2.5 ± 0.0	2.8 ± 0.1	3.1 ± 0.1
Isochrysis galbana	5.2 ± 0.1	5.9 ± 0.1	6.4 ± 0.2	6.8 ± 0.2
Rhodomonas salina	2.4 ± 0.1	2.6 ± 0.1	2.9 ± 0.0	3.2 ± 0.0

2. Materials and Methods

The experiment was accomplished in accordance with the EU Directive 2010/63/EU for animal experiments or the National Research Council's Guide for the Care and Use of Laboratory Animals.

2.1. Facilities. The entire experiment was held in the quarantine areas of the Aquarium of Genoa (Genoa, Liguria, Italy), using the facilities described below.

2.1.1. Phytoplankton Cultures. The marine microalgae *T. suecica* (Kylin) Butcher 1959, *I. galbana* (Parke 1949), and *R. salina* (strain number MCCV118) (Wislouch) D.R.A. Hill & R. Wetherbee 1989 were used in the present study as a food source for copepods and rotifers. They were grown in 80-L polycarbonate cylinders, inoculated into 1 μm filtered seawater (salinity 30%), UV sterilized, and mixed with filtered air. All algae species were grown on a F/2 medium [36]. Microalgae cultures were maintained at 20 ± 1°C with rear illumination based on 10,000 K fluorescent tubes with a 24-hr light regime. The algae were harvested during the log phase. The concentration of algal cultures (cells/mL) (Table 1) was determined daily, using a Neubauer chamber (Marienfeld



(a) (b)

FIGURE 1: Naupliar stage of *Acartia tonsa* 24 hr post-hatch (a), and female of the rotifer *Brachionus plicatilis* (b).

Superior, Germany) under a microscope (DME, LEICA, Germany).

2.1.2. Zooplankton Cultures. Rotifers (*B. plicatilis*), with a size range of 180–250 μm (L size), were cultured on S.parkle® (INVE Aquaculture, Belgium) at 30% ± 1% salinity and 20°C in two transparent cylindrical tanks of 80 L each. Cultures were renewed weekly with a 100% water change, keeping a mean culture density of 200 individuals/mL moved by a gentle airflow. The culture was 12 L/12 D (light/dark) illuminated by two 30 W fluorescent tubes. Density estimations of rotifer, with a mean size of 220 μm (Figure 1(b)), were done daily by averaging three 1 mL^{-1} samples, and each of the samples was checked under a stereomicroscope (EZ4, LEICA, Germany). Nauplii of the calanoid copepod (*A. tonsa*) with a mean size of 70 μm (Figure 1(a)) were obtained by cold stored eggs distributed by Algova® (Germany).

2.1.3. Modular Rearing System (MoRS). The experiment was conducted inside the life support system named MoRS. This was connected to six experimental cylindrical tanks, with a volume of 20 L, and equipped with air line. MoRS filtration system includes a 200 L sump supported by a biological tower, 5 μ mechanical filtration, protein skimmer (Aqua Medic, Germany), and UV sterilization (Panaque, Italy). Water temperature inside MoRS tanks was on average 20 ± 1°C (mean ± standard deviation (SD)) with minimum variation and controlled by a heating device coupled with an automatic thermostat (Panaque, Italy). The whole system was illuminated 12 L/12 D by two 40 W fluorescent tubes, oxygen level ranged from 6 to 7 mg/L, and salinity was established at 30% ± 1%.

2.2. Experimental Design. Cold-stored eggs of *A. tonsa* preserved at 4°C distributed by Algova® (Germany) were hatched following the instructions provided by the producer, while the rotifers *B. plicatilis* were collected from the culture tanks (see Section 2.1.2). Copepods eggs were washed from the buffer solution with 50 μm mesh sieve, inoculated in marine water, and incubated during 24 hr at 24 ± 1°C in a glass flask at the density of 40,000 eggs/L with air bubbling. The newly hatched nauplii of *A. tonsa* at N I stage and the rotifer *B. plicatilis* were immediately concentrated using a 50 μm mesh sieve and counted under a stereomicroscope in triplicate (EZ4, Leica, Germany). The experiment was launched after splitting live preys into three experimental feeding treatments ($n = 5$) as follows: Treatment R (control): mono algal diet based on *R. salina*; Treatment T–R: binary diet (1 : 1 ratio) based on *T. suecica* and *R. salina*; Treatment I–R: binary diet (1 : 1 ratio) based on *I. galbana* and *R. salina* (Figure 2). The three species of algae used in this study were chosen because of their significant differences in LC-PUFAs composition, which allowed a comparison of the effects of the diets on copepods and rotifers FAs composition. *R. salina* was selected as the control diet due to its good nutritional profile and suitable cell size for *A. tonsa* and *B. plicatilis* culture [16, 37–39]. While *T. suecica* and *I. galbana* were selected to generate the binary diets, combined with the control, since they are among the most frequently used species in the commercial mariculture industry [40]. All microalgae treatments were formulated with a ratio of 1 : 1 based on cell count, chosen to facilitate the application of the enrichment and the reintegration of the consumed algae. Each treatment was split into five replicates, incorporated in five tanks of 20 L ($n = 5$), configured without water exchange with the MoRS facility to standardize the culture conditions. Starting culture density was established in 10 ind./mL for *A. tonsa* and 280 ind./mL for *B. plicatilis* by counting individuals as described for zooplankton cultures (see Section 2.1.2). Respectively, there were an estimated 200,000 specimens in each copepod tank and 5,600,000 specimens in each rotifer tank, ensuring a sufficient final sample size for the subsequent biochemical analysis, avoiding overpopulation. During 96 hr, rotifers and copepods were fed with the designated diets after 10% daily water exchange supplied by MoRS connected to the culture tanks. Tanks were kept at 20 ± 1°C, 30% salinity, and 14 L/10 D photoperiod [34], checking water quality parameters daily. Microalgae concentration was checked daily and kept at 1.5×10^5 cells/mL (1 : 1) in each tank following an identical protocol applied for phytoplankton cultures (see Section 2.1.1), ensuring enough feed for live preys and avoiding starvation. High microalgae concentration was chosen as it has been described to support

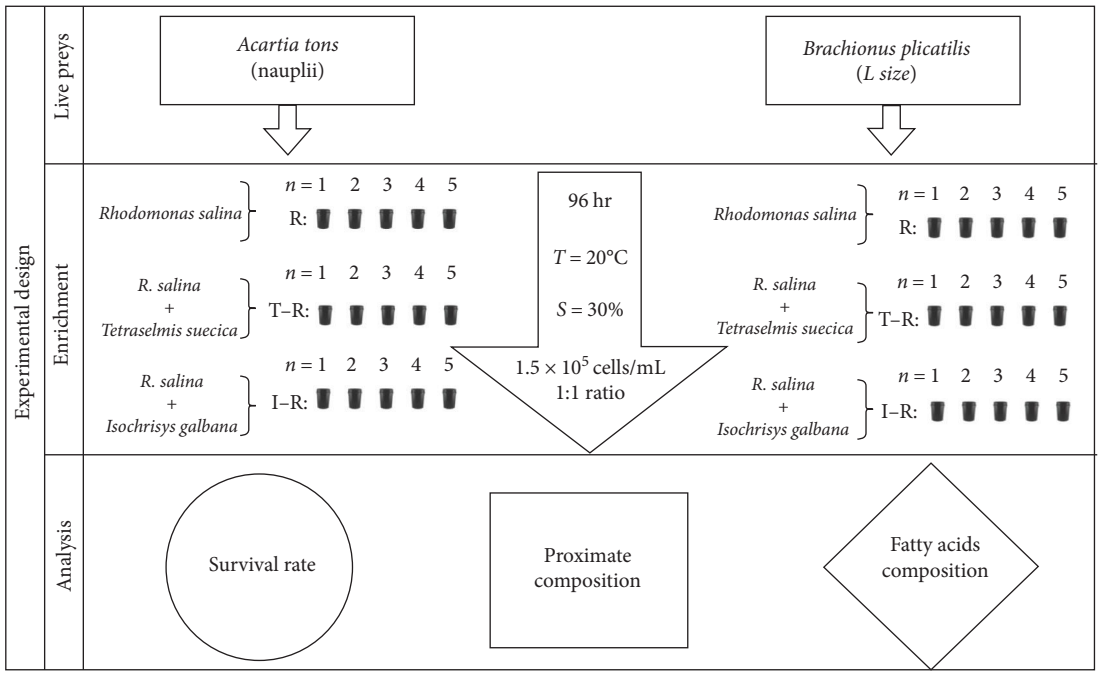


Figure 2: Experimental design flow chart: the copepod *A. tonsa* and the rotifer *B. plicatilis* were divided into three experimental feeding treatments ($n = 5$) and fed with the designated diets during 96 hr. Effects on survival rate, proximate, and fatty acids composition were studied.

Table 2: Concentration of rotifers (*Brachionus plicatilis*) and the number of females, as well as nauplii of calanoid copepods (*Acartia tonsa*) measured in culture tanks during the feeding experimentation using three microalgae treatments (R, *Rhodomonas salina*; I–R, *Isochrysis galbana* + *Rhodomonas salina*; T–R, *Tetraselmis suecica* + *Rhodomonas salina*).

Live feed	Treatment	Day 1		Day 2		Day 3		Day 4	
		Individuals	Females	Individuals	Females	Individuals	Females	Individuals	Females
B. plicatilis	R	280 ± 0.1	13.3 ± 0.8	278.2 ± 0.8	10.2 ± 0.5	277 ± 0.7	15.6 ± 1.1	276.6 ± 0.9	12.3 ± 1.6
	I–R	280 ± 0.1	14 ± 0.2	277.8 ± 1.6	12.6 ± 0.8	277.4 ± 1.5	14.8 ± 0.8	276.6 ± 1.1	18.5 ± 0.8
	T–R	280 ± 0.1	13.8 ± 0.6	274.6 ± 4.7	8.4 ± 0.3	272.2 ± 2.7	8.1 ± 0.6	267.8 ± 1.6	10.4 ± 1.1
		Nauplii		Nauplii		Nauplii		Nauplii	
A. tonsa	R	10 ± 0.1	–	8.8 ± 0.1	–	8.4 ± 0.1	–	8.0 ± 0.1	
	I–R	10 ± 0.1	–	9.6 ± 0.1	–	9.2 ± 0.1	–	8.8 ± 0.1	
	T–R	10 ± 0.1	–	9.6 ± 0.1	–	9.0 ± 0.1	–	8.7 ± 0.1	

All values are expressed as mean values with standard deviation (mean value/mL ± SD).

high survival rates and increase the culture performance of *A. tonsa* and *B. plicatilis* [28, 39, 41, 42]. To estimate the survival rate of each experimental treatment, a daily counting of copepods nauplii and rotifers (Figure 1) was carried out by triplicate in each tank following an identical protocol applied for zooplankton cultures (see Section 2.1.2) (Table 2).

2.3. Samples Collection. Samples of each microalgae species (20 L) employed for live prey diets were collected at the beginning of the experiment from their respective phytoplankton culture tanks. The resulting samples were centrifuged (Rotofix 32°, Hettich, Germany), split in vials per triplicate, lyophilized (Alpha 1-2 LD plus, Christ; Germany), and stored at −20°C for subsequent analysis. The feeding trials were stopped on day 4 (96 hr) when nauplii of *A. tonsa* were molting from the fourth (N IV) to the fifth (N V) naupliar stage, preventing metamorphosis to the copepodite stage [43]. The exogenous feeding was observed starting from 24 hr post-hatch at N II stage. The duration of the experiment (96 hr) ensured to avoid any influence that could come from the endogenous feeding of the first naupliar stage (N I) to the final biochemical composition of *A. tonsa* nauplii. At the end of the feeding trials, each experimental culture tank of copepods and rotifers ($n = 5$) generated a sample (Table 2). Each tank was fully filtered through a 50 μm mesh sieve, the content rinsed with deionized water to remove residual salts, lyophilized (Alpha 1–2 LD plus, Christ; Germany), and stored in the vial at −20°C for subsequent analysis. The collected samples were preserved at the Regional Agency for Environmental Protection–Liguria (ARPAL), Department of Organic Chemistry and Physical Analysis (Genoa, Liguria, Italy).

2.4. Samples Analysis. Samples analysis was carried out at the laboratories of the Highly Specialized Aquaculture and Biotechnology Service (SABE) located at the University Institute of Sustainable Aquaculture and Marine Ecosystems (IU ECOAQUA), belonging to the University of Las Palmas de Gran Canaria (Canary Islands, Spain). Proximate composition of microalgae diets, *A. tonsa* nauplii, and *B. plicatilis* were conducted following standard procedures [44]. Moisture was determined by thermal dehydration until constant mass at 105°C. Ash content was determined by combustion at 600°C for 12 hr. Crude protein content (N · 6.25) was determined by the Kjeldahl method, and crude lipid was extracted following the Folch method [45]. Carbohydrate was estimated by difference (percentage carbohydrate content = 100 − (% moisture + % ash + protein + % fat)). The analysis of FAs was carried out by transmethylation of the total lipids in FA methyl ester as described by Christie and Han [46], separated, and identified by gas chromatography (7820A GC System, Agilent Technologies, United States) following the conditions described by Izquierdo [47]. All gross composition data were expressed as percentages of dry weight (DW ± SD), while the FAs content was expressed as a percentage of the total lipid (DW ± SD). All analyses were conducted in triplicate.

2.5. Data Analysis. All data were analyzed with Levene's test to assess the homogeneity of variances and a Shapiro–Wilk test to assess normality, applying log-transformed percent data when assumptions were not accomplished. Differences in proximate and FAs composition were assessed by one-way analysis of variance (ANOVA). Comparison between groups was calculated by Tukey post hoc test for pairwise comparisons and Games–Howell. To study the effect of diet on the survival of the copepods and the rotifers, a two-way repeated measures ANOVA with two fixed factors, time and diet, was used. The significance level of 0.05 (P value) was tested using the SPSS statistical software package (IBM SPSS for Windows V27.0; SPSS Inc., Chicago, IL, USA). Unless stated otherwise, measurements are presented as mean ± SD.

3. Results

3.1. Analysis of Biochemical Composition of Microalgae

3.1.1. Proximal Composition. All data of microalgae gross composition were expressed as percentages of dry weight (DW ± SD, Table 3). *T. suecica* showed significantly higher protein (29.0% ± 0.7%) and lipid (25.1% ± 0.5%) content in comparison to the other two microalgae tested (ANOVA, $P < 0.05$, Table 3). While *R. salina* showed the highest percentages of carbohydrates (16.1% ± 0.5%) and ash (52.5% ± 0.5%) (ANOVA, $P < 0.05$, Table 3). Moisture content was significantly higher in *I. galbana* (10.6% ± 0.7%) (ANOVA, $P < 0.05$, Table 3); meanwhile, there were no differences (ANOVA, $P \geq 0.05$) between *R. salina* (8.12% ± 0.50%) and *T. suecica* (8.63% ± 0.55%) species.

3.1.2. FA Composition. The FA composition of microalgae was expressed as a percentage of the total lipid DW ± SD (Table 3). In comparison to other algae, *I. galbana* showed the highest levels of total n-3 LC-PUFAs, total monounsaturated (MUFAs), total n-9 FAs, oleic acid (OA; 18 : 1n-9), and DHA (ANOVA, $P < 0.05$, Table 3), while EPA and total saturated FAs (SAFAs) were significantly lower (ANOVA, $P < 0.05$, Table 3). *T. suecica* showed the highest percentage of myristoleic acid (14 : 1n-5), palmitic acid (16 : 0), vaccenic acid (18 : 1n-7), linoleic acid (LA; 18 : 2n-6), gamma-linoleic acid (18 : 3n-6), alpha-linolenic acid (ALA; 18 : 3n-3), stearidonic acid (SA; 18 : 4n-3), arachidonic (ARA; 20 : 4n-6), eicosatetraenoic acid (20 : 4n-3), total n-3 and total n-6 FAs (ANOVA, $P < 0.05$, Table 3). In the same way, high contents in myristic acid (14 : 0) were detected in *R. salina* (ANOVA, $P < 0.05$, Table 3). Finally, all the studied FAs ratios were significantly higher in *R. salina* (ANOVA, $P < 0.05$, Table 3), except for the DHA/EPA and DHA/ARA ratio, which levels were significantly higher in *I. galbana* (ANOVA, $P < 0.05$, Table 3).

3.2. Analysis of Biochemical Composition of Rotifers

3.2.1. Proximal Composition. The gross composition of rotifers was expressed as a percentage of DW ± SD (Table 4). The group fed with treatment R showed the highest lipid content (17.3% ± 1.1%), followed by I–R (15.4% ± 0.8%) and T–R (13.1% ± 1.0%) groups (ANOVA, $P < 0.05$, Table 4). Protein was the major constituent in all groups, but I–R treatment showed the highest content (58.7% ± 1.6%) (ANOVA, $P < 0.05$, Table 4). Carbohydrate did not show any significant difference among all treatments (ANOVA, $P \geq 0.05$, Table 4). While the group T–R showed the higher ash and moisture content (ANOVA, $P < 0.05$, Table 4), no difference was observed between I–R and R treatments (ANOVA, $P \geq 0.05$, Table 4).

3.2.2. FA Composition. The FA composition of the rotifer *B. plicatilis* fed with the three experimental diets was expressed as a percentage of the total lipid DW ± SD (Table 4). Rotifers fed on diet I–R showed significantly higher content in total n-3 LC-PUFAs, total n-6 FAs, OA, LA, and DHA FAs, in comparison to other groups (ANOVA, $P < 0.05$, Table 4). Group fed on diet R showed higher percentage in 14 : 0, 16 : 0, palmitoleic acid (16 : 1n-7), stearic acid (18 : 0), vaccenic acid, ALA, as also total SAFAs and MUFAs series than other groups (ANOVA, $P < 0.05$, Table 4). FAs ratios n-6/n-9, DHA/EPA, and DHA/ARA were significantly higher in rotifers fed on I–R treatment, while n-3/n-6 ratio was higher with R treatment. (ANOVA, $P < 0.05$, Table 4).

3.3. Analysis of Biochemical Composition of Copepods

3.3.1. Proximal Composition. Data of copepods gross composition were expressed as a percentage of DW ± SD in Table 5, where protein represented the major constituent in all groups. The highest value for protein was found in copepods fed with binary diets T–R (71.8% ± 0.7%) and I–R (70.7% ± 0.7%), which showed significantly higher content compared to the group fed on R diet (ANOVA, $P < 0.05$, Table 5). Copepod fed with T–R also showed significantly higher lipid content compared to other diets (18.0% ± 0.3%) (ANOVA, $P < 0.05$, Table 5); meanwhile, no differences were observed in groups fed on I–R and R treatments (ANOVA,

Table 3: Proximal composition (lipid, protein, carbohydrate, ash, and moisture content; g/100 g dry weight) and fatty acid composition (mg/g dry weight) of microalgae used as food for live preys, $n = 3$.

Microalgae	*Tetraselmis suecica*	*Isochrysis galbana*	*Rhodomonas salina*
Lipids	25.1 ± 0.5^{c}	18.8 ± 0.2^{b}	14.2 ± 0.6^{a}
Proteins	29.0 ± 0.7^{c}	22.6 ± 0.4^{b}	17.3 ± 0.5^{a}
Carbohydrates	9.4 ± 0.6^{a}	11.3 ± 0.7^{b}	16.1 ± 0.5^{c}
Ash	36.5 ± 0.7^{a}	47.3 ± 0.5^{b}	52.5 ± 0.5^{c}
Moisture	8.6 ± 0.6^{a}	10.6 ± 0.7^{b}	8.1 ± 0.5^{a}
14 : 0	0.1 ± 0.0^{a}	1.4 ± 0.1^{b}	1.8 ± 0.2^{c}
14 : 1n-5	0.3 ± 0.1^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
16 : 0	3.2 ± 0.2^{b}	0.7 ± 0.0^{a}	0.8 ± 0.2^{a}
16 : 1n-7	0.2 ± 0.1^{a}	0.7 ± 0.1^{b}	0.9 ± 0.2^{b}
16 : 3n-1	3.4 ± 0.1	–	–
18 : 0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.5
18 : 1n-9	1.9 ± 0.5^{b}	3.4 ± 0.2^{c}	0.5 ± 0.0^{a}
18 : 1n-7	0.8 ± 0.1^{b}	0.5 ± 0.1^{a}	0.3 ± 0.2^{a}
18 : 2n-6	1.8 ± 0.1^{c}	1.0 ± 0.0^{b}	0.7 ± 0.1^{a}
18 : 3n-6	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	–
18 : 3n-3	4.8 ± 0.0^{c}	1.7 ± 0.2^{a}	3.7 ± 0.4^{b}
18 : 4n-3	3.7 ± 0.2^{c}	2.8 ± 0.3^{b}	0.7 ± 0.1^{a}
20 : 1n-5	0.4 ± 0.1	–	–
20 : 2n-6	0.1 ± 0.0	–	–
20 : 4n-6 ARA	0.3 ± 0.0^{b}	0.1 ± 0.0^{a}	–
20 : 3n-3	0.1 ± 0.0	–	–
20 : 4n-3	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
20 : 5n-3 EPA	2.2 ± 0.2^{b}	0.6 ± 0.1^{a}	2.1 ± 0.2^{b}
22 : 1n-11	–	0.1 ± 0.0	–
22 : 5n-3	–	0.1 ± 0.0	–
22 : 6n-3 DHA	0.1 ± 0.1^{a}	4.5 ± 0.2^{c}	1.3 ± 0.1^{b}
Σn-3[1]	11.3 ± 0.3^{c}	9.8 ± 0.2^{b}	8.0 ± 0.7^{a}
Σn-6[2]	2.4 ± 0.1^{c}	1.2 ± 0.1^{b}	0.7 ± 0.1^{a}
Σn-9[3]	2.3 ± 0.2^{b}	3.9 ± 0.2^{c}	0.5 ± 0.0^{a}
ΣSAFAs[4]	3.5 ± 0.2^{b}	2.3 ± 0.1^{a}	3.5 ± 0.4^{b}
ΣMUFAs[5]	4.2 ± 0.2^{b}	5.4 ± 0.2^{c}	1.9 ± 0.3^{a}
Σn-3 LC-PUFAs[6]	2.6 ± 0.2^{a}	5.3 ± 0.3^{c}	3.6 ± 0.3^{b}
ratio n-3/n-6	4.7 ± 0.4^{a}	8.2 ± 0.5^{b}	10.8 ± 1.5^{c}
ratio n-3/n-9	4.9 ± 0.4^{b}	2.5 ± 0.2^{a}	15.7 ± 0.8^{c}
ratio n-6/n-9	1.1 ± 0.1^{b}	0.3 ± 0.0^{a}	1.5 ± 0.3^{b}
ratio DHA/EPA	0.1 ± 0.1^{a}	7.6 ± 0.5^{c}	0.6 ± 0.0^{b}
ratio DHA/ARA	0.3 ± 0.4^{a}	71.1 ± 1.2^{c}	32.6 ± 2.0^{b}
ratio EPA/ARA	6.6 ± 0.0^{a}	9.4 ± 0.6^{b}	52.6 ± 2.5^{c}

Values (mean ± SD) followed by different superscripts within a row were ($P < 0.05$) significantly different among the three treatments. *n*, number of replicates; SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; ARA, arachidonic acid. [1]Includes 16 : 3n-3, 16 : 4n-3, 18 : 3n-3, 18 : 4n-3, 20 : 3n-3, 20 : 4n-3, 20 : 5n-3, 22 : 5n-3, and 22 : 6n-3. [2]Includes 16 : 2n-6, 18 : 2n-6, 18 : 3n-6, 20 : 2n-6, 20 : 3n-6, 20 : 4n-6, 22 : 4n-6 and 22 : 5n-6. [3]Includes 18 : 1n-9, 18 : 2n-9, 20 : 1n-9, 20 : 2n-9, 20 : 3n-9 and 22 : 1n-9.[4] Includes 14 : 0, 15 : 0, 16 : 0, 17 : 0, 18 : 0, and 20 : 0.[5]Includes 14 : 1n-5, 14 : 1n-7, 15 : 1n-5, 16 : 1n-7, 16 : 1n-5, 18 : 1n-9, 18 : 1n-7, 18 : 1n-5, 20 : 1n-9, 20 : 1n-7, 20 : 1n-5, 22 : 1n-9 and 22 : 1n-11.[6]Includes 20 : 3n-3, 20 : 4n-3, 20 : 5n-3, 22 : 5n-3, and 22 : 6n-3.

$P \geq 0.05$, Table 5). Carbohydrates constituted the smallest portion of the nauplii proximate composition, with values ranged between $4\% \pm 0.4\%$ in T–R to $9.1\% \pm 0.6\%$ for treatment R. Data of ash and moisture content showed no statistical difference among all treatments (ANOVA, $P \geq 0.05$, Table 5).

3.3.2. FAs Composition. The FA composition of *A. tonsa* nauplii fed with the three experimental diets was expressed as a percentage of the total lipid DW ± SD (Table 5). Total n-3 FAs and total n-3 LC-PUFAs, as well as EPA and DHA, were significantly higher in copepods fed on I–R compared with the other two diets (ANOVA, $P < 0.05$, Table 5). Group fed on diet T–R gave copepod higher percentage of 16 : 0, OA, LA, ALA, vaccenic acid, ARA, as also MUFAs, total n-6 and n-9 FAs series (ANOVA, $P < 0.05$, Table 5). FAs ratios n-3/n-6 and n-3/n-9 were significantly lower (ANOVA, $P < 0.05$, Table 5) in group fed on T–R, while DHA/EPA

Table 4: Proximal composition (lipid, protein, carbohydrate, ash, and moisture content; g/100 g dry weight) and fatty acid composition (mg/g dry weight) of rotifers (*Brachionus plicatilis*) fed with three microalgae diets (R, *Rhodomonas salina*; I–R, *Isochrysis galbana* + *Rhodomonas salina*; T–R, *Tetraselmis suecica* + *Rhodomonas salina*), $n = 5$.

Brachionus plicatilis	T–R	I–R	R
Lipids	13.1 ± 1.0^{a}	15.4 ± 0.8^{b}	17.3 ± 1.1^{c}
Proteins	57.4 ± 0.8^{ab}	58.7 ± 1.6^{b}	56.2 ± 1.7^{a}
Carbohydrates	7.8 ± 0.6	8.7 ± 0.9	7.2 ± 1.0
Ash	21.7 ± 0.4^{b}	17.2 ± 1.7^{a}	19.4 ± 0.6^{a}
Moisture	18.6 ± 2.1^{b}	14.6 ± 1.5^{a}	13.6 ± 2.1^{a}
14 : 0	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.9 ± 0.1^{b}
14 : 1n-5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
16 : 0	1.6 ± 0.4^{a}	1.6 ± 0.2^{a}	2.6 ± 0.3^{b}
16 : 1n-7	0.3 ± 0.1^{a}	0.5 ± 0.1^{ab}	0.7 ± 0.1^{b}
16 : 3n-1	0.2 ± 0.0	–	–
18 : 0	0.4 ± 0.1^{a}	0.5 ± 0.0^{a}	0.8 ± 0.1^{b}
18 : 1n-9	1.3 ± 0.4^{ab}	1.7 ± 0.2^{b}	1.0 ± 0.1^{a}
18 : 1n-7	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}	0.9 ± 0.1^{b}
18 : 2n-6	0.8 ± 0.2^{ab}	1.2 ± 0.3^{b}	0.6 ± 0.0^{a}
18 : 3n-6	0.1 ± 0.0	–	–
18 : 3n-3	1.7 ± 0.5^{a}	1.4 ± 0.2^{a}	2.7 ± 0.3^{b}
18 : 4n-3	0.7 ± 0.3	0.5 ± 0.1	0.7 ± 0.1
20 : 1n-5	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.1
20 : 2n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20 : 4n-6 ARA	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20 : 3n-3	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
20 : 4n-3	1.0 ± 0.3	0.9 ± 0.1	0.9 ± 0.0
20 : 5n-3 EPA	1.1 ± 0.3	1.4 ± 0.1	1.2 ± 0.1
22 : 1n-11	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
22 : 5n-3	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
22 : 6n-3 DHA	0.5 ± 0.1^{a}	1.4 ± 0.1^{c}	0.8 ± 0.1^{b}
Σn-3[1]	5.7 ± 1.3	6.1 ± 0.6	7.0 ± 0.5
Σn-6[2]	1.3 ± 0.2^{ab}	1.7 ± 0.3^{b}	1.2 ± 0.1^{a}
Σn-9[3]	2.0 ± 0.4	2.0 ± 0.2	2.0 ± 0.3
ΣSAFAs[4]	2.4 ± 0.5^{a}	2.5 ± 0.3^{a}	4.4 ± 0.5^{b}
ΣMUFAs[5]	4.0 ± 0.8^{ab}	3.9 ± 0.4^{a}	4.9 ± 0.3^{b}
Σn-3 LC-PUFAs[6]	3.0 ± 0.6^{a}	4.1 ± 0.3^{b}	3.4 ± 0.2^{a}
ratio n-3/n-6	4.4 ± 1.1^{ab}	3.7 ± 0.4^{a}	6.1 ± 0.8^{b}
ratio n-3/n-9	2.9 ± 0.8	3.0 ± 0.2	3.6 ± 0.7
ratio n-6/n-9	0.7 ± 0.2^{ab}	0.8 ± 0.1^{b}	0.6 ± 0.0^{a}
ratio DHA/EPA	0.5 ± 0.1^{a}	1.0 ± 0.1^{c}	0.7 ± 0.0^{b}
ratio DHA/ARA	2.8 ± 0.5^{a}	7.4 ± 2.2^{b}	3.7 ± 0.6^{a}
ratio EPA/ARA	5.7 ± 2.0	7.2 ± 1.7	5.5 ± 1.1

Values (mean ± SD) followed by different superscripts within a row were ($P < 0.05$) significantly different among the three treatments. *n*, number of replicates; SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; ARA, arachidonic acid. [1]Includes 16 : 3n-3, 16 : 4n-3, 18 : 3n-3, 18 : 4n-3, 20 : 3n-3, 20 : 4n-3, 20 : 5n-3, 22 : 5n-3, and 22 : 6n-3. [2]Includes 16 : 2n-6, 18 : 2n-6, 18 : 3n-6, 20 : 2n-6, 20 : 3n-6, 20 : 4n-6, 22 : 4n-6 and 22 : 5n-6. [3]Includes 18 : 1n-9, 18 : 2n-9, 20 : 1n-9, 20 : 2n-9, 20 : 3n-9 and 22 : 1n-9. [4] Includes 14 : 0, 15 : 0, 16 : 0, 17 : 0, 18 : 0, and 20 : 0. [5]Includes 14 : 1n-5, 14 : 1n-7, 15 : 1n-5, 16 : 1n-7, 16 : 1n-5, 18 : 1n-9, 18 : 1n-7, 18 : 1n-5, 20 : 1n-9, 20 : 1n-7, 20 : 1n-5, 22 : 1n-9 and 22 : 1n-11. [6]Includes 20 : 3n-3, 20 : 4n-3, 20 : 5n-3, 22 : 5n-3, and 22 : 6n-3.

and DHA/ARA ratio levels were significantly higher with I–R treatment (ANOVA, $P < 0.05$, Table 5). Finally, copepods fed on R and I–R did not show any significant differences in EPA/ARA ratio (ANOVA, $P \geq 0.05$, Table 5); meanwhile, treatment T–R was significantly lower (ANOVA, $P < 0.05$, Table 5) compared to the other diets.

3.4. Survival. During the feeding trials, survival rates were different among preys. While *B. plicatilis* survival did not change significantly after exposure to each treatment, *A. tonsa* showed different survival peaks over time depending on the type of diet administered (Figure 3, "diet · day", $F_{6.54} = 117.28$, $P < 0.05$). Thus, from day 2, survival of nauplii

Table 5: Proximal composition (lipid, protein, carbohydrate, ash, and moisture content; g/100 g dry weight) and fatty acid composition (mg/g dry weight) of copepods (*A. tonsa*) nauplii, fed with three microalgae diets (R, *Rhodomonas salina*; I–R, *Isochrysis galbana* + *Rhodomonas salina*; T–R, *Tetraselmis suecica* + *Rhodomonas salina*), $n = 5$.

Acartia tonsa	T–R	I–R	R
Lipids	18.0 ± 0.3^{b}	16.4 ± 0.5^{a}	15.9 ± 0.3^{a}
Proteins	71.8 ± 0.7^{b}	70.7 ± 0.7^{b}	68.1 ± 0.6^{a}
Carbohydrates	4.0 ± 0.4^{a}	6.4 ± 0.6^{b}	9.1 ± 0.6^{c}
Ash	6.3 ± 0.4	6.6 ± 0.6	6.9 ± 0.2
Moisture	7.2 ± 0.7	7.6 ± 0.6	8.4 ± 0.7
14 : 0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
14 : 1n-5	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
16 : 0	3.8 ± 0.4^{c}	1.2 ± 0.2^{a}	2.5 ± 0.3^{b}
16 : 1n-7	0.6 ± 0.5	0.2 ± 0.0	0.2 ± 0.0
16 : 3n-1	0.2 ± 0.0	–	–
18 : 0	1.3 ± 0.1^{b}	0.7 ± 0.1^{a}	1.4 ± 0.2^{b}
18 : 1n-9	1.3 ± 0.3^{b}	0.7 ± 0.1^{a}	0.6 ± 0.2^{a}
18 : 1n-7	0.6 ± 0.1^{b}	0.3 ± 0.0^{a}	0.4 ± 0.1^{a}
18 : 2n-6	0.7 ± 0.1^{b}	0.4 ± 0.1^{a}	0.2 ± 0.1^{a}
18 : 3n-6	0.1 ± 0.0	–	–
18 : 3n-3	1.8 ± 0.2^{b}	1.0 ± 0.1^{a}	1.2 ± 0.1^{a}
18 : 4n-3	0.7 ± 0.2	0.5 ± 0.1	0.4 ± 0.1
20 : 1n-5	0.2 ± 0.1	0.1 ± 0.0	–
20 : 2n-6	0.1 ± 0.0	0.1 ± 0.0	–
20 : 4n-6 ARA	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
20 : 3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20 : 4n-3	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
20 : 5n-3 EPA	2.9 ± 0.3^{ab}	2.9 ± 0.1^{b}	2.5 ± 0.1^{a}
22 : 1n-11	0.1 ± 0.0	–	–
22 : 5n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
22 : 6n-3 DHA	2.1 ± 0.3^{a}	6.7 ± 0.2^{c}	4.6 ± 0.2^{b}
Σn-3[1]	7.8 ± 0.8^{a}	11.5 ± 0.3^{c}	9.2 ± 0.4^{b}
Σn-6[2]	1.1 ± 0.2^{c}	0.7 ± 0.2^{b}	0.4 ± 0.1^{a}
Σn-9[3]	1.5 ± 0.3^{b}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}
ΣSAFAs[4]	5.6 ± 0.5^{b}	2.5 ± 0.1^{a}	4.6 ± 0.4^{b}
ΣMUFAs[5]	3.2 ± 0.6^{b}	1.5 ± 0.1^{a}	1.7 ± 0.2^{a}
Σn-3 LC-PUFAs[6]	5.3 ± 0.5^{a}	9.9 ± 0.2^{c}	7.6 ± 0.4^{b}
ratio n-3/n-6	7.2 ± 1.7^{a}	17.2 ± 3.7^{b}	24.5 ± 5.0^{b}
ratio n-3/n-9	5.5 ± 0.9^{a}	14.7 ± 2.0^{b}	12.2 ± 2.1^{b}
ratio n-6/n-9	0.8 ± 0.2	0.9 ± 0.4	0.5 ± 0.1
ratio DHA/EPA	0.7 ± 0.1^{a}	2.3 ± 0.1^{c}	1.9 ± 0.1^{b}
ratio DHA/ARA	12.2 ± 3.2^{a}	96.8 ± 5.0^{c}	80.9 ± 6.7^{b}
ratio EPA/ARA	16.7 ± 3.6^{a}	42.0 ± 2.7^{b}	43.6 ± 3.2^{b}

Values (mean ± SD) followed by different superscripts within a row were ($P < 0.05$) significantly different among the three treatments. *n*, number of replicates; SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; ARA, arachidonic acid. [1]Includes 16 : 3n-3, 16 : 4n-3, 18 : 3n-3, 18 : 4n-3, 20 : 3n-3, 20 : 4n-3, 20 : 5n-3, 22 : 5n-3, and 22 : 6n-3. [2]Includes 16 : 2n-6, 18 : 2n-6, 18 : 3n-6, 20 : 2n-6, 20 : 3n-6, 20 : 4n-6, 22 : 4n-6 and 22 : 5n-6. [3]Includes 18 : 1n-9, 18 : 2n-9, 20 : 1n-9, 20 : 2n-9, 20 : 3n-9 and 22 : 1n-9. [4] Includes 14 : 0, 15 : 0, 16 : 0, 17 : 0, 18 : 0, and 20 : 0. [5]Includes 14 : 1n-5, 14 : 1n-7, 15 : 1n-5, 16 : 1n-7, 16 : 1n-5, 18 : 1n-9, 18 : 1n-7, 18 : 1n-5, 20 : 1n-9, 20 : 1n-7, 20 : 1n-5, 22 : 1n-9 and 22 : 1n-11. [6]Includes 20 : 3n-3, 20 : 4n-3, 20 : 5n-3, 22 : 5n-3, and 22 : 6n-3.

fed with binary diets I–R (96.2% ± 0.8%) and T–R (95.8% ± 0.9%) was significantly higher than nauplii fed on R treatment (87.9% ± 0.7%) (ANOVA, $P < 0.05$, Figure 3). At the end of the experiment, survival was significantly higher in binary diets, especially with I–R treatment. (88.1% ± 0.7%) (ANOVA, $P < 0.05$).

4. Discussion

4.1. Biochemical Composition of Live Preys. The present study showed how different mono and binary diets based on microalgae (T–R, I–R, and R) affect the survival and biochemical composition of *A. tonsa* nauplii and the rotifer

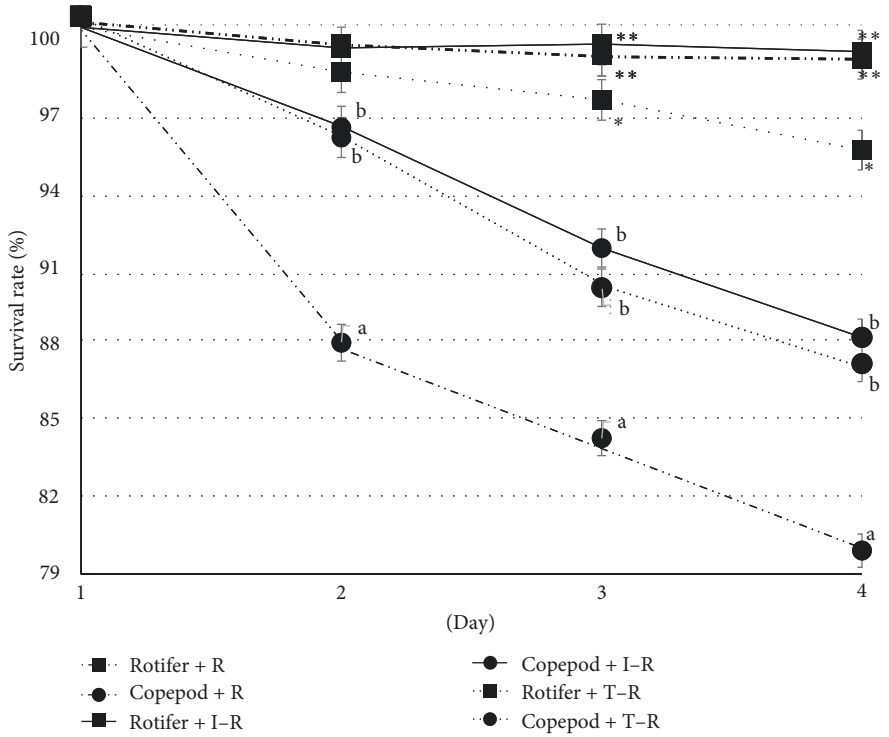


FIGURE 3: Survival (% ± SD) of *Brachionus plicatilis* (rotifer) and *Acartia tonsa* (copepod) nauplii fed with three experimental diets. Values (mean ± SD) followed by different superscripts were ($P < 0.05$) significantly different among the three treatments. R, *Rhodomonas salina*; I–R, *Isochrysis galbana* + *Rhodomonas salina*; T–R, *Tetraselmis suecica* + *Rhodomonas salina*.

B. plicatilis. In both preys, the use of binary diets based on the combination of *I. galbana* and *R. salina* (I–R treatment) improved their protein and LC-PUFAs content as well as the survival of *A. tonsa* nauplii, compared to control and T–R treatment. Indeed, among all microalgae employed in this study, the treatment I–R showed the highest nutritional value, especially in LC-PUFAs. It is plausible that this binary formula took advantage of the content in EPA of *R. salina* and the DHA content of *I. galbana*, potentially generating an LC-PUFAs boosted diet. Although these microalgae strains naturally produce high amounts of PUFAs, their FA content and composition can vary with environmental parameters such as the composition of the growth medium, nutrient availability, or illumination [48]. Therefore, it was important to standardize the microalgae culture conditions, in order to ensure the same FA enrichment day by day. In general, the biochemical composition of *A. tonsa* nauplii fed on I–R treatment was the richest in terms of protein, lipids, total LC-PUFAs, EPA, and DHA, also compared to *B. plicatilis* fed with the same treatment. Despite being a monoalgal diet, *R. salina* (control) has been shown to better improve the nutritional value of *A. tonsa*, especially in EPA and DHA, compared to the binary diet T–R. Indeed, this was probably due to the composition of *T. suecica*, which showed the lowest content in LC-PUFAs, especially in DHA, compared to the other microalgae employed to generate treatments (Table 3). These results agreed with previous studies reporting the excellence of *Rhodomonas* spp. as a diet for calanoid copepods [37, 39, 49, 50], suggesting that *R. salina* is an outstanding candidate to generate binary diet for the calanoid copepod *A. tonsa*. On the contrary, the rotifer *B. plicatilis* was characterized by a lower content in proximate and FAs composition, even after enrichment with I–R treatment, compared to *A. tonsa* nauplii. The retention ability of LC-PUFAs was clearly species-specific [8]. Although *B. plicatilis* is able to synthesize LC-PUFAs, it showed a limited ability to retain EPA and DHA compared to *A. tonsa* [7]. On the contrary, *A. tonsa* nauplii were able to retain a high quantity of n-3 LC-PUFAs acquired exclusively from their phytoplankton diet, especially EPA and DHA. These results are consistent with those obtained by [15], where *A. tonsa* nauplii fed with *Rhodomonas baltica* until 12 days post-hatch reached the highest levels of EPA and DHA after 3 days of culture. The high percentage of DHA and EPA observed in copepods nauplii after 4 days fed with I–R suggested that the combination between *R. salina* and *I. galbana* could be an optimal food source for *A. tonsa*, as diet or enrichment. Besides, results also suggest that *A. tonsa* could retain the portion of saturated FAs (14 : 0, 16 : 0, and 18 : 0), and PUFAs (18 : 3n-3, 18 : 4n-3, 20 : 4n-6, 20 : 5n-3, and 22 : 6n-3), particularly EPA and DHA, which may be catabolized by the copepod as important growth factors [28, 51]. The ability to retain LC PUFAS could be related to the growth and reproduction of calanoid copepods, providing them with a considerable energy store and enabling their growth also during the food-scarce seasons [27, 28, 52]. This high LC-PUFAs retention capacity makes *A. tonsa* an optimal live feed for marine larval cultures, which may replace totally

or partially both rotifers and even *Artemia* throughout all marine fish larvae development. Compared to conventional live preys, *A. tonsa* presents not only a high content in protein, DHA, and EPA but also an attractive swimming behavior and wide size range (*ca.* 70 μm of newly hatched nauplii till 1.5 mm of adult females) [5, 9, 14, 17, 19, 53]. On the other hand, the latest cold storing techniques of *A. tonsa* eggs, resisting at 4°C for several months [35], represent to date the easiest way to manage high quantities of copepods nauplii, although it implies the inevitable decrease in viability and nutritional content [14]. In this sense, the enrichment exposure of *A. tonsa* nauplii to microalgae rich in LC-PUFAs, such as *I. galbana* and *R. salina*, arrange this decrease in nutritional content, improving their nutritional value before being administrated as a live feed to marine fish larvae. Indeed, the increased importance of LC-PUFAs (e.g., EPA and DHA) for marine fish larvae has been reviewed for many fish species [8, 54–56].

4.2. Survival. Finally, none of the diets fed to *B. plicatilis* produced significant mortality; only the group fed on T–R treatment showed reduced survival. That difference could be related to the size and motility of the microalgae employed as enrichment. Indeed, *T. suecica* presented larger cell size and higher motility, around 17 μm in cell length and four flagella, compared to *R. salina* and *I. galbana*, which showed, respectively, 11 and 5 μm cell length, both with two flagella. In contrast, the calanoid copepod *A. tonsa* showed significant differences in survival depending on the treatment supplied, with the highest survival rate when fed on I–R treatment. The mortality observed in all culture trials of *A. tonsa* nauplii could be explained since they pass through the delicate phase of molting where mortality is due not only to the food source but also to many other multifactorial causes such as energy reserves, parents nutrition, eggs storage conditions, storage time, etc. [14, 17]. Despite all treatments showing mortality, these results highlight the importance of using live microalgae binary diets as food sources for *A. tonsa* naupliar stages.

5. Conclusions

The present study provides evidence of the importance of enriching *A. tonsa* nauplii with a binary microalgal diet based on the combination of *I. galbana* and *R. salina* (I–R) in order to supply an adequate content of n-3 LC-PUFAs. Moreover, it showed how the biochemical composition of *A. tonsa* and *B. plicatilis* depended on their FAs retention ability and the diet supplied. The wide differences in nutritional composition observed between both preys revealed *A. tonsa* as a superior live feed, with higher protein content and improved ability to retain LC-PUFAs (EPA and DHA), compared to *B. plicatilis*. In conclusion, this study highlights the biochemical profile of enriched *A. tonsa* nauplii and contributes to the production of high-quality live feeds, which may be able to reach the nutritional needs and enhance the production of new marine fish species, as well as contributing to the improvement of *A. tonsa* culture.

Data Availability

The data in this study are available from the corresponding author upon reasonable request.

Disclosure

This research is part of a doctoral thesis belonging to the PhD program in Sustainable Aquaculture and Marine Ecosystems (ACUISEMAR) of the University of Las Palmas de Gran Canaria (ULPGC).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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