fishes

MDPI

Article

Biochemical Composition of Eggs, Larvae and Tissues of *Macrobrachium tenellum* Females Fed Diets with Different Lipid and Protein Levels

Omar Alejandro Peña-Almaraz [1,2], Manuel Alejandro Vargas-Ceballos [2], Edilmar Cortés-Jacinto [3] and Fernando Vega-Villasante [2,*]

[1] Programa de Doctorado BEMARENA, Centro Universitario de la Costa, Universidad de Guadalajara, Av. Universidad de Guadalajara #203, Puerto Vallarta C.P. 48280, Jalisco, Mexico; omar.pena@alumnos.udg.mx
[2] Laboratorio de Calidad de Agua y Acuicultura Experimental, Centro Universitario de la Costa, Universidad de Guadalajara, Av. Universidad de Guadalajara #203, Puerto Vallarta C.P. 48280, Jalisco, Mexico; manuel.vargas@academicos.udg.mx
[3] Programa de Acuicultura, Centro de Investigaciones Biológicas del Noroeste S.C., Av. Instituto Politécnico Nacional #195, La Paz C.P. 23096, Baja California Sur, Mexico; ecortes04@cibnor.mx
* Correspondence: fvillasante@cuc.udg.mx

Abstract: One way to approach the nutritional requirements of native shrimp, necessary to consolidate their culture, is to know their biochemical composition. The effect of feeding two levels of lipids (4 and 12% L) and four levels of proteins (30, 35, 40 and 45% P) in *M. tenellum* females was evaluated with respect to the biochemical composition of their eggs (EG), larvae (LR), gonad (GO) and hepatopancreas (HP). Total protein (TP), total carbohydrate (TC) and total lipid (TL) were estimated. In EG, L and P levels influence TP and TL; TP increases in diets higher than P35. In LR, there are no differences ($p > 0.05$) in TP and in TL, only diets L4P40 and L12P30 were different ($p < 0.05$). In GO, there is no trend in TP differences; in TC there was variation in the range of the data and TL was higher in L4P30 and L4P35. In HP, the diets with L4 obtained the highest TP values ($p < 0.05$); the L12 diets were higher in TL ($p < 0.05$). In general, diets with an inclusion of L12 showed the highest TP, TC and TL means, within this lipid level the P30 diet stood out; therefore, it is recommended to use a diet with L12P30 in the formulation of balanced feed for the species.

Keywords: shrimp; chemical composition; diets; nutrients; carbohydrates

Key Contribution: Different levels of lipids and proteins in diets affect the biochemical composition of eggs, larvae and tissues of *M. tenellum*. The diet with 12% lipids and 30% proteins obtained the best results.

Citation: Peña-Almaraz, O.A.; Vargas-Ceballos, M.A.; Cortés-Jacinto, E.; Vega-Villasante, F. Biochemical Composition of Eggs, Larvae and Tissues of *Macrobrachium tenellum* Females Fed Diets with Different Lipid and Protein Levels. *Fishes* **2024**, *9*, 145. https://doi.org/10.3390/fishes9040145

Academic Editor: Timothy Bowden

Received: 9 April 2024
Accepted: 18 April 2024
Published: 21 April 2024

1. Introduction

The nutritional contribution of crustaceans to their larvae can significantly influence the profile of nutritional parameters, such as lipids, at hatching and during the first days of life [1]. Lipids are transferred from the ovary to the eggs; therefore, lipid content in eggs can be used to infer the physiological status of breeding females [2,3]. Lipids represent one of the most common energy sources in animal cells [1], which are stored in the form of fatty acids (triacylglycerol). Another important source of energy is glucose, stored as glycogen. Free carbohydrates, in the form of glucose, play a key role in energy metabolism [4]. Glucose is highly mobilized and metabolized because, among other things, a large amount of it is needed after molting to produce the exoskeleton [5]. Proteins, on the other hand, are essential metabolites for the production of hormones and tissues and provide structure and support to the cells [6].

A diet rich in lipids and protein is essential to stimulate sexual maturation and copulation activity in crustaceans [7]. The ideal protein level varies in ranges between 23 and 60% depending on factors such as age, feeding strategies, quality, protein source and energy

levels [8]. On the other hand, biochemical changes that occur in larvae during feeding and starvation are indicators of their nutritional requirements and are an important basis for determining appropriate diets during larval culture [9].

Prawns, also called river shrimp or freshwater shrimp, are freshwater crustaceans valued for their nutritional properties and good taste. Worldwide, research regarding nutritional requirements and reproduction in prawns focuses on *M. rosenbergii*, especially females, because it is the most cultivated species globally and economically important. In this species, it is reported that diets with protein levels of 42% and lipids of 9% promote reproductive performance, egg composition, hatching efficiency and larval survival compared to a commercial diet [10,11]. Regarding Latin American species of prawns, in a study with *M. carcinus* broodstock where different experimental formulations with variation in proteins and lipids were evaluated, greater growth was reported for a diet with 13% lipids and a greater gain in muscle protein for diets with 40 to 45% protein [12]. In *M. acanthurus*, a lipid inclusion of between 15 and 17.5% in the diet of females could be optimal for egg maturation and production and increase their protein and lipid content [13]. In *M. amazonicum*, it is reported that a diet containing 35% protein optimizes the use of energy channeled to growth and minimizes its loss through excretion, which is why it is considered a promising protein level for a diet in this species [14]. Meanwhile, for *M. tenellum*, a protein requirement below 35% is suggested for maturation, gonadal development and spawning [15].

One of the most important shrimp species in the Mexican Pacific is *Macrobrachium tenellum*, due to its importance as a food resource, its local commercial value and its cultural importance [16,17]. At present, adequate protocols and technologies have not been established to develop aquaculture models and commercial-scale production of *M. tenellum* [18]. The lack of postlarvae production of this and other shrimp species is often compensated for by obtaining seed from the wild, but this practice, together with overfishing, could have a devastating impact on the natural populations of this shrimp [19]. To address this problem, several researchers have focused on studying the nutritional requirements in relation to reproduction in freshwater prawns [10,11,15], as well as biochemical profiles of their eggs and tissues [20]. Despite this, information is still limited and scarce, especially in the specific case of *M. tenellum*.

Considering that *M. tenellum* is a native species and that it has attractive characteristics for potential cultivation, it is extremely important to identify its nutritional requirements and to know its biochemical composition. The present study evaluated the effect of feeding different levels of proteins and lipids in *M. tenellum* females in relation to the biochemical composition of their eggs, larvae, gonad and hepatopancreas. The results obtained provide valuable information to increase knowledge of the elaboration of diets that cover the energetic demands to improve the reproductive quality and production of this species. The data on biochemical components also provide an insight into the metabolic processes and nutritional needs during the early stages of development.

2. Materials and Methods

2.1. Shrimp and Experimental Design

Adults of *M. tenellum* were captured from wild populations in the Ameca River, Jalisco-Nayarit, Mexico, using different fishing gear. The shrimp were acclimatized in freshwater ponds at a temperature of 28.0 ± 0.2 °C in the facilities of the Water Quality and Experimental Aquaculture Laboratory of the University of Guadalajara. They were fed daily with a commercial feed Azteca® brand (Aztec, NM, USA) (with 30% protein, 5% lipids and 12% ash) for fourteen days.

An 8 × 3 completely randomized design was used for the experiment, with 8 diets evaluated in triplicate. Each experimental unit (EU) consisted of a 600 L maximum capacity tank, gauged to 200 L, with eight females (24 EU in total). Males were kept separate from females under the same conditions as well as an additional stock of females in order to replace any loss. At the start of the experiment, females and males were selected completely at random from the laboratory stock and biometry (total weight and length from the

base of the eye stalk to the tip of the telson) was performed. Females were taken with stage I ovary maturation (thin, transparent gonad with no apparent formation of ovarian tissue) and early stage II (ovarian tissue is slightly visible near the posterior part of the cephalothorax) [21,22]. The initial weight and total length of females and males were 2.37 ± 0.48 g and 4.69 ± 0.37 cm, and 3.83 ± 0.43 g and 4.92 ± 0.23 cm, respectively. The shrimp were fed once daily at 16:00 h at 5% of the biomass of each pond. Temperature, dissolved oxygen and pH were recorded every day. The leftover feed from the previous day was removed and every two days a 30% water replacement was performed in order to maintain optimal water quality parameters. After 30 days from the first feeding, males were placed in a ratio of four females to one male (4H:1M) in each tank to promote copulation. The experiment lasted 90 days.

2.2. Experimental Diets

The experimental diets were formulated following the protein and lipid levels and manufacturing methods of Peña-Almaraz et al. [23]. Eight diets with two lipid levels (4 and 12% L) and four protein levels (30, 35, 40 and 45% P) were evaluated and the following were used to make the diets: fish meal and oil, corn meal, grenetin, vitamin and mineral premix, vitamin C, sodium benzoate, α-Tocopherol and corn starch in the proportions shown in Table 1.

Table 1. Ingredients (% dry weight) of the eight experimental diets containing two levels of lipids (4 and 12% L) and four levels of protein (30, 35, 40 and 45% P).

Ingredients	**Experimental Diets**							
	L4P30	**L4P35**	**L4P40**	**L4P45**	**L12P30**	**L12P35**	**L12P40**	**L12P45**
Fish meal [1]	35.63	43.19	50.75	58.37	35.63	43.19	50.74	58.31
Corn flour	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Fish oil [1]	1.26	0.68	0.10	0.00	9.26	8.68	8.10	7.52
Corn starch	47.87	40.89	33.92	26.46	39.87	32.89	25.92	18.94
Gelatin	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Vitamins and minerals premix (Rovimix) [2]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin C [2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium benzoate	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Choline chloride [2]	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
α-Tocopherol	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[1] Proteínas Marinas y Agropecuarias, S.A. de CV, Guadalajara, Jalisco, Mexico. [2] Sistemas en Zootecnia (SenZoo) S.A. de C.V., Tlajomulco de Zuñiga, Jalisco, Mexico.

2.3. Proximal Composition of Experimental Diets

Proximate analyses of the diets were performed according to AOAC standard procedures [24] (Table 2). Total protein content was determined by the micro Kjeldahl method and multiplied by a factor of 6.25. Total lipid content was determined by the Soxhlet method, using hexane as carrier solution. Ash was determined by calcining a sample at 550 °C for 6 h in a muffle. The nitrogen-free extract (NFE) was calculated by the difference of dry matter with the formula NFE = 100 − (% crude protein + % total lipids + % ash).

Table 2. Proximal composition of the 8 experimental diets.

Composition Proximal (%)	**Experimental Diets**							
	L4P30	**L4P35**	**L4P40**	**L4P45**	**L12P30**	**L12P35**	**L12P40**	**L12P45**
Lipids	3.87	3.93	4.04	3.78	11.83	12.77	12.54	12.75
Proteins	30.23	35.02	39.98	44.91	30.21	35.63	41.09	45.55
Ashes	10.56	13.03	13.36	15.46	10.58	12.29	14.03	15.68
NFE *	55.34	48.02	42.62	35.85	47.38	39.31	32.34	26.02

The values represented are the means. The analyses were performed in triplicate. * Nitrogen-free extract = 100 − (Proteins + Lipids + Ashes).

2.4. Sampling Protocol

All procedures were performed according to the Mexican Official Standard (NOM-062-ZOO-1999) [25] on technical specifications for the production, care and use of laboratory animals and the Declaration of Helsinki. Samples of eggs, larvae, gonads and hepatopancreas were obtained by the following procedures. Eggs were taken from females seven days after fertilization; for this, the females were removed from the experimental tank (with due precautions to avoid damaging the ovigerous mass), and the whole ovigerous mass was collected with dissecting forceps. To obtain the larvae, 6 ovigerous females with eggs at stage III were randomly selected from each pond [26], with embryos about to hatch and these females were isolated individually in 40 L fish tanks until the hatching of their eggs; the newly hatched larvae were collected in their entirety. Once the egg and larval samples were obtained and the gonad was fully mature (visible dark green ovary occupying the largest dorsal portion of the cephalothorax), the gonads and hepatopancreas were obtained by sacrificing and dissecting six females from each tank; shrimp were euthanized by cold temperature shock, placing them in water and refrigerating them at 4 °C. Death was established by observation every 10 min. The weight of all samples was recorded on a Nimbus® analytical balance (ADAM® NBL:254 d = 0.0001 g) and the samples were placed in 2.0 mL test tubes, frozen at −20 °C, subsequently lyophilized in a Luzeren S/M 15-0825 lyophilizer and stored until the day of biochemical analysis.

2.5. Estimation of Total Proteins (TP), Total Carbohydrates (TC) and Total Lipids (TL) of the Samples

All biochemical analyses were performed by taking 6 samples per replicate of each diet; with these samples two pools were formed and analyzed in triplicate. TP was calculated by the Bradford method [27] from the homogenate with 1.2% saline solution. Aliquots were taken and digestion was performed with 0.5 N NaOH; proteins were quantified using albumin as standard at 595 nm absorbance with spectrophotometer. TC was determined by the anthrone method [28]; from the homogenate with 1.2% saline solution, proteins were precipitated with trichloroacetic acid and the aliquots were centrifuged cold (4 °C) at 4826 rpm for 10 min. From the resulting supernatant, total carbohydrates were quantified, using glucose as standard and reading at 620 nm in a spectrophotometer. TL estimation was performed using the microgravimetry method described in Lu et al. [29] with slight modifications: the homogenate was mixed with chloroform:methanol (2:1), then centrifuged at 13500 rpm for 1 min. The liquid phase was separated and NaCl was added to 20% of the volume, then centrifuged again and the upper phase was discarded and the lower phase was transferred to a clean, tared vial. The vial was aerated for one hour and weighed. The percentage of TL was calculated by dividing the total lipid weight by the initial sample weight and multiplying by 100.

2.6. Statistical Analysis

Data were tested for normality and homoscedasticity using Shapiro–Wilk and Levene tests, respectively. To determine differences in the biochemical composition of eggs, larvae and tissues between diets, one-way ANOVA tests were performed ($p < 0.05$). For cases where differences were found, Tukey tests were applied ($p < 0.05$). Data that did not meet the assumptions of normality and homoscedasticity were analyzed using the Kruskal–Wallis one-way nonparametric test ($p < 0.05$). A two-way analysis of variance was performed to evaluate whether the interactions between the different lipid and protein levels of the analyzed samples are significant as a function of biochemical composition. All statistical analyses were performed with SigmaPlot software (14.5).

3. Results

3.1. Experimental Conditions

During the first 30 days of experimentation, there was a loss of four females from different ponds, probably due to natural causes, since no possible cause was observed.

These females were replaced by others that were in the replacement stock. At the end of the experiment (90 days), the average survival was 80%.

The mean temperature recorded in the ponds was similar, hovering around 28 °C, the mean dissolved oxygen was greater than 5.5 mg/L and the mean pH was 7.4 (Table 3).

Table 3. Water quality parameters recorded in the ponds with the groups of females fed the different experimental diets.

Diets	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (Units)
L4P30	28.0 ± 0.6	5.93 ± 0.6	7.4 ± 0.2
L4P35	28.1 ± 0.8	5.76 ± 0.7	7.3 ± 0.2
L4P40	28.1 ± 0.9	5.71 ± 0.8	7.4 ± 0.2
L4P45	28.3 ± 0.9	5.82 ± 0.6	7.5 ± 0.1
L12P30	28.2 ± 0.8	5.62 ± 0.6	7.4 ± 0.2
L12P35	27.9 ± 0.8	5.73 ± 0.6	7.5 ± 0.2
L12P40	27.9 ± 0.7	5.57 ± 0.6	7.4 ± 0.2
L12P45	28.5 ± 0.8	5.68 ± 0.7	7.2 ± 0.2

The values represented are the means (±standard deviation).

3.2. Biochemical Composition of Eggs

TP per egg varied in different diets, with mean values ranging from 11.26 to 17.72 µg/egg. Diets L12P40 and L12P45 had the highest values and L4P30 and L12P30 had the lowest values (Table 4). The TC did not show much variation, the mean amount of TC did not exceed 3 µg/egg and despite the statistical differences ($p > 0.05$), there is no clear trend in the data; however, the lowest and highest values were found at the extremes, that is, with the lowest lipid and protein levels (L4P30) and the highest levels (L12P45), respectively. On the other hand, the TL data did not seem to obey any pattern with respect to the diets and the means did not exceed the highest lipid level evaluated. It is worth noting that the most notable statistical differences ($p < 0.05$) were in L4P45 and L12P35 versus L12P40. Two-way ANOVA suggested that, in terms of TP, there are significant differences ($p < 0.05$) between protein levels, independent of lipid level, and vice versa; there are also significant differences in TL as a function of protein levels and protein–lipid interactions.

Table 4. Biochemical composition of eggs obtained from *Macrobrachium tenellum* females fed with the different experimental diets.

Diet	Total Protein (µg/egg)	Total Carbohydrates (µg/egg)	Total Lipids (µg/egg)
L4P30	11.32 ± 1.30 ^{a}	1.60 ± 0.20 ^{a}	5.33 ± 0.31 ^{ab}
L4P35	11.86 ± 1.25 ^{ab}	1.97 ± 0.38 ^{ab}	5.89 ± 0.27 ^{a}
L4P40	13.18 ± 1.26 ^{abc}	2.70 ± 0.03 ^{b}	4.83 ± 0.27 ^{b}
L4P45	14.35 ± 0.99 ^{bc}	2.11 ± 0.29 ^{ab}	3.73 ± 0.13 ^{d}
L12P30	11.26 ± 0.61 ^{a}	1.93 ± 0.14 ^{ab}	4.59 ± 0.38 ^{b}
L12P35	11.56 ± 0.74 ^{ab}	2.23 ± 0.45 ^{ab}	3.61 ± 0.11 ^{d}
L12P40	17.72 ± 3.29 ^{c}	2.09 ± 0.98 ^{ab}	6.94 ± 0.16 ^{c}
L12P45	15.53 ± 0.77 ^{cd}	2.43 ± 0.41 ^{b}	5.68 ± 0.36 ^{ab}
Two-way ANOVA (*p*-value)			
Protein	<0.001	0.009	<0.001
Lipid	<0.001	0.561	0.029
Protein × lipid	0.002	0.037	<0.001

The values represented are the means (±standard deviation). Superscripts with different letters within the same columns indicate statistical differences ($p < 0.05$). Two-way ANOVA ($p < 0.05$).

3.3. Biochemical Composition of Larvae

The differences found in the TP eggs are not reflected in the larvae. There were no significant differences in TP counts. There was a large variation in TC counts ($p < 0.05$), from 22.81 to 49.71 µg/mg, but the data did not show a clear trend (Table 5). There were no

significant differences in TL composition, except for the L4P40 and L12P30 diets. Two-way ANOVA showed that the amount of TC present in the larvae is affected by the different levels of proteins, lipids and their interactions. In addition, it was also shown that the level of protein affects the amount of TL.

Table 5. Biochemical composition of larvae obtained from *Macrobrachium tenellum* females fed with the different experimental diets.

Diet	Total Protein (µg/mg)	Total Carbohydrates (µg/mg)	Total Lipids (µg/mg)
L4P30	515.12 ± 69.26	44.57 ± 0.88 ^{c}	72.57 ± 6.92 ^{abc}
L4P35	661.40 ± 61.45	49.71 ± 1.43 ^{c}	73.87 ± 6.37 ^{bc}
L4P40	543.02 ± 63.51	22.81 ± 1.34 ^{a}	87.86 ± 6.73 ^{c}
L4P45	637.09 ± 76.85	33.20 ± 0.93 ^{b}	65.37 ± 4.77 ^{ab}
L12P30	565.75 ± 195.86	64.96 ± 0.68 ^{d}	55.17 ± 5.59 ^{a}
L12P35	544.98 ± 20.93	32.31 ± 5.89 ^{b}	71.37 ± 8.06 ^{abc}
L12P40	656.51 ± 43.20	36.46 ± 2.85 ^{b}	64.78 ± 4.68 ^{ab}
L12P45	651.97 ± 38.82	47.59 ± 8.76 ^{c}	64.51 ± 7.44 ^{ab}
Two-way ANOVA (*p*-value)			
Protein	0.047	<0.001	<0.001
Lipid	0.537	<0.001	0.010
Protein × lipid	0.019	<0.001	0.020

The values represented are the means (±standard deviation). Superscripts with different letters within the same columns indicate statistical differences ($p < 0.05$). Two-way ANOVA ($p < 0.05$).

3.4. Biochemical Composition of the Gonad

There is no trend in the differences found in the biochemical analysis of the gonad in terms of TP composition; the means ranged from 413.19–571.68 µg/mg (Table 6). In the TC, there was variation in the range of the data, with means ranging from 7 to 28 µg/mg and as in previous cases, the data do not seem to have a clear trend.

Table 6. Biochemical composition of gonads obtained from *Macrobrachium tenellum* females fed with the different experimental diets.

Diet	Total Protein (µg/mg)	Total Carbohydrates (µg/mg)	Total Lipids (µg/mg)
L4P30	456.93 ± 53.34 ^{ab}	28.57 ± 5.84 ^{de}	232.35 ± 8.88 ^{cd}
L4P35	413.19 ± 35.55 ^{a}	9.53 ± 0.49 ^{ab}	227.65 ± 7.12 ^{bcd}
L4P40	569.75 ± 45.73 ^{b}	7.41 ± 1.31 ^{a}	213.65 ± 4.95 ^{abc}
L4P45	497.49 ± 40.33 ^{ab}	21.61 ± 8.31 ^{cd}	215.03 ± 5.51 ^{abc}
L12P30	460.03 ± 52.04 ^{ab}	31.40 ± 1.94^{d}	245.00 ± 6.85 ^{d}
L12P35	571.68 ± 96.41 ^{b}	21.23 ± 3.03 ^{c}	205.55 ± 15.81 ^{ab}
L12P40	568.17 ± 90.98 ^{b}	12.05 ± 0.53 ^{ab}	198.46 ± 10.12 ^{a}
L12P45	477.21 ± 55.22 ^{ab}	14.61 ± 1.09 ^{bc}	201.82 ± 8.94 ^{a}
Two-way ANOVA (*p*-value)			
Protein	<0.001	<0.001	<0.001
Lipid	0.060	0.010	0.022
Protein × lipid	0.004	<0.001	0.023

The values represented are the means (±standard deviation). Superscripts with different letters within the same columns indicate statistical differences ($p < 0.05$). Two-way ANOVA ($p < 0.05$).

On the other hand, regarding TL, the data suggest that there is an inverse trend in terms of the increase and decrease in proteins and lipids in the diets, since a higher amount of TL was found in the two diets with lower lipid and protein levels (L4 and P30, P35) and a lower amount in the two diets with higher lipid and protein levels (L12 and P40, P45). The results of the two-way ANOVA show that protein levels mainly affected the biochemical composition of the gonad.

3.5. Biochemical Composition of the Hepatopancreas

Marked differences were observed in lipid levels. The diets with L4 obtained the highest values in the TP estimations ($p < 0.05$) and within these, the diets with higher protein level obtained higher means (Table 7). A similar case occurred with TC, but the same trend was not shown with respect to protein level. The TC data in this tissue showed the greatest variation, 16.37–84.39 µg/mg, compared to the TC of the remaining samples. In TL, there was an inverse relationship with respect to the differences found in TP and TC; the highest values belonged to the diets with L12 ($p < 0.05$) and the lowest, to L4. The two-way ANOVA showed that the levels of proteins and lipids separately, as well as their combinations, significantly affect the biochemical composition of the hepatopancreas.

Table 7. Biochemical composition of hepatopancreas obtained from *Macrobrachium tenellum* females fed with the different experimental diets.

Diet	Total Protein (µg/mg)	Total Carbohydrates (µg/mg)	Total Lipids (µg/mg)
L4P30	422.06 ± 28.82 ^{b}	69.51 ± 12.05 ^{d}	255.16 ± 7.26 ^{c}
L4P35	451.70 ± 42.42 ^{b}	76.00 ± 2.10 ^{de}	213.45 ± 9.36 ^{b}
L4P40	512.00 ± 10.95 ^{c}	56.23 ± 4.16 ^{c}	210.60 ± 15.14 ^{b}
L4P45	619.15 ± 41.92 ^{d}	84.39 ± 1.26 ^{e}	142.11 ± 9.28 ^{a}
L12P30	275.90 ± 23.12 ^{a}	16.37 ± 4.84 ^{a}	456.86 ± 8.77 ^{e}
L12P35	288.03 ± 17.40 ^{a}	56.86 ± 6.67 ^{c}	477.10 ± 9.41 ^{ef}
L12P40	257.96 ± 22.19 ^{a}	51.20 ± 1.10 ^{c}	506.92 ± 10.88 ^{f}
L12P45	286.40 ± 29.02 ^{a}	38.88 ± 1.14 ^{b}	397.99 ± 14.39 ^{d}
Two-way ANOVA (*p*-value)			
Protein	<0.001	<0.001	<0.001
Lipid	<0.001	<0.001	<0.001
Protein × lipid	<0.001	<0.001	<0.001

The values represented are the means (±standard deviation). Superscripts with different letters within the same columns indicate statistical differences ($p < 0.05$). Two-way ANOVA ($p < 0.05$).

4. Discussion

The range of the overall biochemical composition, taking into account the results of the estimations of all diets, were, in eggs, TP 11 to 18 µg/egg, TC 1.5 to 3 µg/egg and TL 3.5 to 7 µg/egg; in larvae, TP 515 to 545 µg/mg, TC 32 to 65 µg/mg and TL 55 to 88 µg/mg; in the gonad, TP 413 to 570 µg/mg, TC 7.5 to 31 µg/mg and TL 198 to 245 µg/mg; and in the hepatopancreas, TP 258 to 619 µg/mg, TC 16 to 84 µg/mg and TL 142 to 507 µg/mg. A variation in TP composition was observed in eggs, larvae and tissues; this has already been documented in muscle and body composition (whole organism) of juvenile and adult marine crabs [30–32] and the prawns *Macrobrachium acanthurus* and *M. nipponense* [13,33]. The same situation occurred with TC, and in this sense, intraspecific variations of this macronutrient are reported in the muscle of other decapods, such as *Charybdis smithii* [34], *Scylla serrata* [30] and *Scylla tranquebarica* [32]. TL was not the exception, it were found to be present in a different range of proportions, as reported in the muscle of the crabs *Portunus trituberculatus* [35] and *S. tranquebarica* [32] and the whole body of the prawns *Macrobrachium rosenbergii* [10,36], *M. acanthurus* [13] and *M. nipponense* [33]. The above demonstrates that decapods can manifest different proportions of proteins, lipids and carbohydrates in their tissues, structures and organs, even if they are of the same species. It is known that the biochemical composition of aquatic animals may vary according to season, animal size, maturity stage, temperature, genetic factors, level of domestication and availability and quality of food [32,37].

This study showed that lipid and protein levels in diets fed to *M. tenellum* broodstock affect the biochemical composition of their eggs, larvae, gonad and hepatopancreas. It has been shown that in some decapods the type of food and protein levels in the diets influence the body protein content [32], as is the case of cultured penaeid shrimp, since they are organisms that have the quality of using protein as a source of energy [38]. In

Macrobrachium americanum, diets with different lipid and protein composition influence TP, TL and TC content [39] and lipid content increases when the lipid level in the diet increases from 6 to 14%. On the other hand, in *M. amazonicum*, the level of protein in body composition is reduced when dietary protein increases from 20 to 35% [40].

In the eggs of *M. tenellum* at the intermediate stage of development, the major organic component is protein, followed by lipids and, to a lesser extent, carbohydrates. Similar proportions have been reported for the eggs of other crustaceans [41–45] and congeneric species [20,46]. Proteins are usually the major component of crustacean eggs, as they are essential for tissue synthesis and serve as a source of energy [47,48]; it is known that the continuous synthesis and degradation of proteins for morphogenesis processes constantly produce variations in their biochemical composition [49,50]. It is typically reported that carbohydrates are the minority component of decapod eggs [51,52]; despite this, they play a key role in metabolic processes and are the main source of energy in the early stage of embryonic development [4,46,49]. In turn, lipids serve as a source of metabolic energy for early tissue formation [13,46,49]. Considering the above, a higher content of proteins, lipids and carbohydrates in eggs could translate into higher egg quality, which would be advantageous for their development and survival under variable environmental conditions.

In the present study, it was demonstrated that lipid and protein levels influence the TP and TL of eggs; within the same lipid levels, TP composition increases when protein in the diets is higher than 35%. For *M. acanthurus*, it is reported that the type of protein and lipids in the diets affect the proximal composition of the egg [53]; this composition is similar to that found in the present study in terms of TP and TL. In contrast, it was also found that for *M. acanthurus*, the egg protein content is not affected by lipid levels [13], which differs with *M. tenellum*. These differences may be due to the specific environmental and feeding conditions for each study, as these variables may affect lipid and protein content to a lesser or greater extent [54,55].

Diets with different proportions of lipids and proteins do not affect the TP composition of newly hatched larvae of *M. tenellum*, but they do affect the composition of TC and TL. It was also shown that the level of protein affects TL, but no relationship was found in the lipid–protein combination. As in eggs, protein is the macronutrient with the highest fraction in larval composition, followed by lipids and finally, carbohydrates. The reason that proteins are noticeably higher than lipids may be because protein catabolism may be more important in the early stages of larval development, while lipid catabolism is a more important energy source for the later stages [56]. The protein composition of larvae in this study is notably higher than reported for spiny lobster (*Jasus edwardsii*), which is 31%, but similar in TC [1]. The lipid proportions found in spiny lobster larvae range from 7 to 12% [1,57,58] and 12% in prawns *(M. amazonicum)* fed L8P43 diets [59]; these percentages are higher than the TL ranges estimated in the present study. In contrast, the percentage of TP found in *M. amazonicum* [59] is notably lower than that reported in *M tenellum*. This study demonstrates that the contribution of the broodstock to the newly hatched larvae through the egg can significantly influence their nutritional parameters, which coincides with what has been reported in other investigations [1,10]. Thus, the quality and quantity of nutrients remaining after embryonic development will have a great impact on larval development and survival, as newly hatched larvae may rely on yolk energy reserves in case food is not immediately available [4,60,61].

The biochemical composition of the gonad followed the same proportion of eggs and larvae, although it is noteworthy that a notoriously higher percentage of TL was found. Similar fractions in TP, TC and TL have been reported in other decapod crustaceans [62–64]. The reason that proteins are the major component in this tissue may be due to the fact that intense protein synthesis occurs during vitellogenesis and oocyte development [65,66], leading to an increase in protein content [22,67]. The reason that TL is higher than in eggs and larvae may be because generating the energy reserves necessary for embryonic development requires high energy expenditure [68]; lipids and, to a lesser extent, carbohydrates will be responsible for covering the energy requirements during ovarian maturation [66,67].

The effect of dietary lipids on the biochemical composition of the ovary has been documented in some decapods. In females of *Cherax quadricarinatus* fed L6P38 diets, values of 47% TL and 33% TP were found [69], while in *Eriocheir sinensis* fed L8P38 diets, TL fractions of 26% and TP of 58.5% were found [64]; in both species, TL was higher than in *M. tenellum*. Studies with the shrimp *M. rosenbergii* report variations in TP as a function of L8 to L10 levels in the diets [10,36], which differs from the present study. In *M. acanthurus*, there is a lower concentration of TP and a higher concentration of TL when dietary lipids are higher, and values of 14 to 15% in TL and 53 to 65% in TP were found in diets with L10 to L12 [13], which indicates that in this species, lipid levels have a clear effect on the biochemical composition of the gonad, contrary to what was found in this study.

The hepatopancreas was the organ that presented the most marked differences in the effect of lipid levels on the composition of TP and TL. Higher TP values were found in L4, while the highest TL means belonged to the diets with L12. This coincides with what was reported for *M. acanthurus* where it was shown that there is a tendency for proteins to decrease in the hepatopancreas when lipid levels increase in the diets [13]. Generally, the proportions of TP and TL in decapods range from 15 to 43% and 45 to 67%, respectively [35,36,55,64], similar to that reported in the present study. The cause of an increase in the proportion of lipid composition compared with other tissues may be due to the fact that this is the organ where, among other functions, lipid absorption, secretion, and metabolism are performed.

5. Conclusions

The biochemical composition of eggs, larvae, ovaries and hepatopancreas of *M. tenellum* is within the proportions and ranges reported for other decapod species and some species of the same genus. This study showed that the inclusion of different levels of lipids and proteins in the diet affects the biochemical composition. Diets with a lipid inclusion of 12% showed the highest TP, TC and TL means; within this lipid level, the diet with 30% protein stood out, since it was the one that obtained the highest means. Therefore, it is recommended to use a diet with L12P30 in the formulation of balanced feed for the females in the reproductive stage of this species. It should be emphasized that these results are a general approximation to the biochemical composition and specific studies of amino acid and fatty acid profiles of the embryos and main organs are required to elaborate diets that perfectly cover the requirements of the species.

Author Contributions: Conceptualization, O.A.P.-A., M.A.V.-C., F.V.-V. and E.C.-J.; methodology, O.A.P.-A. and M.A.V.-C.; software, O.A.P.-A. and M.A.V.-C.; formal analysis, O.A.P.-A. and M.A.V.-C.; investigation, O.A.P.-A., M.A.V.-C., F.V.-V. and E.C.-J.; resources, F.V.-V. and E.C.-J.; writing-original draft preparation, O.A.P.-A. and M.A.V.-C.; writing-review and editing, M.A.V.-C., F.V.-V. and E.C.-J.; supervision, F.V.-V. and E.C.-J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Council of Humanities Sciences and Technologies of México (CONAHCYT), through the O.A.P.-A. Doctoral research fellowship (CVU: 1069870, grant number: 777431) and M.A.V.-C. Postdoctoral fellowship (grant number: I1200/320/2022).

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Animal Welfare Committee of Centro Universitario de la Costa from Universidad de Guadalajara (CUCPV/CBA/02/2023).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors appreciate the invaluable support of Carlos Alfonso Alvarez-Gonzalez and Emyr Saúl Peña-Marín in carrying out the biochemical analyses and the members of LACUIC, CUCosta, UDG for their technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ritar, A.J.; Dunstan, G.A.; Crear, B.J.; Brown, M.R. Biochemical composition during growth and starvation of early larval stages of cultured spiny lobster (*Jasus edwardsii*) phyllosoma. *Comp. Biochem. Physiol. Part A Mol. Integr.* **2003**, *136*, 353–370. [CrossRef] [PubMed]
2. Lavens, P.; Sorgeloos, P. Variation in egg and larval quality in various fish and crustacean species. In *Larvi '91*; Lavens, P., Sorgeloos, P., Jaspers, E., Ollevier, F., Eds.; European Aquaculture Society Special Publication: Gent, Belgium, 1991; Volume 15, pp. 221–222.
3. Cahu, C.L.; Cuzon, G.; Quazuguel, P. Effect of highly unsaturated fatty acids, α-tocopherol and ascorbic acid in broodstock diet on egg composition and development of *Penaeus indicus*. *Comp. Biochem. Physiol Part A Physiol.* **1995**, *112*, 417–424. [CrossRef]
4. Anger, K. *The Biology of Decapod Crustacean Larvae*; A.A. Balkema Publishers: Lisse, The Netherlands; Helgoland, Germany, 2001; pp. 1–420.
5. Palacios, E.; Ibarra, A.M.; Ramirez, J.L.; Portillo, G.; Racotta, I.S. Biochemical composition of eggs and nauplii in white Pacific shrimp, *Penaeus vannamei* (Boone), in relation to the physiological condition of spawners in a commercial hatchery. *Aquac. Res.* **1998**, *29*, 183–189. [CrossRef]
6. Mai, K.; Xue, M.; He, G.; Xie, S.Q.; Kaushik, S.J. Chapter 4. Protein and amino acids. In *Fish Nutrition*, 4th ed.; Hardy, R.W., Kaushik, S.J., Eds.; Academic Press: London Wall, London, 2022; pp. 181–302.
7. González-Ferriol, M.; Betancourt-Aguiar, J.L.; Ramos-Trujillo, L. Endocrinology of reproduction in Decapod Crustaceans (crustacea:decapoda): Scientific advances and future perspectives. *Invest. Mar.* **2018**, *38*, 1–8.
8. Teshima, I.; Koshio, S.; Ishikawa, M.; Alam, S.; Hernandez-Hernandez, L.H. Protein requirements of the fresh-water prawn *Macrobrachium rosenbergii* evaluated by the factorial method. *J. World Aquac. Soc.* **2006**, *37*, 145–153. [CrossRef]
9. Olsen, Y. Lipids and essential fatty acids in aquatic food webs: What can freshwater ecologists learn from mariculture? In *Lipids in Freshwater Ecosystems*; Arts, M.T., Wainman, B.C., Eds.; Springer: New York, NY, USA, 1998; pp. 161–202.
10. Cavalli, R.O.; Lavens, P.; Sorgeloos, P. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. *Aquaculture* **1999**, *179*, 387–402. [CrossRef]
11. Kangpanich, C.; Pratoomyot, J.; Siranonthana, N.; Senanan, W. Effects of arachidonic acid supplementation in maturation diet on female reproductive performance and larval quality of giant river prawn (*Macrobrachium rosenbergii*). *PeerJ.* **2016**, *4*, e2735. [CrossRef] [PubMed]
12. Benítez-Mandujano, M.; Ponce-Palafox, J.T. Efecto de diferentes niveles dietéticos de proteína y lípidos en el crecimiento de reproductores del langostino de agua dulce (*Macrobrachium carcinus*). *Rev. MVZ Córdoba* **2014**, *19*, 3921–3929. [CrossRef]
13. Hernández-Abad, G.Y.; Hernández-Hernández, L.H.; Fernández-Araiza, M.A. Effects of different dietary lipid concentrations on the egg production and egg quality produced by *Macrobrachium acanthurus* females. *Lat. Am. J. Aquat. Res.* **2018**, *46*, 518–524. [CrossRef]
14. Mantoan, P.; Ballester, E.; Ramaglia, A.C.; Augusto, A. Diet containing 35% crude protein improves energy balance, growth, and feed conversion in the Amazon river prawn, *Macrobrachium amazonicum*. *Aquac. Rep.* **2021**, *21*, 100962. [CrossRef]
15. García-Ulloa, M.; Rodríguez, H.; Ogura, T. Egglet quality of two species of prawns (Palemonidae) of the genus *Macrobrachium* (*M. rosenbergii*, De Man 1879, and *M. tenellum*, Smith, 1871) varying broodstock diet: Morphometric indices. *AIA* **2004**, *8*, 1–8.
16. Espinosa-Chaurand, L.; Vargas-Ceballos, M.; Guzmán-Arroyo, M.; Nolasco-Soria, H.; Carrillo-Farnés, O.; Chong-Carrillo, O.; Vega-Villasante, F. Biology and cultivation of *Macrobrachium tenellum*: State of the art. *Hydrobiology* **2011**, *21*, 99–117.
17. García-Guerrero, M.U.; Becerril-Morales, F.; Vega-Villasante, F.; Espinosa-Chaurand, L.D. Prawns of the genus *Macrobrachium* with economic and fisheries importance in Latin America: Current knowledge, ecological role and conservation. *Am. Lat. Am. J. Aquat. Res.* **2013**, *41*, 651–675.
18. De los Santos-Romero, R.; Vega-Villasante, F.; Cortes-Jacinto, E.; García-Guerrero, M. The culture potential and management problems of freshwater prawns (*Macrobrachium americanum* and *Macrobrachium tenellum*) in their native areas: The case for Mexico. *Lat. Am. J. Aquat. Res.* **2021**, *49*, 376–390. [CrossRef]
19. Vega-Villasante, F.; García-Guerrero, M.U.; Cortés-Jacinto, E.; Yamasaki-Granados, S.; Montoya-Martínez, C.E.; Vargas-Ceballos, M.A.; Chong-Carrillo, O.; Guzmán-Arroyo, M.; Carrillo-Farnés, O.V.; Nolasco-Soria, H.G. Chapter 13. Freshwater shrimp of the genus *Macrobrachium*: Biology, ecology and exploitation. In *Temas Sobre Investigaciones Costeras*, 1st ed.; Cifuentes-Lemus, J.L., Cupul-Magaña, F.G., Eds.; Universidad de Guadalajara: Guadalajara, Mexico, 2014; pp. 273–315.
20. Nguyen, T.M.T.; Chen, T.Y.; Shiau, C.Y.; Cheng, Y.T.; Chang, Y.W. Study on biochemical divergences of the meat and egg of freshwater prawns (*Macrobrachium rosenbergii*). *Food Sci. Nutr.* **2019**, *7*, 2017–2023. [CrossRef] [PubMed]
21. Chang, C.F.; Shih, T.W. Reproductive cycle of ovarian development and vitellogen in profiles in the freshwater prawns, *Macrobrachium rosenbergii*. *Invertebr. Reprod. Dev.* **1995**, *27*, 11–20. [CrossRef]
22. Revathi, P.; Iyapparaj, P.; Munuswamy, N.; Krishnan, M. Vitellogenesis during the ovarian development in freshwater female prawn *Macrobrachium rosenbergii* (De Man). *Int. J. Aquat. Sci.* **2012**, *3*, 13–27.
23. Peña-Almaraz, O.A.; Vega-Villasante, F.; Espinosa-Magaña, A.F.; Guerrero-Galván, S.R.; Vargas-Ceballos, M.A. Effect of protein and lipid variation in diets for *Macrobrachium tenellum*, with respect to reproductive indicators. *Ecosis Rec. Agropec.* **2023**, *10*, e3512.
24. Association of Official Analytical Chemists. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2005.

25. NOM-062-ZOO-1999. Mexican Official Standard: Technical Specifications for the Production, Care and Use of Laboratory Animals. 2001. Available online: https://www.gob.mx/cms/uploads/attachment/file/203498/NOM-062-ZOO-1999_220801.pdf (accessed on 8 May 2021).
26. Wehrtmann, I.S. Distribution and reproduction of *Ambidexter panamense* and *Palaemonetes schmittii* in Pacific Costa Rica (Crustacea, Decapoda). *Rev. Biol. Trop.* **1990**, *38*, 327–329.
27. Bradford, M. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
28. Trevelyan, W.; Harrison, K. Studies on yeast metabolism. *Biochem J.* **1950**, *50*, 298–303. [CrossRef]
29. Lu, Y.; Ludsin, S.A.; Fanslow, D.L.; Pothoven, S.A. Comparison of three microquantity techniques for measuring total lipids in fish. *Can. J. Fish. Aquat. Sci.* **2008**, *65*, 2233–2241. [CrossRef]
30. Balasubramanian, C.P.; Suseelan, C. Biochemical composition of the deep water crab *Charybdis smithii*. *Indian J. Fish.* **2001**, *48*, 333–335.
31. Zafar, M.; Siddiqui, M.Z.H.; Hoque, M.A. Biochemical composition in *Scylla serrata* (Forskal) of Chakaria, Sundarban area, Bangladesh. *Pak. J. Biol. Sci.* **2004**, *7*, 2182–2186.
32. Manivannan, K.; Sudhakar, M.; Murugesan, R.; Soundarapandian, P. Effect of feed on the biochemical composition of commercially important mud crab *Scylla tranquebarica* (Fabricius 1798). *Int. J. Anim. Vet. Adv.* **2010**, *2*, 16–20.
33. Li, Y.; Fan, B.; Huang, Y.; Wu, D.; Zhang, M.; Zhao, Y. Effects of dietary vitamin E on reproductive performance and antioxidant capacity of *Macrobrachium nipponense* female shrimp. *Aquac. Nutr.* **2018**, *24*, 1698–1708. [CrossRef]
34. Prasad, P.N.; Neelakantan, B. Proximate and essential amino acid composition in edible crab *Scylla serrata*. *Comp. Physiol. Ecol.* **1989**, *14*, 34–37.
35. Han, T.; Wang, J.; Hu, S.; Li, X.; Jiang, Y.; Wang, C. Effects of different dietary lipid sources on growth performance and tissue fatty acid composition of juvenile swimming crab *Portunus trituberculatus*. *Chin. J. Oceanol.* **2015**, *33*, 957–965. [CrossRef]
36. Cavalli, R.O.; Menschaert, G.; Lavens, P.; Sorgeloos, P. Maturation performance, offspring quality and lipid composition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. *Aquac. Int.* **2000**, *8*, 41–58. [CrossRef]
37. Mairesse, G.; Thomas, M.; Gardeur, J.N.; Brun-Bellut, J. Effects of geographic source, rearing system, and season on the nutritional quality of wild and farmed *Perca fluviatilis*. *Lipids* **2006**, *41*, 221–229. [CrossRef]
38. Brito, R.; Chimal, M.; Gaxiola, G. Effect of different diets on growth and digestive enzyme activity in *Litopenaeus vannamei* (Boone, 1931) early postlarvae. *Aquac. Res.* **2001**, *32*, 257–266. [CrossRef]
39. Méndez-Martínez, Y.; Yamasaki-Granados, S.; García-Guerrero, M.U.; Martínez-Córdova, L.R.; Rivas-Vega, M.E.; Arcos-Ortega, F.G.; Cortés-Jacinto, E. Effect of dietary protein content on growth rate, survival and body composition of juvenile Cauque river prawn, *Macrobrachium americanum* (Bate 1868). *Aquac. Res.* **2016**, *48*, 741–751. [CrossRef]
40. Dos Santos, L.D.; Cagol, L.; Heldt, A.; Campagnolo, R.; Ballester, E.L.C. Níveis crescentes de proteína bruta em dietas práticas para camarão-da-amazônia. *Bol. Inst. Fish.* **2017**, *433*, 417–425.
41. Tian, H.M.; Wang, Q.; Zhao, Y.L.; Luo, W.; Fan, Y.J. Digestive enzyme activities and amino acids composition during embryonic development of *Eriocheir sinensis* (Chinese). *J. Fish. Sci. China* **2003**, *10*, 402–408.
42. García-Guerrero, M.; Racotta, I.; Villareal, H. Variation in lipid, protein, and carbohydrate content during the embryonic development of the crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *J. Crust. Biol.* **2003**, *23*, 1–6. [CrossRef]
43. Figueiredo, J.; Penha-Lopes, G.; Narciso, J.A.L.; Lin, J. Fecundity, brood loss and egg development through embryogenesis of *Armases cinereum* (Decapoda: Grapsidae). *Mar. Biol.* **2008**, *154*, 287–294. [CrossRef]
44. Li, S.; Cheng, Y.; Zhou, B.; Hines, A.H. Changes in biochemical composition of newly spawned eggs, prehatching embryos and newly hatched larvae of the blue crab (*Callinectes sapidus*). *J. Shellfish Res.* **2012**, *31*, 941–946. [CrossRef]
45. Soundarapandian, P.; Ravichandran, S.; Varadharajan, D. Biochemical composition of edible crab, *Podophthalmus vigil* (Fabricius). *J. Mar. Sci. Res. Dev.* **2013**, *3*, 119.
46. Yao, J.J.; Zhao, Y.L.; Wang, Q.; Zhou, Z.L.; Hu, X.C.; Duan, X.W.; An, C.G. Biochemical compositions and digestive enzyme activities during the embryonic development of prawn, *Macrobrachium rosenbergii*. *Aquaculture* **2006**, *253*, 573–582. [CrossRef]
47. Luo, W.; Zhou, Z.L.; Zhao, Y.L.; Yang, Z.B.; Zhang, M.F. Analysis on the contents of protein and amino acids in *Cherax quadricarinatus* during different embryonic development stages. *J. East China Norm. Univ.* **2004**, *1*, 88–92.
48. Samuel, N.J.; Soundarapandian, P. Embryonic development of commercially important portunid crab *Portunus sanguinolentus* (Herbst). *J. Anim. Vet. Adv.* **2009**, *1*, 32–38.
49. Habashy, M.M.; Sharshar, M.K.; Hassan, M.S. Morphological and histological studies on the embryonic development of the freshwater prawn, *Macrobrachium rosenbergii* (Crustacea, Decapoda). *JOBAZ* **2012**, *65*, 157–165. [CrossRef]
50. Soundarapandian, P.; Dinakaran, G.K.; Varadharajan, D.; Dinakaran, G.K. Biochemical composition during the embryonic development and freshly hatched zoea of *Macrobrachium idella idella* (Hilgendorf, 1898). *Adv. Tech. Biol. Med.* **2013**, *1*, 111–115.
51. Figueiredo, J.; Narciso, L. Egg volume, energy content and fatty acid profile of *Maja brachydactyla* (Crustacea: Brachyura: Majidae) during embryogenesis. *J. Mar. Biol. Assoc. UK* **2008**, *88*, 1401–1405. [CrossRef]
52. Khoei, J.K.; Bastami, A.A.; Esmailian, M. The biochemical composition of the eggs blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) in the Persian gulf coasts, Iran. *Middle East J. Sci. Res.* **2012**, *12*, 915–920.
53. Villafuerte Mojica, A.; Hernández-Hernández, L.H.; Fernández Araiza, M.A.; Ángeles-López, O. Contribution to the knowledge of the nutritional requirements of the native shrimp (*Macrobrachium acanthurus*). *Hydrobiology* **2016**, *26*, 15–22.

54. Biesiot, P.M.; Perry, H.M. Biochemical composition of the deep-sea red crab *Chaceon quinquedens* (Geryonidae): Organic reserves of developing embryos and adults. *Mar. Biol.* **1995**, *124*, 407–416. [CrossRef]
55. Hasek, B.E.; Felder, D.L. Biochemical composition of ovary, embryo, and hepatopancreas in the grapsoid crabs *Armases cinereum* and *Sesarma nr. reticulatum* (Crustacea, Decapoda). *Comp. Biochem. Physiol. Part B* **2005**, *140*, 55–463. [CrossRef] [PubMed]
56. Roustaian, P.; Kamarudin, M. Biochemical changes in freshwater prawn *Macrobrachium rosenbergii* (de Man) during larval development. *J. World Aquac. Soc.* **2001**, *31*, 53–59. [CrossRef]
57. Phleger, C.F.; Nelson, M.M.; Mooney, B.D.; Nichols, P.D.; Ritar, A.J.; Smith, G.G.; Hart, P.R.; Jeffs, A.G. Lipids and nutrition of the southern rock lobster, *Jasus edwardsii*, from hatch to puerulus. *Mar. Freshw. Res.* **2001**, *52*, 1475–1486. [CrossRef]
58. Smith, E.G.; Ritar, A.J.; Carter, C.G.; Dunstan, G.A.; Brown, M.R. Photothermal manipulation of reproduction in broodstock and larval characteristics in newly-hatched phyllosoma of the spiny lobster, *Jasus edwardsii*. *Aquaculture* **2003**, *220*, 299–311. [CrossRef]
59. Hayd, L.A.; Anger, K.; Urzúa, A. Growth, elemental and proximate biochemical composition of larval Amazon River prawn, *Macrobrachium amazonicum*, reared under different salinity conditions. *Am. Lat. Am. J. Aquat. Res.* **2017**, *45*, 983–991. [CrossRef]
60. Rotllant, G.; Charmantier-Daures, M.; Charmantier, G.; Anger, K.; Sarda, F. Effects of diet on *Nephrops norvegicus* (L.) larval and postlarval development, growth and elemental composition. *J. Shellfish Res.* **2001**, *20*, 347–352.
61. García-Guerrero, M.; Hendrickx, M.E. External description of the embryonic development of the prawn, *Macrobrachium americanum* Bate, 1868 (Decapoda, Palaemonidae) based on the staging method. *Crustaceana* **2009**, *82*, 1413–1422. [CrossRef]
62. Rosa, R.; Nunes, M.L. Biochemical changes during the reproductive cycle of the deep-sea decapod *Nephrops norvegicus* on the south coast of Portugal. *Mar. Biol.* **2002**, *141*, 1001–1009.
63. Baklouti, S.; Derbali, A.; Dhieb, K.; Jarboui, O. Reproductive biology of the Mediterranean green crab, *Carcinus aestuarii* Nardo, 1847 (Crustacea: Portunidae), in the Gulf of Gabes (Tunisia, Central Mediterranean). *Cah. Biol. Mar.* **2013**, *54*, 411–417.
64. Shao, L.; Wang, C.; He, J.; Wu, X.; Cheng, Y. Hepatopancreas and gonad quality of Chinese mitten crabs fattened with natural and formulated diets. *J. Food Qual.* **2013**, *36*, 217–227. [CrossRef]
65. Abdu, U.; Yehezkel, G.; Sagi, A. Oocyte development and polypeptide dynamics during ovarian maturation in the redclaw crayfish *Cherax quadricarinatus*. *Invertebr. Reprod. Dev.* **2000**, *37*, 75–83. [CrossRef]
66. Cortes-Jacinto, E.; Villarreal-Colmenares, H.; Civera-Cerecedo, R.; Naranjo-Paramo, J. Effect of dietary protein level on the growth and survival of pre-adult freshwater crayfish *Cherax quadricarinatus* (von Martens) in monosex culture. *Aquac. Res.* **2004**, *35*, 71–79. [CrossRef]
67. Rodríguez-González, H.; Hernández-Llama, A.; Villareal, H.; Saucedo, P.E.; García-Ulloa, M.; Rodríguez-Jaramillo, C. Gonadal development and biochemical composition of female crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in relation to the Gonadosomatic Index at first maturation. *Aquaculture* **2006**, *254*, 637–645. [CrossRef]
68. Rosa, R.; Nunes, M.L. Changes in organ indices and lipid dynamics during the reproductive cycle of *Aristeus antennatus*, *Parapenaeus longirostris*, and *Nephrops norvegicus* (Decapoda) from the Portuguese south coast. *Crustaceana* **2003**, *75*, 1095–1105.
69. Castillo-Díaz, F.; Tropea, C.; Stumpf, L.; López Greco, L.S. Effect of food restriction on female reproductive performance in the redclaw crayfish *Cherax quadricarinatus* (Parastacidae, Decapoda). *Aquac. Res.* **2017**, *48*, 4228–4237. [CrossRef]