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Research Article

Nutritional contribution of fish meal and microalgal biomass produced from two endemic microalgae to the growth of shrimp *Penaeus vannamei*

Juan Manuel Pacheco-Vega[1], Julián Gamboa-Delgado[2], Angel G. Alvarado-Ibarra[2]
Martha G. Nieto-López[2], Mireya Tapia-Salazar[2] & Lucía Elizabeth Cruz-Suárez[2]
[1]Escuela Nacional de Ingeniería Pesquera, Universidad Autónoma de Nayarit, Nayarit, México
[2]Programa Maricultura, Departamento de Ecología, Facultad de Ciencias Biológicas
Universidad Autónoma de Nuevo León, (UANL), México
Corresponding author: Julián Gamboa-Delgado (julian.gamboad@uanl.mx)

ABSTRACT. Different sources of microbial biomass have drawn attention as novel ingredients for aquaculture feeds. In the present study, isotopic measurements were applied to determine the contribution of dietary nitrogen supplied by two sources of microalgal biomass and fish meal, to the growth of shrimp *Penaeus vannamei*. Microalgae *Schizochytrium* sp. and *Grammatophora* sp. were isolated from the Sea of Cortez and massively cultured to obtain sufficient biomass. Experimental diets were formulated with low levels of microalgal biomass replacing 5 and 10% of fish meal nitrogen. Nitrogen stable isotope values were determined in ingredients, diets, and shrimp to estimate the relative contributions of the dietary nitrogen and dry matter supplied by these ingredients to the somatic growth. At the end of a feeding trial, significant differences were observed in mean final weight gain. Dietary nitrogen contributions from microalgae were similar to established dietary proportions, but when estimated on a dry matter basis, nutritional contributions were different for a diet containing 10% of *Schizochytrium*, which contributed 24% of dry matter to growth. Results demonstrate that low dietary inclusion levels of microalgal biomass elicit similar or higher growth rates than diets based on a fish meal only. Isotopic data indicated that microalgae actually contributed protein to tissue accretion.

Keywords: microalgae, *Schizochytrium*, *Grammatophora*, fish meal, white shrimp, stable isotopes.

INTRODUCTION

The fast growth that the aquaculture industry has experienced has been concomitant with a steep increase in the demand for aquaculture feeds. Due to its nutritional properties and relative good availability, fish meal has been a widely used ingredient for the manufacture of compound feeds. However, this particular ingredient has brought important concerns on economic and environmental issues. The use of plant proteins have represented efficient partial substitutes for a fish meal but the production of plants is limited mainly due to the restriction of available arable land. The use of plant meals as dietary ingredients for carnivorous fish also impose physiological restrictions. Hence, it is thought that the next generation of ingredients for animal nutrition will be significantly represented by those supplied by different sources of microbial biomass such as microalgae, bacteria, and yeasts (Gamboa-Delgado & Márquez-Reyes, 2016). Microalgal proteins present high digestibility and biological values, comparable to conventional vegetable proteins (Becker, 2007; Teimouri *et al*., 2013). Therefore, recent studies have focused on testing different dietary inclusion levels of microalgal biomass in aquaculture feeds. Li *et al*. (2016) recently reconfirmed that small dietary inclusion levels of microalgae biomass exert positive nutritional and immunological effects on shrimps.

Schizochytrium and *Grammatophora* are genera of widely distributed microalgae. Different species of *Schizochytrium* have been intensively cultured as a well-known source of lipids (Barclay & Zeller, 1996; Li *et al*., 2009). Although the genera *Grammatophora* has been less studied, it has been reported that it performs well as an agent to nutritionally enrich zooplankton (Pacheco-Vega *et al*., 2015). Modern, consistent industrial processes have been able to maintain high production yields of microbial biomass using alternative, less onerous substrates. Several enter-

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prises have focused on the development of high-density microalgal cultures for the production of biofuels, pigments and highly unsaturated fatty acids. Frequently, the remaining defatted material obtained from the latter processes have been proposed and tested as an ingredient for aquaculture diets (García-Ortega *et al*., 2016; Kissinger *et al*., 2016). However, the application of microalgal biomass as dietary ingredients in aquaculture feeds has not been significant yet since there are still technical obstacles to be overcome. On one side, increased production of different sources of microbial biomass is required to ensure lower price and higher availability. On the other side, more studies are needed to demonstrate that aquatic animals have the physiological ability to utilize nutrients supplied by these alternative ingredients.

Dietary tracing analyses can be carried out on the basis of the determination of stable isotopes ratios in an animal's body (Phillips & Koch, 2002; Phillips, 2012). In animal nutrition, the application of isotopic techniques and mixing models has supported the development of studies having the objective of estimating contributions of nutrients supplied by different alternative ingredients derived from plants and microorganisms (Martínez-Rocha *et al*., 2013; Gamboa-Delgado *et al*., 2016). The determination of assimilation efficiencies provides an indicator of the ingestion and digestion of specific dietary components. In the present study, the nitrogen stable isotope values measured in biomass of two microalgae species isolated from the Sea of Cortez, were used to estimate their nutritional contributions to shrimp growth in comparison to fish meal. The nitrogen residency times in shrimp whole bodies were also estimated.

MATERIALS AND METHODS

Experimental animals

Pacific white shrimp (*Penaeus vannamei*) (= *Litopenaeus vannamei*) postlarvae were obtained from a commercial hatchery (Maricultura del Pacífico) located in Mazatlán, Mexico. After the reception, animals were placed in two 500 L tanks and acclimated for 20 days to a bioassay room under the following conditions: seawater temperature 30.2 ± 0.7°C, salinity 35.4 ± 0.7 g L^{-1}, pH 8.4 ± 0.1 and saturated dissolved oxygen. Total ammonia nitrogen (1.19 ± 0.16 mg L^{-1}), nitrite (2.2 ± 1.6 mg L^{-1}), and nitrate (11.7 ± 4.6 mg L^{-1}) were monitored using a commercial kit (FasTest; Aquarium Systems, Sarrebourg, France). A photoperiod was set up to provide a light: the dark ratio of 10:14 h. During the acclimation period, shrimps were exclusively fed a commercial compound diet (35% protein, Grupo Costamar, Hermosillo, Mexico) that established a known isotopic baseline in shrimp tissue before the start of the experiment.

Production of microalgal biomass

The microalgae strains used in this study were isolated from Bahía de La Paz, Sea of Cortez, Baja California Sur, Mexico and characterized as described in Pacheco-Vega *et al.* (2015). Microalgal strains *Schizothyrium* sp. and *Grammatophora* sp. were cultured in previously filtered seawater (10, 5 and 1 µm mesh), which was also UV irradiated. Cultures were maintained in F/2 medium (Guillard, 1975) at 23 ± 1ºC, with continuous illumination at 3000 lux. Seawater and nutrients were autoclaved for 20 min (121°C, 1.02 kg cm^{-2}) and were used for volumes lower than 1000 mL. Larger water volumes were sterilized with 0.1% sodium hypochlorite for 24 h, and any residual chlorine was neutralized with a sodium thiosulfate solution (Pruder & Bolton, 1978). Scaling-up was carried out in 20 L polyethylene carboys and fiberglass tanks (400 L). The microalgal biomass (late logarithmic growth phase) was harvested on the fourth day of cell growth by bio-flocculation through a pH change after addition of 1.8 mL L^{-1} culture of a stock solution of NaOH (1 M) (CAS 1310-73-2, Fermont®, Monterrey, Mexico). The precipitate was collected after one hour and the pH-adjusted to 7.5 using concentrated HCl and continuous stirring. The flocculated biomass was centrifuged at 2320 *g* at 20°C for 15 min (IEC GP8R, UK). The microalgal biomass was subjected to heat shock for 5 min in a water bath (95-100ºC), as it has been suggested that the protein digestibility increases. Finally, the biomass was frozen (-40ºC), freeze-dried (Labconco, USA) and stored until used. The amino acid profile (Table 1) of the microalgal biomass was determined at the Agricultural Experiment Station of the University of Missouri (AOAC, 2006).

Experimental diets

Five isonitrogenous (36% crude protein) and near isoenergetic (3.9 kcal g^{-1}) experimental compound diets were formulated with fish meal (FM, prime Mexican sardine, 68% protein) and dry biomass of microalgae *Schizochytrium* sp. (SC) and *Grammatophora* sp. (GR). Both types of microalgal biomass were used to substitute low levels of FM (estimated on a dietary nitrogen-basis) (Table 2).

Diet 1 (100 FM) contained FM as the only nitrogen source and it was used as an isotopic control to correct for the isotopic differences between diets and consumers (isotopic discrimination factors) after having reached or approached equilibrium. Diets 2 and 3 were formulated with two inclusion levels of *Schizochytrium*, 5 and 10% (95FM/5SC and 90FM/10SC), while diets 4 and 5 included 5 and 10% of *Grammatophora* biomass

Table 1. Amino acid composition (g/100 g protein) of fish meal and microalgal biomass obtained from cultures of *Schizochytrium* sp. and *Grammatophora* sp.

Amino acid	Fish meal	*Schizochytrium* sp.	*Grammatophora* sp.
Taurine	0.77	0.31	0.19
Hydroxyproline	1.30	4.98	0.19
Aspartic Acid	9.50	10.27	10.67
Threonine	4.51	5.21	4.90
Serine	3.68	5.21	5.38
Glutamic Acid	12.65	10.19	12.02
Proline	4.70	4.67	3.94
Glycine	6.89	5.90	5.87
Alanine	6.52	7.66	6.73
Cysteine	0.91	1.30	1.44
Valine	5.41	5.82	5.96
Methionine	2.80	2.07	2.02
Isoleucine	4.53	4.52	5.29
Leucine	7.79	8.97	8.85
Tyrosine	3.65	3.75	3.65
Phenylalanine	4.33	6.44	6.35
Hydroxylysine	0.29	0.31	0.10
Ornithine	0.11	0.31	0.58
Lysine	8.60	4.83	5.19
Histidine	3.49	1.92	1.92
Arginine	6.43	4.67	5.67
Tryptophan	1.16	0.69	0.58

Table 2. Nutritional (g/1000 g diet, dry weight) and isotopic (δ^{15}N ‰) composition of five formulated diets fed to *Penaeus vannamei* to estimate the nutritional contribution of fish meal (FM) and dry biomass of microalgae *Schyzochytrium* sp. (SC) and *Grammatophora* sp. (GR). [a]Alimentos Costamar (Sonora, Mexico), [b]Almidones y gluten S.A. (Monterrey, Mexico), [c]Ragaza Industrias Proteínas Naturales S.A. de C.V. (Monterrey, Mexico), [d]Sigma-Aldrich (St. Louis, MO, USA).

Ingredients	Diet				
	100FM	95FM/5SC	90FM/10SC	95FM/5GR	90FM/10GR
Fish meal[a]	407.6	388.8	370.2	388.7	369.9
Wheat starch[b]	487.1	364.0	241.8	326.3	166.0
Schizochytrium sp. biomass	0.0	140.5	280.0	0.0	0.0
Grammatophora sp. biomass	0.0	0.0	0.0	180.0	360.0
Lecithin[c]	35.5	35.5	35.5	35.5	35.0
Alginate[d]	20.0	20.0	20.0	20.0	20.0
Cellulose[d]	19.0	19.0	19.0	19.0	18.5
Fish oil[a]	21.8	23.2	24.6	21.5	21.6
Vitamin mix[a]	2.5	2.5	2.5	2.5	2.5
Mineral mix[a]	2.5	2.5	2.5	2.5	2.5
Choline chloride[a]	2.0	2.0	2.0	2.0	2.0
Vitamin C[a]	1.0	1.0	1.0	1.0	1.0
Antioxidant[a]	0.5	0.5	0.5	0.5	0.5
Antifungic agent[a]	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000
Proximal and isotopic analysis					
Crude protein (g kg^{-1})	312	325	323	322	351
Lipids (g kg^{-1})	103	104	101	105	104
Ash (g kg^{-1})	95	205	237	171	315
Gross energy (kcal g^{-1})	4.3	4.2	3.6	4.0	3.7
δ^{15}N (‰)	16.7	15.9	15.0	15.9	14.7

(95FM/5GR and 90FM/10GR). Due to the low protein content in the microalgal biomass (12-15%) no negative control diets (*i.e.*, containing only microalgal biomass as a protein source) were manufactured; instead, as indicated below, a correction factor of 3.4‰ ‰ (Minagawa & Wada, 1984) was applied as isotopic discrimination factor for nitrogen. The software Nutrion® (Nutrion, Chapala, Mexico) was used to assist with the formulation of experimental diets. Diet preparation and ensuing bromatological analysis were done as described in Gamboa-Delgado *et al*. (2014).

Experimental design and rearing system

Shrimps having an initial mean wet weight of 110 ± 36 mg were distributed in ten, 60 L capacity tanks. Twenty animals were placed in duplicate tanks and were previously selected in order to distribute animals having the same size distribution pattern in each unit. Tanks were individually fitted with airlifts and the whole tank array was interconnected to a recirculation system holding artificial seawater (Fritz, Chemical Co., USA). Seawater was exchanged in every tank at a rate of 800% d^{-1} and it was treated by mechanical, biological and UV filtration. The feed was supplied at apparent satiety at 8:00, 12:00, 16:00 and 20:00 h for 22 days. Before daily feeding, uneaten feed, feces, and molts were siphoned out. Tank walls were periodically scrubbed off to avoid any possible biofilm growth contributing additional nutrients. The experimental time period and sampling points to collect shrimp samples for isotopic analysis were defined according to the exponential rate of isotopic change previously observed in experiments using small-sized penaeid shrimp (Gamboa-Delgado *et al*., 2014; Martínez-Rocha *et al*., 2013). As an estimate of growth (*k*) is required for the exponential model of isotopic change, the individual wet weight of five animals per replicate was determined on experimental days 0, 4, 8 and 15 using a digital balance. Shrimps were captured with nets and weighed after blotting off excess water with a moist cloth. For isotopic analysis, one or two shrimps (depending on weight) were randomly sub-sampled from each collected batch. Animals were starved overnight to allow clearing of gut contents. Animals were sacrificed in ice/water slurry, rinsed with distilled water and stored in Eppendorf tubes at -80°C until sample pretreatment. At the end of the experiment (day 22), all the remaining animals were treated as previously described.

Sample pretreatment and stable isotope analyses

Samples of whole shrimp bodies and experimental diets were dehydrated at 50°C until constant weight. Samples were manually ground using mortar and pestle to obtain a fine powder. Diet and shrimp samples of 900 to 1100 µg were packed in tin cups (Elemental Microanalysis Ltd., UK). Samples were analyzed at the Stable Isotope Facility of the Department of Plant Sciences, University of California, (Davis, CA, USA) using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., UK). Repeated measurements of calibration standards indicated that instrument precision (SD) was 0.17‰ for $\delta^{15}N$ values. Isotopic results are expressed in delta notation (δ), which is defined as part per thousand (‰) deviations from the $\delta^{15}N$ value of the isotopic standard reference material (atmospheric nitrogen). The term "discrimination factor" ($\Delta^{15}N$) is employed here to denote isotopic differences between a consuming organism and its diet after having reached isotopic equilibrium (a small final isotopic fluctuation of 1‰ between diet and animal). $\Delta^{15}N$ values between shrimps and microalgae were estimated from an average value taken from the literature (3.4‰; Minagawa & Wada, 1984). Such value was used to correct for $\Delta^{15}N$ values when estimating nutritional contributions from microalgal biomass and fishmeal.

Estimation of nutrient contribution and nitrogen residency times

A two-source, one-isotope mixing model (Phillips & Gregg, 2001) was applied to estimate the relative contribution of dietary nitrogen and total dry matter supplied by FM and biomass of SC and GR to the growth of shrimps under the different dietary treatments. The model considers the isotopic differences between the sources (in this particular study represented by the isotopic values of FM, SC and GR ($\delta^{15}N$ = 16.6, -3.8, and -0.8‰, respectively) and the mixture (shrimp whole bodies). Required assumptions of the model such as isotopic equilibrium and knowledge of the elemental concentration (N) of ingredients were met or taken into consideration in interpreting the results (Gannes *et al*., 1997; Post, 2002). Preliminary analysis indicated that elemental contents in FM (N = 10.5%), SC (N = 2.4%) and GR (N = 2.1%) were significantly different. As elemental values are not considered by the mixing model, a correction (Eq. 1) was applied to obtain estimates of the relative contribution of dry matter to growth (Fry, 2006).

$$f_{total1} = f_1 \times W_2/(f_1 \times W_2 + f_2 \times W_1) \text{ and } \quad f_{total2} = 1\text{-}f_{total1} \qquad (1)$$

where f_{total1}= is the total percent contribution of source 1 in a two-source mixing model,

$f_1 = (\delta^{15}N_{sample} - \delta^{15}N_{source2})/(\delta^{15}N_{source1} - \delta^{15}N_{source2})$ and $f_2 = 1-f_1$

where $\delta^{15}N$ is the nitrogen isotopic value of diets/ingredients and consumer, superscripts indicate the heavy isotope mass (N) and W_1 and W_2 represent the elemental content in each of the two sources.

$\delta^{15}N$ values were determined at different times of the experimental period and these values were introduced into an exponential model of isotopic change (Hesslein *et al*., 1993) as described in Gamboa-Delgado *et al*. (2014). The model allows obtaining a quantitative coefficient that allows distinguishing the isotopic change that is due to growth (*k*) and/or metabolic turnover (*m*). Coefficients *k* and *m* provide an indicator of the residency time, the time period necessary for half of the body nitrogen be replaced after animals consume a new diet (halftime, t_{50}) (MacAvoy *et al*., 2006)

$$t_{50} = In2 / m+k \quad (2)$$

Estimation of isotopic discrimination factors ($\Delta^{15}N$) increases the accuracy of the estimated dietary contributions by integrating correction factors into the mixing model (Martínez del Río *et al*. 2009; Phillips, 2012). However, in the present study, measured isotope values were corrected for discrimination factors only for FM as no isotopic control diets were prepared using microalgal biomass.

Statistical analysis

Student's *t*-tests were used to compare nitrogen contents and $\delta^{15}N$ values in FM and biomass from both microalgae. $\delta^{15}N$ values in whole bodies at different times, mean shrimp final weight and survival were analyzed by Kruskal-Wallis tests followed by pair comparisons by Mann-Whitney tests. In order to detect statistical differences between the expected proportions of dietary nitrogen (FM and microalgal biomass) and the observed proportions of dietary nitrogen incorporated in whole shrimp bodies, Chi-square goodness of fit tests (χ^2) were applied. Parameters required by the Hesslein model (Hesslein *et al*., 1993) were estimated by iterative non-linear regression. All tests were conducted using SPSS 17.0 software (SPSS Inc. USA) at a significance level of $P < 0.05$.

RESULTS

Shrimp growth and survival

Throughout the feeding trial, water quality parameters varied slightly in relation to recommended optimal values for this species (Wyban & Sweeny, 1991). Over the second week of the trial, there was a temporary increase in nitrogenous compounds in the seawater, which affected the overall final survival rates (72 ± 2%). As the water quality is simultaneously affected in the tank array, there were no statistical differences in survival among treatments. Animals readily accepted the experimental feeds and at the end of the experiment, shrimps reared under the five dietary treatments showed significant differences in mean final weights, although a relatively high variability was observed (Table 3). Shrimp fed diet 95FM/5SC showed significantly higher mean final weight than animals fed on the control diet (100 FM). The other three mixed diets promoted similar growth gains as those elicited by the control diet. The amino acid profile of the ingredients indicated that both microalgae contained higher contents of the essential amino acids, threonine, valine, leucine, and phenylalanine than FM.

Isotopic influence of diets on shrimp bodies

Isotopic values determined in the microalgal biomass of *Schizochytrium* ($\delta^{15}N$ = -3.8 ± 0.2‰) and *Grammatophora* sp. ($\delta^{15}N$ = -0.8 ± 0.3‰) were significantly different to the isotopic values of fish meal ($\delta^{15}N$ = 16.6 ± 0.4‰). These significant differences allowed formulating diets having ingredients with isotopically contrasting values that in turn elicited different isotopic changes in shrimps treatments (Table 4). All experimental diets exerted a rapid influence on the $\delta^{15}N$ values of shrimp tissue and by day 22, animals in all treatments had reached or approached isotopic equilibrium with their respective dietary treatments. Although the general trend of isotopic change was narrow due to the influence of dietary FM, different isotopic values were still reflected in shrimp bodies. This was due to the different levels of microalgal biomass included in the diets, which in turn allowed assessing dietary nitrogen and total dry matter contributions.

Nitrogen half times in tissue

Nitrogen isotopic shifts followed an expected pattern characterized by an exponential trend of changing iso-

Table 3. Final mean wet weight (FW), weight gain (WG), specific growth rate (SGR) and survival rate (S) of Pacific white shrimp *P. vannamei* reared under diets supplemented with biomass of microalgae *Schizochytrium* sp. (SC) and *Grammatophora* sp. (GR). Mean values ± SD, Different superscripts indicate significant differences for that particular column.

Diet	FW (mg)	WG (%)	SGR	S (%)
100FM	381 ± 120^b	246^b	5.58	72.7^a
95FM/5SC	575 ± 251^a	422^a	7.47	72.7^a
90FM/10SC	402 ± 244^b	266^b	6.06	70.0^a
95FM/5GR	310 ± 189^b	181^b	4.73	70.0^a
90FM/10GR	321 ± 146^b	192^b	4.97	72.7^a

Table 4. Changes in nitrogen stable isotope values (‰) in muscle tissue of white shrimp *P. vannamei* reared on experimental diets containing fish meal and low inclusions (5 and 10%) of the microalgal biomass of *Schizochytrium* sp. (SC) and *Grammatophora* sp. (GR). Corrected isotopic values ($\delta^{15}N$) of ingredients are FM = 16.5‰, SC = -0.2‰, GR = 3.2‰, n = 2 individuals, 10 on final point.

Day	Diet				
	100FM	95FM/5SC	90FM/10SC	95FM/5GR	90FM/10GR
0	9.92 ± 0.44	9.92 ± 0.44	9.92 ± 0.44	9.92 ± 0.44	9.92 ± 0.44
4	10.81 ± 0.34	11.44 ± 0.35	11.84 ± 0.53	12.55 ± 0.23	11.15 ± 1.32
8	12.57 ± 0.42	13.98 ± 0.33	12.97 ± 1.14	12.76 ± 0.12	12.54 ± 1.46
15	15.14 ± 1.01	15.55 ± 0.12	14.46 ± 0.64	14.48 ± 0.83	14.12 ± 1.18
22	16.43 ± 0.36	16.41 ± 0.51	15.33 ± 0.91	15.67 ± 0.82	15.27 ± 0.47

topic values in time. For most treatments, predicted isotopic values fitted well on the observed data and from these data, parameter *m* (metabolic turnover) was estimated by means of iterative non-linear regression. Although isotopic differences between the conditioning diet and the experimental diets were significant, it was not possible to estimate the nitrogen half times for all diets because after applying corrections for isotopic discrimination factors, isotopic differences between shrimp and diets were narrowed down. Parameters *m* and *k* indicated that estimated nitrogen half times in tissue ranged from 4.3 days in shrimp fed diet 90FM/10SC to 5.3 days in shrimp fed on diet 95FM/5SC (Table 5).

Nutritional contributions of *Schizochytrium* and *Grammatophora* biomass

The estimated isotopic discrimination factor between control shrimps and FM was very small ($\Delta^{15}N$ = -0.10‰). The inclusion of asymptotic values (Table 4) into the isotopic mixing model indicated that the contributions of dietary nitrogen from FM, SC, and GR to the growth of shrimps were statistically similar to the expected contributions indicated by the respective proportions of dietary nitrogen established in the dietary formulations (Tables 2, 6).

For example, diet 95FM/5SC contained respective proportions of 95 and 5% of nitrogen from FM and SC, and after the feeding trial, an estimated 98% of nitrogen was derived from FM. However, after corrections were applied to adjust for different nitrogen contents in the ingredients, it was found that observed allocated proportions of dry matter were significantly different to the dietary proportions supplied by ingredients. Diets containing 5 and 10% of *Schizochytrium* sp. contributed 6.6 and 24.6 of dry matter to growth. On the other hand, diets containing 5 and 10% of *Grammatophora* sp. supplied 23.5 and 30.3% of dry matter to growth, respectively. Higher proportions of dry matter were derived from FM than from the microalgal biomass.

Table 5. Mean growth rates (*k*) and estimated half times (t_{50}) of nitrogen in whole bodies of Pacific white shrimp *P. vannamei* reared on diets containing fish meal (FM) and microalgal biomass of *Schizochytrium* sp. (SC) and *Grammatophora* sp. (GR). Mean values ± SD Different superscripts indicate significant differences.

Diet	*k* (d^{-1})	*m* (d^{-1})	half-life (d)	r^2
100FM	0.056 ± 0.009^a	0.080	5.1	96
95FM/5SC	0.075 ± 0.011^b	0.057	5.3	95
90FM/10SC	0.059 ± 0.006^a	0.102	4.3	96
95FM/5GR	0.047 ± 0.010^a	0.101	4.7	97
90FM/10GR	0.049 ± 0.014^a	0.093	4.9	94

DISCUSSION

It has been reported that a dietary protein level above 32% is optimal for early juveniles of this species (Kureshy & Davis, 2002); therefore, it is assumed that the dietary formulations applied in the present study were nutritionally suitable. Although both sources of microalgal biomass contained low protein content, their low dietary inclusions levels did not affect the overall protein content of diets. Likewise, the low dietary inclusion of microalgal biomass had a minimal effect on the amino acid profile of diets in comparison to a diet containing only FM. The slight mortality observed early in the bioassay was attributed to an increase of nitrogenous compounds in the seawater of the tank array; however, this influence simultaneously affected all the experimental rearing tanks, as these were interconnected. Over the experimental period, shrimps increased their body weight from 2 to 4-fold. The observed growth rate, in conjunction with the nitrogen turnover rates elicited by the different diets, was sufficient for the dietary $\delta^{15}N$ values to be reflected in shrimp bodies and approach isotopic equilibrium. The-

Table 6. Estimated relative proportions of dietary nitrogen and total dry matter supplied from the fish meal (FM) and dry biomass of *Schizochytrium* sp. (SC) and *Grammatophora* sp. (GR) contributing to the growth of shrimp *P. vannamei* (mean ± CI, n = 10). Superscripts indicate significant differences between expected and mean observed dietary contributions. *Total dry matter contributions were estimated after correcting for nitrogen concentrations measured in ingredients using the equation proposed by Fry (2006).

		Observed in shrimp bodies		
Diet	Expected	Min.	Mean	Max.
Nitrogen				
95FM/5SC				
FM	95a	96.8	98.4a	100.0
SC	5	0	1.6	3.2
90FM/10SC				
FM	90a	90.5	93.0a	95.5
SC	10	4.5	7.0	9.5
95FM/5GR				
FM	95a	92.0	94.2a	96.4
GR	5	3.6	5.8	8.0
90FM/10GR				
FM	90a	89.0	91.9a	94.8
GR	10	5.2	8.1	11.0
Dry matter				
95FM/5SC				
FM	73.5a	91.7	93.4b	95.1
SC	25.5	4.9	6.6	8.3
90FM/10SC				
FM	56.9a	72.9	75.4b	77.9
SC	43.1	22.1	24.6	27.1
95FM/5GR				
FM	68.4a	74.3	76.5b	78.7
GR	31.6	21.3	23.5	25.7
90F/10GR				
FM	50.7a	66.8	69.7b	72.6
GR	49.3	27.4	30.3	33.2

se growth rates are in agreement with other studies conducted on postlarval or juvenile *P. vannamei* (Martínez-Rocha *et al*., 2013). In the present study, it was observed that addition of 5 and 10% of the microalgal biomass of both species elicited similar or higher growth as the use of FM alone. Glencross *et al.* (2014) recently reported that even small dietary levels of microbial biomass (in this case bacterial) frequently cause positive results in terms of shrimp growth and survival. Authors consider that the microbial biomass contains molecules that facilitate the physiological use of other nutrients. Results from different studies on the use of microalgal biomass as an ingredient in aquaculture diets indicate far more positive than negative effects on parameters such as growth, survival, pigmentation and immune response of aquatic organisms (Gamboa-Delgado & Márquez-Reyes, 2016). For example, a recent study reported that by using microalgal biomass derived from *Haematococcus pluvialis* (2 to 14% inclusion) and *Schizochytrium limacinum* (5% inclusion) in diets for longfin yellowtail *Seriola rivoliana* the growth rate and feed conversion ratios were maintained as those observed in control diets (Kissinger *et al*., 2016). In a similar experiment, García-Ortega *et al*. (2016) also used microalgal biomass of *S. limacinum* and soy products to replace 20, 40 and 80% of animal-derived protein sources in diets for giant grouper *Epinephelus lanceolatus*. Incorporation of this high lipid-yielding microalga allowed for complete fish oil replacement in diets containing 40 and 80% replacement levels.

The isotopic values (uncorrected for isotopic discrimination factors) of the constituent nitrogen of both microalgae types were negative (-0.8 and -3.8‰). Negative or low isotopic values indicate a relative depletion of heavy nitrogen (^{15}N) in a particular substrate. This observation denotes a strong isotopic influence of the fertilizers used to upscale the respec-

tive cultures. $\delta^{15}N$ values in microalgal biomass were very contrasting to that determined in FM, which is a positive characteristic as the isotopic mixing models increase their resolution when nutrient contributions are estimated. In a similar way, it has been reported that the isotopic values of carbon ($\delta^{13}C$ values) in microalgae can be manipulated by imposing specific culture conditions (Gamboa-Delgado *et al*., 2008). The isotopic values of the experimental diets were rapidly reflected in shrimp bodies. Given that relatively high growth rates were observed, it is assumed that shrimps approached isotopic equilibrium mainly through tissue accretion (*k*) instead of metabolic turnover (*m*). The opposite situation is observed in sub-adults, adults or slow-growing individuals (*i.e.*, metabolic turnover rate being the main driver of isotopic change [Hesslein *et al*., 1993; Martínez-Rocha *et al*., 2013]). Estimated half times (t_{50}) of nitrogen in shrimp bodies ranged from 4.3 to 5.3 days and differences were attributed to diet type. Diets containing either type of microalgae biomass elicited different responses in the half times in tissue. Diet 90FM/10SC caused longer nitrogen half times in tissue, while diet 95FM/5SC caused shorter t_{50} values in shrimp whole bodies. The latter observation was probably associated with the higher growth rates observed under this feeding regime.

The introduction of corrected isotopic values into the mixing model, in conjunction with the isotopic values of shrimps, allowed estimating the relative proportional contributions of the dietary nitrogen and total dry matter to growth. The nutritional contributions of dietary nitrogen supplied by FM and both microalgal biomass types were statistically similar to the available dietary proportions. Estimated dietary nitrogen contributions from *Schizochytrium* sp. and *Grammatophora* sp. to shrimp growth were relatively low but consistent with the amounts of nitrogen available in the respective compound diets. However, given that $\Delta^{15}N$ values were estimated from literature values, the confidence intervals for the contributions of microalgal biomass to growth might be wider than those calculated. Similar contributions of dietary nitrogen are not always observed in studies applying isotopic methodologies. In a previous experiment exploring dietary nitrogen contributions from other microbial sources to shrimp, significantly lower contributions of nitrogen derived from yeast (*Candida utilis*) were observed, while those derived from FM were significantly higher (Gamboa-Delgado *et al*., 2016). In the present study, it is thus reasonable to indicate that, from the isotopic evidence, the biomass of *Schizochytrium* and *Grammatophora* is supplying structural nitrogen through their constituent amino acids. Future studies might confirm this supposition as the isotopic techniques have the potential to elucidate not only the transference of dietary nitrogen or carbon but also the transference of specific compounds such as amino acids and fatty acids (Fantle *et al*., 1999; Parrish *et al*., 2007). After correcting for nitrogen content, dry matter contributions from the microalgal biomass increased but were not as high as contributions supplied by FM. It is important to consider that the higher growth rate elicited by diet 95FM/5SC cannot be attributed only to the dietary nitrogen supplied by SC, but also to a potential effect of the microalgae promoting the efficient use of other nutrients supplied by FM. The latter effect has been proposed by Glencross *et al*. (2014) after using low levels of microbial biomass in aquaculture diets. Diet 90FM/10GR supplied higher amounts of dietary nitrogen than the other mixed diets although in similar proportions than those established in the formulated diets. In the present study, the observed contributions of nutrients to shrimp somatic growth and the treatment-dependent weight gain suggest that the dietary addition of 5 and 10% of microalgal biomass, promotes similar growth and survival rates as those observed in shrimps fed only on fishmeal-based diets. The isotopic techniques can yield valuable information on the rates of incorporation of specific, experimental dietary sources and help elucidate their physiological use by marine organisms. Results from the present study also highlight the nutritional benefits that microalgal biomass confers to juvenile shrimp when supplied at low dietary levels.

CONCLUSIONS

In conclusion, the biomass obtained from endemic microalgae *Schizochytrium* sp. and *Grammatophora* sp. constituted a good dietary supplement as results indicate that dietary inclusions as low as 5% promote similar or higher shrimp growth than a control diet containing only fish meal. Isotopic determinations confirmed that the microalgal biomass yields nutrients that are allocated to the growing tissue of shrimps. It is forecast that the biomass or nutrients derived from different microalgal species will be increasingly used as supplements and fish meal replacements in animal feed and aquaculture diets.

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REFERENCES

Association of Official Analytical Chemist (AOAC). 2006. Official Methods of Analysis of AOAC International. Method 982.30 E (a,b,c), chp. 45.3.05.

Association of the Official Analytical Chemist. USA, AOAC International, Gaithersburg, Maryland.

Barclay, W. & S. Zeller. 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia* nauplii by feeding spray-dried *Schizochytrium* sp. J. World Aquacult. Soc., 27: 314-322.

Becker, E.W. 2007. Micro-algae as a source of protein. Biotechnol. Adv., 25: 207-210.

Fantle, M.S., A.I. Dittel, S. Schwalm, C.E. Epifanio & M.L. Fogel. 1999. A food web analysis of the juvenile crab *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia, 120: 416-426.

Fry, B. 2006. Stable isotope ecology. Springer Science, New York, 390 pp.

Gamboa-Delgado, J. & J.M. Márquez-Reyes. 2016. Potential of microbial-derived nutrients for aquaculture development. Rev. Aquacult. (in press). doi: 10.1111/raq.12157.

Gamboa-Delgado, J., J.P. Cañavate, R. Zerolo & L. Le Vay. 2008. Natural carbon stable isotope ratios as indicators of the relative contribution of live and inert diets to growth in larval Senegalese sole (*Solea senegalensis*). Aquaculture, 280: 190-197.

Gamboa-Delgado, J., J.D. Castañeda-Solís, M.G. Nieto-López & L.E. Cruz-Suárez. 2014. Isotopic evaluation of the nutritional contribution of poultry by-product meal and fish meal to the growth of white shrimp, *Litopenaeus vannamei*. J. World Aquacult. Soc., 45: 430-438.

Gamboa-Delgado, J., B. Fernández-Díaz, M.G. Nieto-López & L.E. Cruz-Suárez. 2016. Nutritional contribution of torula yeast and fish meal to the growth of shrimp *Litopenaeus vannamei* as indicated by natural nitrogen stable isotopes. Aquaculture, 453: 116-121.

Gannes, L.Z., D.M. O'Brien & C. Martínez del Río. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology, 78: 1271-1276.

García-Ortega, A., T.J. Trushenski & K. Kissinger. 2016. Evaluation of fish meal and fish oil replacement by soybean protein and algal meal from *Schizochytrium limacinum* in diets for giant grouper *Epinephelus lanceolatus*. Aquaculture, 452: 1-8.

Glencross, B., S. Irvin, S. Arnold, D. Blyth, N. Bourne & N. Preston. 2014. Effective use of microbial biomass products to facilitate the complete replacement of fishery resources in diets for the black tiger shrimp *Penaeus monodon*. Aquaculture, 431: 12-19.

Guillard, R.R.L. 1975. The culture of phytoplankton for feeding marine invertebrates. In: W.L. Smith & M.H. Chantey (eds.). The culture of marine invertebrate animals. Plenum Publishers, New York, pp. 29-60.

Hesslein, R.H., K.A. Hallard & P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in the tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by ^{34}S, ^{13}C and ^{15}N. Can. J. Fish. Aquat. Sci., 50: 2071-2076.

Kissinger, K.R., A. García-Ortega & J.T. Trushenski. 2016. Partial fish meal replacement by soy protein concentrate, squid and algal meals in low fish-oil diets containing *Schizochytrium limacinum* for longfin yellowtail *Seriola rivoliana*. Aquaculture, 452: 37-44.

Kureshy, N. & D.A. Davis. 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 204: 125-143.

Li, Y., G. Xiao, A. Mangott, M. Kent & I. Pirozzi. 2016. Nutrient efficacy of microalgae as aquafeed additives for the adult black tiger prawn, *Penaeus monodon*. Aquacult. Res., 47: 3625-3635.

Li, M., H.E.H. Robinson, C.S. Tucker, B.B. Manning & L. Khoo. 2009. Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. Aquaculture, 292: 232-236.

MacAvoy, S.E., L.S. Arneson & E. Bassett. 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. Oecologia, 150: 190-201.

Martínez-Rocha, L., J. Gamboa-Delgado, M.G. Nieto-López, D. Ricque-Marie & L.E. Cruz-Suárez. 2013. Incorporation of dietary nitrogen from fishmeal and pea meal (*Pisum sativum*) in muscle tissue of Pacific white shrimp (*Litopenaeus vannamei*) fed low protein compound diets. Aquacult. Res., 44: 847-859.

Martínez del Río, C., N. Wolf, S.A. Carleton & L.Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev., 84: 91-111.

Minagawa, M. & E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta, 48: 1135-1140.

Pacheco-Vega, J.M., M.A. Cadena-Roa, F. Ascencio, C. Rangel-Dávalos & M. Rojas-Contreras. 2015. Assessment of endemic microalgae as potential food for *Artemia franciscana* culture. Lat. Am. J. Aquat. Res., 43(1): 23-32.

Parrish, C.C., M. Whiticar & V. Puvanendran. 2007. Is *w*6 docosapentaenoic acid an essential fatty acid during early ontogeny in marine fauna? Limnol. Oceanogr., 52: 476-479.

Phillips, D.L. 2012. Converting isotope values to diet composition: the use of mixing models. J. Mammal., 93: 342-352.

Phillips, D.L. & P.L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia, 130: 114-125.

Phillips, D.L. & J.W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia, 127: 171-179 (see also erratum, Oecologia, 128: 204).

Pruder, G.D. & E.T. Bolton. 1978. System configuration and performance: bivalve molluscan mariculture. Proceedings of the 9th Annual Meeting World Mariculture Society. Louisiana, pp. 747-759.

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Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, 83: 703-718.

Teimouri, M.A., K. Amirkolaie & S. Yeganeh. 2013. The effects of *Spirulina platensis* meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 396-399: 14-19.

Wyban, J.A. & J.N. Sweeney. 1991. The Oceanic Institute shrimp manual: intensive shrimp production technology. The Oceanic Institute, Hawaii, 158 pp.