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The stable isotope of nitrogen in an experimental culture of *Ulva* spp. and its assimilation in the nutrition of white shrimp *Litopenaeus vannamei*, Baja California Sur, Mexico

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Abstract Stable nitrogen isotope ratios have been used to study the incorporation of nitrogen into the food webs of marine systems. Some species of algae can be cocultured with shrimp, resulting in a sustainable alternative to reduce or eliminate the use of commercial food. One option is the development of *Ulva* spp. in open-air ponds under a rigorous control of water quality. Recently, the coculture of *Ulva* spp. and juvenile shrimp (in aquaria and open-air ponds) has shown, under stereomicroscope observation, that the crustaceans were feeding on the *Ulva* spp. The consumption of commercial food and *Ulva* spp. by juvenile shrimp has been evaluated to establish the uptake of nitrogen into tissues of this crustacean. The muscle tissue of juvenile shrimp initially assimilated nitrogen from commercial feed and later the cocultured shrimp assimilated nitrogen from the *Ulva* spp., which demonstrated the potential application of live and fresh diets and the optimization of their use in diets containing very low levels or no commercial food.

Keywords Stable nitrogen isotope · Seaweeds · Shrimp · *Ulva* spp. · Commercial food

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Introduction

Shrimp farming in Mexico has grown 82% in the last decade with increased production from 10,000 to 60,000 tons annually, which is produced on 350 farms in a growing area of 26,000 hectares. The use of balanced commercial food traditionally used in shrimp farming has been changing to increase the efficiency of the crop (e.g., Cruz-Suárez et al. 2008; Aguíñiga-García et al. 2011).

Recently, the Centro Interdisciplinario de Ciencias Marinas Instituto Politécnico Nacional (CICIMAR–IPN) has made studies that show the nutritional value of diets enriched by *Sargassum* spp. (Casas-Valdez et al. 2006) and *Ulva* spp. (Sánchez-Rodríguez et al. submitted for publication) by feeding shrimp under experimental laboratory conditions. These conditions of light, photoperiod, temperature, oxygen, nutrients, and others may enhance shrimp growth and health, although the nutritional benefits that the consumption of fresh macroalgae can bring to shrimp have not been reported. Some studies have shown that the consumption of fresh green seaweed caused maturation of *Penaeus indicus* (Emerson 1980) and light wavelength (Emerson et al. 1983) as well as decreased lipid content in the shrimp carcass and modified the fatty acid profile of the body with a much higher DHA content in shrimp under coculture conditions (Cruz-Suárez et al. 2010).

The carotenoid content in the body of the shrimp was significantly greater in the coculture groups, suggesting that *Ulva* carotenoids were efficiently assimilated and metabolized and also may be involved in growth enhancement. Quantitative analyses of carotenoids from whole shrimp body showed that *Ulva* consumption increased significantly the total carotenoids concentration, resulting in a higher pigmentation of shrimp. Astaxanthin was found as the major carotenoid constituent (76–89% of total carotenoids) in shrimp (Cruz-Suárez et al. 2010). Additionally, some seaweed can provide specific environmental conditions in

shrimp ponds (Porchas-Cornejo et al. 1999), such as water quality improvement caused by nutrient uptake (Hamano et al. 2007; Copertino et al. 2009), physical filtration (Paul and de Nys 2008), and a protective substratum for shrimp.

The diets used can be systematically assessed the assimilation of the ingested and digested food. The types of diets and shrimp tissues have been evaluated based on their chemical composition and biochemical and microbiological assays (e.g., Aguilera-Morales et al. 2005; Cruz-Suárez et al. 2008, 2010). The chemical composition of seaweed (Ito and Hori 1989) may vary as a function of the species, habitat, environmental conditions, and seasons (Marinho-Soriano et al. 2006). In general, green seaweed may have two or three times more protein content than brown seaweed (Burtin 2003). Polyunsaturated fatty acids can compose up to 66% of the total fat of *Ulva lactuca* (Wahbeh 1997). For *Enteromorpha* spp., Aguilera-Morales et al. (2005) reported values of 6.9–9.1% for linoleic acid, 3.5–6.4% for linolenic acid, and 2.8–5.7% for eicosapentaenoic acid. The carotenoids in *Ulva clathrata* meal proved to be good pigmentation precursors for the white shrimp *Litopenaeus vannamei* (Cruz-Suárez et al. 2009), with lutein being the main carotenoid in this alga.

In the exploration of new sustainable alternatives to reduce the use of commercial food to improve shrimp production with lower costs, the green seaweed *Ulva* spp. have been cultured in open-air shrimp ponds recently at the CICIMAR–IPN. Our study was made to evaluate the incorporation of nitrogen in the white shrimp *L. vannamei* feed with the green seaweed *Ulva* spp. and *Ulva* meal in experimental open-air ponds.

Materials and methods

The juvenile shrimp were initially fed with pellets enriched with 4% *Sargassum* spp. and 4% *Ulva* spp. for 30 days. Subsequently, the juvenile shrimp that were fed with 4% *Sargassum* were cocultured in two open-air ponds (8 m^2) containing the green algae *Ulva* spp. for 45 days. The juvenile shrimp fed with *Ulva* pellets were fed with *Ulva* meal for 120 days.

The nitrogen isotopic composition was determined for each of the commercial foods (*Sargassum* and *Ulva* pellets), *Ulva* meal, *Ulva* spp., and the shrimp that were fed each of the different foods. The samples were dried in an oven at 50°C and encapsulated for the analysis of the stable isotopes of N. The analyses were done in an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS) at the CICIMAR–IPN. For every 40 samples, ten validation samples were run, and reported values were defined by the equation:

$$\delta^{15}N(‰) = (({}^{15}N/{}^{14}N_{sample}/{}^{15}N/{}^{14}N_{reference})\text{-}1) \times 1000$$

The analytical precision of isotopic measurements was <0.1‰ for $\delta^{15}N$ determined in primary standards.

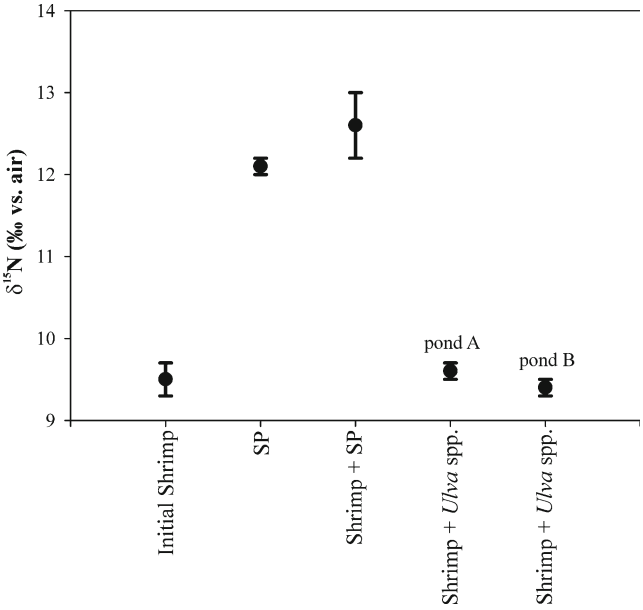

Fig. 1 Nitrogen isotopic composition (vs. air) of the muscle tissue of shrimp before (initial shrimp) and after feeding *Sargassum* pellets (SP), and after coculture in ponds A and B with *Ulva* spp

Results

The isotopic composition of nitrogen in the *Sargassum* pellets and *Ulva* pellets was 12.1±0.1‰ and 12.5±0.4‰, respectively. The *Ulva* meal had a composition of $\delta^{15}N$ of 14±0.1‰. The *Ulva* spp. in ponds A and B had $\delta^{15}N$ values of 3.3±0.1‰ and 3.6±0.3‰. The shrimp had an isotopic composition of nitrogen of 9.5±0.2‰. The shrimp fed with *Sargassum* pellets ($\delta^{15}N$=12.1±0.1‰) showed, after of 30 days of diet, an isotopic composition of 12.6±0.4‰. The shrimp fed with pellets of *Sargassum* were cocultured with *Ulva* spp. ($\delta^{15}N$=3.5±0.2‰) for 45 days in two open-air ponds (A and B), and the values of $\delta^{15}N$ were (A) 9.6± 0.1‰ and (B) 9.4±0.1‰ (Fig. 1). The shrimp fed with *Ulva* pellets ($\delta^{15}N$=12.5±0.4‰) had a composition of $\delta^{15}N$ of 13±0.4‰. The shrimp fed with *Ulva* meal ($\delta^{15}N$=14.0± 0.1‰) had values of $\delta^{15}N$ of 13.9‰ (Fig. 2).

Discussion

Stable nitrogen–isotope ratios have been used to study the food webs of marine systems (e.g., Peterson and Fry 1987). These studies characterize the stable isotopes of nitrogen in various types of food used in shrimp aquaculture in Mexico. Development in open-air ponds of these small organisms with quick growth assumed that the main change in the isotopic composition is simply a dilution of the original nitrogen. That is, the nitrogen of shrimp will be diluted, as it assimilates the new nitrogen supply. The shrimp should

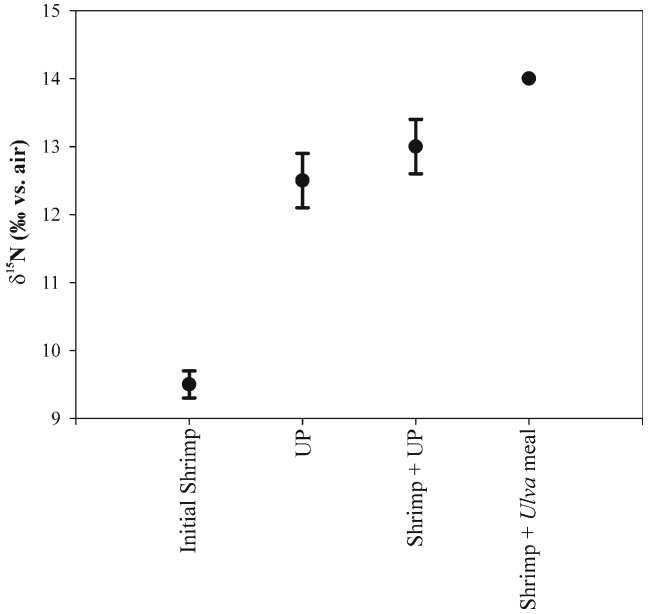

Fig. 2 Nitrogen isotopic composition (vs. air) of the muscle tissue of shrimp before (initial shrimp) and after feeding *Ulva* pellets (UP), and subsequently, to feeding *Ulva* meal

reflect the value of the predominant nitrogen isotope in the food source.

D'Avanzo et al. (1991) created ^{15}N-labeled aggregates from the leachate of four macrophytes, a marsh grass, and three macroalgae common in New England coastal waters. These authors fed the labeled aggregates to two coastal consumers, the grass shrimp *Palaemonetes pugio* and the sheepshead minnow *Cyprinodon variegatus*. Fish and shrimp fed each of the labeled aggregates became labeled with ^{15}N. The result provides direct evidence for nitrogen assimilation from amorphous detritus by marine consumers. In addition, fish fed amorphous marsh grass detritus assimilated 10–40 times more nitrogen from this detritus than from morphous grass detritus. Therefore, amorphous aggregates may be higher-quality food than morphous detrital fragments for coastal consumers.

The ^{15}N-enriched diatoms and *Artemia* were each incorporated into a standard compound pelleted diet. The pellets were fed to juvenile *Penaeus monodon* in the laboratory and in enclosures at a local prawn farm. Analysis of the percentage ^{15}N in the abdominal muscle of juvenile prawns demonstrated rapid assimilation of the enriched isotope from diatoms and *Artemia*. There was a progressive increase in the percentage ^{15}N in the abdominal muscle during the 4–week experiment. The results demonstrated that enriched stable isotope tracers can provide rapid and direct information about the assimilation and retention of nitrogen from individual protein sources in compound diets for shrimp (Preston et al. 1996).

The δ^{15}N in animal or plant tissue reflects the isotopic ratios of their diets and the enrichment factor caused by the different isotopes of an element being incorporated into the tissues at different rates (Martínez del Rio et al. 2009). The discriminating factor is the result of a selective excretion of ^{14}N as the ^{15}N is enriched in each metabolic step (Minagawa and Wada 1984). In animal and plant nutrition, the relationships between the N isotopic discrimination and other factors (supply and quality of dietary protein) are still poorly understood (Robbins et al. 2005). Although it is accepted that the isotopic discrimination factor varies 3‰ in the δ^{15}N at each trophic level, an increasing number of studies show that the isotopic discrimination factors are specific to different species and tissues (DeNiro and Epstein 1981; Yokoyama et al. 2005; Stenroth et al. 2006), and these can also vary during the ontogeny of organisms because of metabolic changes (Hentschel 1998; Rossi et al. 2004), which can even cause a negative discrimination factor.

Juveniles of ghost shrimps *Nihonotrypaea japonica* and *Nihonotrypaea harmandi* were reared on a microalga of a constant isotopic value to quantify their diet tissue isotopic fractionation. Examinations of whole body, muscle, and exoskeleton of the ghost shrimps showed ranges in ^{15}N fractionations for muscles (3.6–4.0‰), only slight effects of acid treatment on ^{15}N fractionation for muscles (≤0.3‰ differences), and 2.3–3.0‰ of ^{15}N fractionation for whole bodies, which were smaller than for muscles due to negative fractionation for exoskeletons (-3.0 to -1.9‰). Although ^{15}N fractionation for ghost shrimp muscle was within the above mentioned accepted range, the highlights are that fractionation is species- and tissue-specific and that the accepted fractionation values may not be universally applicable (Yokoyama et al. 2005). However, there has been some debate on the use of different tissues and treatments before isotope analysis as well as on seasonal effects. These tissues have been different isotope values because come from different sources. For example, adopting this view for a pond food web could render crayfish both predators and detritivores as crayfish prey on nitrogen sources (other invertebrates) and consume large amounts of detritus to satisfy their carbon demand (Stenroth et al. 2006).

The turnover rates of nutrients can be estimated by monitoring the isotopic changes in various tissues and are important in determining how quickly the isotopic profile of a diet is incorporated into the tissue (Pearson et al. 2003). The resolution to estimate rates of metabolic turnover increases when the isotopic differences of the tissue between initial value and that after use of the diet are contrasting (Fry and Arnold 1982; Burford et al. 2002; Gamboa-Delgado 2010).

The average nitrogen isotopic composition of the muscle tissue of white shrimp in the wild was 9.5‰. For 30 days, shrimp were fed *Sargassum* pellets (12.1±0.4‰) and *Ulva* (12.5±0.4‰). The muscle tissue of shrimp acquired an

isotopic composition of 12.6±0.4‰ and 13±0.4‰, similar to the pellets. The change of food suggests that at 30 days, the muscle tissue of shrimp assimilated the isotopic nitrogen composition of commercial feed. Fry and Arnold (1982) and Al-Maslamani (2006) noted that the penaeid shrimp muscle tissue has a rapid isotopic equilibrium between diets and tissues of the shrimp in 15–20 days. Contrasting isotopic values ($\delta^{15}N$) of the fishmeal and soy protein allowed an estimation of the relative contribution of both ingredients to muscle-tissue growth using an isotopic mixing model. Juvenile shrimp fed on the same diet incorporated 73% of nitrogen from fishmeal and 27% from soy protein with an isotopic equilibration time of 20 days (Gamboa-Delgado and Le Vay 2009).

The diet with 4% *Sargassum* was completely replaced by cocultivating juvenile shrimp with *Ulva* spp. for 45 days. The value of the $\delta^{15}N$ in muscle tissue showed a significant change from 12.8‰ to 9.5‰. The shrimp fed with pellets of *Ulva*, thereafter were fed with *Ulva* meal (14±0.1‰), and the nitrogen isotopic composition was unchanged (13.9‰).

The combination of cocultured algae and commercial feed improved shrimp growth significantly, suggesting that the *Ulva* spp. may act as a nutritional supplement and improve the use of nutrients from the commercial feed. The diets were fed to juvenile white shrimp *L. vannamei* (1.6 g) for 28 days. *Ulva* diet resulted in lower loss of dry matter, but a higher loss of protein and also higher distilled water absorption, indicating a modification of the pellet physical quality (better hydro stability). No significant differences in feed consumption and survival were found, but *Ulva* diet resulted in a slightly higher final weight (4.8 for *Ulva* versus 4.6 and 4.3 g for *Ascophyllum nodosum* and *Macrocystis pyrifera*), and better feed conversion ratio (1.7 versus 1.9 and 2.1) and protein efficiency ratio (2.0 versus 1.7 and 1.5), the differences with *M. pyrifera* diet being significant (Cruz-Suárez et al. 2009).

Lombardi et al. (2006) tested the feasibility of coculturing *L. vannamei* and *Kappaphycus alvarezii* in floating cages. After 103 days of growth, no negative interferences between cocultured shrimp and seaweed were found, nor were there significant differences between monoculture and polyculture for shrimp weight gain, survival rate, and feed conversion ratio. Van Tri and Thanh Ha (2004) reported that *L. vannamei* larvae fed fresh seaweed together with a formulated feed had higher growth rate (in length and weight) and higher survival rate (48–53%) than those fed dry seaweed formulated feed or dry seaweed alone.

The initial size average of the shrimp was 3.3 cm changed to 7.3 cm (range of 7.2–8.2 cm) after feeding with 4% *Sargassum* pellets. Subsequently, the cocultured for 45 days shrimp with *Ulva* spp. showed an increase in average size of 11 cm (range of 8–14 cm). In both cases, shrimp had a growth of 4 cm for each type of food. The change of diet had no effect on growth rate of shrimp. So we suggest that N fixation in muscle by shrimp cocultured with *Ulva* spp. has demonstrated the potential of the application of stable isotope analysis of diets and tissue in the evaluation of plant source ingredients and the optimization of their use in diets containing very low or no commercial feed.

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References

Aguilera-Morales M, Casas-Valdez M, Carrillo-Dominguez S, González-Acosta B, Pérez-Gil F (2005) Chemical composition and microbiological assays of marine algae *Enteromorpha* spp. as a potential food source. J Food Comp Anal 18:79–88

Aguiñiga-García S, Casas-Valdéz M, Marín-Álvarez A, Águila-Ramírez RN, Sánchez-Rodríguez I, Sánchez-González A (2011) Contraste de asimilación del fitoplancton natural y alimento balanceado comercial por Litopenaeus vannamei en cultivos semi-intensivos: uso de isótopos de carbono. In: Chávez-Comparan JC y Mimbela-López J (eds) Avances Sobre Investigaciones Marinas y Acuícolas del Pacifico Tropical Mexicano, Universidad de Colima, Manzanillo, Colima, México, Vol. 2 pp 1–12

Al-Maslamani IAMJ (2006) Feeding and nutrition in the marine shrimp *Penaeus semisulcatus*. PhD thesis, University of Wales, UK, 179 pp

Burford MA, Preston NP, Glibert PM, Dennison WC (2002) Tracing the fate of ^{15}N-enriched feed in an intensive shrimp system. Aquaculture 206:199–216

Burtin P (2003) Nutritional value of seaweeds. Electron J Environ Agr Food Chem 2:498–503

Casas-Valdez M, Portillo-Clark G, Águila-Ramírez N, Rodríguez-Astudillo S, Sánchez-Rodríguez I, Carrillo-Domínguez S (2006) Efecto del alga marina *Sargassum* spp. sobre las variables productivas y la concentración de colesterol en el camarón café, *Farfantepenaeus californiensis* (Holmes, 1900). Rev Biol Mar Oceanogr 41:97–105

Copertino MD, Tormena T, Seeliger U (2009) Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. J Appl Phycol 21:31–45

Cruz-Suárez LE, Tapia-Salazar M, Nieto-López MG, Ricque D (2008) A review of the effects of macroalgae in shrimp feeds and co-culture. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Nieto-López MG, Villarreal-Cavazos DA, Gamboa-Delgado J (eds) Avances en nutrición acuícola X — memorias del X simposio internacional de nutrición acuícola, 8–10 de Noviembre, San Nicolás de los Garza, N. L., México. Universidad Autónoma de Nuevo León, Monterrey, México, pp 304–333

Cruz-Suárez LE, Tapia-Salazar M, Nieto-Lopez MG, Guajardo-Barbosa C, Ricque-Marie D (2009) Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. Aquaculture Nutr 15:421–430

Cruz-Suárez LE, León A, Peña-Rodríguez A, Rodríguez-Peña G, Moll B, Ricque Marie D (2010) Shrimp/*Ulva* co-culture: a sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture 301:64–68

D'Avanzo C, Alber M, Valiela I (1991) Nitrogen assimilation from amorphous detritus by two coastal consumers. Estuar Coast Shelf Sci 33:203–209

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Emerson WD (1980) Induced maturation of prawn *Penaeus indicus*. Mar Ecol Prog Ser 2:121–131

Emerson WD, Hayes DP, Ngonyame M (1983) Growth and maturation of *Penaeus indicus* under blue and green light. S Afr J Zool 18:71–75

Fry B, Arnold C (1982) Rapid 13C/12C turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54:200–204

Gamboa-Delgado J (2010) Isótopos estables como trazadores nutricionales naturales en larvas y juveniles de *Litopenaeus vannamei* y *Solea senegalensis*. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Nieto-López MG, Villarreal-Cavazos DA, Gamboa-Delgado J (eds) Avances en nutrición acuícola X - memorias del X simposio internacional de nutrición acuícola, 8–10 de Noviembre, San Nicolás de los Garza, N.L., México. Universidad Autónoma de Nuevo León, Monterrey, México, pp 620–667

Gamboa-Delgado J, Le Vay L (2009) Nitrogen stable isotopes as indicators of the relative contribution of soy protein and fish meal to tissue growth in Pacific white shrimp (*Litopenaeus vannamei*) fed compound diets. Aquaculture 291:115–123

Hamano K, Aue-Umnuoy D, Srisapoome P, Tsutsui I (2007) Profitable environmental remediation with seaweeds in an intensive marine shrimp culture. XIXth international seaweed symposium, March 26–31, Kobe, Japan

Hentschel BT (1998) Intraspecific variations in $\delta^{13}C$ indicate ontogenetic diet changes in deposit-feeding polychaetes. Ecology 79:1357–1370

Ito K, Hori K (1989) Seaweed: chemical composition and potential food uses. Food Rev Int 5:101–144

Lombardi JV, De Almeida Marques HL, Pereira TL, Barreto OJS, De Paula EJ (2006) Cage polyculture of the Pacific white shrimp *Litopenaeus vannamei* and the Philippines seaweed *Kappaphycus alvarezii*. Aquaculture 258:412–415

Marinho-Soriano E, Fonseca PC, Carneiro MA, Moreira WS (2006) Seasonal variation in the chemical composition of two tropical seaweeds. Biores Technol 97:2402–2406

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111

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

Paul NA, de Nys R (2008) Promise and pitfalls of locally abundant seaweeds as biofilters for integrated aquaculture. Aquaculture 281:49–55

Pearson DF, Levey DJ, Greenberg CH, Martinez del Rio C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135:516–523

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Porchas-Cornejo MA, Martínez-Córdova LR, Magallón-Barajas F, Naranjo-Páramo J, Portillo-Clark G (1999) Efecto de la macroalga *Caulerpa sertularioides* en el desarrollo del camarón *Farfantepenaeus californiensis* (Decapoda: Peneidae). Rev Biol Trop 47:437–442

Preston NP, Smith DM, Kellaway DM, Bunn SE (1996) The use of enriched ^{15}N as an indicator of the assimilation of individual protein sources from compound diets for juvenile *Penaeus monodon*. Aquaculture 147:249–259

Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534–540

Rossi F, Herman PMJ, Middelburg JJ (2004) Interspecific and intraspecific variation of $\delta^{13}C$ and $\delta^{15}N$ in deposit and suspension feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenetic changes in feeding mode of *Macoma balthica*. Limnol Oceanogr 49:408–414

Sánchez-Rodríguez I, Sánchez A, Casas-Valdez M, Futema S (submitted for publication). Experimental cultivation of *Ulva* spp.: an option for shrimp nutrition. Rev Latinoam Biotecnol Amb Algal

Stenroth P, Holmqvist N, Nyström P, Berglund O, Larsson P, Granéli W (2006) Stable isotopes as an indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): the influence of tissue, sample treatment and season. Can J Fish Aquat Sci 63:821–831

Van Tri D, Thanh Ha NT (2004) Impacts of feed on the growth of white leg shrimp larvae *Penaeus vannamei* (Boone, 1931). Research Institute for Aquaculture no. 3, Fisheries review no. 9/2004. Fisheries Informatics Centre Internet Web Page license number: 81/GP-BC. http://www.fistenet.gov.vn. Issued on 28 June 2005

Wahbeh MI (1997) Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. Aquaculture 159:101–109

Yokoyama H, Tamaki A, Harada K, Shimoda K, Koyama K, Ishihi Y (2005) Variability of diet tissue isotopic fractionation in estuarine macrobenthos. Mar Ecol Prog Ser 296:115–128