Contribution of the natural biota associated with substrates to the nutritional requirements of the post-larval shrimp, Penaeus esculentus (Haswell), in high-density rearing systems

Author

Burford, MA, Sellars, MJ, Arnold, SJ, Keys, SJ, Crocos, PJ, Preston, NP

Published

2004

Journal Title

Aquaculture Research

DOI

10.1111/j.1365-2109.2004.01052.x

Contribution of the natural biota associated with substrates to the nutritional requirements of the postlarval shrimp, *Penaeus esculentus* (Haswell) in high density rearing systems

Burford, M.A.[1], Sellars, M., J., Arnold, S. J., Keys, S. J, Crocos, P.J., Preston, N.P.
CSIRO Marine Research, PO Box 120, Cleveland, Queensland, 4163, Australia

[1]Correspondence: M.A. Burford, Centre for Riverine Landscapes, Griffith University, Queensland, 4111, Australia.
E-mail: m.burford@griffith.edu.au

Keywords: stable isotopes, epiphytes

Abstract

The contribution of epiphytes associated with physical substrates to the nutritional requirements of postlarval shrimp, *Penaeus esculentus* Haswell was determined in high-density rearing systems (3000, 6000 and 11,000 m^{-3}). Stable isotope signatures of epiphytes on polyethylene mesh substrate, AquaMats^{TM} and tank walls were compared with shrimp signatures. Two methods were used: the determination of carbon and nitrogen natural abundance ratios; and ^{15}N-nitrogen enrichment ratios after the addition of ^{15}N-ammonium to tanks. Using the natural abundance technique and a simple mixing model, epiphytes were found to contribute substantially to the carbon requirements of postlarval shrimp (39 to 53%). This was despite the addition of formulated feed at satiation levels. There was no indication of a reduced contribution of carbon from epiphytes to shrimp nutrition at higher shrimp densities. The lack of a difference in the $^{15}N/^{14}N$ ratios of the two food sources meant that mixing models could not be used to calculate the contribution of nitrogen from epiphytes vs. artificial feed to shrimp nutrition. Using the ^{15}N-nitrogen enrichment method, the amount of nitrogen contributed by epiphytes to shrimp nutrition over 24 h could be determined. This method showed that nitrogen from epiphytes was assimilated by shrimp. ^{15}N-enrichment methods provided a more accurate alternative to natural abundance techniques particularly when the stable isotope signals of the food sources are similar. This experiment has shown the benefits in providing substrates for *P. esculentus* in high density rearing systems to provide an additional food source for shrimp.

Introduction

In intensive pond and tank systems, the nutritional requirements of shrimp are generally met by the addition of formulated feed. However a number of studies have shown that the natural biota can contribute substantially to shrimp growth (Leber & Pruder 1988; Moss & Pruder 1995; Otoshi, Montgomery, Look & Moss 2001). Based on these findings, trials have been conducted to assess the capacity of substrates to promote the growth of epiphytes and microbial biofilms as a food source for the shrimp, *Litopenaeus vannamei* (Boone) and *Farfantepenaeus paulensis* (Pérez-Farfante) (Bratvold & Browdy 2001; Thompson, Abreu & Wasielsky 2002). The presence of substrates has been shown to improve the growth of the freshwater prawn, *Macrobrachium rosenbergii* (De Man) (Tidwell, Coyle, VanArnum & Weibel 2002).

The brown tiger shrimp, *P. esculentus* is an endemic Australian penaeid species (Grey, Dall & Baker 1983). It is a key species in a major Australian fisheries and, more recently, has become a cultured species in Australia. In their natural habitat, juvenile *P. esculentus* are predominantly omnivorous, with gut content analysis showing that protozoa, diatoms, seagrass and zooplankton are the preferred food sources (O'Brien 1994). Much of the microalgal and protozoan species consumed are likely to be seagrass epiphytes (Loneragan, Bunn & Kellaway 1997). Other juvenile and postlarval penaeid species have also been shown to consume considerable amounts of plant material (Newell, Marshall, Sasekumar & Chong 1995; Heales, Vance & Loneragan 1996; Dittel, Epifanio, Cifuentes & Kirchman 1997). However, as *P. esculentus* grow, their feeding

behavior generally shifts from omnivorous to carnivorous (Smith, Dall & Moore 1992; O'Brien 1994).

Measurement of stable isotope ratios in food sources and consumers can be used to determine the importance of different food sources to shrimp nutrition (Gearing 1991; Shearer & Kohl 1993; Phillips & Gregg 2003). The $\delta^{15}N$ and $\delta^{13}C$ ratios are most commonly used. By applying mixing and mass balance models, the relative contribution of each food source can be calculated. This method has been used to determine the contribution of natural foods to shrimp nutrition in aquaculture ponds (Parker & Anderson 1989; Parker, Anderson & Lawrence 1991; Burford, Preston, Minh, Hoa, Bunn & Fry, in press). However, this method has some disadvantages. If the isotopic signatures of food sources are the same or very similar, it is not possible to differentiate between them. Additionally, due to the natural variability in ratios, values for the contribution of food sources have significant errors associated with them.

Another approach is to label the natural food with an enriched form of either $\delta^{15}N$ or $\delta^{13}C$. The enriched nitrogen or carbon can be traced through the food web and into the shrimp by measuring $\delta^{15}N$ or $\delta^{13}C$ ratios in the shrimp and food sources. This approach has been used to determine the contribution of various food sources and ingredients to shrimp nutrition (Preston, Smith, Kellaway & Bunn 1996; Winning, Connolly, Loneragan & Bunn 1999; Burford, Preston, Glibert & Dennison, 2002).

This study used two methods to determine the contribution of natural food to the nutrition of juvenile *P. esculentus* in high-density rearing experiments: $\delta^{15}N$ and $\delta^{13}C$ natural abundance ratios in the natural and artificial feeds, and shrimp; and spiking tanks with ^{15}N-ammonium then tracing ^{15}N-nitrogen into the shrimp.

Materials and Methods

Experimental and tank design

In January 2001, an experiment was conducted to determine the contribution of epiphytes, grown on two types of substrates, to the nutritional requirements of postlarval *P. esculentus*. Tanks (1,750 L rectangular fibreglass tanks, 2.5 m long, 1 m wide and 0.7 m deep) were stocked with *P. esculentus* (PL stage 17) at three stocking densities (Table 1). There were three substrate combinations: straps of buoyant synthetic matting (AquaMat™, similar in shape to seagrass leaves) plus polyethylene mesh substrate (proprietary information); polyethylene mesh substrate (proprietary information); and buoyant and sinking Aquamat™. The three substrate types all varied in the surface area available for epiphyte growth (Table 1). Treatments A, B, C and D are referred to throughout as Polyethylene Mesh + Aquamat™ 3000, Polyethylene Mesh 3000, AquaMat™ 6000 and AquaMat™ 11000 respectively.

Natural food items such as epiphytes were encouraged to grow on the substrates and tank walls by spiking tanks with 55 L unfiltered seawater and fertilizing each tank with: 35 g of plant fertilizer (Yates Gro-Plus Complete Plant Food); and soluble nutrients (1.6 g potassium nitrate, 0.13 g sodium dihydrogen orthophosphate and 0.2 g sodium

metasilicate). Tanks were then maintained for 5 d prior to stocking to allow time for the natural biota to become established on the substrates.

Each tank was supplied with flow-through seawater at an average exchange rate of 80% d^{-1} for the first 2 weeks and 250% d^{-1} for the remaining 5 to 6 weeks. The water temperature was maintained at 27^{o}C. The aeration system consisted of 25 mm PVC pipe running along one wall. The pipe was perforated to produce an even curtain of air bubbles up one side of the tank. The air curtain forced water up the side of the tank creating a laminar flow of water across the surface of the raceway. Particulate waste was removed from tanks periodically when the build up became large enough to become anaerobic, on average once a week.

Two artificial feeds were used: a dried diet which was a combination of a high protein (50%) shrimp feed for juvenile *Marsupopenaeus japonicus* shrimp ground in a hammer mill to a size of 1 – 1.5 mm (Artificial Feed 1), and a commercial feed for postlarval *M. japonicus* shrimp (Artificial Feed 2). This mixture of feeds was chosen because they were regularly used by commercial shrimp farms in the region and resulted in high growth and survival (Keys 2003). There were no commercial feeds available for *P. esculentus,* hence *M. japonicus* feeds were used instead. Both species have a similar, high protein requirement (Keys, 2003). Artificial diets were fed three times daily at 0730, 1200 and 1930 h to satiation. The amount of feed administered each day was approximately 20 to 25% of the estimated shrimp biomass. The combination of post larval shrimp diets and high protein feed comprised 60 and 40% respectively of the total

food fed for the first 7 d of rearing. From days 8 to 14 the ratios were 30 and 70% respectively, and for days 15 to 21 only the ground high-protein shrimp feed was fed. The feed rates and composition were adjusted on a day-to-day basis.

Natural abundance isotope experiment

Samples were taken between days 10 and 13 after stocking when the shrimp were between PL 27 and PL 30 (Table 1). The material sampled from each tank was:

1. epiphytes from the substrates (AquaMat™ and Polyethylene Mesh) and tank walls (~3 g ww)
2. approximately 20 shrimp (~1 g ww)
3. formulated diets (~1 g ea.)

The epiphytes of a known area for each substrate type were collected with a scalpel by scraping the surface of the substrates. The total area collected for each substrate type was: AquaMat™ - one side of a floating and one side of a sinking frond with a total surface area of 80 cm^2; polyethylene mesh – collected from 10 cm x 10 cm area; and walls – collected from 20 cm^2 area. The wall with the curtain of bubbles (Air Wall) was differentiated from the other walls (Other Walls) in each tank. Samples were dried for 24 h at 60^0C.

Shrimp were collected by random netting throughout each tank. Shrimp were stored frozen and later dried at 60^0C for 24 h on acid-washed glass petri dishes. Shrimp and

epiphytes were then ground with mortar and pestle. Shrimp sampled from each tank were combined before grinding.

P.esculentus postlarvae were not sampled for natural abundance signatures prior to commencing the experiment. However, *P. monodon* postlarvae were reared indoors on the same artificial feed as that used for *P. esculentus* postlarvae. Samples were taken at the same size as *P. esculentus*, when stocked into the outdoor tanks, and one and two weeks later to determine the stable isotope signal due to formulated feed.

^{15}N-enrichment experiment

Fourteen days after stocking, when shrimp were PL 32, the ^{15}N isotope ratios were elevated in all tanks by adding ^{15}N-ammonium chloride ($^{15}NH_4Cl$, 99 atom%) at an amount that was approximately 10% of the background ammonia concentration. Ammonia concentrations in the tanks were determined by filtering water samples through 0.45 μm cellulose acetate filters, then analyzing using the phenate method (American Public Health Association 1995). The amount added per tank ranged from 3.63 to 92.65 mg N tank^{-1}. Flow-through water was turned off to allow the epiphytes time to assimilate and cycle the ^{15}N-ammonium. One hour after the addition of ^{15}N-ammonium chloride, biota samples were taken and 24 h later, shrimp samples were taken. Collection and processing protocols for this study were the same as for the natural abundance isotope experiment.

The shrimp biomass was calculated from the stocking density, estimated mortality and average wet weight of shrimp (100 animals tank^{-1} were weighed).

Analyses and calculations

Natural abundance and ^{15}N-enriched samples of epiphytes and shrimp were analyzed for N and C content using an elemental analyzer (Eurovector 3000) and for ^{13}C/^{12}C and ^{15}N/^{14}N ratios using a mass spectrometer (Micromass Isoprime).

A mixing model, assuming only two sources of carbon ie. formulated feeds and epiphytes, was used for the natural abundance samples to determine the contribution of carbon from epiphytes to the nutritional requirements of the shrimp (Phillips & Gregg 2003).

The mixing model used was:

$$C_M = C_x f_x + C_y f_y$$

where:

C_M = δ^{13}C isotope ratio of the shrimp

C_x = δ^{13}C isotope ratio of source *x*

C_y = δ^{13}C isotope ratio of source *y*

F_x = proportion of *x*

F_y = proportion of *y*

To calculate the total amount of nitrogen contributed from the epiphytes using the ^{15}N-enrichment method the following equation was used (Burford, Thompson, McIntosh, Baumann & Pearson, in press):

$$Y = \frac{N * E_s}{W * d * Ep}$$

where:

Y is the total amount of nitrogen from the natural biota consumed by the shrimp (mg N g shrimp^{-1} d^{-1})

N = total nitrogen in shrimp in tank (mg)

E_s = ^{15}N enrichment of the shrimp (atom% excess)

W = wet weight of shrimp in tank (g)

d = number of days

E_p = ^{15}N enrichment of the epiphytes (atom% excess)

Results

Visual examination of the epiphytes showed that there were considerable differences among treatments. Initially, the AquaMat™ treatments had a high level of epiphyte coverage on the walls, especially the wall where the air bubbles were generated. The epiphytes were dominated by a low profile algal turf with short filamentous threads. The epiphytes also varied considerably between substrate types. The Polyethylene Mesh had a low profile algal turf with few filamentous strands whereas the AquaMat™ had a dense mat of short filamentous algae.

There were statistical differences in the $\delta^{15}N$ and/or $\delta^{13}C$ ratios between the three surfaces sampled in each treatment: the wall where the air bubbler was situated ('Air Wall'), the other walls ('Other Walls'), and the substrate (Substrate) for all treatments with the exception of Aquamat™ 11000 (Table 2). However, there were no consistent trends. The 'Other Walls' had a more depleted $\delta^{13}C$ ratio than the other surfaces, but not a lower $\delta^{15}N$ ratio in the AquaMat™ 3000 treatment. The $\delta^{13}C$ ratio for the 'Other Walls' in the AquaMat™ 11000 treatment was also depleted but not the $\delta^{15}N$ ratios. In contrast, the Polyethylene Mesh + AquaMat™ 3000 treatment, the 'Other Walls' had a more depleted $\delta^{13}C$ ratio and a higher $\delta^{15}N$ ratio.

Overall, when data for all surfaces within each treatment were combined, there was a distinct difference in the $\delta^{13}C$ ratios of the epiphytes in the Polyethylene Mesh 3000 treatment compared with the other three treatments (AquaMat™ 6000, Polyethylene Mesh + AquaMat™ 3000, AquaMat™ 11000) (Fig. 1). The $\delta^{15}N$ ratios were similar between treatments.

The two sources of artificial feeds had similar $\delta^{15}N$ and $\delta^{13}C$ ratios, and these were distinctly different from that of epiphytes and shrimp (Fig. 1). The $\delta^{15}N$ and $\delta^{13}C$ natural abundance ratios of shrimp across treatments were similar. This contrasted with the signatures of shrimp reared in the absence of epiphytes. At the end of the experiment, natural food sources were calculated to contribute to shrimp nutrition in all the treatments, ranging from 39% of carbon retained in the Polyethylene Mesh 3000 treatment, to 53% in the AquaMat™ 11000 treatment (Table 3). The contribution of

nitrogen from natural food sources could not be calculated because the $\delta^{15}N/\delta^{14}N$ ratios for the food sources were too similar. The carbon:nitrogen (C:N) ratio of the epiphytic community in the Polyethylene Mesh 3000 treatment was higher than the other treatments while the C:N ratios of the shrimp were similar across treatments (Table 3).

The addition of ^{15}N-enriched ammonium chloride resulted in isotopic enrichment of the epiphytic community within 1 h, and shrimp within 24 h in all the treatments (Table 4). Previous studies have shown that the natural biota in tank systems rapidly assimilated ammonium and reached isotopic equilibration within 1 h of the addition of ^{15}N-ammonium (Burford 2000; Burford, Thompson, McIntosh, Baumann & Pearson, in press). The amount of nitrogen from the epiphytes retained by shrimp was lowest in the Polyethylene Mesh 3000 treatment (7.3 mg N g shrimp^{-1} d^{-1}) and highest in the AquaMat™ 6000 treatment (16.4 mg N g shrimp^{-1} d^{-1}).

Discussion

This study suggests that postlarval *P. esculentus* reared at high densities in tanks can gain considerable nutritional value from epiphytes growing on substrates. The epiphytes contributed irrespective of stocking density. Previous studies have shown that the presence of substrates increased the production of the shrimp, *F. paulensis* and the freshwater prawn, *M. rosenbergii* (Tidwell, Coyle, Weibel & Evans 1999; Tidwell et al. 2002; Thompson *et al.* 2002). The presence of substrates has also been shown to improve water quality in intensive *L. vannamei* culture systems (Bratvold & Browdy 2001).

There have been few previous studies tracing ^{15}N-enriched dietary sources in aquaculture. ^{15}N-enriched formulated feeds have been fed to *P. monodon* to determine short- and long-term assimilation of nitrogen (Preston *et al.* 1996; Burford & Williams 2001; Burford *et al.* 2002). ^{15}N nitrogen labeling of the natural biota has been used to determine the contribution of this source to the nutrition of *Penaeus monodon* (Fabricius) and *L. vannamei* in pond and tank systems without substrates (Burford 2000; Ziemann & Schell 1999). The use of enrichment techniques has the advantage over natural abundance isotope techniques in that more accurate determinations of the contribution of food sources can be made. Natural abundance values can vary markedly within food sources due to a range of factors, increasing the error associated with this technique (Adams & Sterner 2000; Vander Zanden & Rasmussen 2001). The error associated with nitrogen is likely to be higher than carbon because of the additional variable; the fractionation shift in nitrogen isotopic ratios with trophic level. Additionally, natural abundance techniques rely on a degree of differentiation between the food sources, a problem that was encountered in this study.

While stable isotope techniques provide a useful indicator of the contribution of a food source to shrimp nutrition, the variability is often sufficiently high to preclude an accurate measure of the contribution or a comparison between treatments. However, in terms of both carbon and nitrogen, the treatment with polyethylene mesh alone appeared to contribute less than the other treatments. Therefore, the complexity of the structure may be an important factor.

In this study, no attempt was made to adjust the addition rate of formulated feed to account for the contribution of the natural biota. Studies in shrimp ponds have shown that much of the dietary nutrients fed to shrimp are not assimilated and ultimately impact on the health of aquatic waterways when water from farms is discharged (Naylor, Goldburg, Mooney, Beveridge, Clay, Folke, Kautsky, Lubchenco, Primavera & Williams 1998; Burford, Costanzo, Dennison, Jackson, Jones, McKinnon, Preston & Trott 2003). A synthesis of dominant ecological processes in intensive shrimp ponds and adjacent coastal environments in NE Australia. Marine Pollution Bulletin, in press.). Additionally, feed is one of the highest variable costs (Lawrence & Lee 1997). There are, therefore, likely to be considerable advantages to reducing feed wastage by optimizing the growth of the natural biota and adjusting the addition rate of formulated feeds accordingly in nursery systems.

Although carbon and nitrogen from epiphytes contributed to shrimp nutrition, it is unclear from this study how natural food sources contribute to shrimp growth compared with artificial feeds. Previous studies have shown that the presence of natural biota results in substantial improvements in shrimp nutrition over the use of artificial feeds alone (Leber & Pruder 1988; Moss & Pruder 1995; Otoshi et al. 2001). However this effect may be related to the quality of the food source, i.e. amino acid composition, micronutrients, rather than the quantity of protein (N), carbohydrate or lipids (C).

In conclusion, this study has shown that the natural biota in high-density tank systems with substrates can contribute substantially to the carbon and nitrogen requirements of

P. esculentus postlarvae. This study suggests that there are benefits in promoting the epiphytic community in tanks with substrates, at least in the early stages of shrimp growth. It still remains to be established, however, whether the epiphytic community continues to contribute to shrimp nutrition throughout the juvenile phase.

A**cknowledgments**

We thank Stuart Bunn and his staff for stable isotope analyses. This work was supported by CSIRO Marine Research and Fisheries Research and Development Corporation Grant 99/222.

References

American Public Health Association (1995) *Standard Methods for the Examination of Water and Wastewater*. 19th edition (ed. by L.S. Clesceri, A.E. Greenberg & R.R. Trussell), APHA, Washington D.C., USA.

Adams T.S. & Sterner R.W. (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnology and Oceanography* **45**, 601-607.

Bratvold D. & Browdy, C.L. (2001) Effects of sand sediment and vertical surfaces (AquaMats™) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. *Aquaculture* **195**, 81-95.

Burford, M.A. (2000) *Fate and transformation of dietary nitrogen in penaeid prawn aquaculture ponds.* PhD thesis. The University of Queensland, Australia.

Burford M.A. & Williams K.C. (2001) The fate of nitrogenous waste from shrimp feeding. *Aquaculture* **198**, 79-93.

Burford M.A., Preston N.P., Glibert P.M. & Dennison W.C. (2002) Tracing the fate of ^{15}N-enriched feed in an intensive shrimp system. *Aquaculture* **206**, 199-216.

Burford M.A., Costanzo S.D., Dennison W.C., Jackson C.J., Jones A.B., McKinnon A.D., Preston N.P. & Trott, L.A. (2003) A synthesis of dominant ecological processes in intensive shrimp ponds and adjacent coastal environments in NE Australia. *Marine Pollution Bulletin*, **46**, 1456-1469.

Burford M.A., Preston N.P., Minh T.H., Hoa T.T.T., Bunn S.E. & Fry V.M. (200…) Dominant sources of dietary carbon and nitrogen for shrimp reared in extensive rice-shrimp ponds. *Aquaculture Research*, in press.

Burford M.A., Thompson P.J., McIntosh R.P., Bauman R.H. & Pearson D.C. (200….) The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero exchange system. *Aquaculture*, in press.

Dittel A.I., Epifanio C.E., Cifuentes L.A. & Kirchman D.L. (1997) C and N sources for shrimp postlarvae fed natural diets from a tropical mangrove system. *Estuarine, Coastal and Shelf Science* **45**, 629-637.

Gearing J.N. (1991) The study of diet and trophic relationships through natural abundance ^{13}C. In: *Carbon Isotope Techniques* (ed. by D.C. Coleman & B. Fry), pp. 201-218. Academic Press, San Diego.

Grey D.L., Dall W. & Baker A. (1983) A Guide to the Australian Penaeid Prawns. Northern Territory Government, Darwin, 140 pp.

Heales D.S., Vance D.J. & Loneragan N.R. (1996) Field observations of moult cycle, feeding behaviour, and diet of small juvenile tiger prawns, *Penaeus semisulcatus* in the Embley River, Australia. *Marine Ecology Progress Series* **145**, 43-51.

Keys S.J. (2003) Aspects of the biology and ecology of the brown tiger prawn *Penaeus esculentus*, relevant to aquaculture. *Aquaculture* **217**, 325-334.

Lawrence A.L. & Lee P.G. (1997) Research in the Americas. In: *Crustacean Nutrition. Advances in World Aquaculture, Vol.* 6. (ed. by L.R. D'Abramo, D.E. Conklin & D.M. Akiyama), pp. 566-587. World Aquaculture Society, Baton Rouge, Louisiana.

Leber K.M. & Pruder G.D. (1988) Using experimental microcosms in shrimp research: The growth-enhancing effect of shrimp pond water. *Journal of the World Aquaculture Society* **19**, 197-203.

Loneragan N.R., Bunn S.E. & Kellaway D.M. (1997) Are mangroves and seagrasses sources of organic carbon for penaeid prawns in a tropical Australian estuary? A multiple isotope study. *Marine Biology* **130**, 289-300.

Moss S.M. & Pruder G.D. (1995) Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* Boone, reared under intensive culture conditions. *Journal of experimental Marine Biology and Ecology* **187**, 175-191.

Naylor R.L., Goldburg R.J., Mooney H., Beveridge M., Clay J., Folke C., Kautsky N., Lubchenco J., Primavera J. & Williams M. (1998) Nature's subsidies to shrimp and salmon farming. *Science* **282**, 883-884.

Newell R.I.E., Marshall N., Sasekumar A. & Chong V.C. (1995) Relative importance of benthic microalgae, phytoplankton, and mangroves as sources of nutrition for penaeid prawns and other coastal invertebrates from Malaysia. *Marine Biology*, **123**, 595-606.

O'Brien C.J. (1994) Ontogenetic changes in the diet of juvenile tiger prawns *Penaeus esculentus* (Decapoda: Penaeidae). *Marine Ecology Progress Series* **112**, 195–200.

Otoshi C.A., Montgomery A.D., Look A.M. & Moss S.M. (2001) Effects of diet and water source on the nursery production of Pacific White Shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society* **32**, 243-249.

Parker P.L. & Anderson R.K. (1989) A $\delta^{13}C$ and $\delta^{15}N$ tracer study of nutrition in aquaculture: *Penaeus vannamei* in a pond growout system. In: *Stable Isotopes in Ecological Research*. (ed. by P.W. Rundel, J.R. Ehleringer, K.A. Nagy). pp. 288-303. Springer-Verlag, New York.

Parker P.L., Anderson R.K. & Lawrence A.L. (1991) Stable isotope methodology for evaluation of nutritional requirements of shrimp. In: *Frontiers of Shrimp Research*. De Loach P.F., Dougherty W.J., Davidson M.A. (Eds.). pp. 157-171. Elsevier, Amsterdam.

Phillips D.L. & Gregg J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia* **136,** 261-269.

Preston N.P., Smith D.M., Kellaway D.M. & Bunn S.E. (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.

Shearer G. & Kohl D.H. (1993) Natural abundance of ^{15}N: Fractional contribution of tow sources to a common sink and use of isotope discrimination. In: *Nitrogen Isotope Techniques* (ed. by R. Knowles, T.H. Blackburn) pp. 89-125, Academic Press, San Diego.

Smith D.S., Dall W. & Moore L.E. (1992) The natural food of some Australian Penaeids. In: *Proceedings of the Aquaculture Nutrition Workshop*, pp. 95-96. NSW Fisheries, Salamander Bay, NSW, Australia..

Thompson F.L., Abreu P.C. & Wasielesky W. (2002) Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* **203**, 263-278.

Tidwell J.H., Coyle S., Weibel C. & Evans J. (1999) Effects and interactions of stocking density and added substrate on production and population structure of freshwater prawns *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society* **30**, 174-179.

Tidwell J.H., Coyle S.D., VanArnum A. & Weibel C. (2002) Effects of substrate amount and orientation on production and population structure of freshwater prawns *Macrobrachium rosenbergii* in ponds. *Journal of the World Aquaculture Society* **33**, 63-69.

Vander Zanden M.J. & Rasmussen J.B. (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography* **26**, 2061-2066.

Winning M.A., Connolly R.M., Loneragan N.R., Bunn S.E. (1999) ^{15}N enrichment as a method of separating the isotopic signatures of seagrass and its epiphytes for food web analysis. *Marine Ecology Progress Series* **189**, 289-294.

Ziemann D.A. & Schell D.M. (1999) Tracking nitrogen and carbon flows and sinks in experimental shrimp culture systems using stable isotope additions. In: *World Aquaculture Society Book of Abstracts*, p. 832. Sydney, Australia,.

Figure Legend

Figure 1: Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ ratios in formulated feed, epiphytes and shrimp in the four treatments. PE mesh refers to polyethylene mesh substrates.

Table 1. Experimental design for high density rearing treatments for *P. esculentus* with a range of stocking densities and substrate comparisons. Surface area refers to area of substrates. Mean weights (g) (± SD) given for individual shrimp. Superscripts denote statistical difference ($P < 0.05$)

	Stocking density (# m^{-3})	Initial wt (g)	Final wt (g)	Substrate (m^2)	Surface area
Substrate	2,860	0.017 (0.005)	0.092 (0.033)[a]	A. AquaMat™ + Mesh	5.1+18.0
	2,860	0.017 (0.005)	0.088 (0.033)[a]	B. Mesh	24.7
Density	5,720	0.017 (0.005)	0.075 (0.030)[b]	C. AquaMat™	7.9
	11,430	0.017 (0.005)	0.052 (0.022)[c]	D. AquaMat™	7.9

Table 2: Natural abundance $\delta^{15}N$ and $\delta^{13}C$ ratios for the surfaces sampled for epiphytes in each treatment at the end of the experiment. Superscripts denote statistical differences within $\delta^{15}N$ and $\delta^{13}C$ ratios.

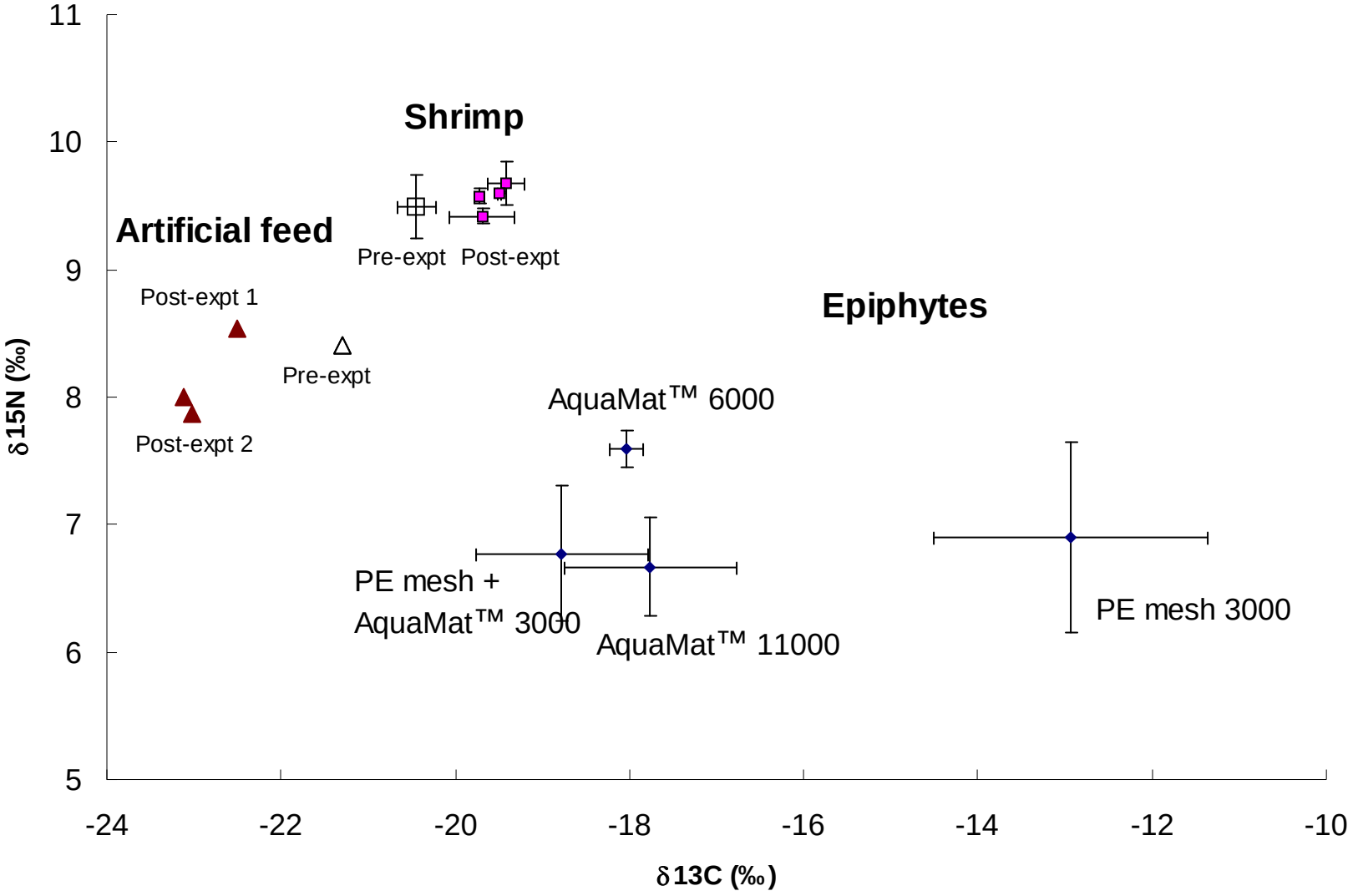
	$\delta^{15}N$			$\delta^{13}C$		
Treatment	**Air Wall**	**Other Wall**	**Substrate**	**Air Wall**	**Other Wall**	**Substrate**
Polyethylene Mesh 3000	7.43^{a}	9.14^{a}	6.38^{a}	-16.46^{a}	-13.38^{a}	-11.90^{b}
Polyethylene Mesh + AquaMat™ 3000	6.69^{a}	8.81^{b}	6.29^{a}	-16.42^{a}	-14.28^{a}	-18.71^{b}
AquaMat™ 6000	7.37^{a}	7.92^{b}	7.60^{a}	-15.87^{a}	-14.21^{b}	-18.41^{a}
AquaMat™ 11000	6.56^{a}	8.19^{a}	6.71^{a}	-15.68^{a}	-14.21^{a}	-19.27^{a}

Table 3: Contribution of the epiphytes to carbon nutrition of *P. esculentus* in tanks as determined by natural abundance stable isotope ratios, and molar C:N ratios of the epiphytes and the shrimp.

Treatment	**% carbon from the epiphytes (Mean ± SE)**	**C:N ratio of the epiphytes**	**C:N ratio of shrimp**
Polyethylene Mesh 3000	39 (7)	8.7 (0.5)	4.9 (0.1)
Polyethylene Mesh + AquaMat™ 3000	53 (12)	7.7 (0.5)	4.8 (0.1)
AquaMat™ 6000	47 (10)	6.7 (0.4)	4.8 (0.1)
AquaMat™ 11000	49 (11)	6.2 (0.1)	4.9 (0.2)

Table 4: Mean (± SD) $\delta^{15}N$ ratios (‰) in the epiphytic community and shrimp in four treatments in the tanks enriched with ^{15}N-nitrogen, and the amount of nitrogen from the epiphytic community retained by the shrimp.

Treatment	**Epiphytes $\delta^{15}N$ (‰)**	**Shrimp $\delta^{15}N$ (‰)**	**Nitrogen retained by shrimp from epiphytes (mg N g shrimp^{-1} d^{-1})**
Polyethylene Mesh 3000	42.4 (7.8)	14.4 (0.1)	7.3
Polyethylene Mesh + AquaMat™ 3000	29.3 (5.2)	11.9 (0.6)	13.0
AquaMat™ 6000	21.1 (3.5)	12.1 (0.3)	16.4
AquaMat™ 11000	55.3 (7.5)	18.9 (1.8)	9.8


11
10
9
8
7
6
5
δ15N (‰)
-24
-22
-20
-18
-16
-14
-12
-10
δ13C (‰)
Shrimp
Artificial feed
Pre-expt
Post-expt
Post-expt 1
Pre-expt
Post-expt 2
Epiphytes
AquaMat™ 6000
PE mesh +
AquaMat™ 3000
AquaMat™ 11000
PE mesh 3000