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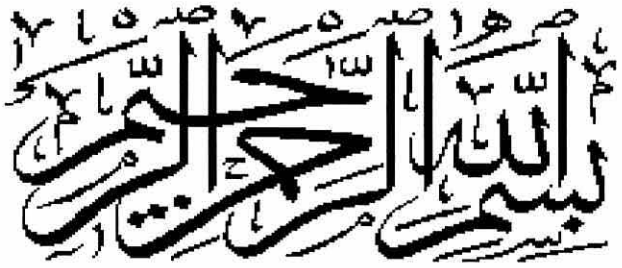

In the name of Allah The Most Gracious
The Most Merciful

Feeding and nutrition in the marine shrimp *Penaeus semisulcatus*

A thesis submitted to the University of Wales

By

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DEDICATION

To

My parents
My Wife
My son
My daughter

Ibrahim A.M.J AlMaslamani
University of Wales, Bangor

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ABSTRACT

This study examined the feeding and nutrition of *Penaeus semisulcatus* using a combination of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis and gut contents analysis. Field collections included post larvae, juvenile and adult shrimp inhabiting intertidal and subtidal habitats on the coast of Qatar. Post-larvae were collected in shallow water seagrass beds while juveniles and adults were caught in deeper macroalgal beds. Post-larvae and juvenile shrimp in seagrass beds were found to feed mainly on benthic organism such as foraminifera, polychaetes, diatoms and small crustaceans (amphipods, isopods and ostracods), whereas larger shrimp in the macroalgal beds fed mainly on bivalve molluscs and to a lesser extent polychaetes. Post larval stages of *Penaeus semisulcatus* were found in seagrass beds, in contrast to *Metapenaeus affinis* which settle at a comparatively younger age and were found in seagrass beds and over the intertidal zone where microbial mats were present. Stable isotope analysis indicated that primary production in mangroves ($\delta^{13}C$ -28.5 to -27.4 ‰ and $\delta^{15}N$ 0.8 to 0.9 ‰) did not contribute to nutrition of either shrimp species, but suggested an influence from seagrass beds ($\delta^{13}C$ ‰ -7.9 ± 1.3and $\delta^{15}N$ 1.5 ± 0.42 ‰) and microbial mats ($\delta^{13}C$ -7.7 ‰ and $\delta^{15}N$ -0.9 to -0.2 ‰). In laboratory studies, the very earliest stage *P. semisulcatus* post-larvae, not completely adapted to a benthic life style, exhibited reduced survival when fed on microbial mat. However, within a few days of metamorphosis microbial mats supported high growth and survival. The possible role of intertidal mudflats in supporting shrimp populations merits further investigation. Analysis of stable isotope ratios in sieved size-fractions of the microbial mat and the shrimp tissue supports the hypothesis that the shrimp are gaining most of their nutrition from the associated infauna, primarily nematodes. The results indicate that both the C and N isotope contents in shrimp muscle tissue take about 2-3 weeks to equilibrate after a change in dietary isotopic signature. A simple dilution model showed that most of this change was explained by the high growth rate in early life stage shrimp, rather than turnover. A wide range of trophic enrichment in tissue stable isotope signatures relative to diet in the laboratory, supporting interpretation of field studies which confirm the linkage between sensitive shallow water habitats and early life stages of penaeid species and indicate the need for suitable assessment of the potential indirect impacts of coastal developments involving dredging and land reclamation.

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General introduction

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1.1 Introduction

In many countries, the consumption of marine shrimps has grown over recent years, while the natural stocks of these decapod crustaceans have been increasingly depleted. For this reason, global aquaculture of penaeid shrimp has grown rapidly during the last 20 years (Bainy 2000), with a range of species farmed throughout the world, primarily in marine and brackish water. The major regions for shrimp production, at the present time, are China, Thailand, Vietnam, Indonesia, Philippines, India and Ecuador. About 75% of farmed shrimp is produced in Asia, with greatest production in China and Thailand. The remaining 25% is mainly produced in Latin America, primarily in Ecuador. The largest farmed shrimp exporting nation is Thailand, which produced 220,000 tons of farmed shrimp in 1995, twice as much as it produced in 1990 (Hagler 1997). The production of farmed penaeid shrimp worldwide has increased exponentially to the most recently recorded values of 1.8 million MT from 1950 to 2003, outstripping wild fisheries which have increased linearly to 1.4 million MT over the same time period (Figure 1, FAO 2004). Many penaeid shrimp have been commercially cultured, with the most widely farmed species including the giant tiger prawn (*Penaeus monodon*), western white prawn (*Litopenaeus vannamei*), western blue prawn (*Litopenaeus stylirostris*), Chinese white prawn (*Fenneropenaeus chinensis*), Indian white prawn (*Fenneropenaeus indicus*), and Japanese kuruma prawn (*Marsupenaeus japonicus*). There has been some interest in the farm production of the grooved tiger shrimp *Penaeus semisulcatus*, particularly in the Eastern Mediterranean and the Arabian Gulf (Tom *et al.* 1984; Browdy *et al.* 1986; Seidman and Issar 1988; White 1988; Colorni 1989; Kumlu *et al.* 2003), but production to date has been extremely limited.

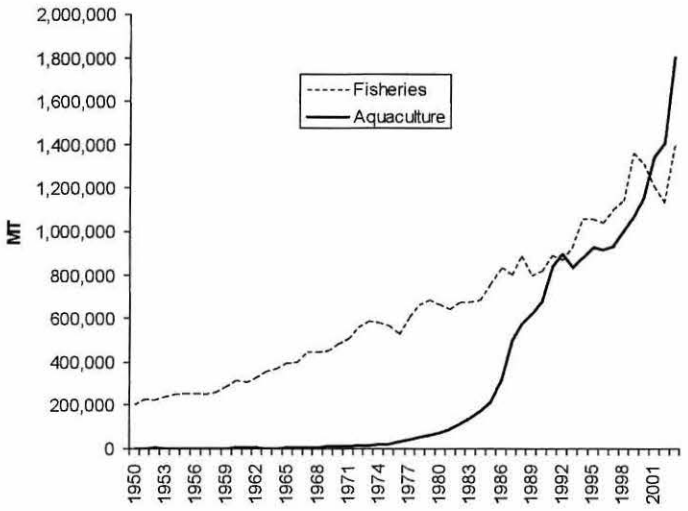

Figure (1) Increase in aquaculture and fisheries production of penaeid shrimp from 1950-2003 (FAO 2004).

1.2 Feeding in the natural environment

Many field and laboratory studies have been carried out on the feeding and nutrition of penaeid shrimp, as development of a better understanding of nutrient requirements, nutrient sources and feed management is necessary for successful production of commercial marine and freshwater aquaculture. In semi-extensive and intensive production systems, adequate nutrition is critical and provision of feeds represents 40-50% of the production costs. Furthermore, research into nutritional ecology is important, as effective management of fishery resources requires an understanding of feeding ecology and trophic dependencies on specific habitats.

In the wild, penaeid shrimp feed predominantly on small invertebrates and detritus. Dall *et al.* (1990) and McTigue and Zimmerman (1998) reported that penaeid shrimp browse through sediment surfaces probing and handling items they encounter, possible suggesting selective feeding (Karim 1970; Gleason and Zimmerman 1984). Food selection is determined by food palatability, prey size and catchability. For

example there is some evidence that foraminifera, copepods and brachyuran megalopae are commonly selected by juvenile shrimp but not larger potential prey items such as sergestids, mysids and juvenile fishes which are also abundant (Sheppard *et al.* 1992). Heales (2000) found that the common food items present in the gut of juvenile *P. semisulcatus* in a tropical Australia estuary containing intertidal seagrass and subtidal algal beds were copepods, diatoms and filamentous algae. In addition, Wassenberg and Hill (1987) reported that the most abundant food items for *P. esculentus* and *P. semisulcatus* in a similar environment were bivalves, gastropod, ophiuroids, crustaceans and polychaetes. Penaeid shrimp species may exhibit different feeding habits (Table 2). For example Wassenberg and Hill (1987) found apparent different responses of *P. semisulcatus, P. esculentus* to seagrass beds that may be related to behavioral and feeding differences between the two species. They found that juvenile *P. semisulcatus* eat mainly benthic infauna while juvenile *Penaeus esculentus* of a similar size eat mainly seagrass leaf epifauna. Similarly, *Litopenaeus setiferus* and *L. aztecus* have been classified as bottom feeders who consume any available organic material (Young 1959; Darnell 1961), while other species such as the *P. duorarum* are known to be more selective and consume mainly infauna (Nelson and Copone 1990). However, Chong and Sasekumar (1981) suggested that feeding may be opportunistic and that the diet of *P. merguiensis* is related to food availability. However, for all penaeid shrimp species, McTigue and Zimmerman (1991) pointed out that feeding habits are difficult to determine, partly because the digestive process hampers identification of gut content material and may lead to an over-representation of less-digestible food items. For example, Mayer (1985) found that the gut contents of juvenile *P. setiferus* usually contained remains of polycheates, tanaids, copepods, foraminifera, ostracods and fish but also a high percentage of unrecognizable matter.

There may be some change in diet with shrimp age and with seasons. Wassenberg (1990) showed that diet may change seasonally, depending on prey availability. Rothlisberg (1998) reported that small juvenile shrimp feed mainly on microinvertebrates and some plant material (mangrove detritus, epiphytes on seagrasses and even seeds), but as they grow they eat larger invertebrates and less plant material.

1.3 Nutritional requirements

The nutrients required to support growth and development in shrimp species can be generally classified as proteins, carbohydrates, lipids, vitamins and minerals. However, the optimum levels of these nutrients vary from one species to another. Recently, the requirements of macronutrients such as protein and carbohydrate for penaeid shrimp species in captivity have been better understood, but only limited information is available for natural diets consumed in the wild. Dall *et al.* (1991) found that the typical natural diet was high in protein (70 to 80 %), low in carbohydrate (11 %) and low in lipid (12 %). The nutritional requirements of penaeid shrimp post larvae and juveniles have been studied in more detail in relation to aquaculture production, using experimental compound diets (New 1976). These experiments were primarily conducted to develop formulations that minimise the cost of the ingredients of a diet and its ecological impact (Rosas *et al.* 2001). The protein requirement of penaeid shrimp is one of most important components for consideration in aquaculture because the protein is the main dietary component supporting growth and the most expensive bulk ingredient used in artificial diets. Protein requirements can vary between species (Allan and Smith 1998; Wouters *et al* 2001). The optimum protein levels for *Marsupenaeus joponicus* and *Fenneropenaeus penicillatus* range

between 50% to 55% (Teshima and Kanazawa 1984), for *Litopenaeus aztecus* between 25% to 40% (Balazs and Ross 1976; Venkataramiah *et al.* 1975), while for *Penaeus mondon* the optimum dietary protein level was 40 % (Alava and Lim 1983). A recent study of the optimum dietary protein level for *P. semisulcatus* (Alam 2004) showed that shrimp fed on a 40% protein diet exhibited better growth than those fed diets of 20, 30 or 50 % protein content. In contrast, some species such as *Litpenaeus vannamei* can grow well on diets containing as little as 20 % protein (Lawrence *et al* 1998). Other studies have shown that protein requirements also depend on physiological stage, dietary characteristics and size of the animal (Guillaume 1997). Harrison (1990) reported that protein requirements are higher during maturation and reproduction compared to the non reproductive stages.

The optimal level of dietary carbohydrate for marine aquatic animals ranges from 20 to 40 % (Sick and Andrews 1973; Cuzon *et al.* 1994). Several studies in penaeids have shown that the level and type of dietary carbohydrate can influence growth rate (Andrews *et al.* 1972; Abdel-Rahman *et al.* 1979). Penaeid shrimp also require dietary lipids to supply a variety of metabolic functions. For example, dietary lipids are an important source of essential fatty acids and phospholipids which are required for growth and survival. The optimal dietary level for lipids in marine shrimp ranges from 6 to 7.5 % (Andrews *et al.* 1972: Akiyama *et al.* 1992). There is an upper limit to the amount of that energy can be supplied in the form of lipids, that is generally recognised to be around 12% of the diet as crustacean can only store lipids in the hepatopancreas. Chuntapa *et al.* (1999) found the best growth rate and survival in *Penaeus monodon* fed an artificial diet containing a lipid-carbohydrate ratio of 1:4.6,

while diets containing low carbohydrate and high lipid levels supported relatively lower growth and survival.

1.4 Nursery habitat utilization

Penaeid postlarvae use the shallow estuarine and lagoon habitats for their early nursery development (Minello and Zimmerman 1991) as these habitats are rich in nutrients and organic matter and provide suitable substratum for different elements of a complex tropic chain (Albertoni 1998). Nursery habitats utilized by post larvae and juveniles are characterized by the presence of vegetated substrates such as mangrove forests, saltmarshes, seagrass beds and algal beds (Rönnbäck *et al.* 2002) (Table 1). Primary producers such as microbial mat, mangroves and seagrass are important contributors to coastal food webs as well as providing nursery habitats for young shrimp. However, the extent to which shrimp species depend on these different habitats for their nutrition is not well-understood.

Table (1) The nursery ground habitats ultilised by penaeid shrimps.

Species	Nursery habitat	References
Fenneropenaeus indicus	Mangroves	de Freitas (1986); Chong *et al.*(1990); Sasekumar *et al.* (1992).
Fenneropenaeus merguiensis	Mangroves	Staples *et al.* (1985); Robertson and Duke (1987); Sasekumar *et al.* (1992); Primavera and Lebata (1995).
Penaeus duoraum	Seagrass and algal beds	Sheridan (1992)
Penaeus esculentus	Seagrass and algal beds	Staples, *et al.*(1985); de Freitas(1986); Robertson and Duke (1987) Haywood *et al.* (1995).
Penaeus semisulcatus	Seagrass and algal beds	Staples, *et al.* (1985); de Freitas (1986);Robertson and Duke (1987). Shappard *et al.* (1992). Haywood *et al.* (1995) Wassenberg and Hill (1987); Heales *et al.* (1996).
Penaeus monodon	Mangroves	de Freitas (1986)
Metapenaeus monoceros	Seagrass, mudflat and mangrove channels	de Freitas (1986)
Metapenaeus affinis	Mangrove, mud flat and seagrass	Sasekumar *et al.* (1992); Al-Zaidan (2002).
Metapenaeus ensis	Seagrass, mudflat and mangrove channels	Staples, *et al.*(1985); Robertson and Duke (1987).
Litopenaeus setiferus	Salt marshes	Zimmerman and Minello (1984).
Farfantepenaeus. aztecus	Salt marshes	Zimmerman and Minello (1984) Fry *et al* 2003

Bishop and Khan (1999) reported that shallow waters are likely provide a degree of protection from predation by preventing access of larger individuals and / or removing the competitive edge of manoeuvrability for smaller would be predators. Also, Clayton (1986) demonstrated that during flood tide large numbers of marine organisms, including young shrimps invade the mudflats feeding at the edge of the advancing tide. In the Arabian Gulf, during the winter months the lowest tide occurs during the day, while in the summer lowest tides occur at the night (Bishop and Khen 1999). Thus, Jones (1986) reported that intertidal biota are spared the effected of seasonal temperature extremes.

1.5 The role of benthic primary producers

A high proportion of marine primary production takes place in coastal areas (Graco *et al.* 2001) and phytoplankton such as dinoflagellates and diatoms are particularly important producers in the planktonic food webs. However, high primary production and the often delayed response of herterotrophs also results in much of the pelagic organic matter being exported to the benthos in sinking particles which become incorporated into the sediment (Suess 1980). Much of this primary production is subject to nutrient recycling back into the water column as a result of the action of benthic microbial communities (Pelegri and Blackurn 1995). However, in oligotrophic subtropical seas such as the Arabian Gulf, benthic communities are themselves important sources of primary production.

Where mudflats are present, benthic microalgae are a source of primary production and may contribute significantly to benthic shelf food webs (Cohen *et al.* 1990).

Schaaf and Peters (1992) showed that algal production on mudflats is produced by edaphic microalgae and epiphytic microalgae. These benthic floras are typically dominated by various species of diatoms and blue green algae, but also microscopic green, yellow-green, red and brown algae may be abundant. However, Brook (1977) reported that these highly productive algae are heavily grazed by a host of organisms including: nematodes, ostracods, harpacticoid copepods, isopods, amphipods, molluscs, shrimp and various fish. Benthic diatoms are an important component of the microphytobenthos that inhabit intertidal mudflats where they are usually the main primary producers present (Brouwer and Jody 2002). Diatoms often show considerable patchiness in biomass distribution (Taylor *et al.* 1999). In some mudflat areas diatom mats stabilize the surface sediment layers of the mudflat against resupension (Paterson 1995; Decho 2000).

Vance *et al.* (1990) and Rönnbäck *et al.* (2002) reported that many coastal species spend their juvenile phase in or associated with vegetation such as mangrove forests or seagrass beds. Seagrass and algal beds have been identified as important nursery areas for *P. semisulcatus* in Australia and the Arabian Gulf (Basson *et al.* 1977; Young 1978; Jones and Al Attar 1981; Staples *et al.* 1985; Wassenberg and Hill 1987; Sheppard *et al.* 1992; Hill and Wassenberg 1993; Haywood *et al.* 1995; Heales *et al.* 1996; Loneragan *et al.* 1998; Jackson *et al.* 2001). Some animals in seagrass communities use seagrasses directly as a food source, and the importance of seagrass primary production as direct or indirect food source of post and juvenile shrimp has been recognized. However, in some studies the role of seagrass primary production is less clear. Fry (1984) found that in some shallow seagrass-dominated areas carbon

from planktonic and benthic algae was more important to consumers than seagrass detritus.

Stable isotope studies indicate that primary production in both seagrasses and adjacent mangroves contribute to nutrition of juveniles, though the relative importance of these sources can be seasonally variable (Loneragan *et al.* 1997). The $\delta^{13}C$ in animals associated with seagrass beds are usually heavier than those in animals found offshore, reflecting the difference in isotope ratios between primary producers within and away from seagrass meadows (Parker and Calder 1970; Thayer *et al.* 1978; Fry and Parker 1979).

Positive correlations between the extent of mangrove forests and commercial yield of fisheries have been found in several studies (eg.Turner 1977; Sasekumar and Chang 1987; Robertson and Blaber 1992; Loneragan *et al* 1997; Sheridan and Hays 2003). Mangroves are very important nursery grounds for many organisms due to the export of dissolved and particulate nutrients to coastal waters. They also provide a complex sheltered habitat for juvenile fish and invertebrates (Chong *et al.* 1990). Penaeid species are attracted to nursery habitats of high heterogeneity such as the intertidal mangrove forest and several studies have shown that some penaeid species prefer mangroves as nursery grounds, including *Fenneropenaeus indicus*, *F. merguiensis* and *Penaeus monodon* (de Freitas 1986; Chong *et al.* 1990; Sasekumar *et al.* 1992; Staples *et al.* 1985). Rönnbäck *et al.* (2002) identified the role of mangrove-dominated environments as nursery habitats for penaeid shrimp in Mozambique. However, the same study found no differences in abundance of *Fenneropenaeus indicus* and *Metapenaeus monoceros* between sand flats and mangrove habitats,

suggesting either movement between and utilisation of both habitats. Some previous studies of stable isotopes in food webs in mangroves systems have shown that mangroves can be important sources for nitrogen and carbon for many organisms (e.g. Rodelli *et al.* 1984; Odum and de la Cruz 1963: Odum and Heald 1975), including penaeid shrimp (Martosubroto and Naamin 1977; Gedney *et al* 1982; Staples *et al.* 1985; Chong 1995; Loneragan *et al.* 1997). For instance, in Malaysian mangroves Rodelli *et al.* (1984) found that juvenile shrimp species assimilated about 65% of mangrove carbon and Chong *et al.* (2001) have demonstrated a strong influence of mangrove primary production on shrimp tissue composition.

Intertidal mudflats in Kuwait are dominated by microbial mats (Ahmed 1975; Smythe 1979; Glayzer *et al.* 1984; Clayton 1986; Jones 1986). Jones *et al.* (2002) and Al-Zaidan *et al.* (2006) used stable isotopes ratio techniques to show that there is a link between microbial mat production and commercial fish and shrimp species, which are supported by primary production emanating from the benthic cyanobacteria-diatom complex. Similarly, in Malaysia, Newell *et al.* (1995) concluded that benthic microalgae production on mudflats, just offshore from mangroves contributed more to secondary production than detrital input from mangrove leaves. The same study however, highlighted the important role that mangrove forests serve in trapping and stabilizing the sediment of extensive intertidal mudflat.

Microbial mats probably are the oldest structured ecosystem on Earth. Gemerden (1993) described them as vertically laminated structures developing on solid surface. Schopf *et al.* (1983) reported that microbial mats found in modern hot springs provide examples of the range of mat-forming communities that might have existed at

different times during the Precambrian era when the biological and chemical environment of Earth changed. Furthermore, Cohen (1984) demonstrated that laminated microbial sediment ecosystems develop under a wide range of environmental conditions and may be found in different areas such as hypersaline coastal lagoons, hot springs, alkaline lakes and marine intertidal flats. In addition, Margulis *et al.* (1980) described that microbial mats develop in time as the result of microbial growth and activity sediment trapping and binding in the organic matrix. According to Navarrete *et al.* (2000) a microbial mat is a community composed primarily of different population of bacteria, which form thin horizontal layers. They also reported that these layers have active growth and can be from several millimetres to a few centimetres thick. The layers develop along microgradients established at fluid interfaces on solid substrate. Microbial mats developing in shallow water are composed primary of aerobic cyanobacteria in the upper layers; purple and green sulphur bacteria in the middle layers, and sulphate-reducing bacteria at the bottom layers (Navarrete *et al.* 2000).

The importance of the intertidal zone in the productivity of the Arabian Gulf is well documented (Jones 1985, 1986; Sheppard *et al.* 1992; Price *et al.* 1993). In the Arabian Gulf, Kinsman (1964) was the first to report the existence of intertidal microbial mats. Exhaustive studies of those microbial communities were later carried out by numerous researchers in the surrounding area of Abu Dhabi (Kendall and Skipwith 1968; Cardos *et al.* 1978) and the east of Saudi Arabia where microbial mats are dominated by cyanobacteria (Basson *et al.* 1977). Furthermore, Hoffmann (1996) indicated that there were three different type of mats in the intertidal zone of the Jubail Marine Wildlife Sanctuary in the Saudi Arabia, with the most important being

being the blister or folded type, which in many areas occurs in the upper intertidal zone. These types of cyanobacterial communities on mudflats are also known from other coasts of Arabian Gulf (Clayton 1986; Al-Hassan and Jones 1989) and are important components of the coastal ecology (Evans *et al.* 1964; Golubic 1992). Cyanobacteria are the main producers in microbial mats in the intertidal zone, but diatoms are also important components. Jones *et al.* (2002) suggested that the mudskipper (*Boleophthalmus boddarti)* and crabs (*Uca ucasindensis, Macrophthalmus dentipes and Leptochryseus kuwaitense*) are able to selectivity feed upon microbial mats rejecting diatoms, or that they ingest both but selectively digest *Cyanophyta* rather than diatoms. Preliminary investigation of gut contents reveals the presence of both algal groups in the gut, suggesting that selective digestion may occur. Casanova *et al.* (1993) suggested that shrimp might assimilate dissolved matter from the bacteria, whereas Polz *et al.* (1998) proposed that autotrophic bacteria in the gut might contribute to nutrition.

1.6 Application of stable isotopes in food web analysis

Both gut content analysis and stable isotopes have been used to study trophic linkages in marine food webs. Gut content analysis has been used quite widely in early studies but, as McTigue and Zimmerman (1991) pointed out, penaeid shrimp feeding habits are difficult to determine partly because the digestive process hampers identification of gut content material and may lead to an over-representation of less-digestible food items. For example, Mayer (1985) found that the gut contents of juvenile *P. setiferus* usually contained remains of polychaetes, tanaids, copepods, foraminifera, ostracods and fish but also a high percentage of unrecognizable matter. Stable isotope technique have increasingly been used and are becoming a standard analytical tool in food web

ecological studies (Cohen and Plisnier 2002) and several authors have studied carbon and nitrogen isotope content for primary producers and other components of coastal food webs (Table 2 & 3).

Stable isotopes of elements often occur naturally in different forms, such as nitrogen ($^{15}N/^{14}N$), carbon ($^{13}C/^{12}C$), sulphur ($^{34}S/^{35}S$). The isotopic ratios C, N, S in the organic matter of producers and in consumers have proved useful in describing the organic flow and food web relationships in estuaries and benthic communities (Machas and Santos 1999). Fry and Sherr (1984) reported that the most frequently used signatures are those derived from the natural abundance ratios of ($^{13}C:^{12}C$), ($^{15}N:^{14}N$) which are being increasingly used to delineate trophic relationships and to trace the origins of nutrients or pollutants in complex food webs (Michener and Schell 1994). Grey and Jones (2001) suggested that signatures could then be traced through the food web because the isotope ratios of organism reflect those of the diet in a dependable manner. However, the same authors showed that the stable isotope analysis approach requires the different basal resources available to a food web to show distinct and robust isotope signatures.

The ratio of isotopes is measured against a standard (marine fossil limestone and atmospheric air for carbon and nitrogen respectively). Photosynthesis introduces $\delta^{13}C$ variations among different plant species (Smith and Epstein 1971) and the $\delta^{13}C$ values of animals may be interpreted in terms of the relative carbon contributions from plants at the base of food chains. An isotopic ratio of an organism is usually understood to represent its diet.

Table (2) Stable carbon and nitrogen isotope signatures for six different sources of primary productivity in marine systems.

Primary producers	$\delta^{13}C$ ‰	Reference	$\delta^{13}N$ ‰	Reference
Phytoplankton	-19.6	Fontugne and Duplessy (1981) .	9.9 ± 0.9	Moncreiff and Sullivan
	-21	Haines and Montague (1979).		(2001).
	-21.64	Jones *et al.* (2002)	4.3±0.8	Hart *et al.* (2003)
	-19.9 ± 1.26	Loneragan *et al.* (1997).		
	-21.8 ± 0.7	Moncreiff and Sullivan (2001).		
	-20.59 ± 0.03	Thimdee *et al.* (2003).		
	-19.6 ± 0.12	Mohan *et al.* (1997).		
	-23.8	Primavera (1996)		
	-19.54±0.2	Hart *et al.*(2003).		
Seagrass	-16	Fry and Parker 1979.	3.4± 1.96	Loneragan *et al.* (1997).
	-8	Fry 1984	6.0 ± 1.1	Moncreiff and Sullivan
	-11.68± 2.2	Loneragan *et al.* (1997).		(2001).
	-6.7	Kharlamenko *et al.* (2001).	1.5 ± 0.9	Marguillier *et al.* (1997).
	-12.2 ± 1.2	Moncreiff and Sullivan (2001).		
	-10.5	Thimdee *et al.* (2003).		
	-16.23 ± 4.35	Marguillier *et al.* (1997).		
Mangrove	-27.1	Rodelli *et al.* (1984).	2.7 ± 0.91	Loneragan *et al.* (1997).
	-26.5	Mohan *et al.* (1997).	4.6 ±1	Bouillon *et al.* (2002).
	-28.25 ± 0.49	Bouillon *et al.* (2002).	1.9 ±0.38	Marguillier *et al.* (1997).
	-28.09	Jones *et al.* (2002).	2.55 ± 0.07	Primavera (1996)
	-28.1±0.65	Loneragan *et al.* (1997).		
	-28.60 ± 1.96	Thimdee *et al.* (2003).		
	-28.17 ± 2.8	Marguillier *et al.* (1997).		
	-26.9 to -30	Primavera (1996).		
Algae	-22.3 ± 2	Loneragan *et al.* (1997).	0.7	Loneragan *et al.* (1997).
	-16.8±0.5	Moncreiff and Sullivan (2001).	8.0±1.13	Bouillon *et al.* (2002).
	-15.6 ± 0.45	Thimdee *et al.* (2003).	7.0 ±2.8	Moncreiff and Sullivan (2001).
Epiphytic algae	-18.4	Rodelli *et al.* (1984	2.4 ± 0.63	Loneragan *et al.* (1997).
	-19.3	Fry 1984	5.9±0.9	Moncreiff and Sullivan
	-17.13	Jones *et al.* (2002).	6.0	(2001).
	-13.3	Loneragan *et al.* (1997).		Primavera (1996).
	-13.5 ± 0.3	Kharlamenko *et al.* (2001)		
	-17.5 ± 1.7	Moncreiff and Sullivan (2001).		
	-24±0.1	Primavera (1996)		
Microbial mat	-8.4	Barghoorn *et al.* (1977).		
	-6	Schidlowski *et al.* (1984).	-9.6 to +1.6	Van Dover (1994).
	-9.5	Schidlowski *et al.* (1995).	-1.13 to+0.39	Mueller (2005).
	-15.45	Jones *et al.* (2002).		
	-22.30 to -16.63	Mueller (2005)		

Table (3) Stable carbon and nitrogen isotope signatures for penaeid shrimp and other selected organisms from coastal food webs.

Organisms	$\delta^{13}C$ ‰	Reference	$\delta^{15}N$ ‰	Reference
Penaeid shrimp	-12.8	Van Dover (2002).	5.38 in seagrass	Fry *et al.* (1999).
	-12.11 in seagrass	Fry *et al.* (1999).	6.96 in	Fry *et al.* (1999).
	-21.75 in	Fry *et al.* (1999).	mangrove	Primavera (1996).
	mangrove	Mohan *et al.* (1997).	6.9	Sherwood and
	-20.37 ± 3.34	Fry (1984).	11.4 ± 1.1	Rose (2005)
	-18.1 ± 1.26	Primavera (1996).		
	-15.5 to -19.6	Jones *et al.* (2002).		
	-14.67	Rodelli *et al.* (1984).		
	-18.4 to -23.3	Sherwood and Rose		
	-18.2±0.25	(2005)		
	-13 to -8.5 in	Loneragan *et al.*		
	seagrass	(1997).		
	-22 to -16 in	Loneragan *et al.*		
	mangrove	(1997)		
Gastropoda	-20.25±0.63	Kurata *et al.* (2001).	9.9 ± 0.35	Kurata *et al.*
	-10.6 ± 1.2	Kharlamenko *et al.*	7.2 ± 0.42	(2001)
		(2001).		Bouillon *et al.*
	-25.7 ± 0.6			(2002)
	-18.59 ± 0.23	Bouillon *et al.*		
	-19.4 to -26.1	(2002).		
		Fry (1984).		
		Rodelli *et al.* (1984).		
Polychaeta	-13.9 to -22	Nithart (2000)	11.3 to 17.9	Nithart (2000)
	-16.4 ± 4.17 to	Iken *et al.* (2001)	13.7 ± 2.08	Iken *et al.* (2001)
	13.7 ± 2.08			
	-21.35 ± 0.07	Hsieh *et al.* (2002).		
	-16.36 ± 0.78	Fry (1984).		
Foraminifera	-21.32 ± 1.02	Iken *et al.* (2001).	9.55 ± 1.68	Iken *et al* (2001)
Isopoda	-20.9 ± 0.85	Sherwood and Rose	4.69 ± 0.26	Sherwood and
		(2005)		Rose (2005)
	-17.72	Iken *et al.* (2001).	14.35	Iken *et al.* (2001)
	-19.08.± 2.12	Fry (1984).		

The stable isotopic composition of an animal reflects that of its diet (Fry and Parker 1979; Checkley and Entzeroth 1985; Fenton and Ritz 1987) allowing for trophic shifts of ~ 3.5‰ for nitrogen and around 1‰ for carbon at each step in a food chain (DeNiro and Epstein 1978; Minagawa and Wada 1984). According to Cohen and Plisnier (2002), carbon provides information on the primary energy source such as benthic vs. pelagic photosynthesis whereas nitrogen allows discrimination between

trophic levels. Michener and Schell (1994) recorded the most abundant species in a pelagic food web from a linear food chain from phytoplankton to the copepod *Tropodiaptomus simplex* to the zooplanktivorous fish *Stolothrissa tanganicae* to the predatory fish *Lates stappersi*. They proposed that this linear food chain should be apparent as a distinct sequential enrichment in the carbon and nitrogen isotopes with increasing trophic level.

Stable isotopes can also be used to track migration and changes in utilization of different habitats with life stage. This can be a useful approach in many commercial marine species for which migration and movement are difficult to follow and for which the factors that control or promote movements and migrations are not well known (Fry 1981; Fry *et al.* 1999). Sheridan (1996) reported that *Penaeus duorarum* could move substantial distances in their lifetime, drifting inshore as young post larvae and migrating back offshore as large juveniles. Fry (1981) investigated the shift in $\delta^{13}C$ values in tissues of *Penaeus aztecus* as they moved between inshore seagrass beds enriched in ^{13}C compared with more depleted phytoplankton-based food web during their offshore pelagic phase. Harrigan *et al.* (1989) showed that *P. duorarum* found in seagrass meadows are 10-15‰ enriched in ^{13}C. Furthermore, the carbon in animals is generally isotopically similar, within a range of ±2 ‰ to the carbon in the diet (Fry *et al.* 1978).

Nitrogen is often used to determine trophic level (Fry and Sherr 1984; Peterson and Fry 1987) and the change in isotope ratio between diet and consumer between 2 to 4 ‰ (DeNiro and Epstein 1981; Minagawa and Wada 1984) For invertebrate food webs, it appears that differing enrichment in $\delta\ ^{15}N$ ($\delta\ ^{15}N_{organism}$ - $\delta\ ^{15}N_{diet}$) does not

always mean that the food chains have variable length (Vander Zanden *et al.* 1999a; Post *et al.* 2000). Adams and Sterner (2000) demonstrated that $\delta^{15}N$ enrichment varied with dietary nitrogen content for *Daphnia magna* by a range of 0 to 60 ‰ $\delta^{15}N$ depending on the C: N ratio of a single algal food. Also, Macko *et al.* (1982) showed the potential for difference in $\delta^{15}N$ fractionation between species of aquatic invertebrates. Vander Zanden and Rasmussen (1999) found that baseline $\delta^{15}N$ varied between habitats within the same aquatic ecosystems. The food sources of juveniles and adult shrimp species have been identified by using the stable isotopes of carbon and nitrogen in mangrove and seagrass systems (Fry and Arnold 1982; Primavera 1995; Macia 2004; Mutchler *et al.* 2004). On the other hand, in laboratory and pond experiments with *Litopenaeus vannamei*, Parker *et al.* (1989) used diets with enriched stable isotopes to determine the assimilation efficiency of ingredients in formulated diets.

Stable isotopes have also been used to measure protein turnover in aquatic animals (Hesslein *et al.* 1993; Sakano *et al.* 2005). Houlihan (1991) reported that protein turnover could by divided into three main processes: 1- protein synthesis 2- protein growth 3- protein degradation. Millward *at el.* (1976) described protein growth as a net balance between protein synthesis and protein degration at any particular time and in shrimp an increase in growth rate is a result of increased protein synthesis and reduced protein turnover (Mente *et al.* 2002). In general metabolic and growth rate are the main factors reported that influence protein turnover in muscle tissue of animals. Thus, temperature may play an important role in controlling the rate of carbon and nitrogen turnover in the tissue of organisms (Frazer *et al.* 1997), as organisms with a higher metabolic rate will equilibrate faster to a new diet than those with a low metabolism signature over larger periods (Tieszen *et al.* 1983). Also the quality and

quantity of diet may be affect the rate of protein turnover. Bosley *et al* (2002) found that turnover rates may also vary with life stage. For example, animals which are growing or developing quickly may have higher turnover rates than animals that are growing less rapidly or not at all. Similarly, Fry and Arnold (1982) reported that rapidly growing postlarvae of *Litopeneus aztecus* had higher carbon turnover rates than post larvae that grew more slowly.

1.7 Study area

Qatar is a peninsula situated located half way a long the western coast of the Arabian Gulf (Figure 2) The shoreline is irregular and has many bays and lagoons. The extent of the coastline, including the islands, is over 700 km in length (Al-Ansi 1995). The history of shrimp fisheries in the State of Qatar was described in detail by Al-Ansi (1995). Due to a decline in catches the Ministry of Municipal Affairs and Agriculture issued an order to stop shrimp fishing in 1993, until the shrimp stock recovers. From that time until present (2006) commercial shrimp fishing in Qatar has been banned. According to Van Zalinge (1984), most Gulf countries introduced an annual closure of differing length at the beginning of the fishing season during the period February-June.

Al-Ansi (1995) described the main shrimp fishing ground of Qatar as Doha and Al-Khor (Figure 2). The Doha shrimp grounds is an area of sand, mud and sand-shell bottom, whereas at Al-Khor the sediment is mainly sand–shell, with a hard bottom substrate and some muddy finer sediments. A total of six penaeid species have been recorded in Qatari waters (Al-Ansi 1995), and all of those species were found in Al-Khor, whereas the only four were found in Doha (Table 4).

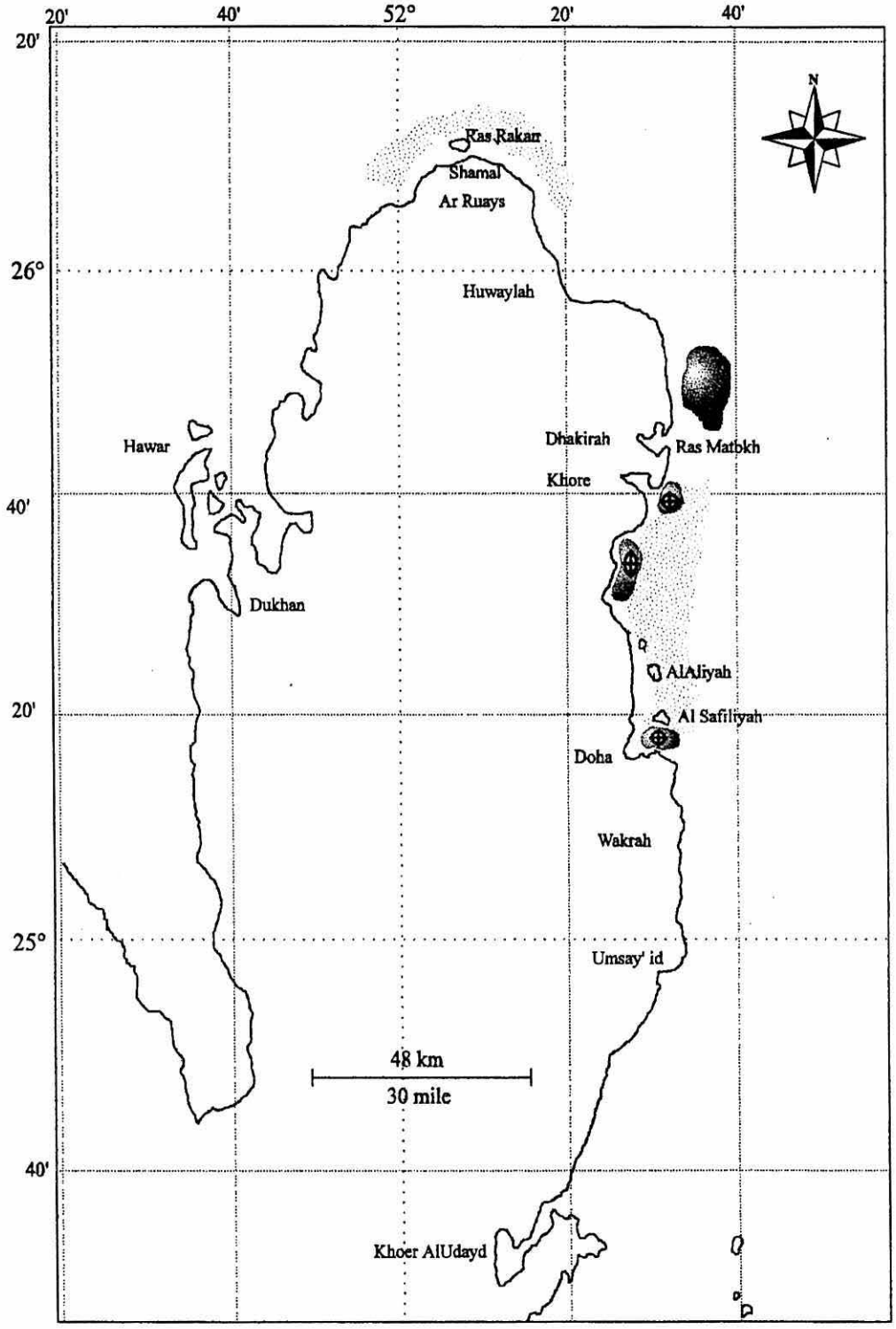

Figure (2) The main shrimp fishing ground around the Qatari coastline (from Al-Ansi 1995).

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Table (4) Common shrimp species in Doha and Al-Khor (Al-Ansi 1995). √ = present × = absent.

Species	Doha	Al-Khor
Penaeus semisulcatus	√	√
Penaeus latisulcatus	√	√
Metapenaeus affinis	√	√
M. stebbingi	√	√
M. mogiensis	×	√
Trachypenaeus curvirostris	×	√

Al-Ansi (1995), concluded that *Penaeus semisulcatus* is the most common shrimp species in Qatar waters, averaging 97.5% of the shrimp catch, with five other species that were less common in the market (Table 4). The main shrimp fishery in Qatar off Doha Harbour (Figure 3) has been impacted by long term damage to coastal habitats resulting from development activities such as building of new hotels, coastal engineering and dredging projects. All these operations have been carried out in the main nursery ground of the shrimp fishery. As a result, the shrimp fishery in Qatar is currently in a complex situation.

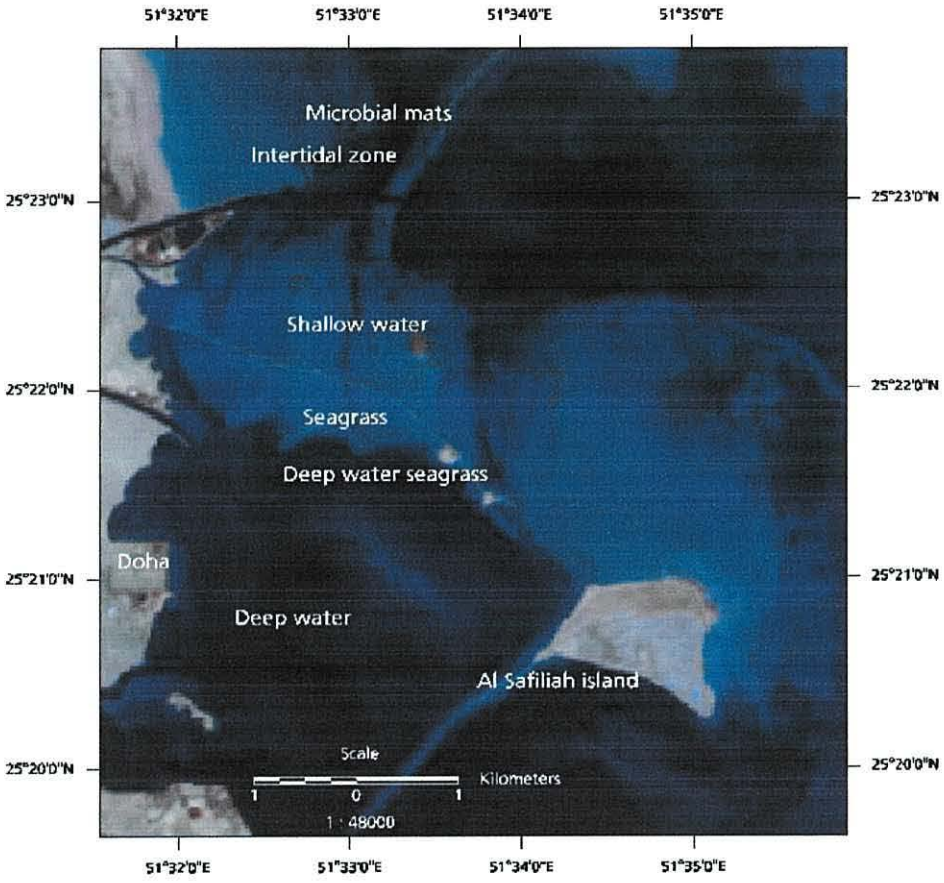

Figure (3) Satellite image of the Doha shrimp fishing grounds showing principal sublittoral habitats.

After Doha, Al-Khor, located in the northern part of the Qatar peninsula is the second main shrimp ground in Qatari waters (Figure 4).

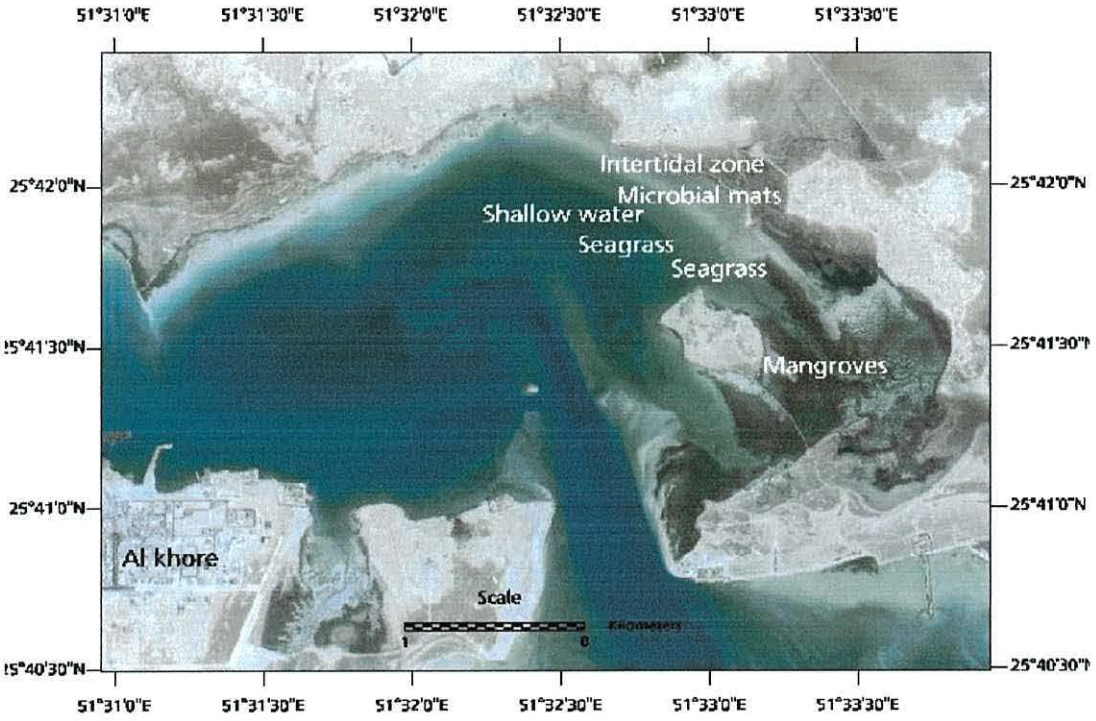

Figure (4) Satellite image of Al-Khor bay.

1.8 Distribution and ecology of *Penaeus semisulcatus*

The taxonomic classification of *Penaeus semisulcatus* is presented in Figure 5. *P. semisulcatus* is one of the larger penaeid shrimp and is distributed widely throughout the Indian and Pacific Oceans, ranging from South Africa to Japan (Grey *et al.* 1983; Rothlisberg *et al.* 1987). It is also found in the Red Sea and in the Mediterranean where it has migrated through the Suez Canal (Holthuis 1980). In Australia and many Arabian / Persian Gulf countries such as Kuwait, Bahrain, Saudi Arabia and Qatar *P.*

semisulcatus sustains important commercial fisheries. *P. semisulcatus* is also cultured in the Eastern Mediterranean in Turkey and Israel (Tom *et al.* 1984; Browdy *et al.* 1986; Seidman and Issar 1988; Colorni 1989; Kumlu *et al.* 2003).

Phylum: Arthropoda
Class :Crustacea
Series: Eumalacostraca
Superorder: Eucarida
Order :Decapoda
Suborder: Natantia
Infraorder: Penaeidea
Superfamily: Penaeoidea
Family: Penaeidae
Genus: *Penaeus*
Subgenus*: Penaeus*
Species*: semisulcatus*
Authority: De Haan, 1844

Figure (5) The taxonomic classification of *Penaeus semisulcatus*.

As in other penaeid shrimp species, the life cycle of *P. semisulcatus* has the following stages: (1) pelagic offshore larvae (2) settlement as benthic post larvae (3) juvenile recruitment to inshore nursery grounds, followed by later movement offshore as they grow to (4) development as subadult and adults in nearshore and offshore habitats (Dall *et al.* 1990; Sheppard *et al.* 1992). This is consistent with the very early observation of Viosca (1920) who proposed that the life cycle of most commercially important penaeids involves a predictable inshore juvenile phase and an offshore adult phase. After spawning, eggs are pelagic and hatch in less than 42 hours. Larvae (protozoa to mysis) undergo several development stages before post-larvae settle into shallow inshore water (Sheppard *et al.* 1992). Females may produce 50,000 to several

hundred thousand eggs, depending on size and temperature, whether they are wild or captive-bred, and on the number of times they have previously spawned (Rosenberry 1999). Jackson *et al.* (2001) reported that in Australia adult *P. semisulcatus* spawn in relatively deep water, at around 40 - 70 m. The close season for *P. semisulcatus* in the Gulf area varies regionally, depended on spawning season. For example, in Bahrain from December to March, in Saudi Arabia from October to April (Price and Jones 1975), and from December to May (Price 1979). Enomoto (1971) reported that the spawning season in Kuwait is from January to May (Al-Attar and Ikenous 1974), and from January to April (Mohamed *et al.* 1980). In Qatar, Al-Ansi (1995) reported that female *P. semisulcatus* became sexual mature in the Doha area between December to January and at Al-Khor mature females were found between October to January. The abundance of juvenile *P. semisulcatus* is closely tied to the distribution of submerged vegetation (Price and Jones 1975). Juvenile in shallow waters are often associated with seagrass beds (Grey *et al.* 1983) and adults in soft-sediment mud-sand substrates at depths of 2-130m (Holthuis 1980). Jones and Al Attar (1981) reported that seaweed, especially *Sargassum* sp. is an important habitat for post larvae and juvenile *P. semisulcatus* in the Arabian Gulf. This supports the suggestion by Basson *et al.* (1977) that *P. semisulcatus* is dependent on algae and seagrass beds during the post larvae and juvenile stages, and that the life cycle is synchronised to coincide with the period of maximum algal development and seasonal growth of seagrass beds.

1.9 Aims of the thesis

The aims of this thesis were :

1) To identify coastal nursery grounds for *P. semisulcatus* in Qatar and to confirm the link between microbial mats, seagrass beds and mangroves and post larval and juvenile shrimp using stable isotope methodology.

2) To use a range of techniques in an experimental approach in determining the potential role of microbial mats in the nutrition of the early settlement stages of the penaeid shrimp *P. semisulcatus*.

3) To determine the effect of diets on stable isotope (^{13}C and ^{15}N) content of shrimp tissue post larvae over time and to use stable isotopes to study C and N turnover.

Chapter 2

Feeding ecology of the grooved tiger shrimp *Penaeus semisulcatus* De Haan (Decapoda: Penaeidae) in inshore waters of Qatar, Arabian Gulf

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2.1 Introduction

The grooved tiger shrimp *Penaeus semisulcatus* is an Indo-Pacific species that is distributed from southeast Africa to Japan and North Australia. In some areas, for example northern Australia and the Arabian Gulf, it is an important component of commercial shrimp fisheries (Somers 1994; Al-Ansi 1995). In the Arabian Gulf, *P. semisulcatus* represented 97.5 % of the shrimp catch in Qatari waters in 1992, with five other species making up the rest of shrimp landings (Al-Ansi 1995). More recently, declining landings due to overfishing and habitat loss have resulted in closure of the commercial shrimp fishery in several countries in the Arabian Gulf, including Qatar in 1993 (Al-Ansi 1995; Anon. 2001) and the United Arab Emirates since the 1970's (Anon. 1997). In Kuwait and Bahrain shrimp landings have declined in recent years due to both over-fishing and military conflict (Anon. 1997; Mohammed *et al.* 1998).

As with other penaeid species, *P. semisulcatus* is mostly found in shallow, soft-sediment, coastal habitats with early life stages closely linked with sheltered productive habitats (Fry and Parker 1979; Coles *et al.* 1987; Heales 2000). Despite the urgent need for conservation of critical habitats to support recovery of shrimp populations in the Arabian Gulf, relatively little is known about the feeding and nutrition of *P. semisulcatus* postlarvae and juveniles in the natural environment. In general, nursery grounds for post-larvae and juvenile penaeid shrimp are usually characterized and influenced by the presence of vegetation such as mangrove forests and seagrass beds (Rönnbäck *et al.* 2002). In both Australia and the Arabian Gulf, seagrass and algal beds have been identified as important nursery areas for *P.*

semisulcatus (Basson *et al.* 1977; Jones and Al Attar 1981; Sheppard *et al.* 1992; Haywood *et al.* 1995; Loneragan *et al.* 1998). Stable isotope studies indicate that primary production in both seagrass and adjacent mangrove are as contribute to nutrition of juveniles, though the relative importance of these sources can be seasonally variable (Loneragan *et al.* 1997). In the Northern Arabian Gulf, particularly in Kuwait waters, seagrass beds and mangroves are scarce but shrimp (*P. semisulcatus* and *Metapeneus* spp.) are abundant. In these areas, intertidal microbial mats appear to be a major source of primary production, supporting benthic and epibenthic invertebrates, including penaeid shrimp (Jones *et al.* 2002; Al-Zaidan *et al.* 2006). Laboratory feeding studies have shown that *P. semisulcatus* postlarvae may feed on microbial mats from first settlement, supporting high growth rate and survival (Al-Maslamani *et al.* 2005). This is consistent with observations that other penaeid species feed by browsing through the sediment surface, ingesting a range of epibenthos and infauna (McTigue and Zimmerman 1991). For example, gut contents of juvenile *Penaeus setiferus* in subtropical tidal creeks in South Carolina, USA, have been found to comprise mostly remains of plant material, polychaetes, small crustacean and fish together with a significant proportion of unrecognisable detritus (Hunter 1984; Mayer 1985; Nelson and Capone 1990). Similarly, juvenile *P. semisulcatus* feeding in intertidal seagrass and subtidal algal beds in tropical Australia estuaries have been found to ingest a range of benthic invertebrates, diatoms and filamentous algae (Heales 2000; Wassenberg and Hill 1987).

However, determination of dietary components from stomach contents may be biased due to different digestibility of soft and hard tissues (O'Brien 1994; Schwamborn and Criales 2000) and food items may be hard to identify due to mechanical grinding by

the mandible and gastric mill (Chansang 1984; Dall *et al.* 1990; McTigue and Zimmerman 1991). Given the limitations of stomach content analysis, other dietary measures such as stable isotope analysis present a significant advantage in that the isotope ratios yield time-integrated dietary information that reflects assimilated, and not solely ingested, food. Stable isotopes have been widely used in studies of aquatic food web structure and function.

The stable isotope ratios for C, N and S in the organic matter of primary producers and in consumers have proved useful in describing the organic flow, nutrient sources and food web relationships (Cohen and Plisnier 2002). Such studies have largely been based on applying estimates for trophic shifts of ~ 3.5‰ for nitrogen and around 1‰ for carbon (DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984), though interpretation of possible diet and trophic relationships can be problematic (Vanderklift and Ponsard 2003; McCutchan *et al.* 2003; Yokoyama *et al.* 2005). However this technique has been widely used in studying trophic relationships in aquatic food webs, including penaeid shrimp, in relation to several sources of primary production (e.g. Fry 1981; Fry and Parker 1979; Rothlisberg 1998; Chong *et al.* 2001; Stoner and Zimmerman 1998; Primavera 1996; Loneragan *et al.* 1997). The aim of the present study was to use both gut content and stable isotope approaches to assess the relative importance of nursery habitats for postlarval and juvenile shrimp *P. semisulcatus* where microbial mats, seagrass and macroalgal beds are present in adjacent areas.

2.2 Materials and Methods

The study was carried out in the intertidal and subtidal zones of the main shrimp fishery grounds of the State of Qatar, northeast of Doha harbour near Al-Saflia Island (Figure 1). In this area, the seabed sediment is mainly sand, mud and shell-gravel. Subtidal benthic communities include those dominated by seagrasses and macroalgal beds, while intertidal mud flats are dominated by microbial mats. No mangroves are present on the adjacent coastline. Samples of post larvae, juvenile and adult shrimp were collected from start of the spawning season (January – June). Three areas were sampled that represented two subtidal habitats (Figure 1). Sites 1 and 2 had sand, sandy-mud & shell gravel sediments, which were mainly covered by seagrass beds. At site 1, water depth ranged from 0.3-1.0 m, while site 2 extended to a depth of 2-5 m. Site 3 mainly comprised deeper macroalgal beds (depth 5 – 10m) on sand and sandy mud. All samples of shrimp were collected at high tide. In the subtidal zone at all three sites, a small otter trawl net was towed by a small boat with outboard engine. Mesh size of the main net was 50 mm, with 5 mm in the cod end. In shallow water at sites 1 & 2, at high tide, a small hand-pulled seine net of mesh 0.5 mm was used. These methods will collect all shrimp down to the earliest post-larval stages. During the sampling period, surface seawater temperature and salinity were monitored using mercury bulb thermometer and a portable refractometer.

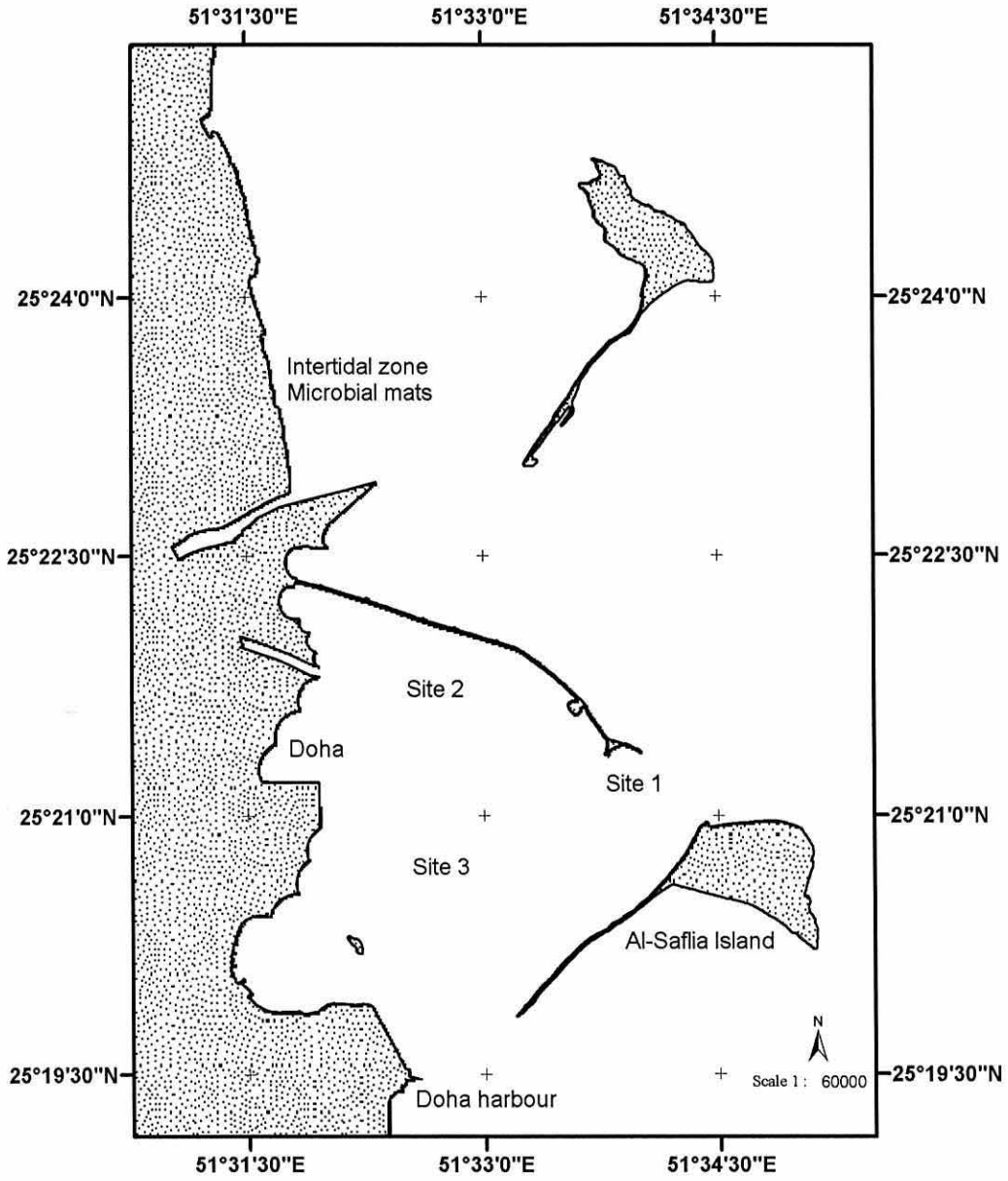


Figure (1) Location of the study area and sampling sites, showing the main habitats.

All shrimp samples were placed on ice in the field, before sorting and preservation in the laboratory. Species were identified and samples were measured (total length and wet weight). From shrimp collected at sites 1 and 3, specimens for gut content analysis were stored in plastic or glass jars in 5% buffered formalin. Shrimp stomachs

were later dissected under a binocular microscope, contents identified and relative abundance recorded as frequency of occurrence.

Benthic invertebrates were collected by grab sampling of sediments at Sites 1 and 2, washing over 0.25mm and 0.5 mm meshes, and subsequently sorted under binocular microscope. Samples of primary producers from the general study area were taken for stable isotope analysis, including microbial mats, macroalgae, phytoplankton, seagrass and epiphytic algae scraped from blades of seagrass.

Dried (50-60 °C, 24 hrs) homogenised, samples of primary producers, muscle tissue excised from the tails of shrimp and other whole organisms were weighed into precombusted silver boats (500°C, 3hrs) and carbonate material was removed through a combination of HCl (10%) additions and drying at ~50°C. For isotopic analysis, the decalcified samples were placed in pre-combusted (910°C, 3 hours) quartz tubes with copper and copper oxide.

The stable C and N isotopic composition was determined on CO_2 and N_2 generated by vacuum combustion and is reported in the δ notation as the ratio of the heavy to the light stable isotope in the material, R_{sample}, relative to that of a standard, $R_{standard}$, with standard = Vienna Pee Dee Bellemnite (VPDB) and air for carbon and nitrogen respectively, i.e

$$\delta_{sample} = 1000\left(\frac{R_{sample}}{R_{std}} - 1\right)$$

The gases were separated and collected by vacuum distillation from the same sample and analyzed on a EUROPA-PDZ GEO 20/20 isotope ratio mass spectrometer ($\delta^{13}C$) and VG SIRA II dual inlet isotope ratio mass spectrometer ($\delta^{15}N$).

2.3 Results

During the sample collection period, surface seawater temperatures increased from 18.5 °C in January up to a maximum of 32 °C in June, while salinity declined from 42 to 35.5 ‰ (Table 1). Different sizes of *P. semisulcatus* were caught at each of the three sites, with post larvae more abundant in samples from the shallowest and adults more abundant in samples from the deeper sites (Table 2).

Table (1) Mean monthly surface temperature and salinity in the study area during the sampling period.

	January	February	March	April	May	June
Temperature °C	18.7	19.7	22	26.5	28	32
Salinity ‰	42	42	44.5	41	36.5	35.5

Table (2) Location and length ranges of *Penaeus semisulcatus* samples collected from the study area.

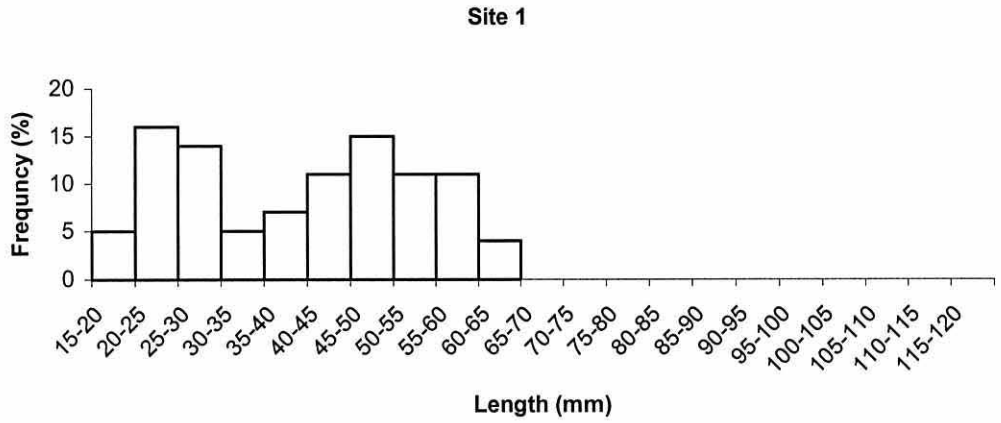
Site	Habitat	Depth (m)	Life stage	Total length (mm)
1	Shallow water seagrass (sand-mud)	0.3-1	Post larvae , Juvenile and early post larvae	18.6 - 64.20
2	Deep water seagrass (sandy-mud, sand and shell gravel)	2 - 5	Juvenile and adult	52- 100
3	Deep water (sand, sandy-mud with macroalgal beds)	5 - 10	Adult	60- 120

The length frequency distributions for *P. semisulcatus* collected from three sites are shown in Figure 2. The mean total length of *P. semisulcatus* from the Site 1 was 41.9

± 13.4 mm, while shrimp from Site 3 were significantly larger at 81 ± 14.3 mm ($p<0.05$, ANOVA). Shrimp collected from Site 2 were of intermediate in size, 77± 10.9 mm, and significantly different in size to those from the other two sampling sites ($p<0.05$, ANOVA).

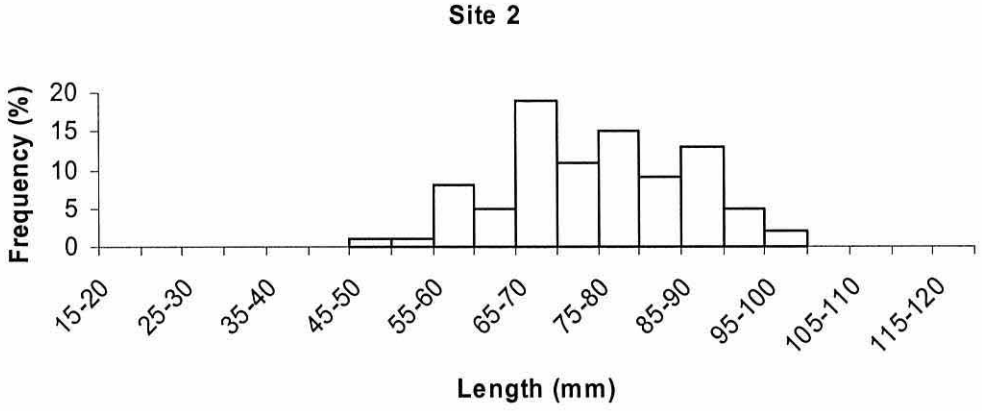
Gut contents of 693 post-larvae, juvenile and adult shrimp from sites 1 and 3 were examined. Of these, 55 % were caught at Site 1 and 45 % at Site 3, At Site 1 most shrimp were post-larvae and juveniles and the most frequent components of the gut were a diverse range of benthic epifauna and infauna including molluscs (19.6%), Foraminifera (30.3%) and Crustacea (19.3%) together with benthic diatoms (7.8 %), green algae (0.7 %,) and fish (0.3 %). Unidentified debris made up the remaining 18.6 % (Figure 3).

At Site 3, *P. semisulcatus* were mainly large juvenile and adults and the most common food items in the gut were bivalve shells (41.9%), with less frequent occurrences of polychaetes (8.6%), and crustaceans (9.7%) and green algae (7%). At this site unidentified debris contributed 32.3% of gut contents (Figure 4).



(a)

(b)



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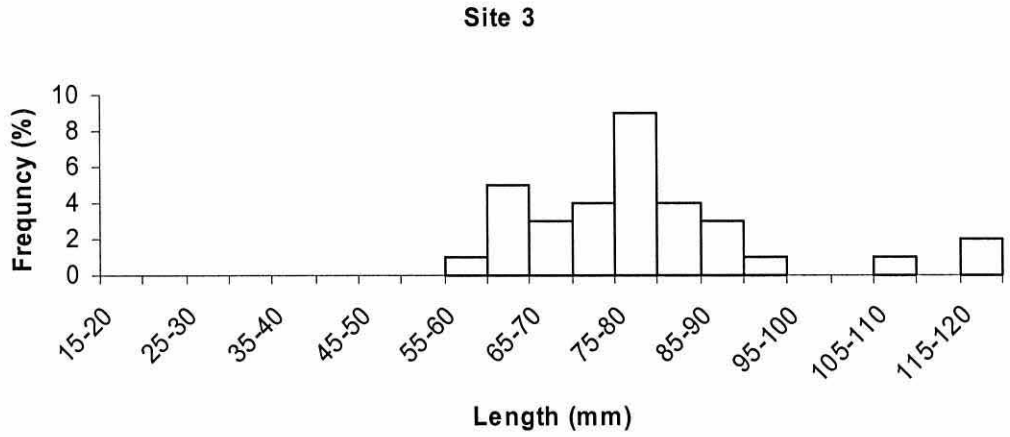

Figure 2 (a,b,c) Length-frequency distributions (total length) for *P. semisulcatus* sampled in seagrass beds (Sites 1 & 2) and deeper algal beds (Sites 3).

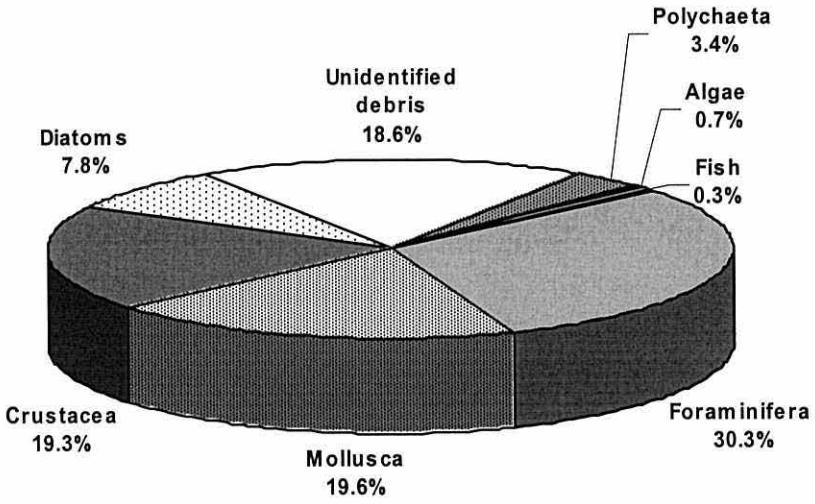

Figure (3) Percentage of food items by frequency of occurrence in gut contents for postlarval and juvenile *P. semisulcatus* in shallow water seagrass beds (Site 1).

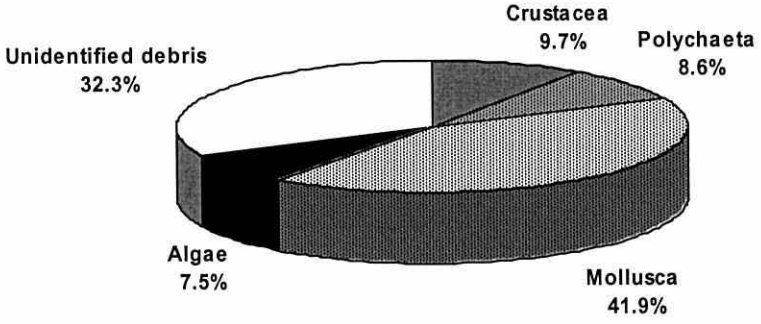

Figure (4) Percentage of the main food items by frequency of occurrence in gut contents of *P. semisulcatus* in deeper water algal beds (Site 3).

P. semisulcatus of differing ages and from the three sites had a narrow range of $\delta^{13}C$ values from -9.5 ± 0.26 to -12.7 ± 0.05 ‰ and $\delta^{15}N$ values ranging from 5.4±1.1 ‰ to 7.7 ± 0.1‰ in. (Table 4). When divided into life stages the most negative $\delta^{13}C$ values were found in juvenile and adult *P. semisulcatus* from the deeper water seagrass beds (DJAS) at Site 2 and the adult *P. semisulcatus* (DA) from Site 3 (Figure 5). The least negative $\delta^{13}C$ values were recorded in post larvae and juveniles from shallow seagrass beds (SSE, SSP and SSJ) at Site 1 (Figure 5). For $\delta^{15}N$ the post-larvae and juveniles at site 1 were lighter than the juveniles and adults collected at sites 2 and 3 (Figure 6). There were significant differences between $\delta^{13}C$ and $\delta^{15}N$ values for shrimp from the three sites (one way ANOVA), and could be divided into three groups (Tukey's method, pairwise comparisons). Group 1 included only early post larvae from Site 1. Group 2 included older postlarvae and juveniles from Site 1, and Group 3 included juveniles & adults from Site 2 and adults from Site 3.

The $\delta^{13}C$ values of the primary producers covered a larger range of 11.4‰, with two distinct groupings (Table 3). The $\delta^{13}C$ values for phytoplankton (PH), epiphytic algae (Es) and the green algae *Chaetomorpha aerea* (G), (-19 ± 1.4‰, -14.6 ± 0.2 and -15.7 ± 0.2‰, respectively) were more negative than values for microbial mats (M) and seagrasses (S1 and S2), ($\delta^{13}C$ -7.7 ± 0.1‰ and -7.9 ± 1.3 ‰ for sites 1 and 2 respectively). The $\delta^{15}N$ values for the primary producers also divided into similar groupings, but with more positive $\delta^{15}N$ values for the phytoplankton , green algae and epiphytic algae (4.1 ± 0.0‰ , 3.4 ± 0.1‰ and 3.2 ± 0.05‰, respectively) than for the microbial mat and seagrass (-0.2 ± 0.9 ‰ and 1.5 ± 0.4‰, respectively).

Of the benthic organisms collected from seagrass beds, foraminifera (FS), polychaetes (PL) and gastropods (GS) had a relatively small range of $\delta^{13}C$ values (6.9‰) (Table 3). Gastropods were the most ^{13}C enriched (-10.9 ± 0.1‰) and close to the $\delta^{13}C$ values of polycheates (-11.0 ± 0.1‰) and foraminifera (-13.3 ± 0.8). The bivalves (principally *Amiantis umbonelle*) in shallow water were the least ^{13}C enriched with a $\delta^{13}C$ values of -17.8 ± 0.5‰. The bivalves, gastropods and polychaetes had similar $\delta^{15}N$ values (4.±0.2, 5.6±0.9, 6.4±0.2‰ respectively) while the foraminifera had much lighter values (1.9 ± 0.1‰).

Table (3) Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for primary producers and other selected components collected from the study area. Values are means ± s.d.

Sample type/ Site	Abbreviation	No. of samples	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
Primary producers				
Phytoplankton	PH	3	-19.1 ±1.4	4.1
Epiphytic algae from shallow-	Es	3		
water seagrass (Site 1)			-14.6 ± 0.2	3.2 ± 0.1
Green alga, *Chaetomorpha aerea*	G	3		
(Site 3)			-15.7 ± 0.2	3.4 ± 0.1
Seagrass *Halodule uninervis*	S1	3		
(Site 1)			-6.9 ± 0.6	1.2 ± 0.2
Seagrass *Halophila stipulacea*	S2	3		
(Site 1)			-8.8 ± 0.5	1.8 ± 0.8
Microbial mat (intertidal zone)	M	3	-7.7 ± 1	-0.2 ± 0.9
Consumers/prey				
Foraminifera in seagrass beds	FS	3	-13.3 ± 0.8	1.9 ± 0.1
Polychaetes in seagrass beds	PL	3	-11.0 ± 0.1	6.4 ± 0.2
Bivalve *Amiantis umbonelle.*	BV	3	-17.8 ± 0.5	4.5 ± 0.2
Gastropods in seagrass beds	GS	3	-10.9 ± 0.1	5.6 ± 0.9

Table (4) Stable isotope values ($\delta^{13}C$ ‰ and $\delta^{15}N$‰) and total length (mm) for samples of *P. semisulcatus* collected from the study area. Values are means ± s.d. For stable isotopes values, rows bearing different superscripts are significantly different (one way ANOVA & Tukey's pairwise comparisons, $p < 0.05$).

Sample type/Site	Abbreviations	Life stage	Total length (mm)	No. of individual	No. of samples	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰.
Shallow water seagrass early postlarvae (Site 1)	SSE	Postlarvae	19.9 ± 1.6	20	3	-11.3 ± 0.3^{c}	5.4 ± 1.1^{c}
Shallow water seagrass postlarvae (Site 1)	SSP	Postlarvae	28.3 ± 4.0	50	3	-9.7 ± 0.25^{b}	5.6 ± 0.17^{b}
Shallow water seagrass juvenile (Site 1)	SSJ	Juvenile	51.4 ± 6.9	>100	3	-9.5 ± 0.26^{b}	6.4 ± 0.05^{b}
Deep water juvenile and adult in seagrass (Site 2)	DJAS	Juvenile/ adult	77.0 ± 10.7	96	3	-12.7 ± 0.05^{a}	7.4 ± 0.00^{a}
Deep water adult shrimp (Site 3)	DA	Adult	80.2 ± 14.2	35	3	-12.5 ± 0.10^{a}	7.7 ± 0.11^{a}

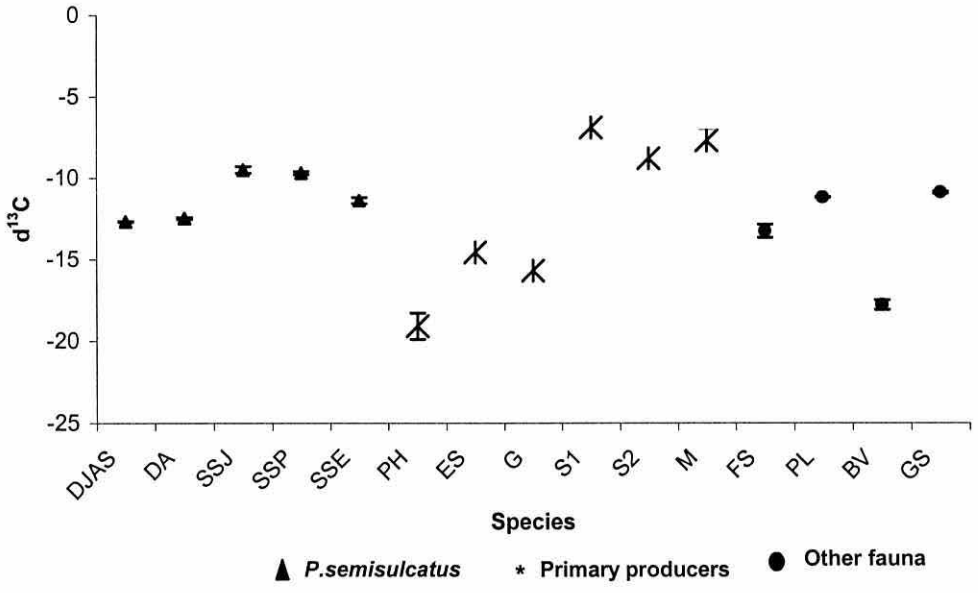

Figure (5) $\delta^{13}C$ values of *P. semisulcatus* primary producers and prey species (abbreviations are explained in Tables 3 and 4). Values are mean ± s.d., n = 3 for each point.

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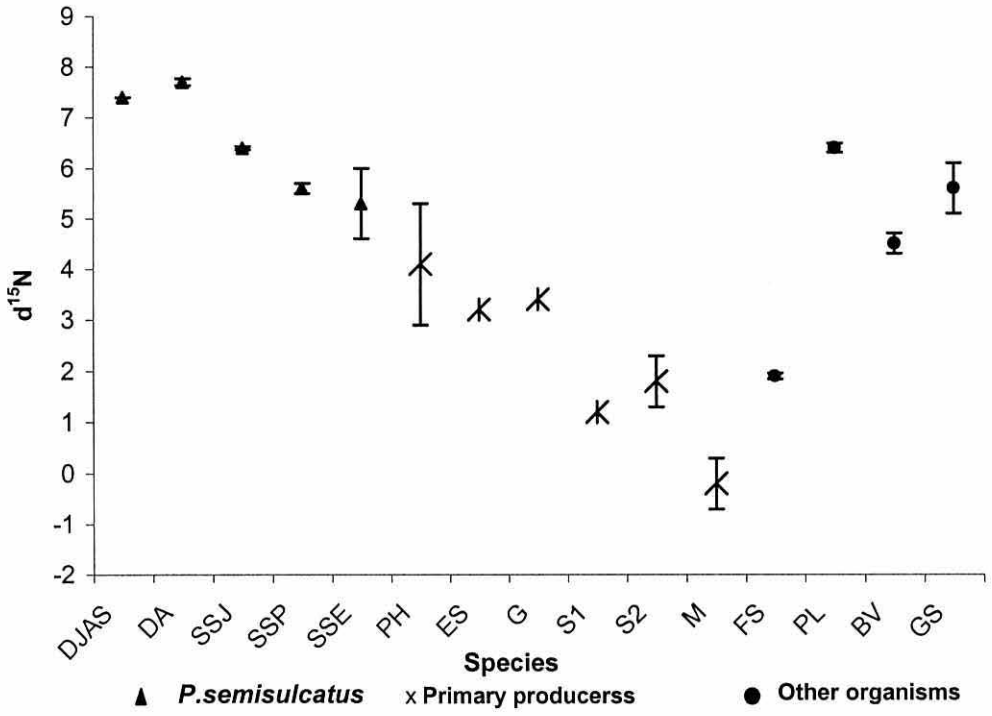

Figure (6) $\delta^{15}N$ values of *P. semisulcatus*, primary producers and other selected prey species (abbreviations are explained in Tables 3 and 4). Values are mean ± s.d., n = 3 for each point.

2.4 Discussion

The length-frequency distribution of *P. semisulcatus* (20 to 120 mm) suggests that not all life stages of the shrimp were found during this study. The earliest post-settlement post-larval stages (5 to 15 mm) were not found in any of sampled areas. However, Al-Maslamani *et al.* (2005) have shown in experimental studies that microbial mats can support excellent survival and growth in early-stage *P. semisulcatus* post-larvae and it is possible that the main habitat for the earliest stages is closer inshore where microbial mats are the predominant intertidal benthic community.

The data collected indicate that seagrass beds are the main nursery grounds for post-larval and juvenile *P. semisulcatus* in the study area. This is consistent with studies of this species in other areas where seagrass beds are present (Young and Carpenter 1977; Zimmerman *et al.* 1984; Staples *et al.* 1985; Coles *et al.* 1987; Dall *et al.* 1990; Sheppard *et al.* 1992: Hill and Wassenberg 1993; Loneragan *et al.* 1994; Haywood *et al.* 1995; Heales *et al.* 1996; Loneragan *et al.* 1998). The presence of older shrimp in Site 3 is consistent with migration of subadult and adult *Penaeus* sp. to deeper water where macroalgal beds predominate (Dall *et al.* 1990; Somers and Kirkwood 1991; Crocos and Van der Velde 1995; Jackson *et al.* 2001).

Many studies have indicated both omnivorous and carnivorous feeding in juveniles of a range of penaeid shrimp species, with the most commonly observed prey being crustaceans, molluscs, polychaetes and other benthic invertebrates (Chong and Sasekumer 1981; Leber 1985; Wassenberg and Hill 1987, Hill and Wassenberg 1993; Nelson and Capone 1990; O'Brien 1994; Schwamborn and Criales 2000) and the prevalence of benthic invertebrates in the gut of *P. semisulcatus* in the present study is consistent with others studies of this species in Australian waters (Wassenberg and Hill 1987; O'Brien 1994; Haywood *et al.* 1995; Heales *et al.*1996). In the present study, comparison of the gut contents indicates a clear transition in the diet of shrimp as they grow and move from shallow water seagrass beds to deeper water. At the shallow site, small organisms such as diatoms and foraminifera constitute up to half of the apparent diet. In contrast, at the deeper site, these food items were absent from shrimp stomachs and a bivalve was most common (41.3 %).

Overall, the gut contents data suggest that juvenile and adults of *P. semisulcatus* are benthic omnivores but develop a predominantly carnivorous diet as they grow, feeding on one or two dominant prey species, as has been observed in this other penaeid species (Hunter 1984; Mayer 1985; Nelson and Capone 1990; Schwamborn and Criales 2000; Heales 2000). However, examination of gut contents alone may not accurately reflect dietary intake due to under-representation of readily homogenised and digested items, such as epiphytic algae, relative to components such as gastropod and bivalve shells that are more resistant to mechanical and enzymatic digestion (Hill 1976; Schwamborn and Criales 2000). At both sites, a significant proportion of gut contents was unidentifiable debris (Site 1 18.6% and Site 2 32.2 %). This may reflect partially-digested food and also detrital material ingested in the non-selective feeding behaviour of postlarval *P. semisulcatus* (Al-Maslamani *et al.* (2005).

Stable isotopes have been used to determine the primary source(s) of carbon to coastal food webs as they reflect the propagated $\delta^{13}C$ values, with little step-wise enrichment, along the food chain. Estimates of the isotopic shift between diet and consumer have indicated a shift in $\delta^{13}C$ ranging between -1.5 and +2.7‰ with trophic level, but of +1.3±0.3‰ for consumers analysed as muscle tissue McCutchan *et al.* (2003). For *Litopenaeus vannamei,* in experimental studies, Parker *et al.* (1989) found a $\delta^{13}C$ trophic enrichment of a similar magnitude (+1.7‰) , while Yokoyama *et al.* (2005) reported values of +2.0‰ in the ghost shrimps *Nihonotrypaea japonica* and *N. harmandii.* Previous studies to determine food sources of juveniles and adult penaeid shrimp in mangrove, seagrass and mud flats systems have found that $\delta^{13}C$ values range between -14.67 to -21.75 ‰ (Primavera 1996; Mohan *et al.* 1997; Fry *et al.*1999 ; Jones *et al.* 2002; Van Dover 2002; Rothlisberg 1998). The large isotopic

range in shrimp tissue values reflects the diversity of their environmental settings and hence the varying primary sources of carbon supporting the food webs in the different habitats (Loneragan *et al.* 1997). In most cases, it has been concluded that mean shrimp $\delta^{13}C$ values are probably a reliable index of local feeding conditions (Fry 1981), while taking into account the trophic shift for carbon (see p 17).

The primary producers at Doha indicate that they have isotopically distinct values of $\delta^{13}C$ in the range reported in other studies for phytoplankton (Fontugne and Duplessy 1981), seagrasses (Hemminga and Mateo 1996) and microbial mats (Jones *et al.* 2002, Abed-Navandi and Dwarschak 2005). In the present study, there was no difference in $\delta^{13}C$ between post-larvae and juveniles from the same site, but there were significant differences between sites that may reflect both diet and life stage. The post-larvae and juveniles at site 1 (-9.5 ±0.26 to -11.3±0.3‰) were heavier than the adults collected at sites 2 and 3 (-12.5±0.1 to-12.7±0.05‰). The $\delta^{13}C$ in animals associated with seagrass beds are usually heavier than those in animals found offshore, reflecting the difference in isotope ratios between primary producers within and away from seagrass meadows (Parker and Calder 1970; Thayer *et al.* 1978; Fry and Parker 1979). Taking a $\delta^{13}C$ trophic enrichment of between 1.3 to 2‰, the potential diet of the post-larvae and juveniles at site 1 could be between -7.5 and -9‰.

In the potential sources (Table 3) of carbon (seagrasses and microbial) mat have $\delta^{13}C$ values that cover a similar range, while the major stomach content (foraminifera, 30.3%) and other prey associated with the seagrass beds have slightly more negative values (Table 3). Given the large proportion of un-identifiable detritus and diatom frustules (26.4% of gut contents), the $\delta^{13}C$ of the post-larvae and juveniles shrimp at

site 1 may be indicative that they are feeding on, and assimilating, the sedimentary material in the seagrass beds. However, some contribution to their diet from microbial mats cannot be discounted. Previous field studies have suggested that there is a link between microbial mat productivity and commercial fish and shrimp species on the Kuwait coast, in the Northern Arabian Gulf (Jones *et al.* 2002) and controlled laboratory experiments have shown also that intertidal microbial mats appear to be important sources of nutrition for shrimps (Al-Maslamani *et al.* 2005; Al-Zaidan *et al.* 2006). Although no identifiable components of the algal tissues from the microbial mats were observed in the gut contents, the presence of the relatively refractory diatom frustules, which are also an important component of the microbial mat, may indicate some recent reliance on this carbon source.

The adults collected at sites 2 and 3 (-12.5±0.1 to-12.7±0.05‰) were heavier in terms of $\delta^{13}C$ than the post-larvae and juveniles at site 1. Taking the same $\delta^{13}C$ trophic enrichment, the potential diet of the adults at sites 2 and 3 could be between -10.5 and -11.4‰. These $\delta^{13}C$ values are still commensurate with prey associated with the seagrass beds, but the gut contents suggest a lower reliance on prey predominantly found associated with the seagrass beds (i.e. foraminifera) and there is a larger proportion of unidentified material (32.3%). The more negative values of the adults as compared to postlarvae and juveniles are also consistent with a greater contribution of other primary sources of carbon (e.g. phytoplankton and green algae), found at the deeper sites but not specifically identified in the gut contents and/or an increasing contribution of animals such as bivalves in the diet (19.6% for site 1 versus 41.9% for site 3). The carbon isotopic signature of *Amiantis umbonelle* ($\delta^{13}C$ -17.8‰) reflects that of the particles it filters and so the ^{13}C depletion in *P. semisulcatus* as they grow

and move from site 1 to 2 and 3 may reflect both a change in the primary source of carbon to the food chain as well as a change in diet.

Measurements of $\delta\ ^{15}N$ in consumers show that they are generally heavier (3‰) than their diet and that the degree of nitrogen isotope enrichment is a measure of the animals trophic status (DeNiro and Epstein 1981; Peterson and Fry 1987; Fry and Quiñones 1994; Loneragan *et al.* 1997). Thus the $\delta^{15}N$ values depend both on those of the primary producers and on the N pathway through the food chain. However, the trophic shift in $\delta^{15}N$ can be quite variable (Vanderklift and Ponsard 2003; McCutchan *et al.* 2003; Yokoyama *et al.* 2005). A review of isotopic shifts between diet and consumers has found that the $\delta^{15}N$ trophic shift for consumers ranges from -0.8‰ to +5.9‰, with an average value of +2‰ McCutchan *et al.* (2003). To illustrate the complexity in interpreting trophic enrichment, Yokoyama *et al.* (2005) have recently shown that the trophic shift in $\delta^{15}N$ for the ghost shrimps fed a single type of food was at the higher end of the range at ~ +4‰, while, in general, animals raised on invertebrate diets can exhibit the lower values for the $\delta^{15}N$ shift (+1.4 ± 0.2‰) (Vanderklift and Ponsard 2003, McCutchan *et al.* 2003).

The $\delta^{15}N$ values of the primary producers in the present study were less isotopically distinct than for $\delta^{13}C$ but are commensurate with ranges reported elsewhere for phytoplankton (Moncreiff and Sullivan 2001) and seagrasses (Marguillier *et al.* 1997; Moncreiff and Sullivan 2001) and microbial mats (Abed-Navandi and Dwarschak 2005).

There were significant differences in $\delta^{15}N$ of the post larvae and juveniles between sites that may reflect both diet and life stage. The post-larvae and juveniles at site 1 (5.4±1.1 to 6.4±0.05‰) were lighter than the juveniles and adults collected at sites 2 and 3 (7.4 to 7.7±0.11 ‰). Taking a trophic enrichment of between 1.4 to 4‰, the diet of the post-larvae and juveniles at site 1 could be between +1.4 to + 5‰. This range describes values that are much heavier than those observed for either seagrass or microbial mats but which are similar to those of the animals inhabiting the seagrass meadows (Table 3). Again, the gut contents of shrimps at site 1 contained large amounts of unidentifiable (18.6%) or recalcitrant (diatom frustules 7.8% and foraminifera tests 30.3%) material and microbial alteration of this material may be a contributory factor that has influenced the $\delta^{15}N$ assimilated by the shrimp.

Heterotrophic processes do not fractionate stable carbon isotopes but cause an increase in the variability of stable nitrogen ratios, making them less useful to distinguish between fresh and senescent material as a food source (Fellerhoff *et al.* 2003). The adult shrimp at sites 2 and 3 had heavier $\delta^{15}N$ values than the post-larvae and juveniles at Site 1 (Table 3). Taking the same trophic enrichment of between 1.4 to 4‰, the diet of the adults at sites 2 and 3 could be between +3.4 to + 6.3‰. The more positive values of the adults as compared to postlarvae and juveniles are (as for $\delta^{13}C$), consistent with a greater contribution of other primary sources of carbon (e.g. phytoplankton and green algae), found at the deeper sites but not specifically identified in the gut contents. There was still a large proportion of unidentifiable detritus in the gut contents (32.2%) of these shrimp and an increasing contribution of animals such as bivalves in the diet. Hence the ^{15}N enrichment in *P. semisulcatus* as

they grow and move from site 1 to 2 and 3 may reflect both a change in the primary source of nitrogen to the food chain as well as a change in trophic level.

Overall, the ages, location (habitat) and diet of shrimp are important in determining stable isotope ratios in their tissues. Establishing trophic links by stable isotopes alone is difficult given the uncertainty over enrichment levels for carbon and nitrogen (Vanderklift and Ponsard 2003; McCutchan *et al.* 2003; Yokoyama *et al.* 2005). However, the combination of gut content and stable isotope data demonstrates that seagrass beds are important habitats for post-larvae and juvenile *P. semisulcatus* in Doha Bay, while the transition to deeper water habitats in older shrimp involves a change in diet and source of carbon and nitrogen that is reflected in shrimp tissue stable isotope ratios.

The results of this study confirm the significance of shallow water seagrass beds as both habitat and a source of primary production to support juvenile populations of commercial shrimp in the Arabian Gulf. In addition there is some evidence to support the role of intertidal mudflats and microbial mats in supporting the first post-settlement stages, which is consistent with the findings of Jones *et al.* (2002) in Kuwait. Both seagrass beds and mudflats habitats on the western coast of the Arabian Gulf are at risk from modification of the coastline through land reclamation to create marinas, islands and beaches (Khan *et al.* 2002; Al-Jamali *et al.* 2005) and the linkage between such sensitive habitats and inshore fisheries should be included any assessment of the potential impacts of such developments.

Chapter 3

Nutrition of penaeid shrimp in Al-Khor Bay; A stable isotope study of the role of mangroves, seagrasses and microbial mats.

3.1 Introduction:

Mangrove forests cover approximately 180000 km^2 worldwide, of which 41.5% are found in Asia (Spalding *et al.* 1997; Primavera 2002). However, a large proportion of Asian mangrove areas have been replaced by a variety of other land uses such as agriculture, aquaculture, salt production and human settlements. Overexploitation by coastal inhabitants has caused mangroves to decline, with aquaculture (mainly shrimp ponds) being one of the major causative factors (Chan *et al.* 1993; Primavera 1995).

Avicennia marina is the only mangrove species present in the Arabian Gulf (Satchell 1978), due to its tolerance of high salinity and wide temperature extremes (Macnae 1968; Ghowail *et al.* 1993; Al-Khayat 1996), though the lack of rainfall and high salinities experienced have been reported to restrict the growth and development of planted *Avicennia marina* in some areas (Al-Khayat 1996). Along the western coast of the Arabian Gulf, natural mangroves stands are limited in area, covering 30 km^2 in the UAE (Embabi 1993; Tamaei *et al.* 2002), 9 km^2 in Qatar (Al-Khayat 1996), 20 km^2 (Sheppard *et al.* 1992) and 292 km^2 in Saudi Arabia (Spalding *et al.* 1997). In the northeast Arabian Gulf, natural mangroves are absent from the coasts Kuwait and Iraq (Al-Khayat 1996). However, in recent years, some areas of *Avicennia marina* have successfully been planted in the Kuwait coastal zones, using seedlings derived from natural stands in Bahrain and UAE (AboEl-Nil 2001). Due to perceived beneficial effects of mangrove forests in terms of biodiversity and associated fisheries, mangrove conservation is considered a key issue in coastal environmental management in the region (AboEl-Nil 2001), particularly in the context of loss of intertidal habitats due to coastal infrastructure development in many Arabian Gulf countries (Abou-Seida and Al-Sarawi 1990).

The importance of mangrove areas as nursery grounds for many tropical juvenile marine fish and Crustacea is well-recognised (Day *et al.* 1981; Odum *et al.* 1982; Thayer *et al.* 1987; Hatcher *et al.* 1989; Robertson and Blaber 1992; Bouillon *et al.* 2002; Sheridan and Hays 2003). Most penaeid shrimp species have life cycles in which they spawn and undergo larval development in coastal waters before the post-larvae settle inshore (Dall *et al.* 1990) where the juvenile phase may be spent in or associated with vegetation such as seagrass beds and mangroves (Rönnbäck *et al.* 2002). Several studies have shown that in some penaeid shrimp species post larvae and juveniles have a preference for mangroves as nursery habitats. These species include *P. merguiensis* in Australia, India and the Philippines (Staples *et al.* 1985; Robertson and Duke 1987; Vance *et al.* 1996; Mohen *et al.* 1997; Meager *et al.* 2003; Kenyon *et al.* 2004), *P. monodon* in South Africa and India (de Freitas 1986; Mohen *et al.* 1997), *F. indicus* in Mozambique, South Africa and Malaysia (de Freitas 1986; Chong *et al.* 2001; Rönnbäck *et al.* 2002), *M. anchistus* in Philippine (Primavera 1998) and also *M. ensis* in the Philippines (Primavara 1998). Seagrass and macroalgal beds have also been recognized as important nursery grounds for some penaeid species, including *P. semisulcatus* and *P. esculentus* in Australia and *P. semisulcatus* in the Arabian Gulf (Basson *et al.* 1977; Young and Carpenter 1977; Staples *et al.* 1985; Coles *et al.* 1987; Jones and Al Attar 1981; Sheppard *et al.* 1992; Loneragan *et al.* 1994; Haywood *et al.* 1995; Heales *et al.* 1996; Loneragan *et al.* 1998). Similarly *Metapenaeus* spp utilize a range of habitats including seagrass beds, mangroves channels and mud flats (de Freitas 1986). These habitats can provide a refuge from predators and shelter from physical disturbance and there is a range of evidence

indicating their greater or lesser significance as sources of primary production supporting shrimp populations.

Although gut content examination has been used to indicate feeding patterns and habitat utilization by consumers, it cannot always be taken as a true reflection of dietary sources because ingestion does not necessary equate with assimilation of these items (Zieman *et al.* 1984). Also, identification and quantification of food items is difficult in crustacea due to the mechanical grinding by the mandibles and the gastric mill (Dall *et al.* 1990). In Australia, Wassenberg and Hill (1987) reported that the most abundant food items found in the gut of *P. semisulcatus* are molluscs, crustacean and polychaetes. In the Doha area of Qatar the main food items in the gut of *P. semisulcatus* were similar, being foraminifera, molluscs, polychaetes and small crustaceans, with considerable amounts of unidentified detritus (Al Maslamani *et al.* in press). In contrast, Su and Liao (1984) found that organic detritus represent 72-100% of food in the stomachs of *P. semisulcatus* and *M. affinis* in Taiwan and concluded that such material may be an important food source.

Stable isotope analysis is an important technique for tracing aquatic food webs. Generally, carbon provides information on primary energy sources while nitrogen allows discrimination of trophic levels. While some previous studies of stable isotopes in food webs in mangroves systems have shown that mangroves can be important sources for nitrogen and carbon for many organisms (e.g. Rodelli *et al.* 1984; Odum and de la Cruz 1963: Odum and Heald 1975), including penaeid shrimp (Martosubroto and Naamin 1977; Gedney *et al.* 1982; Staples *et al.* 1985; Chong 1995; Loneragan *et al.* 1997), others have shown that penaeid shrimp may derive their

carbon from phytoplankton and benthic algae rather than from mangroves detritus (Stoner and Zimmerman 1988; Newell *et al.* 1995; Primavera 1996). The relative importance of mangrove primary production may also vary seasonally. For example stable isotope studies of estuarine populations of penaeids in Australia indicate that primary production from mangroves only contributes to nutrition of juveniles during the rainy season, with no evidence of mangrove-source nitrogen during the dry season (Loneragan *et al.* 1997).

In the Arabian Gulf, chiefly in northern Kuwait waters, mangroves are rare but penaeid shrimp (*P. semisulcatus* and *Metapeneus* spp.) are abundant. In these areas, Jones *et al.* (2002) and Al-Zaidan *et al.* (2006) found that intertidal microbial mats appear to be a major contributor of primary production, supporting benthic and epibenthic invertebrates, including penaeid shrimp. In addition, recent stable isotope studies in the Doha area of Qatar, where there are no mangrove present, have shown that both seagrasses and microbial mats may be important sources of primary production for postlarval and juvenile *P. semisulcatus* (Al-Maslamani *et al.* in press).

In Al-Khor Bay, on the northeastern coast of Qatar, approximately 40 km north of Doha city *Avicennia marina* forms a natural mangrove forest (Al-Khayat 1996; Sadooni and El-Kassas 1998). This acts as an important habitat as nursery ground for many species of fish, shrimp, crab and molluscs with *Penaeus semisulcatus* and *Metapenaeus affinis* being the most common shrimps in the area (Al-Ansi 1995). The semi-enclosed bay of Al-Khor not only contains mangroves, but supports seagrass beds and large areas of microbial mat, all of which may act as a source of primary production to penaeid shrimp. Thus the aim of this study was to investigate the

relative contribution of the different potential sources of primary production to supporting growth in juvenile shrimp in the bay.

3.2 Material and Methods

3.2.1 Field site description:

The study was carried out in the intertidal and subtidal zones of one of main shrimp fishery grounds of the State of Qatar Al-Khor Bay (Al-Ansi 1995), approximately 40 km from Doha, along the east coast of Qatar. According to Al-Khayat (1996) the total natural mangrove area in the northeast of Qatar at Al- Khor Bay and adjacent Al-Dhakhira totals 8.6 km^2 and for Al-Khor alone approximately 3.1 km^2.

The maximum depth in Al-Khor Bay is 5 m with an average of 3 m. Adjacent to the mangrove are areas of intertidal soft sediments covered with microbial mat. Seagrass beds are also present in close proximity to the mangroves forest. Three areas were sampled that represented two intertidal and one subtidal habitat (Figure 1). Sites 1 and 2 were intertidal, with water depths at high tide ranging from 0 – 1.5 m. Site 1, covered by microbial mats, had sand and sandy-mud sediments while Site 2 was mainly covered by mangroves *Avicennia marina*. The distance between Sites 1 and 2 was just over 100 m. Site 3 was sub-tidal, over 200 m from Site 1 and over 100 m from Site 2, with water depth ranging from 0.3 – 2 m covering shallow seagrass beds growing in mainly sandy-mud (Table 1).

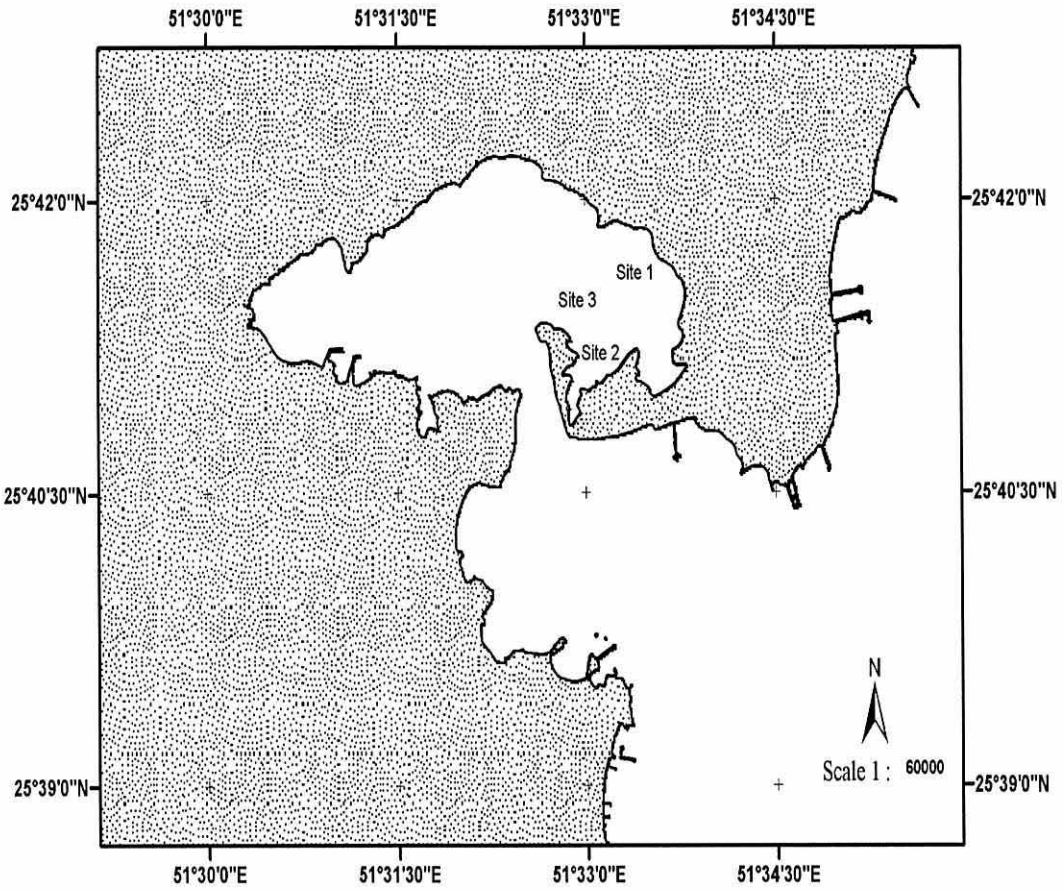

Figure (1) Map of the Al-Khor Bay study area showing the three sampling sites.

Table (1) Sites and sizes for penaeid and caridan shrimp collected from Al-Khor Bay, Qatar.

Site	Habitat	Depth (m)	Life stage	Total length(mm)
1	Intertidal mud flat (sandy-mud).	0 - 1.5	Post larvae (*M. affinis*).	19-24
2	Inertidal mangroves forest	0 - 1.5	Juvenile and adult (*Palaemon khori*).	10-40
3	Seagrass beds (sandy-mud, sand with shell gravel)	0.3 - 2	Post larvae and juvenile (*P. semisulcatus).*	21.3 -57.8
			Early post larvae and juvenile (*M. affinis*).	11.2-43.2

Surface temperature and salinity were measured at each site using a mercury bulb thermometer and a portable salinity meter. Samples of post larvae and juvenile shrimp and other selected food web components were collected during the start of the shrimp spawning season (January – June). In the intertidal zone (Sites 1 and 2) all samples of shrimps were collected at high tide. At site 3, samples were collected at low tide. At all sites the same hand-pulled seine net was used. Overall the net was 6 m long and 1.5 m high with internal net mesh size of 0.5 mm, and external net mesh size of 5mm. All samples of shrimp were placed on ice in the field and then frozen at -20 °C on return to the laboratory at the Marine Sciences Department, Qatar University.

The species, size and life stage of the shrimp were identified and the shrimp were measured for total length (rostrum to telson) and wet weight. Specimens for gut content analysis were stored in plastic or glass jars in 5% buffered formalin. Stomachs were later dissected under a binocular microscope and contents identified and relative abundance recorded as frequency of occurrence. The large numbers of *Metapenaeus affinis* caught at site 3 allowed for gut content examination but there were not sufficient numbers of *P. semisulcatus* for stomach contents analyses.

For stable isotope analysis, muscle tissue was excised from the tails of subsamples of shrimps taken from April to June and oven-dried at (50-60 °C for 24 hrs) before being stored in plastic bags or glass vials. A range of other organisms from the study areas were taken for stable isotope analysis. Benthic invertebrates from seagrass beds (Site 3) were collected by washing sediment over 0.25 and 0.5 mm mesh sieves and then identified using a binocular microscope.

Primary producers, such as microbial mats, macroalgae, phytoplankton, seagrass, mangroves (roots & leaves) and epiphytes algae scraped from blades of seagrass and from mangroves roots were also collected. Subsamples of the microbial mats were removed to determine their faunal and floral composition using a binocular microscope.

3.2.2 **Stable isotope analysis.**

Samples (~ 2mg for elemental and 10-15mg) of primary producers, muscle tissue excised from tails of shrimp and other whole organisms were a finely ground. Sub-samples were weighed into pre-combusted silver boats (500°C, 3hrs), and carbonate material was removed through a combination of HCl (10%) additions and drying at ~50°C. The remaining, acidified, sub-samples were used for stable isotopic analysis and were placed in pre-combusted (910°C, 3 hours) quartz tubes with copper and copper oxide. The stable C and N isotopic composition was determined on CO_2 and N_2 generated by vacuum combustion and is reported in the δ notation as the ratio of the heavy to the light stable isotope in the material, R_{sample}, relative to that of a standard, $R_{standard}$, with standard = Vienna Pee Dee Bellemnite (VPDB) and air for carbon and nitrogen respectively, i.e.

$$\delta_{sample} = 1000\left(\frac{R_{sample}}{R_{std}} - 1\right)$$

The gases were separated and collected by vacuum distillation from the same sample, and were analyzed on a EUROPA-PDZ GEO 20/20 isotope ratio mass spectrometer ($\delta^{13}C$) and a VG SIRA II dual inlet isotope ratio mass spectrometer ($\delta^{15}N$).

3.3 Results

During the sampling period surface seawater temperature increased from 17°C in January up to a maximum of 27.5 °C in June, while salinity increased from 41.5 ‰ to 44 ‰ (Figure 2). Only postlarval *M. affinis* were caught at site 1 (microbial mats), neither *M. affinis* or *P. semisulcatus* were caught at site 2 (mangroves), while both species were caught in the seagrass beds of Site 3.

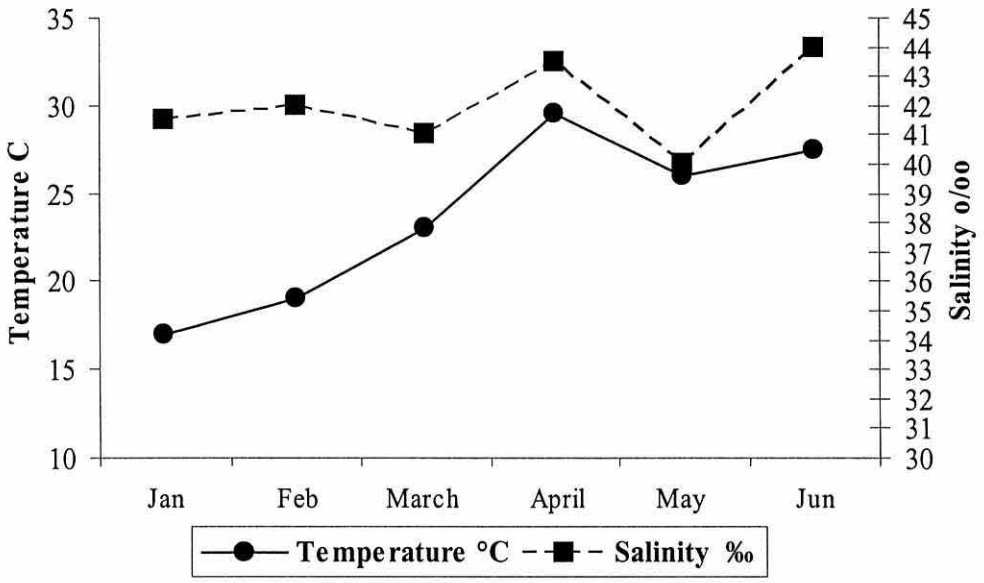

Figure (2) Mean surface of temperature and salinity in Al-Khor Bay during the sampling period.

The main food items occurring in the gut of *M. affinis* were foraminifera (25.1 %), crustacean (17.5%), diatoms (11.5%), polychaetes (8.7%) and dinoflagellates 8.2%. The less frequent food items were algae (4.4%) and invertebrate eggs (2.2%). Unidentified debris made up the remaining 16.9 % (Figure 3). It was found that there were more than one species of foraminifera and diatom present in the gut of *M. affinis*, while the most commonly occurring crustaceans in the gut were copepods.

The hard parts of organisms such as foraminifera and crustaceans were more common in the gut (42%) than soft tissue food items such as algae (4.4%) and polychaetes (8.7%). The high frequency of occurrence of dinoflagellates and diatoms, together represented up to 19% of organisms present in the gut contents, suggesting some non-selective grazing on the sediment surface.

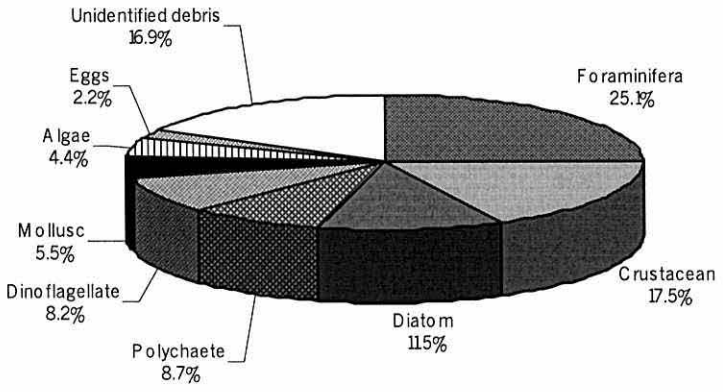

Figure (3) Food items by frequency of occurrence in gut contents of postlarval and juvenile *M. affinis* in shallow water seagrass beds in Al-Khor Bay.

The carbon and nitrogen isotopic measurements of the various samples collected from Al-Khor Bay are shown in the Tables 2 and 3. *P. semisulcatus* and *M. affinis* of differing sizes and from the various sites had a narrow range of $\delta^{13}C$ values from -10.6 ± 0.06‰ to -11.9 ± 0.6‰ and $\delta^{15}N$ values ranging from 3.7 ± 0.06‰ to 4.4 ± 0.1‰ (Table 4, Figure 4 and 5). There were small but significant differences in $\delta^{13}C$ and $\delta^{15}N$ values for penaeid shrimp from the three sampling sites ($P< 0.05$, one way ANOVA). *M. affinis* larvae at Site 1 (microbial mat) had significantly more positive $\delta^{13}C$ and $\delta^{15}N$ values than *P. semisulcatus* at site 3 (seagrass). The $\delta^{13}C$ values for *M.*

affinis postlarvae at Site 1 (microbial mats) were indistinguishable from postlarvae of the same species at site 3 (seagrass) but were significantly more positive than for the juveniles at site 3. Conversely the $\delta^{15}N$ values for *M. affinis* postlarvae at Site 1 (microbial mats) were indistinguishable from values for juveniles at site 3 (seagrass) but were significantly more negative than values for early postlarvae at site 3.

The $\delta^{13}C$ and $\delta^{15}N$ values for primary producers from sites 1-3 ranged from -28.5 and -7.6 ‰ and -0.9 to 3.2‰ respectively and reflected the different primary producers that predominated at each site. Site 1 (microbial mat) was characterised by negative (light) $\delta^{15}N$ values and relatively heavy $\delta^{13}C$ values (Table 3, Figure 4). Site 2 (mangroves) had the most negative $\delta^{13}C$ values and low to intermediate $\delta^{15}N$ values (Table 3, Figure 4 and 5). Site 3 (seagrass) displayed the widest range of $\delta^{13}C$ values (-7.9 to -14.6‰) and intermediate to high $\delta^{15}N$ values (Table 3, Figure 4).

Table (2) Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of primary producer, and other selected components collected from Al-Khor Bay study area.

Sample type/ Site	Abbrevi-ation	No. of individual	No. of samples	$\delta^{13}C$‰	$\delta^{15}N$ ‰
Primary producer					
Mangrove leaves *Avicennia marina* (Site 2)	ML	_	3	-28.5 ±0.45	0.8 ±0.81
Mangrove roots *Avicennia marina* (Site 2)	MR	_	3	-27.4 ±1.22	0.9 ± 0.5
Red algae *Polysiphonia* sp (Site 2)	R	_	3	-22 ± 0.98	2.5 ± 1.4
*Epiphytic algae in shallow water seagrass beds	ES	_	3	-14.6 ± 0.2	3.2 ± 0.05
*Seagrass	S	-	3	-7.9±1.3	1.5±0.42
Microbial mats (Site 1)	M	_	3	-7.6 ± 0.99	-0.9 ±0.51
Other selected components					
Gastropods in shallow water seagrass beds (Site 3)	G	10	3	-11.8 ± 0.4	3.8 ± 1.7
Polychaetes in shallow water seagrass beds (Site 3)	PL	_	3	-11.9 ± 0.4	4.1±0.00
Isopod + amphipod on mangrove roots (Site 2)	IA	_	3	-18.7±0.5	2.9 ± 0.7
Caridean shrimp *Palaemon khori* on mangrove roots (Site 2)	CS	Over 100	3	-14.9 ± 0.05	5.7 ± 0.00
*Foraminifera in seagrass beds	FS	-	3	-13.3 ±0.8	1.9 ± 0.1
Bivalves *Amiantis cimbonelle* (Site 1)	BV	10	2	-16.05±0.35	2.8±0.84

* From Doha Bay

Table (3). Stable isotope values ($\delta^{13}C$ ‰ and $\delta^{15}N$‰) and total length (mm) for *P. semisulcatus* and *M. affinis* collected in Al-Khor Bay. Values are means ± s.d. For stable isotopes values, rows bearing different superscripts are significantly different (one way ANOVA & Tukey's pairwise comparisons, $p < 0.05$).

Species/Site	Abbrevia -tions	Life stage	Mean length ±sd (mm)	$\delta^{13}C$ ‰ ± sdn=3	$\delta^{15}N$ ‰ ± sdn=3
M. affinis Site1,microbial mat.	MI	Post larvae	21.1 ± 4.4	-10.6 ± 0.3^{b}	3.7 ± 0.06^{b}
M. affinis. Site3, seagrass.	ME	Postlarvae	16.3 ± 2.3	-10.7 ± 0.3^{b}	3.9 ± 0.2^{a}
M. affinis Site 3, seagrass.	MS	Juvenile	35.9 ± 4.3	-11.5 ± 0.3^{a}	3.8 ± 0.2^{b}
P.semisulcatus, Site3, Seagrass.	SSP	Postlarvae	28.03 ± 1.7	-11.5 ± 0.02^{a}	4.4 ± 0.11^{a}
P.semisulcatus, Site 3, seagrass.	SSJ	Juvenile	50.17 ± 5.5	-11.9 ± 0.60^{a}	4.1 ± 0.30^{a}

The stable isotope contents of other consumers (Table 3) generally reflected those of the primary producers at the location in which they were collected, when allowance for trophic enrichment in $\delta^{13}C$ and $\delta^{15}N$ had been made. The polychaetes and gastropod spp. from the seagrass beds (site 3) were the most ^{13}C enriched (-11.9 ± 0.4 and -11.8 ± 0.4 ‰ respectively), while the isopods and amphipods collected from amongst mangroves roots at Site 2 were the least ^{13}C enriched with a values of (-16.5 ± 0.05 ‰ and -18.7 ±0.5 ‰ respectively). The caridean shrimp (*Palaemon khori*) which was also collected from amongst mangrove roots had intermediate $\delta^{13}C$ values (-14.9±0.05‰) as did the bivalves (*Amiantis umbonelle*) from the intertidal mud flat (Table 3). The isopods and amphipods and bivalves had similar $\delta^{15}N$ values (2.9 ± 0.7 ‰ and 2.8 ± 0.8‰, respectively) while the polychaetes and gastropod had much higher values (4.1‰ and 3.8±1.7‰, respectively). The caridean shrimp (*Palaemon*

khori) had the highest $\delta^{15}N$ values, 5.7‰, compared to the other animal species analysed (Table 3).

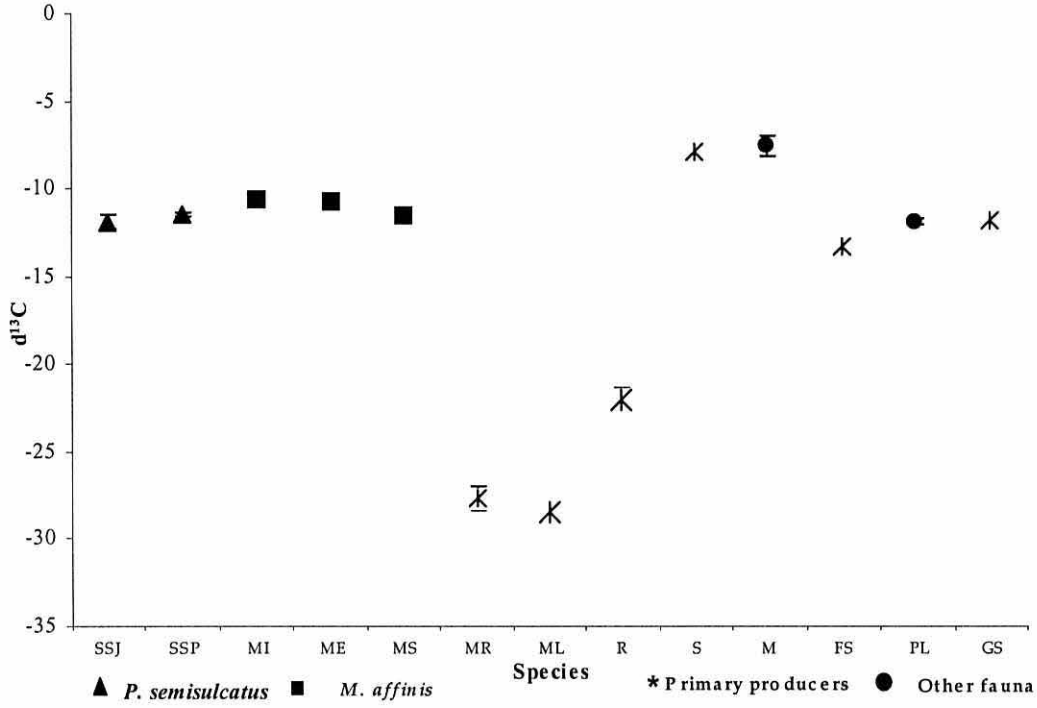

Figure (4) $\delta^{13}C$ values of *P. semisulcatus and M. affinis* primary producers and prey species from Al-Khor Bay (abbreviations are explained in Tables 1 and 2). Values are mean ± s.d., n = 3 for each point.

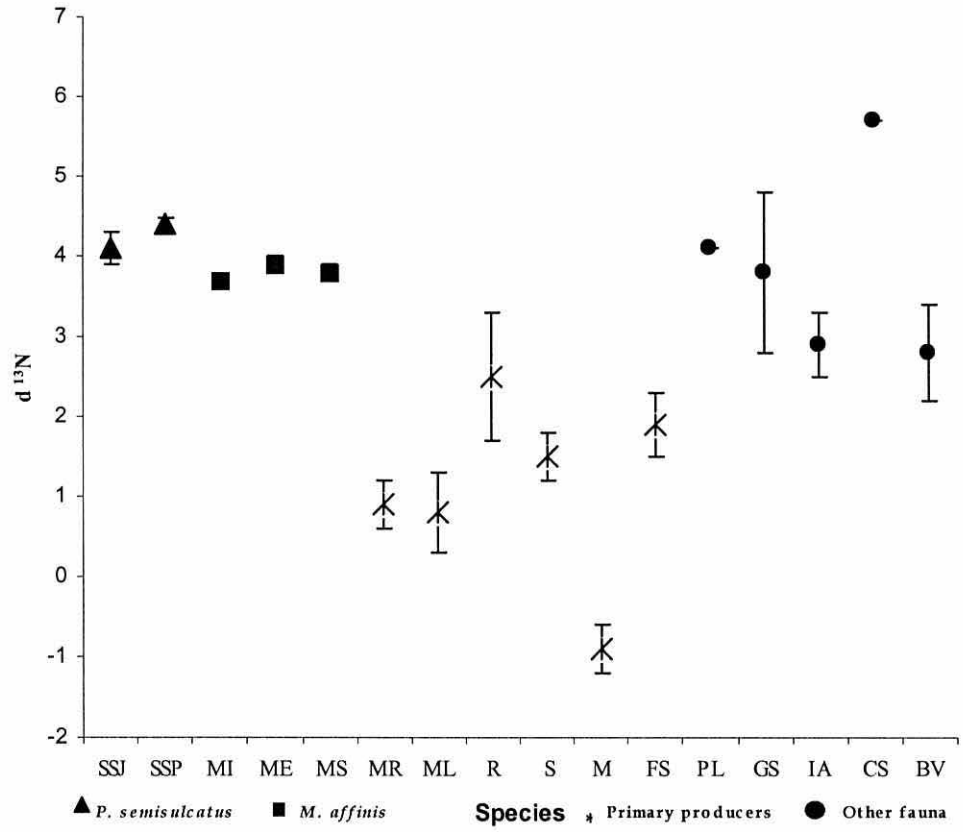

Figure (5) $\delta^{15}N$ values of *P. semisulcatus* and *M. affinis* primary producers and other selected prey species from Al-Khor Bay (abbreviations are explained in Tables 1 and 2). Values are mean ± s.d., n = 3 for each point.

3.4 Discussion

In general, penaeid shrimp post-larvae settle in shallow water habitats (Dall *et al.* 1990; Rothlisberg *et al.* 1996; Condie *et al.* 1999) and they are usually associated with complex habitats with high primary productivity such as seagrass beds, algal beds and mangroves (Robertson and Blaber 1992; Blaber 2000). In both Australia and Malaysia penaeid shrimp post larvae have been recorded to settle amongst all types of aquatic vegetation (Robertson and Duke 1987; Chong *et al.* 1990; Vance *et al.* 1990). In northern Australia, for example, mangroves provide a critical nursery habitat for post larval and juvenile *P. merguiensis* (Loneragan *et al.* 1997) whereas seagrass beds and macroalgae are nursery habitats for the brown tiger shrimp *P. esculentus*, *P. semisulcatus*. and *Metapenaus* spp (Staples *et al.* 1985; Haywood *et al.* 1995; Loneragan *et al.* 1997). *P. semisulcatus* was only found in seagrass in Al Khor Bay, and this consistent with other studies that have shown that this is the primary habitat for the early life stages of this species in Qatari waters (Al-Maslamani *et al.* in press) and in Australia (Haywood *et al.* 1995; Loneragan et al 1997). In contrast, in the present study, *M. affinis* were caught as they moved over the intertidal mud flat zone from the seagrass beds during high tide, indicating possible foraging on microbial mats. This is consistent with several other studies have shown that *M. affinis* juveniles are found over intertidal mud flats and that have indicated feeding on microbial mats (Clayton 1986; Jones *et al.* 2002).

While comparison of feeding habits of two different species at two different sites should be interpreted with caution, the results of gut contents examination of post larval and juvenile *P. semisulcatus* from the Doha Area (Al Maslamani *et al.* in press) and *M. affinis* from Al-Khor Bay in the present study suggest that there are

differences between these species in terms of the food ingested. In both *M. affinis* and *P. semisulcatus* foraminifera and molluscs were the most frequently occurring food items in the gut. However, such hard parts may be evacuated from the gut at a slower rate than soft food, and thus may be over-represented in the gut contents (Hill 1976) and trituration and homogenization of soft items in the feeding mill may hamper the identification of soft food items (O'Brien 1994). It seems likely that soft-bodied fauna such as nematodes are also under-represented. Of the hard-bodied fauna, molluscs represented 19.6% of gut contents in *P. semisulcatus* in seagrass beds in Doha while molluscs represented only 5.5% of the diet of *M. affinis* in seagrass beds in Al-Khor Bay. Some groups that were present in the gut of *M. affinis* in seagrass beds in Al-Khor Bay, such as dinoflagellates were absent from the gut contents of *P. semisulcatus* in Doha Bay. The occurrence of both diatoms and dinoflagellates in the gut contents of *M. affinis* are consistent with feeding on microbial mats during high tides, as the microbial mat community in Al Khor Bay comprised mostly cyanobacteria, diatoms (*Navicula sp, Amphora sp, Diploneis stroemi, Mastogloia sp* and *Oestropia sp)*, dinoflagellates such as *Gymnodinum sp* and associated faunal components dominated by nematodes and foraminifera. Many studies emphasise the carnivorous feeding behaviour of penaeid shrimp juveniles due to the presence of benthic invertebrates in the gut (Chong and Sasekumer 1981; Leber 1985; Wassenberg and Hill 1987; Nelson and Capone 1990; Schmidt 1993; Schwamborn and Criales 2000). However, a similarly diverse diet has been observed in juvenile *Litopenaeus setiferus* (Hunter 1984; Mayer 1985; Nelson and Caopone 1990) and in *P. semisulcatus* and *M. affinis* in Taiwan (Su and Liao 1984).

The penaeid shrimp species in this study were caught in the seagrass beds and on the intertidal mud-flats covered by microbial mats, none were found in the mangroves. While the seagrass and mangroves can supply ecosystem functions such as protection from predators and physical disturbance, this is not true of the mud flats. However all three environments have the potential to provide food, without a nursery shelter function. The extent to which the shrimp have a dietary rather than nursery dependence on one rather than other primary producing habitats can be tested using stable isotope analysis. The $\delta^{13}C$ values of primary producers such as seagrass, mangroves and microbial mat recorded in the present study are comparable to previous studies for mangroves (Rodelli *et al.* 1984; Mohan *et al.* 1997; Bouillon *et al.* 2002; Jones *et al.* 2002) microbial mats (Barghoorn *et al.* 1977; Schidlowski *et al.* 1984; Jones *et al.* 2002) and seagrasses (Hemminga and Mateo 1996). Table 4 summarises previous studies and the results of present study for carbon and nitrogen stable isotopes in penaeid shrimp in different habitats.The observed $\delta^{13}C$ value for microbial mats was heavier (-7.6‰) than those recorded (-15.3‰) by Al-Zaidan *et al.* (2006) in Kuwait, but both $\delta^{13}C$ and $\delta^{15}N$ are indistinguishable from those in Doha, Qatar (-7.7 and -0.2‰ respectively) (Chapter 2).

The $\delta^{13}C$ values for *P. semisulcatus* and *M. affinis* (-10.6 ± 0.3 to -11.9 ± 0.6‰) along with estimates of trophic $\delta^{13}C$ enrichment, can be used to derive the isotopic composition of the presumed diet. Recently, McCutchan *et al.* (2003) has shown that the trophic shift for $\delta^{13}C$ ranged between -1.5 and +2.7‰, but that for consumers analysed as muscle tissue the trophic shift was +1.3 ± 0.3‰. Experimental studies with penaeid (*Litopenaeus vannamei*) and ghost shrimps (*Nihonotrypaea japonica* and *N. harmandii*) have shown similar trophic shift of 1.7 - 2.0‰ respectively (Parker et

al. 1989; Yokoyama *et al.* 2005). Using a trophic enrichment of 1.63 – 4.03 ‰ (live diets, chapter 5) leads to the presumed diet having $\delta^{13}C$ value within the range of -12.23 – -15.93 ‰ (Figure 4). Neither *P. semisulcatus* and *M. affinis* in Al-Khor Bay appear to derive carbon from mangroves roots and leaves ($\delta^{13}C$ -26.6 to -28.5 ‰) but may be more likely to depend in the main carbon originating from outside the mangrove area from either or both from the seagrass ecosystem ($\delta^{13}C$ -7.9 to -14.6 ‰) or microbial mats ($\delta^{13}C$ -7.6 ‰). The trophic shift in $\delta^{15}N$ for consumers ranges from -0.8‰ to +5.9 (McCutchan et al., 2003). Trophic enrichment for shrimp is currently poorly defined with values ranging from +1.4 to 2.1‰ from the field studies (Vanderklift and Ponsard 2003, McCutchan *et al.* 2003) to ~4‰ in experimental studies (Yokoyama *et al.* 2005). Using the full range of potential values 0.12 – 3.47 ‰ (live diets, chapter 5) for $\delta^{15}N$ trophic enrichment means that recent diet ranges from -0.23 – 4.28.‰. Given the large error in determining the isotopic signature of the presumed diet, these values cannot be used to support or confute the interpretation of diet based on $\delta^{13}C$ values.

In the Doha area of the Qatar coast (Chapter 2) there was no direct evidence that *P. semisulcatus* utilised the intertidal mud flat and at Al-Khor, despite sampling at all sites from January onwards, only post larvae and juveniles were found (in April/May) and so the first settlement stages were not observed (Chapter 2). These early settlement stages may prefer bare muddy or sandy substrates in preference to seagrass beds (Loneragan *et al.* 1998) and in support of this, both studies show that there is some indication of potential trophic linkage to microbial mats from the stable isotope data. However the gut contents suggest that *M. affinis* post-larvae may have a stronger dietary reliance on microbial mats than do *P. semisulcatus*.

Although $\delta^{15}N$ values of *P. semisulcatus* and *M. affinis* could not be used to distinguish between the three potential sources of primary production comparison with other studies show that both $\delta^{13}C$ and $\delta^{15}N$ values are not characteristic of shrimp associated with mangroves (Table 4). In South-east Asia and Australia several studies have shown links between mangrove forests and food webs supporting coastal fisheries (Macnae 1974; Staples *et al.* 1985; Camacho and Bagarinao 1987; Chong *et al.* 1990; Loneragan *et al.* 1997; Primavera 1998). Mangroves may be linked to adjacent food webs by several processes (Schwamborn *et al.* 2002) such as trophic coupling through detritus (Odum 1971), mass production of planktonic larvae by major invertebrate which contribute to the food chains (Robertson *et al.* 1988) and also direct use of mangrove organic material by some species (Schwamborn and Bonecker 1996). For example, *Metapenaeus monoceros* feed directly on mangrove leaf detritus (Sumitra-Vijayarahavan *et al.* 1980), but the significance of such direct utilisation is unclear as mangrove detritus is relatively refractory material (Odum and Heald 1972; Robertson *et al.*1988).

The processes of production and breakdown of mangrove leaves have been extensively reviewed by Ashton *et al.* (1999) and their breakdown and decay is an important step in the link to detritivores (Fell *et al.* 1980; Fell *et al.* 1984; Rajendran and Kathiresan 2004). Odum and Heald (1975) reported that the main flow energy is via mangrove leaf detritus, to bacteria and fungi, to detrital consumers, to low carnivores and to high consumers. There is also evidence of nutrient cycling in Indo-Pacific mangroves, largely through the leaf litter processing by sesarmid crabs (Malley 1978; Dahdouh-Guebas *et al.* 1999; Sheaves and Molony 2000).

Table (4). Summary of studies on carbon and nitrogen stable isotope values for penaeid shrimp in different habitats.

Species	Habitats/Site	$\delta^{13}C$ ‰ mean ± SD	$\delta^{15}N$ ‰ mean ± SD	Reference
Penaeus semisulcatus *Penaeus merguiensis* *Metapenaeus spp.*	Mangrove Seagrass	-22 to -16 -13 to -8.5	- -	Loneragan *et al.* (1997)
P. semisulcatus	Seagrass	-11.7±0.3	4.3 ± 0.2	Current study
P. semisulcatus	Seagrass	-10.2±0.9	5.8 ± 0.5	Chapter 2
P. semisulcatus	Deep water	-12.5±0.1	7.7 ± 0.1	Chapter 2
P. semisulcatus	Seagrass	-16.7 to -13.7	5.1 to 11.8	Macia (2004)
P. semisulcatus	Mangrove	-13.8	10.6	Bouillon *et al.* (2002)
Metapenaeus japonicus	Mudflat	-13.7±0.4	6.0 ± 0.2	Macia (2004)
P. merguiensis	Mangrove	-21.1±1.75	9.3 ± 0.6	Newell *et al.* (1995)
P. merguiensis	Mangrove	-25.2±0.2	9.7 ± 0.7	Chong *et al.* (2001)
P. merguiensis	Mudflat	-18.0±0.5	9.4 ± 0.2	Chong *et al.* (2001)
P. merguiensis	Offshore	-16.3±1.3	10.8 ± 0.3	Newell *et al.* (1995)
P. merguiensis	Mangrove	-24.03±0.8	9.1 ± 0.6	Hayase *et al.* (1999)
P. merguiensis	Mangrove	-20.7±1.4	-	Rodelli *et al.* (1984)
P. merguiensis	Offshore	-16.7±0.8	9.6 ± 0.2	Chong *et al.* (2001)
P. penicillatus	Mangrove	-20.5±0.4	-	Rodelli *et al.*(1984)
Penaeus monodon	Mangrove	-18.9±3.75	-	Mohan *et al.* (1997)
Farfantepenaeus aztecus	Offshore	-17.8±0.36	15.2 ± 0.33	Sherwood and Rose (2005)
Penaeus sculptilis	Offshore	-15.0± 0.96	11.0 ± 0.7	Newell *et al.* (1995)
Metapenaeus affinis	Seagrass	-11.1±0.6	3.9 ± 0.07	Current study
M. affinis	Mudflat	-10.6±0.3	3.7 ± 0.06	Current study
M. affinis	Mudflat	-23.9	10.9	Chong *et al.* (2001)
M. affinis	Offshore	-16.0±0.2	9.7 ± 0.2	Chong *et al.* (2001)
Metapenaeus ensis	Mangrove	-25.03±0.03	8.8 ± 0.02	Hayase *et al.* (1999)
Metapenaeus mutatus	Mangrove	-18.4	-	Rodelli *et al.*(1984)
Metapenaeus brevicornis	Mangrove	-26.6±0.03	8.4 ± 0.02	Hayase *et al.* (1999)
M. brevicornis	Mangrove	-22.3	-	Rodelli *et al.*(1984)
Metapenaeus monoceros	Mudflat	-19.7±0.6	5.4 ± 0.5	Macia (2004)
Metapenaeus stebbingi	Mudflat	-16.1±0.4	5.0 ± 0.4	Macia (2004)
Fenneropenaeus indicus	Mangrove	-21.2±3.5	-	Mohan *et al.* (1997)
Fenneropenaeus indicus	Mangrove	-19.5	-	Rodelli *et al.* (1984)
Fenneropenaeus indicus	Mudflat	-19.9±0.3	5.9 ± 0.3	Macia (2004)
Litopenaeus schmitti	Mangrove	-18.7	-	Schwamborn *et al.* (2002)
Farfantepenaeus schmitti	Mangrove	-18.2	-	Schwamborn *et al.* (2002)
Litopenaeus subtilis	Mangrove	-19.7	-	Schwamborn *et al.* (2002)
Farfantepenaeus duorarum	Seagrass	-18.1±1.3	-	Fry (1984)
F. duorarum	Mangrove	-13.4	10.7	Kieckbusch *et al.* 2004

Some other studies are contradictory and suggest that mangroves do not make a major contribution to coastal food webs (Stoner and Zimmerman 1988; Newell *et al.* 1995; Primavera 1996). For example, Primavera (1996) studied the stable isotope contents of shrimp tissue and those of primary producer, and concluded that juvenile shrimp in the mangroves derive their carbon from plankton and epiphytic algae rather than though the mangrove detritus pathway. However, a few studies have shown a strong influence of mangrove primary production on shrimp species tissue (Chong *et al.* 2001). Similarly, in Malaysian mangroves Rodelli *et al.* (1984) found that juvenile shrimp species assimilated about 65% of mangrove carbon. In the present study, mangroves leaves and roots appears to have not had any measurable input to the food webs supporting post larvae and juvenile penaeid shrimps in Al-Khor Bay. This may reflect the lower productivity of mangroves in the Arabian Gulf which are characterised by monospecific stands of *Avicennia marina,* with relatively small trees at low densities (Sheppard *et al.* 1992). It seems likely also that the extremely low rainfall and the low tidal range in the Gulf may greatly limit the export of mangrove-derived particulate organic matter (POM) and dissolved organic matter (DOM) to even closely adjacent habitats. A similar significant weakening of the linkage between mangroves and penaeid shrimp in nearby seagrass beds has been seen in the dry season on the coast of northern Australia (Loneragan *et al.* 1997). Interestingly, the caridean shrimp, *Palaemon khori,* which was found exclusively amongst the roots of mangrove trees in Al-Khor Bay, and that has not been previously reported elsewhere was more enriched with ^{15}N than either of the penaeid shrimp species studied. For this species, $\delta^{13}C$ ratios were closer to those of isopods and amphipods associated with epiphytic red algae on the mangrove roots.

Loneragan *et al.* (2005) found that the highest landings of shrimp in Malaysia were recorded in the region of the largest and most stable, extent of mangrove forest reserve (400 km^2) and the largest area of shallow water (< 5 m deep), implying that both factors played a part in determining the abundance of *Penaeus merguiensis*. At Al-Khor, the mangrove forest is much smaller (3.13 km^2) and the seagrass meadows replace mangroves as the major nursery, as observed for *P. semisulcatus* in Australia (Loneragan *et al.* 1997*)*. The seagrass can provide a refuge from predators and shelter from physical disturbance but may or may not provide their major food resources.

In arid environments such as the Arabian Gulf, the evidence from the present study suggests that there is little export of primary production from mangrove. Thus mangove in such areas may be more important as nursery habitats and in supporting mangrove-dependent fauna, rather than as sources of coastal productivity. Further study of influence of mangroves at a range of sites in the Arabian Gulf and Red Sea should be undertaken to confirm this hypothesis.

Chapter 4

Potential significance of intertidal microbial mats in the nutrition of post-settlement tiger shrimp, *Penaeus semisulcatus* **De Haan (Decapoda: Penaeidae)**

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4.1 Introduction

Intertidal microbial mats are composed of a matrix of cyanobacteria, with associated green algae, diatoms and infauna. Where they occur on soft-sediment shores, they form a surface layer over the upper intertidal zone but are also subject to continuous grazing lower on the shore (Al-Zaidan 2002). The high levels of primary production associated with microbial mats (Hoffmann 1996) lead to the accumulation of excess organic matter in the sediment, which is in turn thought to be an important input to coastal food webs. For example, Al-Zaidan (2006) describe in detail the role of microbial mats as a source of primary production on mud flats on the Kuwait coast and reported that microbial mats act as the major nutritional source supporting associated mobile epifauna, macro- and meio-infauna (Jones *et al.* 2002; Al-Ziadan 2002).

Penaeid shrimp post-larvae commonly feed on a range of benthic macrofauna such as gastropods, bivalves, amphipods, tanaids and polychaetes (Dall 1968a; Wassenberg and Hill 1987; Dall *et al.* 1991). Early-stage post-larvae of some penaeid shrimp, for example *Metapenaeus* spp., have been reported to forage over mud flats and associated microbial mat habitats during high tide, especially in areas where seagrass beds and mangroves are absent (Jones *et al.* 2002), indicating a important nursery-habitat function of mud flats in such areas. While *Penaeus semisulcatus* juveniles are known to be associated with seagrass beds (Young and Carpenter 1977; Coles *et al.* 1987; Sheppard 1992; Hill and Wassenberg 1993; Loneragan *et al.* 1994; Heales *et al.* 1996; Loneragan *et al.* 1998), very little is known about the response of early post-

larvae to different habitats (Liu and Lonergan 1997) and hence the significance of intertidal mudflat habitats may have been underestimated.

The use of stable isotopes is based on the fact that they either fractionate in a predictable fashion between trophic levels and so reflect trophic position ($\delta^{15}N$), or display little fractionation ($\delta^{13}C$) and so can be used to indicate isotopic signatures of the carbon sources that feed through to the higher trophic levels. Significant advantages to using the stable isotope approach to augment more conventional dietary techniques include the fact that the isotope ratios yield time integrated dietary information that reflects assimilated, and not just ingested foods. Trophic shifts of ~ 3.5‰ have been reported for nitrogen and around 1‰ for carbon (DeNiro and Epstein 1978; Minagawa and Wada 1984). While use of this technique may give an indication of possible diet and trophic relationships, interpretation can be problematic (Vanderklift and Ponsard 2003; McCutchan *et al.* 2003; Yokoyama *et al.* 2005). However this technique has been widely used in studying trophic relationships in aquatic food webs and several authors have studied stable C and N isotopes in the tissues of marine invertebrates, including penaeid shrimp, in relation to several sources of primary production (e.g. Fry 1981; Fry and Parker 1979; Chong *et al.* 2001; Stoner and Zimmerman 1996; Primavera 1996; Loneragan *et al.* 1997).

The aim of the present study was to use a range of techniques in an experimental approach to determining the potential role of microbial mats in the nutrition of the early settlement stages of the penaid shrimp *Penaeus semisulcatus.* Hence, the present study is linked to parallel field studies applying stable isotope analyses to identification of linkages between *P. semisulcatus* populations and critical habitats in

the Arabian Gulf (Al-Maslamani *et al.* in press) and provides a laboratory-based experimental approach in supporting determination of the potential role of intertidal microbial mats as an food source for *P. semisulcatus* at first settlement.

4.2 Materal and Methods

4.2.1 Production of postlarvae

Wild-caught broodstock *P. semisulcatus* originating from the State of Qatar, Arabian Gulf, were kept in a dimly-lit 8 m^3 raceway on a 14:10 L:D photoperiod, maintained at 28 °C and 33‰ salinity. Water was circulated through in a biofilter, fluidised bed sand filter, foam-fractionater and ultraviolet lamp array, with a turnover of approximately 100% d^{-1}. Mature eye-stalk-ablated females were spawned in darkened, aerated 60 l tanks with 5 μm filtered seawater at 28 °C and 33‰. The protozoea larvae were reared through the larval stages with algae (*Skeletonema costatum*: *Tetraselmis* 35:20 cell/μL) and the mysis stages were fed *Artemia* nauplii (5 nauplii ml^{-1}).

4.2.2 Microbial mats

Daily, freshly-collected samples of microbial mat were obtained from an area of sheltered high intertidal zone of the Menai Strait, North Wales. At low tide, areas of microbial mat were cut from the shore, and circular discs of mat were cut and placed in Petri dishes, so that the mat completely filled each dish. The Petri dishes (91 mm diameter) were positioned on the bottom of experimental aquaria for different periods depending on the experimental procedure (see later sections). Subsamples of the microbial mats were removed to determine their faunal and floral composition using a binocular microscope.

4.2.3 Gut contents analysis

Postlarvae at a range of ages (Table 1) were used to determine whether postlarval *P. semisulcatus* were able to feed on the microbial mats, as presented, and to determine the composition of food ingested. Post larvae were fed on mussel but were fasted a day before the feeding experiment started. Approximately 10 post larvae for each group age were stocked in 7L plastic aquaria (18 x 28.5 x17cm), one replicate was made for each group. Freshly collected microbial mat was introduced for a period of 2 h, after which all the shrimp were collected and preserved in 5% of formalin. Gut contents were assessed by dissection and visual examination under binocular microscope and the percentage occurrence of food items calculated.

Table (1). Mean total length and carapace length (mm) for different ages of postlarval *Penaeus semisulcatus* presented microbial mat in the gut content analysis.

Post Larvae age (Days)	No. of samples	Total length ± s.d. (mm)	Carapace length ± s.d. (mm)
17	10	12.4±1.6	2.9± 1.6
35	10	34.9 ± 4.5	8.6±1.08
42	10	17.3±1.3	4.1±0.4
42	10	24.8±3.4	6.1±0.2

4.2.4 Growth experiments

P. semisulcatus postlarvae, at different stage of development following settlement as day 1 postlarval stage (PL1), were studied to determine (1) whether they utilised microbial mats as a food source and (2) if microbial mats conferred any dietary advantage compared with other food sources. Three separate experiments were run and in each experiment, 15 to 20 *P. semisulcatus* were introduced into 7l plastic aquaria (18 x 28.5 x17cm), with three replicate tanks assigned to each of three dietary treatments. In the first experiment (Experiment 1, 7 days) 1 postlarvae (PL1) (initial mean total length 5.57± 0.14 mm and mean weight 0.30 mg) were fed microbial mat alone, microbial mat combined with 5 *Artemia* nauplii ml^{-1} and *Artemia* nauplii alone (5 nauplii ml^{-1}) as control. The postlarvae were fed once daily with newly-hatched *Artemia* nauplii and/or freshly collected microbial mat. The food was left in the tank for a whole day. At the start of the experiment the total length of the postlarvae was measured, and on the final day the total length (mm) was measured and the survival calculated. In the remaining two experiments (Experiments 2 and 3, 21days) postlarvae (PL3) (initial total length 7.13 ± 0.17 mm, mean weight 1.0 mg) and postlarvae (PL7) (initial total length 8.97±0.15 mm, mean weight 1.8 mg) respectively were offered either microbial mat or fresh mussel (*Mytilus edulis*) as a control , both offered to excess. At the start of the experiment the total lengths of the postlarvae were measured and thereafter total length (mm) of 15 randomly selected post larvae from each treatment were measured every 7 days to the nearest 0.1 mm and their survival calculated.

4.2.5 Identification of nutritional value of microbial mat components.

A fourth experiment (Experiment 4, 21 days) was conducted to determine which components of the microbial mat were contributing to growth and survival. Freshly-collected microbial mat was separated into sizes fractions of <38 μm, 38-125μm, 125-250μm, 250-500μm, 500-1000μm by washing with seawater over a series of sieves. Sub-samples of each size fraction of the microbial mat were taken to determine their floral and faunal composition and carbon and nitrogen content. In each treatment 20 post larvae (PL6, initial total length 9.1 ± 0.21 mm, mean weight 2.6mg) were stocked in the experimental tanks, as previously. Three replicate tanks were fed either intact mat or one size fraction of microbial mat, added to the tanks daily to excess. The total length (mm) of 5 shrimp in each tank were taken and measured every 5 days. On the final day all the post larvae total length (mm) were measured and the survival calculated. After 30 days feeding, postlarvae from each treatment were sampled and analysed for stable isotope content. Prior to sampling, the post-larvae were fasted for one day to ensure that there was no food in the gut. Muscle tissue was excised from the tail and was oven-dried at 50-60 °C for 24h before being stored prior to analysis.

4.2.6 Carbon and nitrogen concentration and stable isotopic composition.

For analysis of samples of intact freshly-collected microbial mat, and the various size fractions, dried material (~ 2mg for elemental and 10-15mg for isotopic analysis) used in experiment 4, were dried (~50°C) and finely ground. Sub-samples were weighed into precombusted silver boats (500°C, 3hrs), and carbonate material was removed through a combination of HCl (10%) additions and drying at ~50°C. One set of

subsamples were combusted and analyzed using CARLO ERBA NA 1500 elemental analyzer, using acetanilide as a standard with a precision better than 5%. The remaining, acidified, sub-samples were used for stable isotopic analysis and were placed in pre-combusted (910°C, 3 hours) quartz tubes with copper and copper oxide. The stable C and N isotopic composition was determined on CO_2 and N_2 generated by vacuum combustion and is reported in the δ notation as the ratio of the heavy to the light stable isotope in the material, R_{sample}, relative to that of a standard, $R_{standard}$, with standard = Vienna Pee Dee Bellemnite (VPDB) and air for carbon and nitrogen respectively, i.e.

$$\delta_{sample} = 1000\left(\frac{R_{sample}}{R_{std}} - 1\right)$$

The gases were separated and collected by vacuum distillation from the same sample, and were analyzed on a EUROPA-PDZ GEO 20/20 isotope ratio mass spectrometer ($\delta^{13}C$) and a VG SIRA II dual inlet isotope ratio mass spectrometer ($\delta^{15}N$) The precision of the carbon and nitrogen isotopic measurements was better than 0.1‰ based on internal standards..

4.2.7 Statistical analysis

Data were checked for normality and homogeneity of variances, before statistical analysis using one-way ANOVA, two sample t-tests or Kruskal-Wallis tests, as appropriate.

4.3 Results

4.3.1 Microbial mat and gut content analysis

The microbial mats comprised mostly cyanobacteria, with filamentous green algae and diatoms (*Frustule* sp, *Paralia* sp, *Cocconeis* sp, *Pleurosigma* sp and *Coscinodiscus* sp.) also present. Associated faunal components were dominated by nematodes, with a few marine insect larvae and egg/cysts also present (Table 2). Examination of gut contents suggests that post-larvae feed indiscriminately on the microbial mat, with the percentage occurrence of ingested food components largely reflecting the composition of the microbial mats themselves. Gut contents were dominated by algae and cyanobacteria (40-45%) and diatoms (20%) with a lesser contribution by faunal components including nematodes (5-15%), eggs/cysts (9-10%) and foraminifera (0-3%). Examination of faeces indicated that the faunal components were most readily digested, with intact cyanobacteria and algae predominant in faecal samples. The content of the size-fractions are shown in Table 2. Most of the faunal component, largely nematodes, was retained in the > 500 μm fraction. The 125 – 500 μm fractions contained mostly cyanobacteria, while the smaller fractions comprised mostly diatoms and fine sediment.

Table (2) Composition of microbial mat size fractions

Mesh size	Type of organisms	Nitrogen (mmol/g) Mean ± SD	Carbon (mmol/g) Mean ± SD	C/N Mean ± SD	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰
	Intact microbial mat	-	-	-	6.8	-21.3
>500 µm	Mostly large nematodes, some cyanobacteria and filamentous green algae. A few marine insect larvae.	0.5 ± 0.1	6.6 ± 1.0	11.3 ± 1.01	6.5	-21.4
250-500 µm	Mostly cyanobacteria, eggs/cysts. Some very small nematodes.	0.8 ± 0.1	9.8 ± 2.5	12.6 ± 2.8	6.9	-22.2
250-125µm	Mostly cyanobacteria and eggs/cysts.	0.9 ± 0.1	12.7 ± 1.7	12.9 ± 0.0	6.7	-22.6
125-38µm	Mostly diatoms, eggs/cysts and some cyanobacteria.	0.4 ± 0.1	4.7 ± 0.2	13.4 ± 3.01	6.5	-22.6
< 38µm	Fine sediment, detritus and some diatoms.	0.4 ± 0.1	2.1 ± 0.1	6.2 ± 1.2	-	-
Nematodes		4.0 ± 0.3	21.2± 1.0	5.3 ± 0.1	10.4	-19.0
Post-larvae		-	-	-	10.7	-17.0 ±0.01

4.3.2 Growth experiments

Post larvae (PL1) fed on *Artemia* alone in experiment 1 grew significantly better over the seven day experimental period than either those feeding on microbial mat or a mixture of *Artemia* and microbial mat ($p<0.001$) (Figure 1). The group feeding on *Artemia* alone also exhibited significantly better survival (100%), compared to post-larvae feeding on microbial mat (43.3 ±5.8 %) and those feeding on microbial mat supplemented with *Artemia* (63.3 ± 10.4 %) (Figure 2)

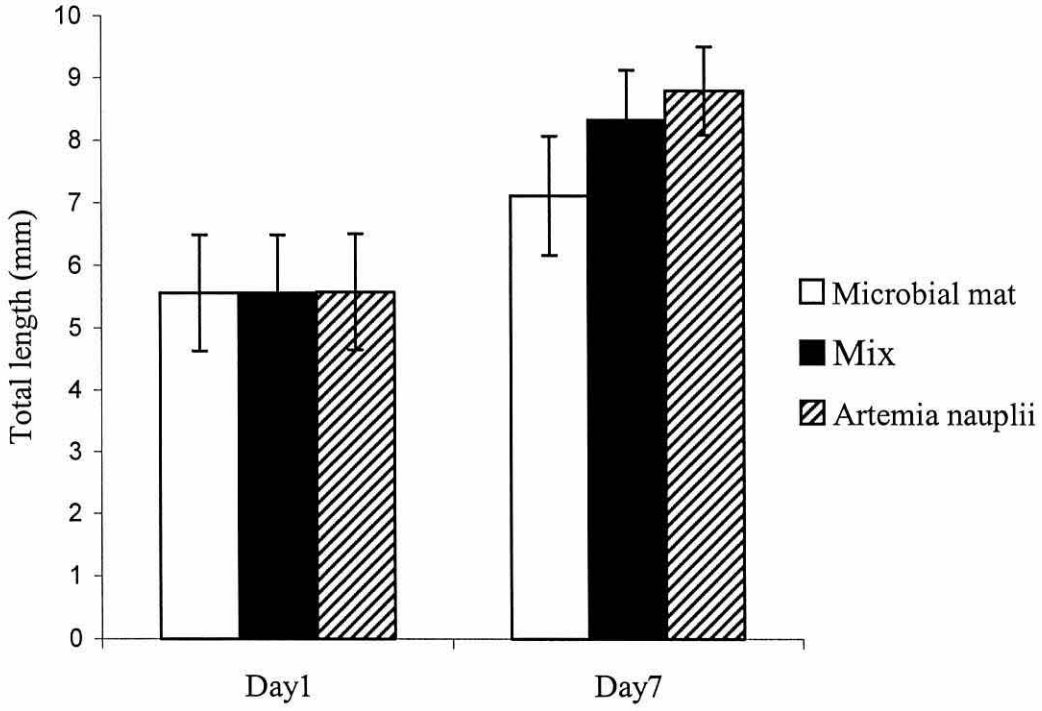

Figure (1) Initial and final lengths of *P. semisulcatus* fed microbial mat, *Artemia* nauplii or a mixture of both for seven days from PL1 (Experiment 1).

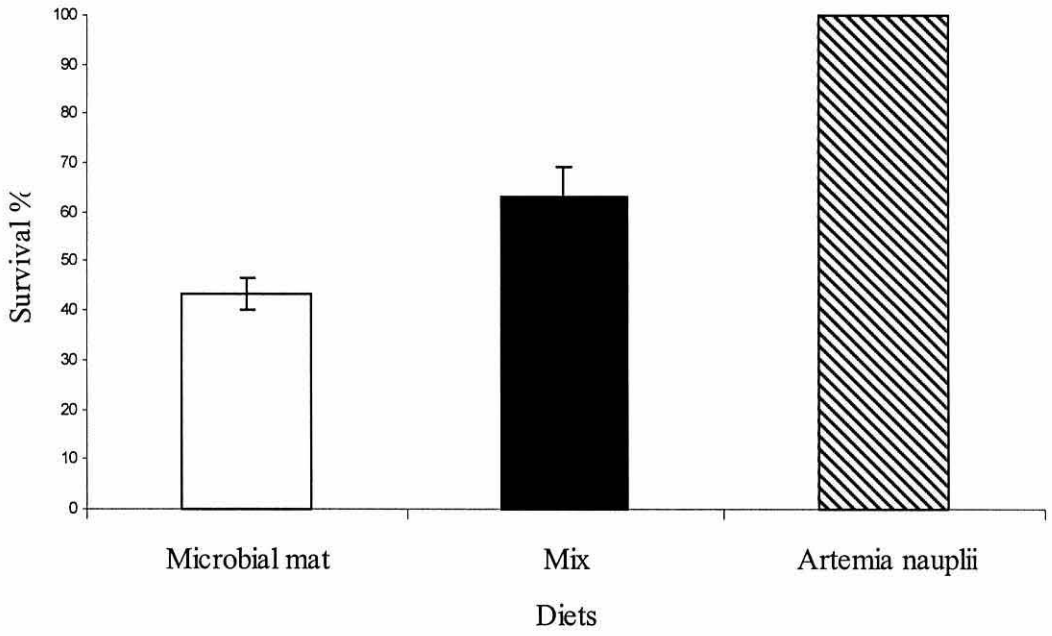

Figure (2) Percentage survival of *P. semisulcatus* fed either microbial mat, *Artemia* nauplii or a mixture of both from Day 1 post-metamophosis for seven days from PL1 (Experiment 1)

Post-larvae (PL3) feeding on microbial mat in experiment 2 exhibited significantly greater growth over the 20 day experimental period than those feeding on mussel *Mytilus edulis* ($P<0.05$) (Figure 3). Survival in both treatments was good, though survival in post-larvae fed microbial mats was significantly higher on the final day (93.3 ± 6.7 % compared to 82.2 ±13.9%) on the final day. Postlarvae from PL3 onwards exhibited burying behaviour, sheltering in the surface layer of the microbial mat. Post-larvae (PL7) fed on either microbial mat or mussel in experiment 3 exhibited similar growth rates (Figure 4), but with those feeding on mussel having a slightly but significantly greater final length (24.3 ± 3.07 mm compared to 22.9± 3.56 mm, $P< 0.05$). Survival was 100% for the post larvae on both diets.

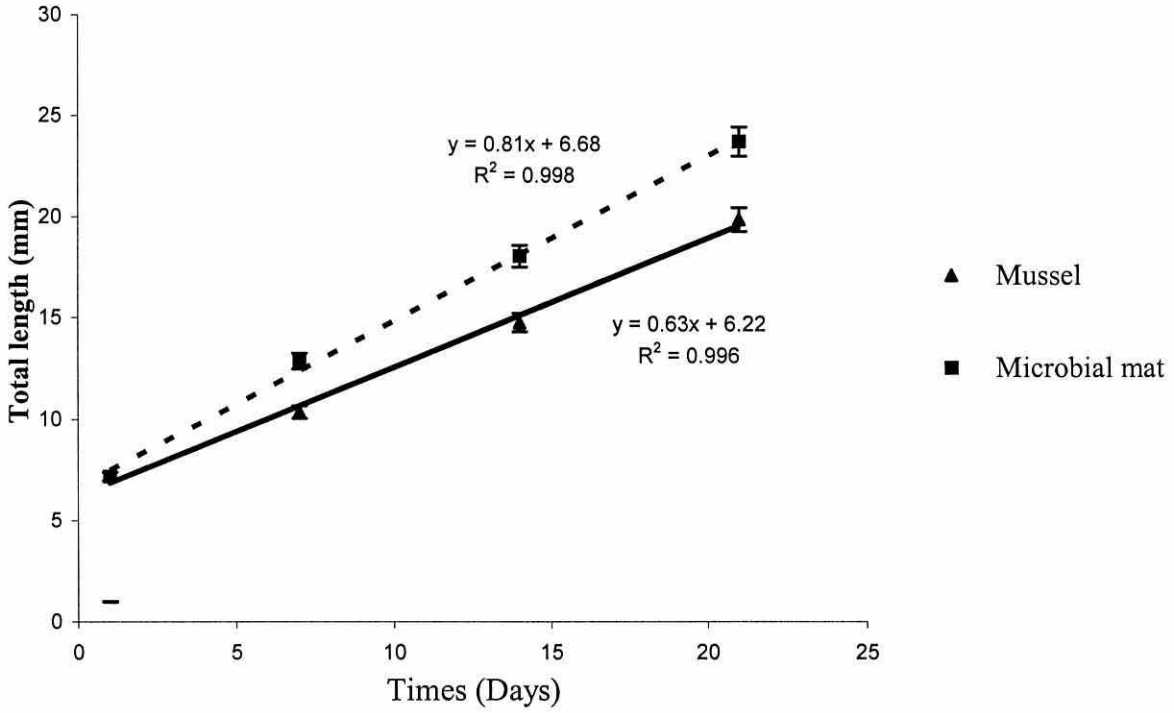

Figure 3 (a) Growth of *P.semisulcatus* postlarvae fed either on microbial mat or mussel flesh from PL3 (Experiment 2).

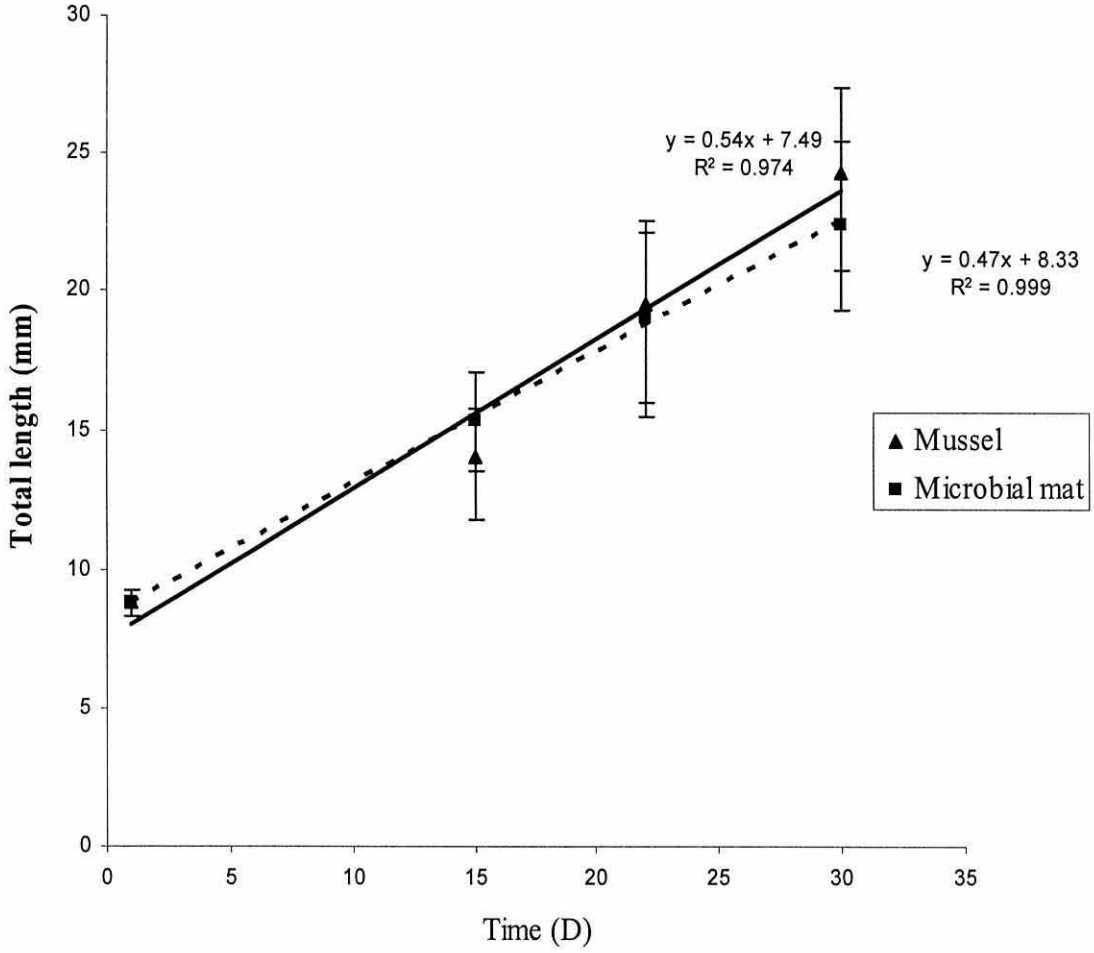

Figure (4) Growth of *P. semisulcatus* fed either microbial mat or fresh mussel from PL7 (Experiment 3).

4.3.3 Identification of nutritional value of microbial mat components.

The growth rates of the groups of post-larvae fed (PL6) on the various microbial mat fractions in experiment 4 were significantly different over the 20 day experimental period ($P < 0.05$, Figure 5). The group feeding on intact microbial mat and the size fraction > 500 μm had the highest final mean total lengths (20.46 ± 3.61 mm and 20.67 ± 2.43 mm respectively). The lowest final mean total length (10.67± 1.29 mm) of surviving post-larvae was recorded in those feeding on the size fraction between 38-125 μm, with intermediate mean length in the post-larvae fed on the 125-250μm.

The groups feeding on the intact microbial mat, >500μm and 125-250μm size fractions had a high final percentage rates of survival (98.3 ± 2.3%, 80.0 ± 22.9% and 95 ± 5% respectively), compared to groups feeding on the 38-125 μm size fraction (38 ± 16%) and the < 38μm fraction (0%).

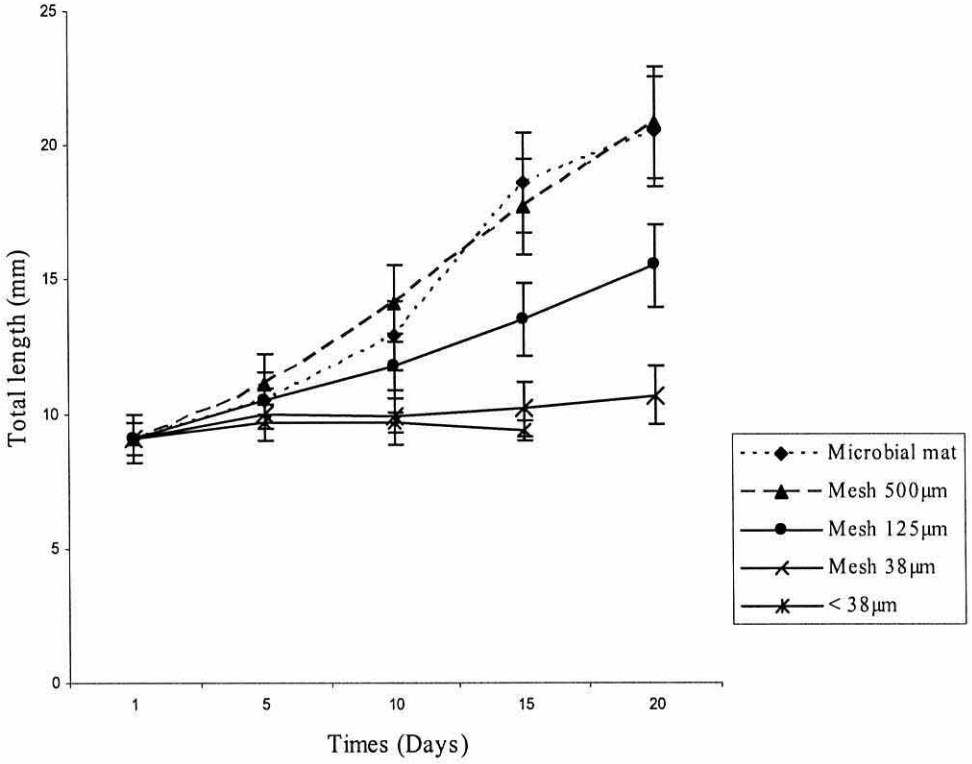

Figure (5) Growth of *P. semisulcatus* fed different size-components of microbials mats from PL6 (Experiment 4).

The results of biochemical analyses of the various microbial mat components (Table 2) shows that there was a significant difference in carbon and nitrogen content and C:N ratio between size fractions ($P < 0.05$). Carbon and nitrogen contents of isolated nematodes were relatively high (21.2 ± 1.0 mmol C g^{-1} and 4.0 ± 0.25 mmol N g^{-1} respectively), with the lowest values (2.1 ± 0.06 mmol C g^{-1} and 0.4 ± 0.1 mmol N g^{-1}) recorded for the fine fraction (< 38 μm). Stable isotope analysis showed significant differences in $\delta^{13}C$ values between the size fractions and isolated nematodes ($P<0.05$).

Nematodes had the least negative $\delta^{13}C$ value (-19.0‰), compared to values of -21.3‰ to -22.6 ‰ for intact and fractionated microbial mat. Isolated nematodes also had a highest $\delta^{15}N$ value (10.4 ‰), compared to values of between 6.5 to 6.9 ‰ for intact and fractionated microbial mat (Table 2). The $\delta^{15}N$ and $\delta^{13}C$ values for postlarvae fed on intact microbial mat for 30 days were 10.7 ± 0.0 ‰ and -17 ± 0.1 ‰ respectively.

4.4 DISCUSSION

Examination of the microbial mats in the present study showed that they comprised mainly cyanobacteria, filamentous green algae and diatoms and that associated faunal components were dominated by nematodes, with a few marine insect larvae and invertebrate eggs also present (Table 2). Microbial mat composition has been studied by several authors (e.g Urmeneta and Navarrete 2000; Wieland *et al.* 2003; Sørensen *et al.* 2005) The present are consistent with previous reports microbial mats from the Arabian Gulf are composed mostly of cynobacteria, diatoms and green algae, with infauna being mainly nematodes (Basson *et al.* 1977; Navarrete *et al.* 2000; Jones *et al.* 2002; Al-Ziadan 2002; Al-Maslamani *et al.* in *prep*). Al-Ziadan (2002), found that the predominance of each of these groups depended on shore height. For example, the higher intertidal region which is exposed to high temperatures in the day during low tide, is dominated by cyanobacteria, while diatoms dominate in the lower intertidal at low temperature. Distribution of primary producers may also influence meiofaunal abundance. For example, distribution of nematodes may be controlled by patchiness in diatom abundance (Admiraal 1984).

The results of the present study demonstrate that, under laboratory conditions, microbial mats can support good growth and survival of *P. semisulcatus* postlarvae.

In subtropical coastal areas, where the intertidal zone is dominated by microbial mats, Jones *et al.* (2002) have suggested that invertebrate and fish populations at all trophic levels may be supported by primary production originating from microbial mats and benthic diatoms. In the wild, *P. semisulcatus* post-larvae are typically associated with seagrass beds (Young and Carpenter 1977; Mohamed *et al.* 1981; Jones and Al-Attar, 1982; Coles *et al.* 1987; Sheppard 1992; Hill and Wassenberg 1993; Loneragan *et al.* 1994; Heales *et al.* 1996; Loneragan *et al.* 1998). In laboratory studies, Liu and Loneragan (1997) have indicated that post-larval *P. semisulcatus* are likely to settle, becoming epibenthic in seagrass beds within 1 - 2 days of metamorphosis. This is consistent with the behaviour of the earliest post-larval stages in the present study, which to some extent were still planktonic. At this stage they were still zooplanktivorous, and best growth and survival were observed in the treatment that provided a planktonic prey in the form of *Artemia* nauplii. Liu and Loneragan (1997) suggested that settlement preferences depend on the size and age of post-larvae, with larger post-larvae rarely found on bare substratum. In contrast, the current study demonstrates that, where there no seagrass is present, postlarval *P. semisulcatus* will settle and feed on microbial mats. This is consistent with several studies that have shown that mud flats, including areas of microbial mat, are an important habitat for postlarval penaeid shrimps, particularly *Metapenaeus affinis* and to a lesser extent *P. semisulcatus* (Bishop and Khan 1991; Al-Zaidan *et al.* 2006).

Shrimp feeding on microbial mats were able to survive and grow at rates comparable to a control diet of fresh mussel (Figures 3,4), which has been shown to be an excellent diet for penaeid shrimp post-larvae (Deshimaru and Shigueno, 1972; Beard and Wickins, 1980; Cuzon *et al.* 1981; Galgani *et al.* 1989; Ribeiro 1998). This

supports the suggestion that intertidal microbial mats can support nutriment for a range of invertebrate grazing species (Garrett 1970b; Gerdes and Krumbein 1984; Al-Zaidan *et al.* 2006). Several studies have shown that microbial mats are important for the nutrition of marine invertebrate as well as for providing habitat (Rodina 1949: Baker and Bradnam 1976: Barid and Thistle 1986; Bärlocher and Mudroch 1989; Decho and Moriarty 1990; Decho and Lopez 1993; Philips and Kilambi 1994). For example, microbial mats are important components of the feeding ecology of polychaetes and copepods (Decho and Lopez 1993; Decho and Moriarty).

Microbial mat has also been identified as an important source of carbon for chironomids (Fry 1982) and bacterial mass occurring within microbial mats is thought to be necessary for chironomids to digest and absorb other microbial mat constituents (Rodina 1949). Hoskins *et al.* (2003) reported that microbial mats are enriched in carbohydrate which are produced by bacteria and microalgae. On the other hand, bacteria in microbial mat may also be used as an important nutrition source (ZoBell and Feltham (1938).

The natural diet of penaid shrimp post-larvae is known to include small infaunal and epifaunal invertebrates (Dall 1968a; Moriarty 1977; Wassenberg and Hill 1987; Dall *et al.* 1991). Heales *et al.* (1996) found that food items in the foregut of small juvenile *P. semisulcatus* consisted mainly of copepod, insect larvae, diatoms, filamentous algae and unidentified matter. Chong and Sasekumar (1981) suggested that the diet of *P. merguiensis* post-larvae is related to food availability, within which there may be some evidence of prey selection. In contrast, the analysis of stomach contents in the present study suggests that *P. semisulcatus* post-larvae graze indiscriminately. This is

more consistent with the observations by McTigue and Zimmerman (1991) that post-larvae of other penaeid species feed by browsing the sediment surface, with *Litopenaeus aztecus* and *Litopenaeus setiferus* observed to ingest infauna, epifauna, microalgae and vascular plant detritus. However, the use of stomach contents to deduce feeding preference is problematic due to the rapid digestion of soft tissue (O'Brient 1994; Schwamborn and Criales 2000) and mechanical grinding by the penaeid shrimp mandible and gastric mill (Chansang 1984; Dall *et al.* 1990; McTigue and Zimmerman 1991). This may have resulted in over-representation of the significance of cyanobacteria and algae in the diet of post-larvae in the present study.

C:N ratios in animal tissues have been used as indicators of protein:lipid ratios (Omari 987; Anger, 1988) and the very high C:N ratios for the microbial mat size-fractions in the present study are indicative of their very low N and hence protein content (estimated as 4.2-7.6 protein as % of dry weight). Protein is the most important feed element in the growth of cultured penaeid shrimp species (Jones *et al.* 1996a) and in general postlarve are considered to have higher dietary protein requirements than older shrimp (Goddard 1996). Several studies have indicated protein requirements for penaeid shrimp juveniles of 27 - 60 %, depending on species (Akiyama *et al.* 1992; Allan and Smith 1998). Alam (2004) found that *P. semisulcatus* post larvae exhibited better growth when fed a formulated diet containing 40% protein, compared to those containing either 30% and 50 % protein diet. This suggests that, in the present study, the shrimp were unlikely to be deriving protein nutrition from the microbial mat flora and cyanobacteria, or from the accumulated organic sediments, even those these are all ingested. It is more likely that the infauna within the microbial mat, which mostly comprised nematodes, were the principal protein

source. This is supported by the observation that only the >500μm size-fraction, which selected most of the nematodes, was able to support growth equivalent to that supported by intact microbial mat. This is consistent with studies that have shown that, under culture conditions, free-living nematodes can support growth in penaeid shrimp larvae (Wilkenfeld *et al.* 1984; Biedenbach *et al.* 1989; Kumlu and Fletcher 1997; Kumlu *et al.* 1998).

The stable isotopic composition of muscle tissue of post larvae fed on microbial mat and its various size-fractions and components is consistent with nematodes representing a major dietary component. The $\delta^{13}C$ enrichment between diet and consumer has commonly been used to identify food sources for animals. A recent review by McCutchan *et al.* (2003) has shown that the trophic shift for $\delta^{13}C$ ranged between -1.5 and +2.7‰, but that for muscle tissue the apparent trophic shift was +1.3 ± 0.3‰. In experimental studies, $\delta^{13}C$ enrichment of a similar magnitude (+1.7‰) has been reported for the penaeid shrimp, *Penaeus (Litopenaeus) vannamei* (Parker *et al.* 1989) and for ghost shrimps *Nihonotrypaea japonica* and *N. harmandii* of +2.0‰ (Yokoyama *et al.* 2005). Thus in the current study, the $\delta^{13}C$ value of -17 ± 0.01‰ for tissue of post-larvae would be predicted to have been derived from assimilated carbon with an isotopic composition of between -18.3 and -19.0‰.

Thus carbon isotopic value of the presumed diet is close to that of the nematodes (-19‰) and different from other components of the microbial mat, ranging from -21.3 to -22.‰ (Table 2). Measurements of $\delta^{15}N$ can also be used to track dietary trends on the basis that consumers usually have more enriched $\delta^{15}N$ values than their diet (DeNiro and Epstein, 1981). However the trophic shift in $\delta^{15}N$ can be quite variable,

with differences occurring due to the major form of nitrogenous waste, the C:N ratio or protein content of the diet and the taxonomic class (Vanderklift and Ponsard 2003, McCutchan *et al.* 2003). The trophic shift in $\delta^{15}N$ for consumers ranges from -0.8‰ to +5.9‰, with an average value of +2‰ (McCutchan *et al.* 2003). However the trophic shift tends to be lower than average for those animals raised on invertebrate diets (+1.4±0.21‰) and those, like crustacean that excrete ammonia (2.08‰), (Vanderklift and Ponsard 2003, McCutchan *et al.* 2003). In contrast, Yokoyama *et al.* (2005) recently reported that the trophic shift in $\delta^{15}N$ for the ghost shrimps fed a single type of food was much larger at ~4‰. In the present study, the $\delta^{15}N$ of the post larvae was 0.3‰ higher than that of the nematodes and between 3.8 and 4‰ higher than that of other components of the microbial mat. While it is difficult to interpret the $\delta^{15}N$ values of the post larvae in relation to the possible dietary components, the very low nitrogen content of the dietary size fractions and the poor growth attained on them, suggests that the nematodes are the principal source of dietary nitrogen. Hence, a $\delta^{15}N$ enrichment of only 0.3‰ seems likely.

In conclusion, the results of the present study demonstrate that, under laboratory conditions, microbial mat communities may support high levels of growth and survival in early post-settlement post-larvae of *P. semisulcatus*, though not during the first 1-3 days post-metamorphosis, before the shrimp are fully benthic. There is evidence that the postlarvae feed indiscriminately on the microbial mats, but size-fractionation and stable isotope ratios suggest that the associated infauna, primarily nematodes, are preferentially assimilated and are the main source of C and N to support shrimp growth.

Chapter 5

Influence of live and formulated diets on stable isotope ratios in the tiger prawn *Penaeus semisulcatus* under controlled laboratory conditions.

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5.1 Introduction

Stable isotope analysis is an important technique that has been used to study food web structure and trophic relationships between animals and primary producers in aquatic ecosystems (e.g. DeNiro and Epstein 1978; Wada *et al.* 1987; Rundel *et al.* 1988; Persson *et al.* 1992; Vander Zanden *et al.* 1999a; Sakano *et al.* 2005). Changes in the stable isotope composition of organisms generally result from dietary shifts, though the timescales associated with such changes are not well known. There is a lag time before the isotope signature of new food sources can be detected in animal tissues and often the stable isotope signature of animal is intermediate between that of alternative food items (Frazer *et al.* 1997). There is also a trophic shift in isotope signature, which is the difference in the isotopic ratios between the tissue of a consumer and its diet (Fry and Sherr 1984). Thus stable isotope signatures in tissues may reflect an animal's diet over a period of time (Peterson and Fry 1987; Vander Zanden *et al.* 1997) and can be used as tracers for carbon and nitrogen flow in food webs. Carbon isotope ratios in consumers are usually considered to be similar to the isotope ratios of their diet, plus an enrichment for carbon of approximately 1‰ to 2‰ (DeNiro and Epstein 1987; Fry and Sherr 1984). Stable nitrogen isotope ratios in tissue provide information on trophic relationships based on interpretation as an integrated signal from assimilated diet over time (e.g. DeNiro and Epstein 1981; Tieszen *et al.* 1983; Minagawa and Wada 1984; Ambrose and DeNiro 1986; Hobson and Montevecchi 1991; Hobson and Welch 1992; Hesslein *et al.* 1993). Nitrogen isotope ratios of organisms are considered to reflect their position within food webs, increasing by a enrichment factor of 3 - 4 ‰ per each trophic level (DeNiro and Epstein 1981; Minagawa and Wada 1984; Hobson and Welch 1995; Wainwright *et al.* 1993; Kelly 2000). Both on the type of tissue and on the nature of the diet are important in

determining the relationship between the carbon isotope ratio of a consumer's tissue and carbon isotope ratio of the diet (DeNiro and Epstein 1978). The same authors found that carbon-enrichment may be variable with species, life stage and tissues analysed. Similarly, Minawaga and Wada (1984) showed that the enrichment with tropic level for a range of consumers ranges from 1.3 to 5.3 ‰ for nitrogen. Factors such as food supply can also have an effect; for example, Gaye-Siessegger *et al.* (2004) reported that $\delta^{13}C$ values in lipids and $\delta^{15}N$ in fish (*Cyprinus carpio*) is reduced with increasing feeding rate.

Changes in carbon and nitrogen isotopic composition over time can be attributable to growth and metabolic tissue replacement (turnover) components (Hesslein *et al.* 1993; Maruyama *et al.* 2001). Several studies have shown that the rate of change in nitrogen and carbon isotopic composition increases with increasing growth rate (e.g. Fry and Arnold 1982; Hesslein *et al.* 1993; Frazer *et al.* 1997; Herzka and Holt 2000; Sakano *et al.* 2005). To record the incorporation of nutrients into various tissues and to quantify the relative contribution of growth and metabolic turnover in governing isotope change, the experimental transfer of animals to diets with different stable isotope ratios has been used (Jardine *et al.* 2004). Hobson and Sealy (1991) reported that the turnover rates of isotopes may vary between tissues. For example, Tieszen *et al.* (1983) found in mammals that carbon isotope turnover were low in brain and hair, are high in fat and liver. Recently, Logan *et al.* (2006) found that liver tissue of the salt marsh mummichog, (*Fundulus heteroclitus)* had nitrogen turnover faster than muscle tissue. Several studies have also, shown that isotope turnover rates are usually related to metabolic rate (eg.Voigt *et al.* 2003).

As protein synthesis is essential to provide a supply of structural proteins to promote growth, many studies have focused on protein turnover, that is the ratio of protein accretion to protein degradation. Houlihan (1991) showed that protein turnover can be divided into three processes: protein synthesis, protein growth and protein degradation. A variety of methods, including use of stable isotope and radioactive isotope labelling have been used to measure protein turnover (Jones *et al.* 1986; Hayden *et al.* 1992). In marine animals, stable isotope have been used to determine protein turnover (Tieszen *et al.* 1983; Hesslein *et al.* 1993; Frazer *et al.* 1997; Bosley *et al.* 2002; Jardine *et al.* 2004;Sakano *et al.* 2005), though there have been few studies in crustaceans (Fry and Arnold 1982). Previous studies of turnover rates using carbon and nitrogen isotope analysis suggested that turnover rates are specific to the taxon and tissue being analysed (Fry and Arnold 1982; Hobson and Clark 1992). Many studies have also suggested that the major factors affecting the turnover rates are the water temperature (Frazer *et al.* 1997; Herzka and Holt 2000; Bosley *et al.* 2002; Witting *et al.* 2005; McIntyre and Flecker 2006; Logan *et al.* 2006), quality and quantity of diets (Mente *et al.* 2002), life stage, body size and growth (Hesslein *et al.* 1993; McIntyer and Flecker 2006) and metabolic replacement (MacAvoy *et al.* 2001). For example, Bosley *et al.* (2002) showed that animals which are growing and developing quickly have more rapid turnover than those growing less or not at all. Fry and Arnold (1982), working with brown shrimp *Farfantepenaeus aztecas* found that the most rapidly growing animals had higher turnover rates than post larvae that grew more slowly. Also, it has been reported that the availability of individual free amino acids may influence rates of protein turnover (Mente *et al.* 2001) and Claybrook (1983) reported that in most invertebrates the concentration of a free amino acids is higher than in vertebrates.

There have been relatively few studies on the effect of diet on tissue isotopic signatures in penaeid shrimp, and thus field studies such as those reported in Chapters 2 and 3 must rely on estimates of isotopic enrichment and rates of change of tissue isotopic signatures that may not accurately reflect conditions for the particular species, habitat or food web being studied. To address this problem, the aims of this study were:

1) To determine the effect of a range of formulated and natural diets on stable isotope signature ($\delta^{13}C$ and $\delta^{15}N$) in postlarval *Penaeus semisulcatus* under controlled laboratory conditions.

2) To estimate rates of changes in N and C isotope signatures in tissue over time and to separate the effects of growth and turnover components

3) To determine enrichment factors for $\delta^{13}C$ and $\delta^{15}N$ in shrimp fed a range of diets under constant conditions.

5.2 Material and Methods

5.2.1 Artificial diet formulation

5.2.1.1 Diet composition:

Two different formulated diets were prepared (Table1), formulated to be approximately iso-nitrogenous, iso- lipidic and iso-energetic. The sources of carbon and nitrogen in each diet were selected to give overall differences in $\delta^{13}C$ and $\delta^{15}N$. Thus Diet 1 was based on fishmeal and cornflour, while Diet 2 was based on soya flour, wheat gluten and wheat starch. Details of diet composition are shown in Table 1 and micro-nutrients are shown in Table 2.

5.2.1.2 Diet preparation:

The ingredients were weighed using a Metler balance (0.001g) and then mixed in a Kenwood mixer. A small amount of water was then slowly added to the mix until the ingredients formed dough. This was then passed through a mincer die, forming "spaghetti" strands. The diet was oven-dried for 24h at 50 °C and then stored in plastic bags at –20 °C until used. Prior to use, the strands were crumbled by hand to a size suitable for the shrimp being fed.

5.2.2 Natural diets

Fresh samples of microbial mat were collected daily from the intertidal zone (see Chapter 4). At low tide, the microbial mat was cleaned by removal of filamentous algal overgrowth, using a small knife. Circular discs of mat were cut and placed in Petri dishes, so that the mat completely filled each dish. The Petri dishes (91diameter x 7.5 mm depth) were positioned on the bottom of experimental aquaria for different periods depending on the experimental procedure. Fresh live mussels were collected

from the Menai Strait, opened and placed in the experimental tanks on half shells. Both microbial mat and fresh mussel (*Mytilus edulis*) were added once daily, to excess, to each of the respective treatments.

Table (1) Composition of formulated feeds used in experimental studies.

Diet 1 **High ^{13}C + High ^{15}N**	**%**	**Diet 2** **Low^{13}C + Low ^{15}N**	**%**
Fishmeal	60	Soya flour	22
Corn flour	25	Wheat gluten	22
Fish oil	2.5	Wheat starch	22
Corn oil	2.5	Fish oil	6.5
Cholesterol	1	Cholesterol	1
Lecithin	1	Mineral and vitamins	8
Mineral and vitamins	8	Lecithin	1
		Kaolin	19
		CMC	1
		Attractants	1

Table (2) Vitamin and mineral mix.

Vitamin	mg/kg
Niacin	200
p-amiobenzoic acid	100
Folic acid	10
Biotin	1
Inositol	400
Nicotinic acid	400
Ca-pantothenate	75
Pyrixodine-HCl	50
Ribooflavin	40
Thiamine HCl	60
Choline chloride	500
Cyanocobalamin	0.2
Astaxanthin (Carophyll Pink™ 8%)	364
Vitamin C (Stay C 35%)	700
Alpha tocopherol	200
Vitamin A	2
Calciferol	0.1
Menadione	100

Mineral	g/100g
Potassium monophospate	2
Dicalcium phospate	2.5
Mg sulphate	2.5
Sodium monophospate	1

5.2.3 Larval rearing procedure

Wild-caught broodstock *P. semisulcatus* originating from the State of Qatar, Arabian Gulf, were kept in a dimly- lit 8 m^3 raceway on a 14:10 L:D photoperiod, maintained at 28 °C and 33‰ salinity. Water was circulated through in a biofilter, fluidised bed sand filter, foam-fractionater and ultraviolet lamp array, with a turnover of approximately 100% d^{-1}. Mature eye-stalk-ablated females were spawned in darkened, aerated 60 l tanks with 5 µm filtered seawater at 28 °C and 33‰. The protozoea larvae were reared through the larval stages with algae (*Skeletonema costatum*: *Tetraselmis* 35:20 cell/µL) and the mysis stages were fed *Artemia* nauplii (5 nauplii per ml^{-1}). Early post larvae were fed *Artemia* nauplii (5 nauplii per ml^{-1}) until the start of the feeding experiments.

5.2.4 Effect of artificial diets on stable isotope content of shrimp muscle tissue.

From PL7, shrimp were maintained in one of two stock tanks (35.5 x 46 x 45cm), each group being fed either the diet of high $\delta^{13}C$ and $\delta^{15}N$ or the diet of low $\delta^{13}C$ and $\delta^{15}N$. Shrimp were fed 3 times d^{-1} (0900, 1300 and 1700h). After 65 days, 20 shrimp from each dietary pre-treatment were transferred to each of 3 replicate aquaria (18 x 28.5 x17cm). From this point, the dietary treatments were reversed, so that each group received a diet of higher or lower $\delta^{13}C$ and $\delta^{15}N$ content than the diet they had been raised on previously. Every 7 days, three animals from each tank were sampled, measured (total length and total weight). Before taking the samples the shrimp were starved for one day to ensure that there was no food present in the gut. The muscle tissue was dissected from the tail of each animal and gently rinsed with distilled

water. The samples were then oven-dried at 50 °C for 24 h before being stored in plastic bags .

5.2.5 Effect of natural diets on stable isotope content of shrimp muscle tissue.

Post larvae PL1 were fed on *Artemia nauplii* until PL 6. Once they were fully benthic, they were fed microbial mat for 30 days. The stock animals were then split into two groups, with 60 post larvae stocked into each of two glass aquaria (33 x 46 x 27 cm). One group continued to feed on microbial mat, while the other was changed to a diet of fresh mussel. Following the change of diet, subsamples of post larvae feeding on the microbial mat were taken sampled every 7 days, while post larvae feeding on mussel were sampled every three days. Following the procedure described for animals fed the artificial diets. Due to the initial small size of the shrimp used in this experiment, whole body samples were used for analysis rather than muscle tissue.

5.2.6 Stable isotope analysis

Samples of post larvae and diets were dried (~50°C) and finely ground. Sub-samples were weighed into precombusted silver boats (500°C, 3hrs), and carbonate material was removed through a combination of HCl (10%) additions and drying at ~50°C. The remaining, acidified, sub-samples were used for stable isotopic analysis and were placed in pre-combusted (910°C, 3 hours) quartz tubes with copper and copper oxide. The stable C and N isotopic composition was determined on CO_2 and N_2 generated by vacuum combustion and is reported in the δ notation as the ratio of the heavy to the light stable isotope in the material, R_{sample}, relative to that of a standard, $R_{standard}$, with standard = Vienna Pee Dee Bellemnite (VPDB) and air for carbon and nitrogen respectively, i.e.

$$\delta_{sample} = 1000\left(\frac{R_{sample}}{R_{std}} - 1\right)$$

The gases were separated and collected by vacuum distillation from the same sample, and were analyzed on a EUROPA-PDZ GEO 20/20 isotope ratio mass spectrometer ($\delta^{13}C$) and a VG SIRA II dual inlet isotope ratio mass spectrometer ($\delta^{15}N$)

5.2.6 Calculation of turnover of carbon and nitrogen.

Change in tissue stable isotope composition over time may be due to both growth and turnover. In order to separate these, the model used by Hesslein *et al* (1993) was applied. This requires an estimate of the growth constant (k) from the experimental growth equation:

$$W = W_{0.}e^{(kt)}$$

Where k is the growth rate, W_0 is the initial wet weight and t is the time (days). Weight data for shrimp after each dietary transition was fitted to the model using iterative non-linear regression in the SPSS statistical software package, Version 12

The model defining the relationship between tissue isotopic values (R) at time (t) was:

$$R\ sample = R_n + (R_0 - R_n)\ e^{(k+m)\,t}$$

where R_0 is the initial isotope content, R_n the final assymptotic isotope content and m is the turnover rate constant (Hesslein *et al.* 1993). For each diet transition, isotope content data were fitted to the model using iterative non-linear regression in SPSS. Only m was not defined, with R_0 and R_n entered as the initial and final isotope content

for each group of shrimp. K was taken from the growth equation for shrimp for the same dietary treatment. All graphs were plotted by using Gent Stat, 8th Edition.

5.3 Results

5.3.1 Stable isotope content of the diets.

Table 3 shows the stable isotope content of the artificial and live diets used in the experimental studies. The stable isotope content of the two artificial diets were very different. Diet 1, which contained fishmeal and corn flour had high $\delta^{13}C$ and $\delta^{15}N$ (-16.4 ± 0.11 ‰ and 12.83 ± 0.15 ‰, respectively) compared to Diet 2 which contained soya, wheat gluten and wheat starch (-24.9 ± 0.00 ‰ and 3.56 ± 0.03 ‰, respectively) Of the fresh diets, $\delta^{13}C$ and $\delta^{15}N$ values for microbial mat were substantial lower than those for mussel (-21.36 ± 0.11 and 6.83 ± 0.35, compared to -17.93± 0.30 ‰ and 11.06 ± 0.35 ‰, respectively), while values for *Artemia* nauplii were higher again (-16.23 ± 0.05 ‰ and 12.7 ± 0.15 ‰, respectively).

Table (3) Stable isotope content of artificial and natural diets used in experiments.

Diets	Number of sample	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
Artificial Diet 1	3	-16.4 ± 0.11	12.83 ± 0.15
Artificial Diet 2	3	-24.9 ± 0.00	3.56 ± 0.03
Artemia nauplii	3	-16.23 ± 0.05	12.73 ± 0.15
Microbial mat	3	-21.4 ± 0.11	6.83 ± 0.35
Mussel *M. edulis*	3	-17.93 ± 0.30	11.06 ± 0.35

5.3.2 Growth on artificial diets

The wet weight of shrimp after 65 d feeding on Diet 2 was 0.206 ± 0.080 g, increasing to 1.311 ± 0.061g after 21 days following the change to Diet 1. After the initial 65 d, the wet weight of shrimp raised on Diet 1 was 0.328 ± 0.100 g. Following the change to Diet 2, this increased to a final wet weight of 1.181 ± 0.26 g after a further 21 d. Figure 1 (a,b) shows fitted growth curves for each data set. The growth rate in post larvae feeding on Diet 1 was substantially higher ($k = 0.096 \pm 0.03\ d^{-1}$, $r^2 = 0.59$) than in those feeding on Diet 2 ($k = 0.052 \pm 0.01\ d^{-1}$, $r^2 = 0.66$).

5.3.3 Growth on natural diets

During the initial 30 days feeding on microbial mat, the shrimp increased in mean weight from 0.001 g to 0.0803 ± 0.03 g. Those that continued to feed on microbial mat reached a final mean weight of 0.3173 ± 0.61 g after a further 17 days. Those that were switched to feeding on mussel grew faster, reaching a mean weight of 1.181 g after 27 days from the change in diet. Figure 2 (a,b) shows the growth curves fitted to data from shrimp fed microbial mat for 47 days ($k = 0.093 \pm 0.01\ d^{-1}$) and in shrimp after the change in diet from microbial mat to mussel ($k = 0.069 \pm 0.008\ d^{-1}$).

5.3.4 Effect of artificial diets on stable isotope content of shrimp tissue.

At the start of the experiment, after 65 days of feeding on each diet, the initial $\delta^{13}C$ for muscle tissue for shrimp raised on Diet 1 was -13.96 ± 0.25 ‰ compared to -21.4 ± 0.9 ‰ for those raised on Diet 2. After the change in diets, the $\delta^{13}C$ content of shrimp muscle tissue changed over time, reflecting the $\delta^{13}C$ content of the respective new diet. After 21 days from the diets being switched, the $\delta^{13}C$ content of shrimp now

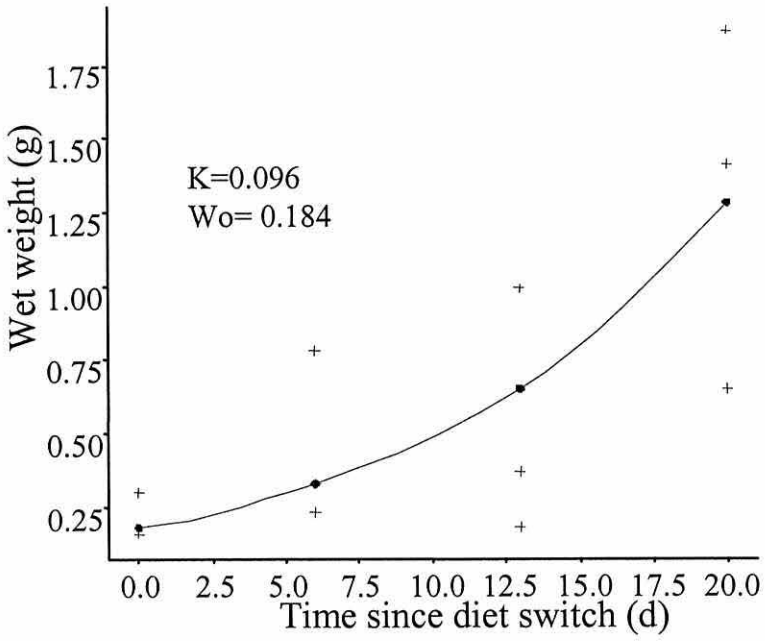
feeding on Diet 1 reached -17.63 ± 1.2 ‰ compared to a $\delta^{13}C$ -18.15 ± 0.77 ‰ for those feeding on Diet 2 (Figure 3 a,b).

The initial $\delta^{15}N$ for muscle tissue in shrimp raised on Diet 1 for 65 d was 14.90 ± 0.17‰ compared to 8.86 ± 0.95 ‰ for those raised on Diet 2. After the change in diets, the $\delta^{15}N$ content in shrimp muscle changed over time reflecting the $\delta^{15}N$ content of the respective new diet. The final $\delta^{15}N$ content for muscle tissue in shrimp now feeding on Diet 1 was 11.93 ± 1.0 ‰ compared to 11.45 ± 0.2 ‰ in shrimp feeding on Diet 2 (Figure 4 a,b).

5.3.5 Effect of natural diets on stable isotope content of shrimp tissue.

At the start of the experiment, after 12 days of feeding on *Artemia* nauplii, the initial $\delta^{13}C$ for shrimp tissue was -14.6 ± 0.00 ‰. Once feeding on microbial mat was initiated, the $\delta^{13}C$ content of shrimp tissue changed over time reflecting the $\delta^{13}C$ content of the new diet, reaching -17.0 ± 0.1 ‰ after 30 days. After 47 days, the final $\delta^{13}C$ content of muscle tissue for shrimp fed only microbial mat was –17.33 ± 0.15 ‰. For shrimp feeding on mussel from d 30, the $\delta^{13}C$ content increased reflecting the change in diet and reached a final value of -15.1 ± 0.1 ‰ 27 days after the change in diet (Figure 5).

The initial $\delta^{15}N$ content for shrimp after 12 days of feeding on *Artemia* nauplii was 12.85 ± 0.07‰. After 30 d feeding on microbial mat, this had decreased to 10.7 ± 0.00 ‰ and reached 10.36 ± 0.36 ‰ after 47 days. For shrimp feeding on mussel from d 30, $\delta^{15}N$ content increased to a final value of 12.9 ± 0.1 ‰ after 27 days from the change in diet (Figure 6).



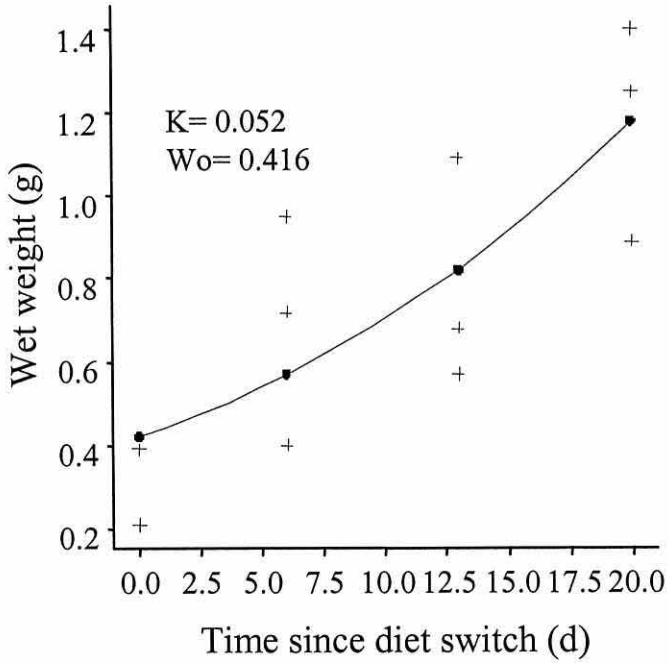


Figure (1) Growth in wet weight in *P. semisulcatus* following the transitions in artificial diet treatments (a) from Diet 2 to Diet 1 and (b) from Diet 1 to Diet 2.

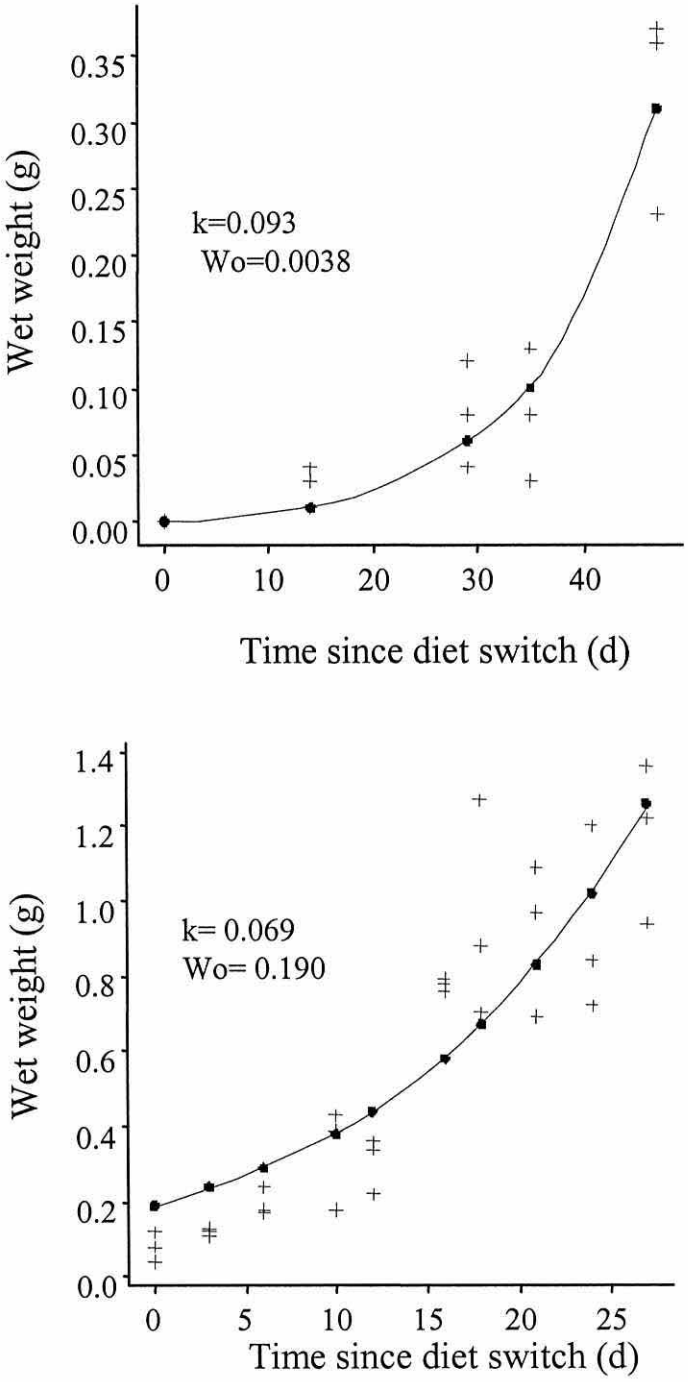


Figure (2) Growth in wet weight in *P. semisulcatus* following (a) the transition from an initial diet of *Artemia nauplii* to microbial mat and (b) the subsequent transition from microbial mat to mussel.

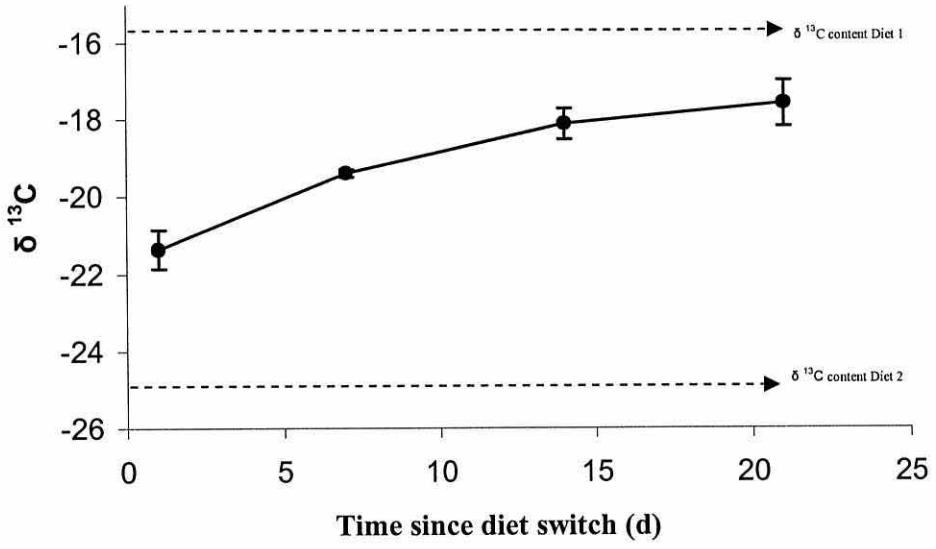


Figure 3 (a) Change in δ ^{13}C in *P. semisulcatus* tissue over time, following a change in formulated diet from lower (Diet 2) to higher (Diet 2) δ ^{13}C values.

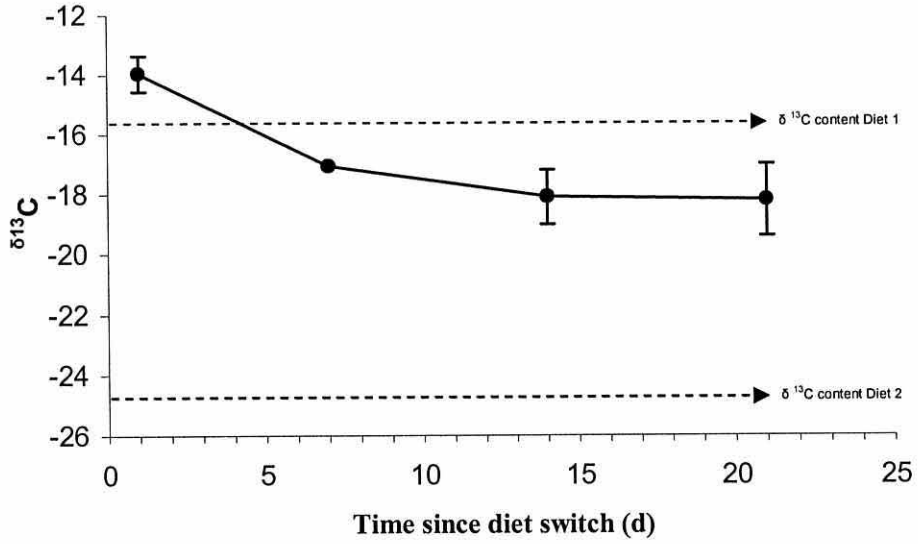


Figure 3 (b) Change in δ ^{13}C in *P. semisulcatus* tissue over time, following a change in formulated diet from higher (Diet 1) to lower (Diet 2) δ ^{13}C values.

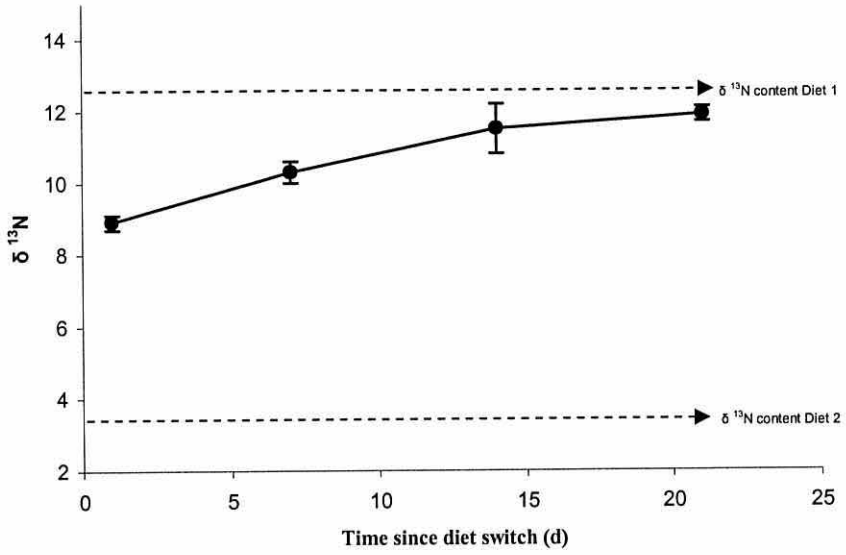


Figure 4 (a) Change in δ ^{15}N in *P. semisulcatus* tissue over time, following a change in formulated diet from lower (Diet 2) to higher (Diet 1) δ ^{15}N values.

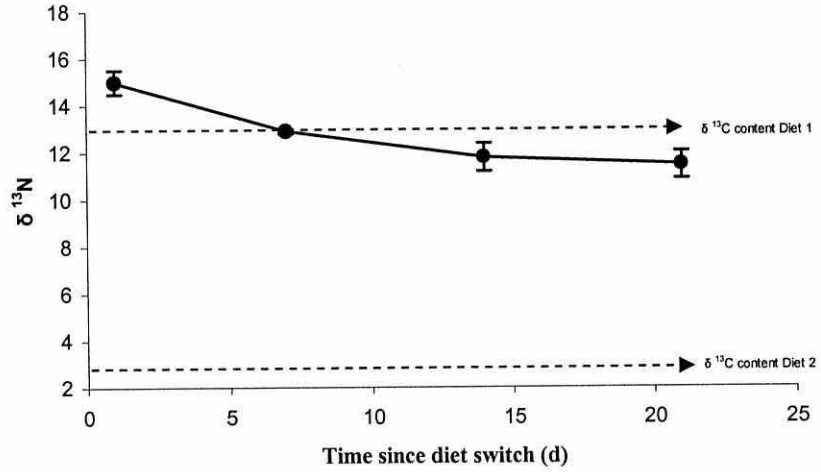


Figure 4(b) Change in δ ^{15}N in *P. semisulcatus* tissue over time, following a change in formulated diet from higher (Diet 1) to lower (Diet 2) δ ^{15}N values.

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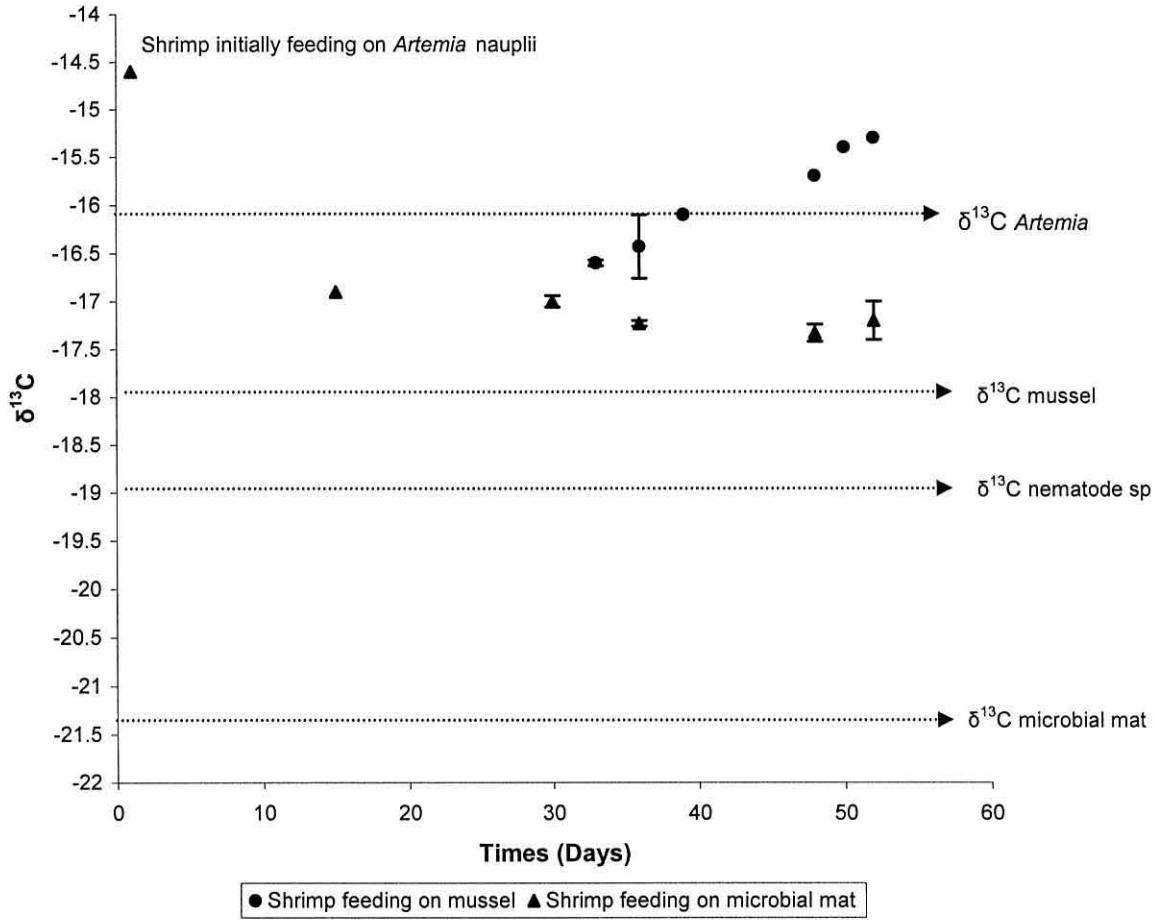


Figure (5) Change in $\delta^{13}C$ in *P. semisulcatus* tissue over time following the transition from an initial diet of *Artemia nauplii* to microbial mat and the subsequent transition from microbial mat to mussel.

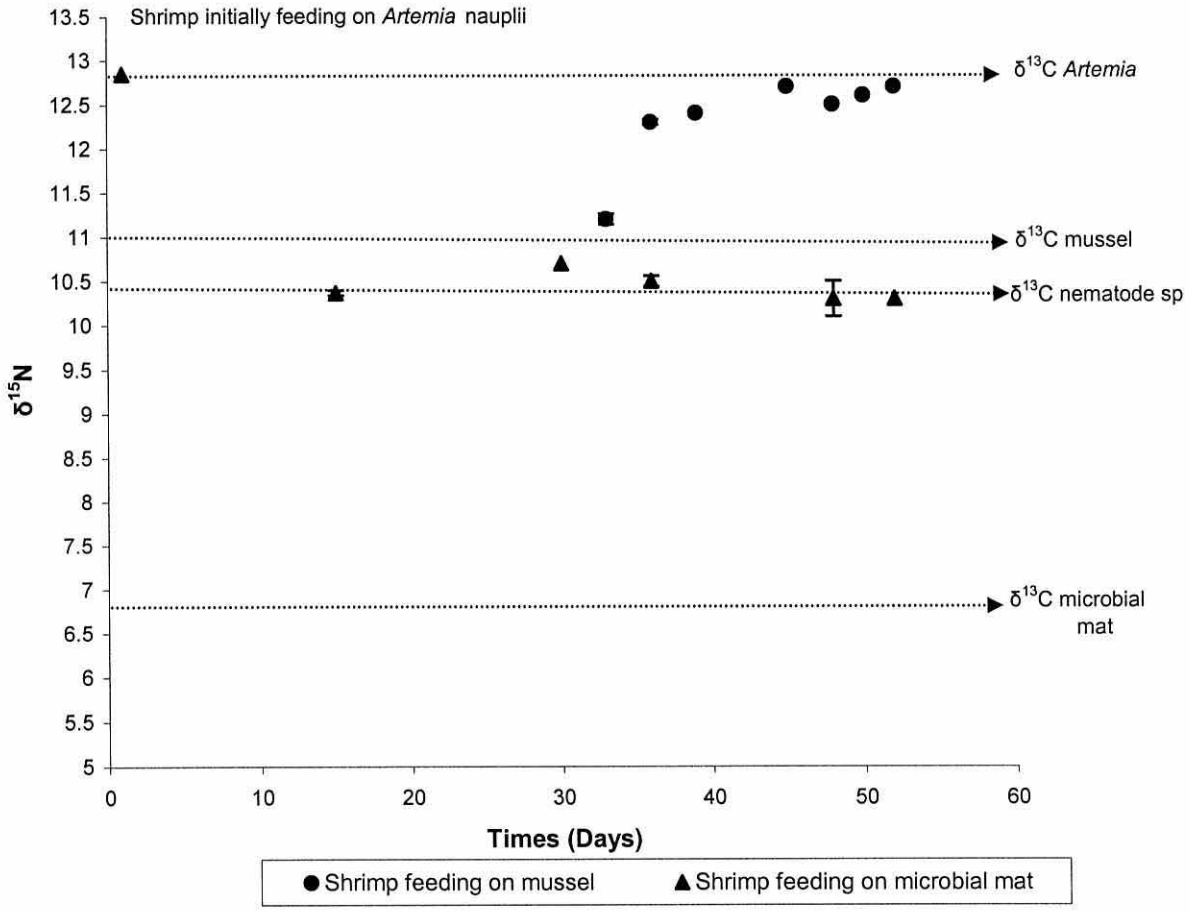


Figure (6) Change in $\delta^{15}N$ in *P. semisulcatus* tissue over time following the transition from an initial diet of *Artemia nauplii* to microbial mat and the subsequent transition from microbial mat to mussel.

5.3.6 Estimation of C and N turnover

5.3.6.1 *Artificial diets*

Figure 7 and 8 show changes in $\delta^{13}C$ and $\delta^{15}N$ over time, with fitted regression lines, following the reversal of dietary treatments. In general the model shows a good fit to the data with r^2 values ranging from 0.74 – 0.93 Table 4 showed the results of estimated turnover of carbon and nitrogen (m). The turnover of carbon in the muscle tissue of the shrimp changing from Diet 2 to Diet 1 ($0.048 \pm 0.03\ d^{-1}$) was lower than that in shrimp changing from Diet 1 to Diet 2 ($0.185 \pm 0.04\ d^{-1}$). Similarly, estimated nitrogen turnover of muscle tissue of shrimp changing from Diet 1 to Diet 2 was higher ($0.109 \pm 0.03\ d^{-1}$) than that in shrimp changing from Diet 2 to Diet 1 ($0.022 \pm 0.03\ d^{-1}$).

5.3.6.1 *Live diets*

As with the artificial diets the model shows a good fit to the isotope data, with r^2 values ranging from 0.81 – 0.98. In general the carbon and nitrogen turnover in the shrimp fed both live diets was lower than the turnover in the shrimp fed the artificial diets (Table 4). Shrimp changing diet from microbial mat to mussel had the lowest carbon turnover ($0.008 \pm 0.1\ d^{-1}$) compared to $0.016 \pm 0.009\ d^{-1}$ in shrimp feeding on microbial mat (Figure 9). In contrast, there was less difference in nitrogen turnover between shrimp feeding on microbial mat and mussel ($0.041 \pm 0.03\ d^{-1}$ and $0.03 \pm 0.02\ d^{-1}$), respectively (Figure 10).

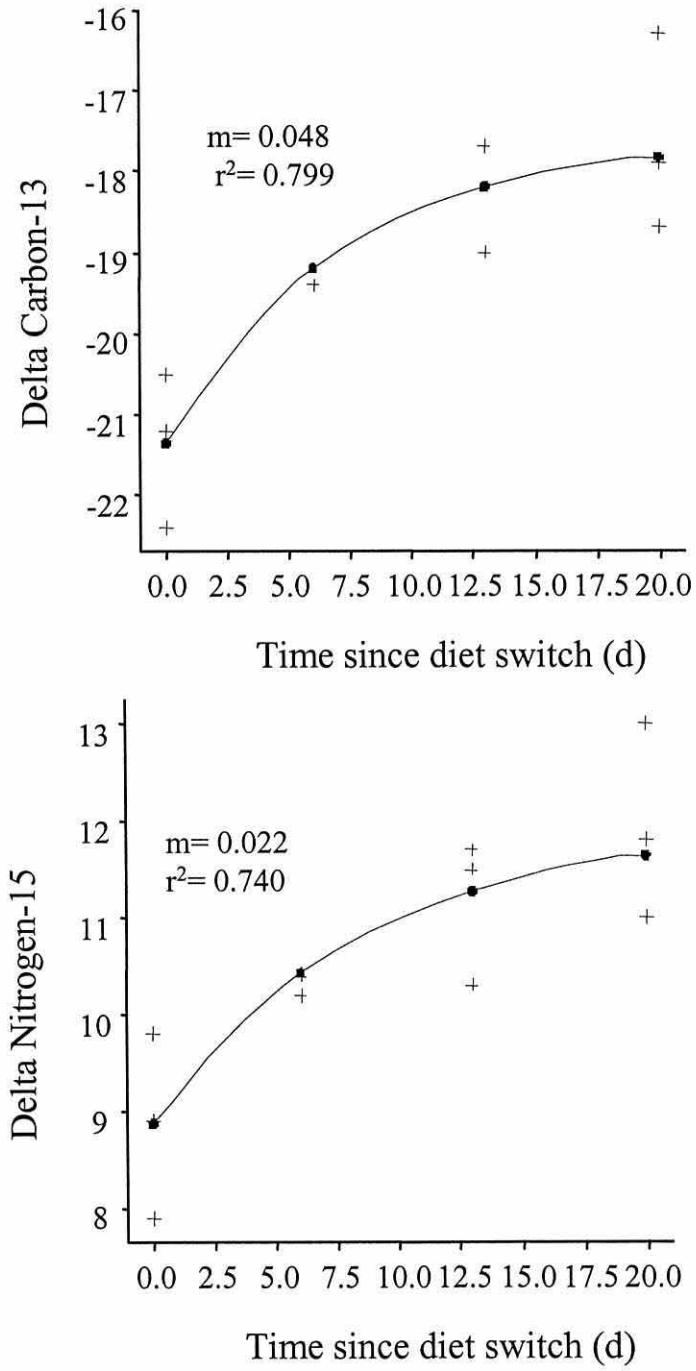
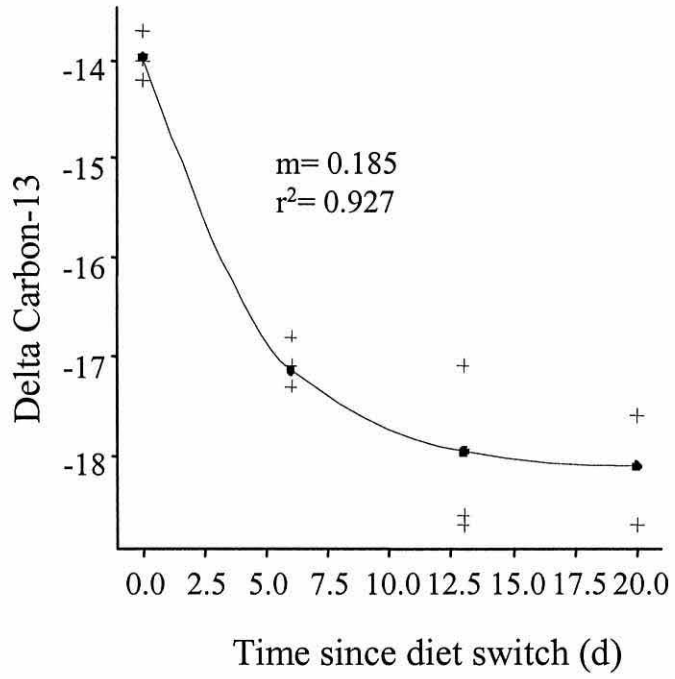


Figure (7) Fitted models of change in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in *P. semisulcatus* tissue following the transition in artificial diet treatments from lower $\delta^{13}C$ and $\delta^{15}N$ (Diet 2) to higher $\delta^{13}C$ and $\delta^{15}N$ (Diet 1).



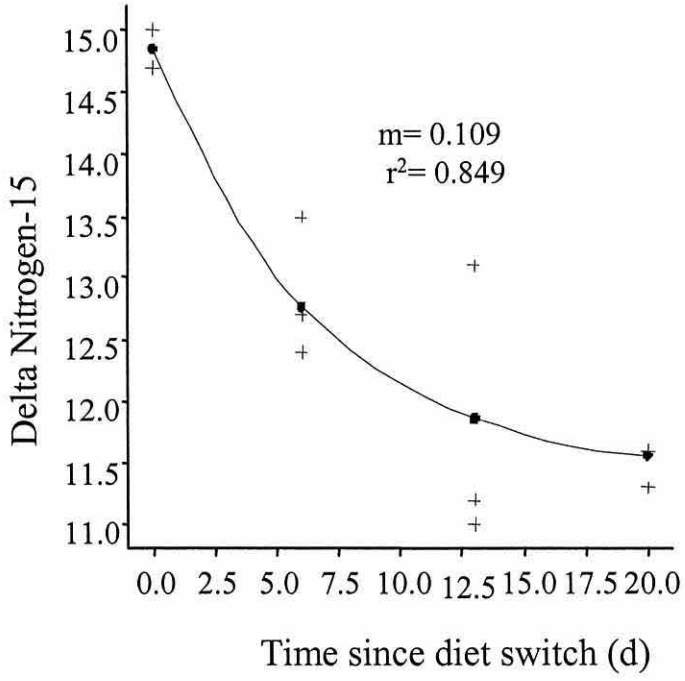


Figure (8) Fitted models of change in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in *P. semisulcatus* tissue following the transition in artificial diet treatments from higher $\delta^{13}C$ and $\delta^{15}N$ (Diet 1) to lower $\delta^{13}C$ and ^{15}N (Diet 2).

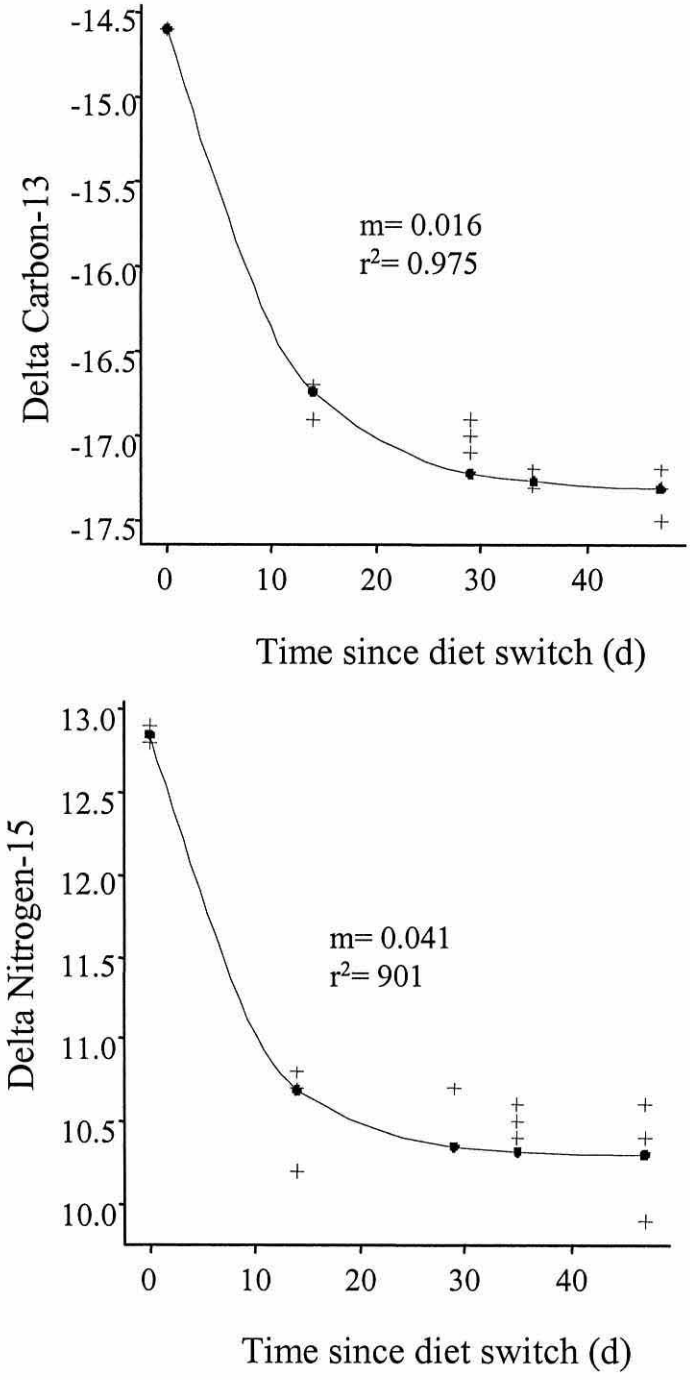
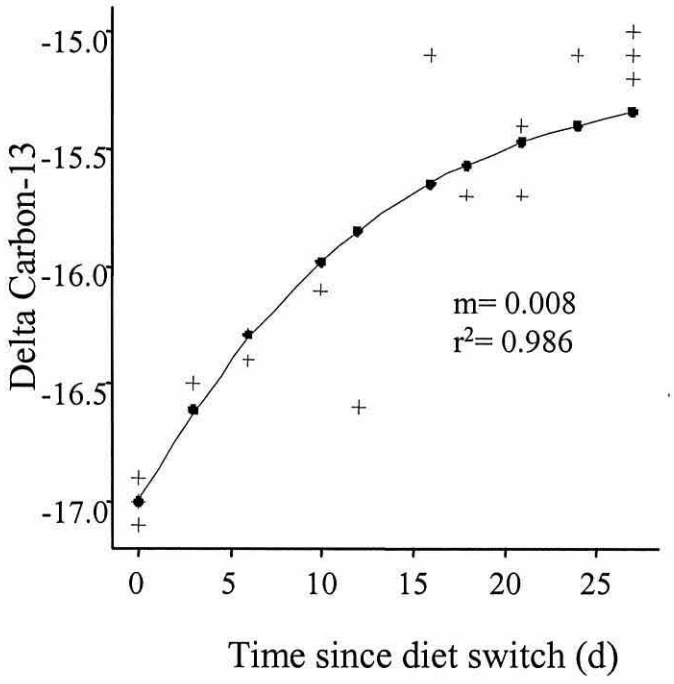


Figure (9) Fitted models of change in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in *P. semisulcatus* tissue following the transition from an initial diet of *Artemia nauplii* to microbial mat



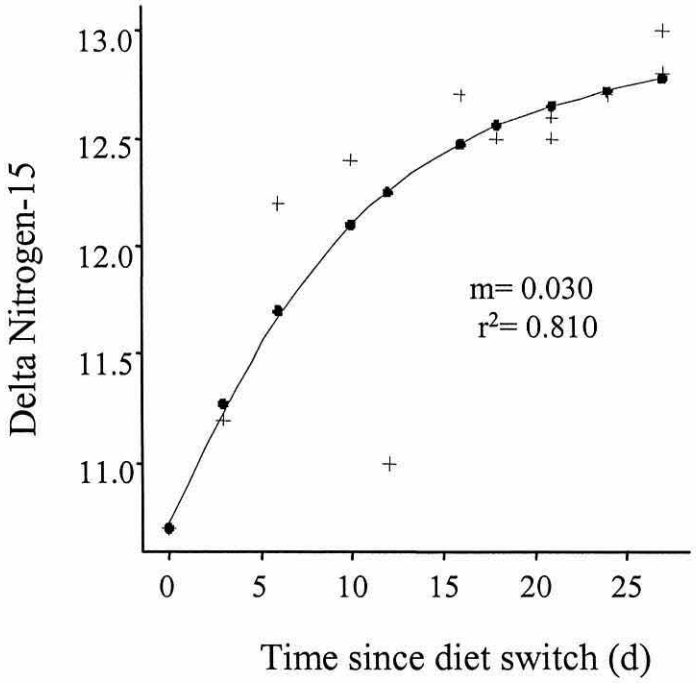


Figure (10) Fitted models of change in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in *P. semisulcatus* tissue following the transition in diet from microbial mat to mussel (*Mytilus edulis).*

Table (4) Estimated carbon and nitrogen turnover (m) in *P. semisulcatus* tissue.

Experiment	K (d^{-1})	Wo (g)	m Carbon (d^{-1})	m Nitrogen (d^{-1})
Artificial diets				
Transition from Diet 2 to Diet 1	0.096 ± 0.03	0.184 ± 0.11	0.048 ± 0.03	0.022 ± 0.03
Transition from Diet 1 to Diet 2	0.052 ± 0.01	0.416 ± 0.09	0.185 ± 0.04	0.109 ± 0.03
Live diets				
Transition from *Artemia* to microbial mat	0.093 ± 0.01	0.0038 ± 0.002	0.016 ± 0.009	0.041 ± 0.03
Transition from microbial mat to mussel	0.069 ± 0.008	0.190 ± 0.04	0.008 ± 0.01	0.030 ± 0.02

5.4 Discussion

The present study examined the shift in isotopic ratios for C and N in shrimp tissue in response to changes in diet. Such studies may provide some insights into the changes in isotope content in wild shrimp associated with changes in habitat with age (see Chapters 2 & 3). They also quantify the relative contribution of growth and metabolic turnover in governing such isotope changes (Jardine *et al.* 2004). In addition, they provide a range of enrichment values for ^{13}C and ^{15}N under laboratory conditions. Previous laboratory studies have shown that the stable isotope values in Crustacea can be very variable and may be affected by factors such as species, age and size, quality of food, use whole body of the shrimp or muscle tissue (without exoskeleton) for analysis and the stable isotope content of diet used in the experiment (Fry and Arnold 1982; Parker *et al.* 1989; Dittel *et al.* 1997; Yokoyama *et al.* 2005).

Apart from the early work by Parker *et al.* (1989), there have been no previous studies which have used stable isotope analysis of tissues to determine carbon and nitrogen turnover in marine shrimp. This approach to estimation of tissue turnover has been more widely used in fish (eg. Hesslein *et al.* 1993; Bosley *et al.* 2002; Jardine *et al.* 2004; Sakano *et al.* 2005; McIntyre and Flecker 2006). The results of the present study indicate that, in the size range studied, both the C and N isotope contents in shrimp muscle tissue take about three weeks to equilibrate after a large change in dietary isotopic signature. This is consistent with the observation of Parker *et al.* (1989) that the $\delta^{13}C$ content of *Litopenaeus vannamei* reached equilibrium in muscle tissue several weeks after a change in dietary isotope content. Similarly, McIntyre and Flecker (2006) found that stable isotope signature of four primary consumer species in

tropical freshwater systems (*Ancistrus triradiatus*, *Tarebia granifera*, *Rana palmipes* and *Lavigeria grandi*) can change noticeably within weeks following a shift in diet.

Effectively, growth represents a dilution of the existing tissue by the retention of newly-assimilated carbon and nitrogen and several studies have shown that growth is the most important factor causing stable isotopic change in muscle tissue of animals, especially fish, following a change in diet (Hesslein *et al.* 1993; Vander Zanden *et al.* 1998; Herzka and Holt 2000; McEvoy *et al* 2001; Maruyama *et al* 2001; McIntyre and Flecker 2006). Thus species and life stages with high growth rates tend to exhibit rapid levels of isotopic change; Bosley *et al.* (2002) suggested that turnover rates during periods of high growth have faster turnover rates than when growth is less rapid or static. For example, Herzka and Holt (2000) found that rapid changes in tissue isotopic composition in larval red drum *Sciaenops ocellatus* was due to their high growth rate. In recent studies in fish, Sakano *et al.* (2005) and McIntyre and Flecker (2006) found that the contribution of growth to isotope turnover is higher in young and faster-growing than older and slower-growing animals. Tieszen *et al.* (1983) reported that animals with a high metabolic rate will equilibrate faster to a new diet than those with low metabolism. Thus the rapid change in isotopic composition of shrimp in the present study reflects the rapid growth rate, with substantial relative weight gain over the experimental period, and possibly also the high metabolic rate associated with tropical seawater temperatures (28 °C).

In the present study, the Hesslein *et al.* (1993) model was used to separate changes in carbon and nitrogen isotopic signatures caused by turnover and growth rate, respectively. The observed growth rate in *P. semisulcatus* fed on artificial and live

diets was comparable to normal growth rates recorded in previous studies for the same species (Kumlu and Eroldogan 2000; Soyel and Kumlu 2003). However, growth was slower in shrimp fed on the soya/gluten-based Diet 2 that those fed the fishmeal-based Diet 1. This is consistent with other studies in penaeid shrimp that have shown that complete replacement of marine meals with plant protein sources resulted in reduced growth rates (Akiyama 1991; Lim 1997; Sudaryono *et al.* 1999; Paripatananont *et al.* 2001). This may in part be due to lower ingestion rates resulting from lower palatability of plant-based diets (Day *et al.* 2000; Paripatananont *et al.* 2001). Soya and wheat gluten have similar protein and energy digestibility compared to fishmeal (Fox *et al.* 2004). For example Akiyama *et al.* (1989) reported that in shrimp, soyabean meal has a higher apparent protein digestibility (90%) than fishmeal (80.7%). Although soya protein is low in methionine (FAO 1983; Dersijant-Li 2002) and wheat gluten is low in lysine (Bassat and Mokady 1985), it is unlikely that levels of these essential amino acids in Diet 2 would have impaired growth, as the combined use of the two protein-source ingredients was selected to provide a balanced amino-acid profile.

Several studies have shown that the variation in isotope turnover rates in animals appears to be associated with body size (Vander Zanden *et al.* 1998; Bosley *et al.* 2002; Hesslein *et al.* 1993; McIntyre and Flecker 2006) and temperature, both of which may influence growth and metabolic rates and thus play an important role to control the rate of carbon and nitrogen retention and turnover in the tissue of organisms (Frazer *et al.* 1997; Bosley *et al.* 2002; McIntyer and Flecker 2006). The lower C and N turnover rates in shrimp feeding on Diet 2 seems likely to reflect the lower growth rate in this group. This is consistent with previous studies that have

suggested that high specific growth rates may be associated to low rates of protein turnover. For example Mente *et al.* (2002) showed that increasing growth rate in *Litopenaeus vannamei* shrimp was a result of increased protein synthesis and reduced protein turnover. The decline in both carbon and nitrogen turnover rates with age observed in postlarval *P. semisulcatus* feeding on microbial mats and then mussel, probably reflects the decrease in growth rate observed during this period of development. Similarly, Fry and Arnold (1982) reported that rapidly-growing post larval *Farfantepenaeus aztecas* had higher changes in carbon composition than post larvae that grew more slowly.

The isotopic composition of shrimp tissue following diet transitions did not match exactly that of the diet to which the animals had equilibrated, reflecting some level of enrichment in all cases, though the magnitude of the difference varied between diets. In general animals are enriched in carbon and nitrogen isotopic ratios relative to their diet, but the factors underlying isotope enrichment in animal tissue are not completely understood (Frazer *et al.* 1997). Table 5 shows the different final enrichment values for shrimp fed each experiment diet. Enrichment values for $\delta^{15}N$ ranged from -1.27 to + 6.75 ‰ and $\delta^{13}C$ from -0.9 to +7.89 ‰. The highest and lowest values for both C and N enrichment are in shrimp fed the artificial diets, for tissue samples taken 21 days after the reversal of the dietary treatments. It might be argued that there was insufficient time for the tissue isotopic content to reach truly asymptotic values. However, examination of Figures 3 and 4 suggests that the final values on day 21 for shrimp feeding on both formulated diets are close to asymptotic. For the group feeding on Diet 1, the final $\delta^{13}C$ and $\delta^{15}N$ content are lower than might be expected relative to the diet (-1.27‰ and -0.9 ‰ enrichment respectively) despite a six-fold

increase in body weight and respective carbon and nitrogen turnover rates of 4.8% d^{-1} and 2.2% d^{-1}. Thus there is likely to have been very little scope for further increase in $\delta^{13}C$ and $\delta^{15}N$ content in shrimp fed this dietary source of carbon and nitrogen and the disparate enrichment values for both $\delta^{13}C$ and $\delta^{15}N$ between this group and those shrimp reared from settlement for 65 days on the same diet is likely to reflect real variability in the relationship between diet and tissue isotopic composition.

Table 6 reviews published values of carbon and nitrogen enrichment in marine shrimp reared on different experimental diets. For example, in the most directly comparable study, Parker *et al.* (1989) found that the carbon enrichment for muscle tissue of *Litopenaeus vannamei* under laboratory conditions over 3 weeks was +1.7 ‰ for $\delta^{13}C$ and +2.4 ‰ $\delta^{15}N$. In present study, carbon and nitrogen enrichment values varied more widely. Several previous studies have shown that the $\delta^{15}N$ enrichment is variable. $\delta^{15}N$ enrichment through trophic levels in a range of species has been estimated at 2.54 ‰ (Vanderklift and Ponsard 2003), 3.4 ‰ (Mangawa and Wada 1984; Post 2002) and 2.9 ‰ (Vander Zanden and Rasumussen 2001). Lower values have been reported by Macko *et al.* (1982) in experimental studies of two species of marine amphipods reared on macroalgae (*Ulva* sp. and *Gelidium* spp), where a negative enrichment in $\delta^{13}C$ and $\delta^{15}N$ -1.5 to -0.4‰ and -0.7 to -0.1‰, respectively, was found higher $\delta^{15}N$ (up to 5.8 ‰) enrichment has been estimated for invertebrates such as copepod feeding on phytoplankton (Checkley and Harkness, 1987). Thus the observed enrichment values in the present study cover the range of published values for other Crustacea, though these represent responses in diverse species.

Some previous studies suggest that the degree of $\delta^{15}N$ enrichment between a consumer and its diet may depend on the protein content of the diet (Fantle *et al.* 1999). Vander Zanden and Rasmussen (2001) reported that $\delta^{15}N$ enrichment is lower and/or more variable in herbivores, while Post (2002) found no difference between carnivores and herbivores. Some studies have reported that under conditions of nutritional stress the degree of $\delta^{15}N$ enrichment may be greater and conversely that when growth rates are higher then $\delta^{15}N$ enrichment may be lower (Hobson *et al.* 1993). In the present study there was a considerable difference in the level of $\delta^{15}N$ and $\delta^{13}C$ enrichment between shrimp feeding on the two formulated diets (Table 5), with consistently higher values for shrimp feeding on the soya/gluten-based Diet 2. To some extent this may reflect higher C & N turnover rates and lower growth rates in shrimp fed Diet 2, as well as other undetermined differences dietary quality (eg amino-acid balance, plant versus animal protein digestibility).

The intermediate enrichment values observed in shrimp feeding on microbial mat may reflect the role of infaunal nematodes representing the major source of nutrition due to differential digestion and assimilation (see Chapter 4, McCutchen *et al.* 2003). The use of whole body samples, including exoskeleton, for early postlarvae may also have some effects on the stable isotope values. McCutchan *et al.* (2003) reported a higher trophic enrichment in terms of carbon when muscle tissue was analysed compared to analysis of whole body. Yokoyama *et al.* (2005) found that the exoskeleton of two ghost shrimps *Nihonotrypaea japonica* and *N. harmandi* reared on microalgae (*Chaetoceros gracilis*) had negative $\delta^{15}N$ enrichment ranging from -3.0 to -1.9 ‰. They suggested that carbonate derived from seawater may affect the isotopic signature of the exoskeleton and suggested that the exoskeleton should be removed

from tissues by acid treatment since it may lead to erroneous estimation of the isotopic composition of muscle. This might suggest lower than recorded, possibly negative, enrichment values for both carbon and nitrogen in muscle tissue in postlarvae feeding on *Artemia* nauplii.

In the second part of the present study, the sequential change in live diets, from *Artemia* to microbial mat to *Mytilus edulis,* reflects a similar change in diet observed in wild postlarvae (from zooplankton to benthic microbial mats to infaunal invertebrates, Chapters 2 and 3). These results were intended to support confirmation of the interpretation of dietary sources of C & N in tissue of shrimp sampled in the field. However, the results in Table 5 show that stable isotope enrichment is highly dependent on diets under constant environmental conditions. The rate and magnitude of such changes may in turn be affected by rate of metabolic turnover, growth rate and temperature, suggesting even greater levels of variation in the wild where a diverse diet and seasonal changes in environmental conditions are typical. This indicates that interpretation of trophic links to primary sources of carbon and nitrogen based on field data should be undertaken with considerable caution, though the range of enrichment values applied in Chapters 2 and 3 were taken from the highest and lowest values for carbon and nitrogen enrichment for live foods from the present study (+2.8‰ in both cases). The laboratory results do, however, indicate consistently that equilibrium in stable isotope composition of muscle tissue in *P. semisulcatus* is achieved in 2-3 weeks at 28°C. Recruitment and subsequent growth of shrimp in the field was observed from April onwards, when seawater temperatures ranged from 26.5 – 32 °C, suggesting that a similar time period for tissue isotopic changes associated with changes in age, diet or habitat to become apparent. Thus the differences in isotopic

signatures between shrimp sampled in wild can be considered very likely to reflect integration of dietary carbon and nitrogen sources over a relatively short time frame and thus to reflect dietary intake for that particular life stage and habitat. The range of trophic shifts in $\delta^{13}C$ and $\delta^{15}N$ observed in the present study are considerably greater than previously reported. The results should be confirmed in further studies, also investigating the effects of age, growth rate, temperature and dietary protein: energy ratios.

Table (5) Summary of stable isotope carbon and nitrogen enrichment for *P. semisulcatus* feed on different diets experiment in the present study.

Experimental diet	Time feeding on diet (d)	Initial length (mm)	Final length (mm)	Initial wet weight (g)	Final wet weight (g)	Diet $\delta^{13}C$ ‰	Diet $\delta^{15}N$‰	Tissue $\delta^{13}C$ ‰	Tissue $\delta^{15}N$‰	Enrichment $\delta^{13}C$‰	Enrichment $\delta^{15}N$‰
Artificial diet											
Diet1	65	8.79 ± 0.48	37.50±3.58	0.00139±0.00	0.328 ± 0.10	-16.36±0.36	12.83±0.15	-13.96±0.25	14.9±0.17	2.4	2.07
Diet1	21	36.90±5.73	60.27±7.29	0.2061 ± 0.08	1.311±0.61	-16.36±0.36	12.83±0.15	-17.63±1.2	11.93±1.0	-1.27	-0.9
Diet2	65	8.79±0.48	36.90±5.73	0.00139±0.00	0.206±0.08	-24.9±0.0	3.56±0.03	-21.36±0.96	8.86±0.95	3.54	5.3
Diet2	21	37.50±3.58	56.57±4.12	0.3282 ± 0.10	1.181±0.26	-24.9±0.0	3.56±0.03	-18.15±0.77	11.45±0.2	6.75	7.9
Live diet											
Artemia	12	*Mysis**	8.79±0.48	--	0.0014±0.00	-16.23±0.05	12.73±0.15	-14.6±0.0	12.85±0.07	1.63	0.12
Microbial mat	47	8.79 ± 0.48	39.25±3.77	0.00139±0.00	0.3173±0.61	-21.36±0.1	6.83±0.35	-17.33±0.15	10.3±0.36	4.03	3.47
Mytilus edulis	27	16.43±2.49	55.73±3.06	0.0803±0.03	1.181±0.21	-17.93±0.3	11.06±0.35	-15.1±0.1	12.93±0.1	2.83	1.87

Table (6) Summary of previous studies of isotopic enrichment for shrimp based on feeding experiments.

Shrimp species	Diet	Enrichment $\delta^{15}N‰$	Enrichment $\delta^{13}C‰$	Reference
Litopenaeus vannami	Artificial diet	2.4	1.7	Parker *et al.* 1989
L. vannami	Zooplankton	2.7	0.4	Dittel *et al.* 1997
Farfantepenaeus azatecus	Shrimp, squid, brine shrimp	-0.9 to 1.1	-	Fry and Arnold 1982
Nihonotrypaea japonica	Microalgae (*Chaetoceros gracilis*)	-1.9 to 3.9	-0.5 to 2.1	Yokoyama *et al.*2005
N. harmandi	Microalgae (*Chaetoceros gracilis*)	-2.7 to 4	-1.7 to 2.2	Yokoyama *et al.*2005

Chapter 6

Summary and conclusions

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Summary and conclusions

The present study was divided into two phases. The field studies aimed to identify tropic dependence on specific habitats in coastal nursery grounds for a commercially important shrimp, *Penaeus semisulcatus*. The study used carbon and nitrogen stable isotope and stomach content analysis to confirm the link between primary producers and post larval and juvenile shrimp from intertidal and subtidal habitats. Two different areas with contrasting habitats were surveyed; Al-Khor Bay, characterized by a variety habitats such as mangrove, seagrass beds and microbial mat and Doha Bay, where seagrass beds, algal beds and microbial mat were present, but not mangrove. The experimental studies investigated the nutritional value of microbial mats as a food source for shrimp post larvae and quantified the changes in stable isotope signatures in shrimp tissues under controlled laboratory conditions, to support field data by demonstrating the time scale over which stable isotope signatures accumulate in different shrimp tissues and the range of trophic enrichment values.

In tropical coastal environments, primary production from benthic habitats such as seagrass beds, mangroves, algal beds and intertidal microbial mats are very important important links in coastal food webs and support many commercial fisheries. In particular for penaeid shrimps, the nursery role of seagrass beds and algal beds have been widely recognized. Mangrove forests are also important nursery grounds for many species of commercial fish and crustaceans. Finally, microbial mats are important in soft-sediment intertidal habitats in both temperate and sub-tropical coastal ecosystems and have been demonstrated to support the nutrition of a range of benthic and epibenthic invertebrates.

During the present study, the earliest post-settlement post-larval stages *P. semisulcatus* were not found at any of the sampling sites in Doha and Al-Khor bays. Later-stage postlarval *P. semisulcatus* were only found in seagrass habitats in both bays, which is consistent with other studies that have shown that seagrasses beds are the primary habitat for the early life stages of this species (Haywood *et al.* 1995; Loneragan *et al.* 1997). Interestingly, in Al-Khor Bay the first settlement stage post larvae of *Metapenaeus affinis* were found in both seagrass beds and on intertidal microbial mats, while neither *P. semisulcatus* nor *M. affinis* were found in the mangrove. This is consistent with several other studies have shown that *M. affinis* juveniles are found over intertidal mud flats and that have suggested feeding on microbial mats (Clayton 1986; Jones *et al.* 2002; Al-Zaidan 2002;Al-Zaidan *et al.* 2006). Chapter 4 demonstrated that under laboratory conditions microbial mats can support excellent survival and growth in very early stage *P. semisulcatus* post-larvae and it is suggested that the microbial mats represent the most likely principal habitat for the earliest post-settlement stages of this species, in both Doha and Al-Khor bays.

Overall, seagrass beds in both bays are the main nursery ground for late post larvae and juvenile *P. semisulcatus* and this is consistent with several previous studies (eg. Young and Carpenter 1977; Zimmerman *et al.* 1984; Staples *et al.* 1985; Coles *et al.* 1987; Dall *et al.* 1990; Sheppard *et al.* 1992: Hill and Wassenberg 1993; Loneragan *et al.* 1994; Haywood *et al.* 1995; Heales *et al.* 1996; Loneragan *et al.* 1998). Adult *P. semisulcatus,* as with other penaeid species, are consistently found in deeper water where macroalgal beds dominate (eg. Dall *et al.* 1990; Somers and Kirkwood 1991; Crocos and van der Velde 1995; Chapter 2).

In the present study, the analysis of gut contents showed that post-larvae and juvenile *P. semisulcatus* in shallow water seagrass beds at Doha Bay fed mainly on benthos such as foraminifera, polychaetes, diatoms and small crustaceans (amphipods, isopods and ostracoda), whereas the examination of gut content of larger *P. semisulcatus* shrimp in deep water (where macroalgal beds are predominant) indicated fed mainly on bivalve molluscs and to a lesser extent polychaetes. On the other hand, the gut contents of *M. affinis* at Al-Khor Bay in shallow water seagasss beds indicated that they fed mainly on molluscs (bivalves) and polychaetes, foraminifera, while the presence in the gut of diatoms, dinoflagellates and some fine organic matter suggested some non-selective grazing on microbial mats.

Overall, the examination of gut contents of post larval and juvenile *P. semisulcatus* from the Doha Area (Chpter 2) and *M. affinis* from Al-Khor Bay in the present study suggests that there are differences between these species in terms of the food ingested. In both *M. affinis* and *P. semisulcatus* foraminifera and molluscs were the most frequently occurring food items in the gut. However, such hard parts may be evacuated from the gut at a slower rate than soft food, and thus may be over-represented in the gut contents (Hill 1976) and trituration and homogenization of soft items in the feeding mill may hamper the identification of soft food items (O'Brien 1994). It seems likely that soft-bodied fauna such as nematodes are also under-represented. Consequently comparison of feeding habits between species and sites should be interpreted with a degree of caution.

Many studies have indicated both omnivorous (indiscriminate) and selective carnivorous feeding in juveniles of a range of penaeid shrimp species. The most commonly observed prey item are crustaceans, molluscs, polychaetes and other benthic invertebrates (eg. Chong and Sasekumer 1981; Leber 1985; Wassenberg and Hill 1987, Hill and Wassenberg 1993; Nelson and Capone 1990; O'Brien 1994; Schwambron and Criales 2000). The prevalence of benthic invertebrates in the gut of *P. semisulcatus* in the present study is consistent with others studies of this species in Australian waters (Wassenberg and Hill 1987; O'Brien 1994; Haywood *et al.* 1995; Heales *et al.*1996). Overall, the gut contents data form Doha Bay suggest that juvenile and adults of *P. semisulcatus* are benthic omnivores but develop a predominantly carnivorous diet as they grow, feeding on one or two dominant prey species, similar to other penaeid species (Hunter 1984; Mayer 1985; Nelson and Capone 1990; Schwamborn and Criales 2000; Heales 2000). The comparison of the gut contents of shrimp in Doha Bay indicates a clear transition in the diet as shrimp grow and move from shallow seagrass beds to deeper algal beds.

In this study, the primary producers at Doha and Al-Khor bays have isotopically distinct values of $\delta^{13}C$ similar to previously reported values for phytoplankton (Fontugne and Duplessy 1981), seagrasses (Hemminga and Mateo 1996), microbial mats (Barghoorn *et al.* 1977; Schidlowski *et al.* 1984; Jones *et al.* 2002, Abed-Navandi and Dwarschak 2005) and mangroves (Rodelli *et al.* 1984; Mohan *et al.* 1997; Bouillon *et al.* 2002; Jones *et al.* Stable isotope analysis of shrimp from Doha Bay indicated that there was no difference in $\delta^{13}C$ between *P. semisulcatus* post-larvae and juveniles from the same site, but there were significant differences between sites that may reflect both diet and life stage differences. For example, the post-larvae

and juveniles at shallow water seagrass beds (-9.5 ± 0.26 to -11.3 ± 0.3‰) were enriched compared to adults collected at seagass beds and algae beds at deep water (-12.5 ± 0.1 to -12.7 ± 0.05‰), reflecting the differences in isotope ratios between primary producers (Parker and Calder 1970; Thayer *et al.* 1978; Fry and Parker 1979).

In Al-Khor Bay, stable isotope analysis indicated that primary production in mangroves ($\delta^{13}C$ -28.5 to -27.4 ‰ and $\delta^{15}N$ 0.8 to 0.9 ‰) did not contribute to nutrition of either shrimp species, but suggested an influence from seagrass beds ($\delta^{13}C$ ‰ -7.9±1.3and $\delta^{15}N$ 1.5±0.42 ‰) and microbial mats ($\delta^{13}C$ -7.7 ‰ and $\delta^{15}N$ -0.9 to -0.2 ‰). Primavera (1996) studied the stable isotope contents of shrimp tissue and those of primary producer, and concluded that juvenile shrimp in the mangroves derive their carbon from plankton and epiphytic algae rather than though the mangrove detritus pathway. On the other hand, several studies have shown links between mangrove forests and food webs supporting coastal fisheries (Macnae 1974; Staples *et al.* 1985; Camacho and Bagarinao 1987; Chong *et al.* 1990; Loneragan *et al.* 1997; Primavera 1998). The present study in Al Khor clearly shows that despite the presence of quite extensive areas forested areas, mangrove productivity made no measurable contribution to nutrition of shrimp in seagrass areas within a distance of even a few hundred metres. This may be attributed to the low rainfall in the Gulf, resulting in limited mangrove productivity and minimal the export of DOM and POM to adjacent habitats. This is consistent with the previously observed weaker influence of mangrove production on shrimp isotopic composition during the dry season in northern Australia (Loneragan *et al.* 1997)

It was clear from the laboratory studies that once *P. semisulcatus* post-larvae are fully-benthic, at 3d post-metamorphosis, microbial mat supported high growth and survival rates, equivalent to that of control diets. Earlier-stage post-larvae, which were still some extent planktonic, exhibited significantly poorer survival when only the microbial mat was available as a food source. Examination of gut contents in laboratory study indicated that benthic post-larvae feed indiscriminately on the microbial mat and their gut content was dominated by algae, cyanobacteria and diatoms with a lesser contribution by faunal components including nematodes, eggs/cysts and foraminifera. However, rearing post-larvae on separated size-fractions of the microbial mat showed that only the fraction containing a high concentration of infauna (mainly nematodes) was able to support the same growth as intact microbial mat. This is consistent with previous rearing trials were free-living nematodes were successfully used to raise penaeid shrimp larvae (Wilkenfeld *et al.* 1984; Biedenbach *et al.* 1989; Kumlu and Fletcher, 1997; Kumlu *et al.* 1998). Analysis of total carbon and nitrogen content suggested that both the overall protein content of the microbial mat and the various size fractions is insufficient to support growth in shrimp. Analysis of stable isotope ratios in the dietary size-fractions and the shrimp tissue supports the hypothesis that the shrimp are gaining most of their nutrition from the associated infauna. This may be due to selective feeding that is not apparent from stomach contents due to rapid digestion of infauna soft tissues. Alternatively it could be due to differential assimilation of nematode tissue relative to other microbial mat components such as cyanobacteria (blue green algae), diatoms and organic fractions of sediment. The carbon isotopic value of the presumed diet is close to that of the nematodes (-19‰) and different from other components of the microbial mat ranging from -21.3 to

-22.‰, representing a trophic shift in $\delta^{13}C$ for post larvae fed on microbial mat of 2‰.

Examination of the shift in isotopic ratios for C and N in shrimp tissue in response to changes in diet indicated that stable isotope signatures of shrimp tissue take about 2-3 weeks to equilibrate after a large change in dietary isotopic signature. This is consistent with the observation of Parker *et al.* (1989) that the $\delta^{13}C$ content of *Litopenaeus vannamei* reached an equilibrium in muscle tissue 3 weeks after a change in dietary isotope content. In the two artificial diet treatments, the slower growing shrimp exhibited higher turnover of carbon and nitrogen. This is consistent with previous studies which have suggested that high specific growth rate may be associated with low rates of protein turnover. In addition several studies have shown that growth is the most important factor causing stable isotopic change in fish following a dietary shift (Hesslein *et al.* 1993; Herzka and Holt 2000). The results of the enrichment values for both $\delta^{13}C$ and $\delta^{15}N$ (Chapter 5, Table 5) may be helpful to interpretation of the field study data. The high range of both carbon and nitrogen stable isotope enrichment supports the conclusion that nutrition of *P. semisulcatus* post larvae and juvenile could be derived from both seagrass beds and microbial mats. There is considerable scope for further work applying stable isotope techniques to shrimp in the laboratory. These might include more detailed study of the effect of temperature and life stage on isotopic turnover, which could be linked to further field studies. From an aquaculture point of view the technique offers potential for examination of protein turnover, in studies determining optimal dietary protein levels and sources.

Overall, the results presented in this study suggest that the combined use of stable isotope analysis and gut contents examination is an effective approach to studying the nutrition and feeding ecology for selected species. This approach confirms the linkage between sensitive shallow water habitats and the key life stages of important commercially-exploited species. Unfortunately, recently many of the sampling areas used in this study in Doha Bay, included much of the segrass beds have been lost of damaged due to land reclamation operations. To protect the natural habitats supporting populations of shrimp and many other marine organisms, the government have to consider very carefuly any type of operations such as digging, drilling and dredging of coastal zone which may negatively influence sensitive habitats. A positive step would be to conduct coastal habitat surveys in areas under consideration for further development to allow mitigation of any impact that could cause permanent damage to essential habitats, compromising the long term sustainable use of important marine resources in Qatar. Continued development of the coastal zone at the present rate, over the next few years, runs the serious risk of endangering local populations of shrimp, reduced this traditional source of revenue for the coastal communities in Qatar. Based on the present study, it is recommended that public institutions such as the Department of Fisheries and the Ministry of Municipal Affairs and Agriculture should establish a long-term strategy to prevent the loss of essential habitats while developing infrastructures in the area. Identification and conservation of important nurseries along with the restoration of damaged seagrass beds (Al-Jamali *et al.* 2006) should be carefully contemplated. It would also be wise also to determine the status of the reproductive stocks, and to consider augmentation through revision of fishery regulations and/or enhancement programs.

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