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The Potential Environmental Impacts from Spiny Lobster Aquaculture in Sea-cages

Sooxi Lee

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Marine Science

The University of Auckland, 2015
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<td>Andrew Jeffs</td>
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<td>Neil Hartstein</td>
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<td>Ke Yee Wong</td>
<td>Development of depositional models</td>
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Extent of contribution by PhD candidate (%): 90

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Chapter 4/ Characteristics of faecal and dissolved nitrogen production from tropical spiny lobster, Panulirus ornatus

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</tr>
<tr>
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Abstract

Spiny lobsters are one of the world’s most valuable seafood species and they have been aquacultured in floating sea-cages since the 1970’s, mainly in Asian-Pacific countries. Despite the growing interest and rapid expansion of spiny lobster aquaculture, knowledge on the potential environmental impacts of this activity are limited. In an effort to begin to understand the environmental impacts of spiny lobster aquaculture, the waste products of two spiny lobsters species (*Jasus edwardsii* and *Panulirus ornatus*) were quantified, including the settling velocity of faecal material and DIN output from lobsters of a range of sizes that were fed two dietary treatments (seafood and artificial diet). Quantified particulate (faeces and waste feed) and soluble wastes (dissolved inorganic nitrogen, DIN) were then used as model inputs for a hydrodynamic numerical model to predict potential benthic carbon deposition as well as DIN elevation from hypothetical lobster farming operations. Multiple scenarios with various combinations of stocking density (3 and 5 kg m\(^{-3}\)), feed conversion ratios (FCR) using seafood or artificial diet (FCR 1.28 - 28) and three different sizes of farm layout were used in the model simulations. The overall mean settling velocity of faecal strands produced by spiny lobsters (both *J. edwardsii* and *P. ornatus*) fed with seafood ranged from 0.30 ± 0.01 (mean ± S.E.) cm s\(^{-1}\) to 0.9 ± 0.05 cm s\(^{-1}\), which was significantly higher than those from lobsters fed with artificial diet (0.22 ± 0.01 cm s\(^{-1}\)). However, the settling velocity of faeces did not differ among the lobsters of different sizes for each diet. There was a positive correlation between both the weight and density of faecal strands and their settling velocity. Conversely, neither the length nor the surface area of faecal strands was correlated with the settling velocity. Diet also had significant influence on the DIN excretion. Lobsters of both species (*J. edwardsii* and *P. ornatus*) fed with seafood produced more DIN (9.07 ± 0.90 to 12.66 ± 1.98 μg N g\(^{-1}\) hr\(^{-1}\)) than those fed with artificial diet (2.11 ± 0.41 to 11.39 ± 2.36 μg N g\(^{-1}\) hr\(^{-1}\)). Hydrodynamic modelling showed that carbon deposition and DIN elevation mainly occurred directly beneath the sea-cages and can be dispersed up to 200 m away from the perimeter of the sea-cage. Modelled results showed that the carbon deposition directly beneath the sea-cages ranged from 0.15 to more than 0.80 kg C m\(^{-2}\) yr\(^{-1}\) while elevated DIN ranged from 5.4 to 25 μg N L\(^{-1}\). Both deposited carbon mass and elevated DIN increased proportionately with higher stocking density and FCR values. The type of diet had the largest effect on both carbon deposition and DIN elevation generated from a lobster farm, with seafood diet producing more extensive impacts than artificial diet. Therefore, the elimination of the use of trash fish as a feed would
greatly reduce the environmental impacts of spiny lobster aquaculture. Overall, the results indicate that spiny lobster aquaculture in sea-cages is unlikely to cause adverse environmental impacts on the seabed and water column unless the lobsters are heavily stocked and supplied with poor quality feed.
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I am grateful to the Danish Hydraulics Institute (DHI) in Malaysia as well as New Zealand for welcoming me to their offices and providing me with the necessary modelling software to simulate my models. I would like to thank the DHI staffs for assisting me with the modelling work especially Alan Kerroux and Rachel Wong.

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Chapter 1: General Introduction

1.1 Overview of aquaculture

Overfishing of wild fish stocks and increasing demand for seafood has led to aquaculture becoming the world’s fastest growing food production industry. Annual global aquaculture production volumes have increased 6.6 % per annum on average since 1970 (FAO 2011) (Figure 1.1) so that in 2011 aquaculture contributed 40.1 % to the world total fish production (FAO 2013). The global aquaculture production in 2011 reached 62.7 million tonnes with a value of US$130 billion, where the majority (approximately 80%) of the world’s aquaculture production is in Asia Pacific region (FAO 2013). Such activity has benefited both the social and economic status of many developing countries (FAO 1997). Aquaculture is undertaken either in land-based systems in ponds and tanks, or in aquatic systems in pens and cages. A wide variety of fish, mussels, oysters, seaweed, prawns, as well as spiny lobsters, are among the species currently being cultured.

![Figure 1.1. Total global aquaculture production volumes for 1970-2011 (FAO 2013).](image)
1.2 Spiny lobster aquaculture

One of the world’s most valuable seafood species are the spiny lobsters (Crustacea: Decapoda: Palinuridae). The most commercially important genera are Panulirus and Jasus. These spiny lobsters have a high market appeal in over 90 countries across Asia, Europe and America (Williams et al. 2001). Panulirus species are found in the warm temperate to tropical regions while the Jasus species are found in the cooler temperate regions of the Southern Hemisphere (Lipcius and Eggleston 2000). Many wild stocks of spiny lobsters have been over-exploited through intensive commercial, recreational and traditional fishing, and therefore wild populations are unable to meet the increasing market demand (Hooker et al. 1997). Aquaculture of these species is believed to provide a route for relieving pressure on limited spiny lobster fisheries in the longer term by providing an alternative supply (Cox and Johnston 2003; Williams 2007b).

There has been interest in spiny lobster aquaculture for over 100 years, yet at present, a variety of spiny lobster species are commercially cultured on a relatively small scale in the Americas, Europe, as well as in the Asia-Pacific region (Phillips and Matsuda 2011). Vietnam has pioneered commercial scale aquaculture of spiny lobsters starting firstly in their Khanh Hoa province in 1992 before spreading through many sheltered coastal areas in the southern regions of the country (Tuan and Mao 2004; Hart 2009; Petersen and Phuong 2010). The success of spiny lobster aquaculture in Vietnam has encouraged further development of this activity in other countries in the Asia-Pacific region, especially the Philippines, Thailand, Malaysia, India, Indonesia and Australia (Table 1.1) (Jones 2010; Rao et al. 2010; Phillips and Matsuda 2011; Phillips et al. 2013). Commercially important spiny lobster species used in aquaculture includes Panulirus ornatus, P. homarus, P. stimpsoni, P. polychagus, P. longipes, P. penicillatus, P. versicolor and Jasus edwardsii (Jeffs and James 2001; Phillips and Matsuda 2011).
Table 1.1. Total annual aquaculture production of spiny lobsters (1998 – 2009) by key countries involved in this aquaculture activity. (Phillips et al. 2013)

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1.2.1 Husbandry methods

Husbandry methods in lobster aquaculture include land-based aquaculture systems using tanks with either recirculating water systems or flow-through systems as well as sea-cage systems deployed in shallow coastal waters (Crear et al. 2003; Hung and Tuan 2009). Sea-cage aquaculture of spiny lobsters is still in its infancy and is not practiced widely. However, it is emerging as the preferred method for culturing spiny lobsters in the Asia region with substantial growth in this activity in recent years (Phillips and Matsuda 2011). Traditional aquaculture cages such as wooden fixed pens in shallow water, floating sea-cages where wooden frames are attached to buoys, as well as submerged cages made of netting deployed in shallow coastal water (up to 30 m depth) close to shore are used in spiny lobster grow-out operations (Arcenal 2004; Tuan and Mao 2004; Priyambodo and Sarifin 2008; Hung and Tuan 2009). The grow-out period of spiny lobster aquaculture is generally separated into a nursery phase and a grow-out phase (Priyambodo and Sarifin 2008; Petersen and Phuong 2010). The nursery phase is from puerulus (post-larva) to juveniles of approximately 2 cm total length (i.e., 10-80 g) while the grow-out phase is from juveniles to a marketable size (i.e., 100 – 1000 g) (Priyambodo and Sarifin 2008; Petersen and Phuong 2010). Average culture duration of nursery and grow-out phases is approximately 18 months, depending on the lobster species, aquaculture location and the final market size of the lobsters (Petersen and Phuong 2010). Stocking densities of lobsters in sea-cages are approximately 85 - 100 individuals m⁻³ for nursery phase and 8 – 10 individuals m⁻³ for the grow-out phase (Arcenal 2004; Priyambodo and Sarifin 2008; Petersen and Phuong 2010).
1.2.2 Seed stock

Spiny lobsters have a long and complex larval period and the duration of the larval cycle varies from species to species (Table 1.2) (Jeffs and Davis 2009; Phillips and Matsuda 2011). Although there has been success in completing the larval development from egg to juvenile for some spiny lobster species (e.g. *Jasus edwardsii*, *Panulirus ornatus*, *Panulirus argus*), the high mortality rates during the complex phyllosoma phase of development makes hatchery production uneconomic at present (Kittaka 1997; Goldstein et al. 2008). The high rates of larval mortality may be due to a poor understanding of larval nutrition, disease, as well as suitable designs of larval culture tanks (Kittaka 1997; Ritar 2001; Matsuda et al. 2009). Thus, the development of technology to support commercial scale hatchery production of juveniles from larvae to provide sufficient seed stock is still lacking. At present, almost all seed stock for aquaculture of spiny lobsters comes from harvesting puerulus and early juveniles from the wild which are then reared in sea-cages until reaching marketable size, i.e., 100 – 1000 g (Jones 2011).

**Table 1.2. Larval cycle of spiny lobsters in laboratory (adapted and modified from Phillips and Matsuda (2011))**

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration of phyllosoma stages (months)</th>
<th>Duration of puerulus stages (days)</th>
<th>Author(s)</th>
</tr>
</thead>
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<tr>
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<td>10.5 – 13.4</td>
<td>19</td>
<td>Kittaka (1988); Ritar &amp; Smith (2005); Kittaka et al. (2005)</td>
</tr>
<tr>
<td><em>Jasus lalandii</em></td>
<td>10</td>
<td>11</td>
<td>Kittaka (1988)</td>
</tr>
<tr>
<td><em>Panulirus japonicas</em></td>
<td>7.5 – 12.6</td>
<td>9 – 26</td>
<td>Kittaka &amp; Kimura (1989); Yamakawa et al. (1989); Sekine et al. (2000); Matsuda &amp; Takenouchi (2005)</td>
</tr>
<tr>
<td><em>Panulirus longipes bispinosus</em></td>
<td>17</td>
<td>9.1 – 9.5</td>
<td>Matsuda &amp; Yamakawa (2000)</td>
</tr>
<tr>
<td><em>Panulirus argus</em></td>
<td>4.5 – 6.5</td>
<td>11 – 26</td>
<td>Goldstein et al. (2006)</td>
</tr>
</tbody>
</table>
1.2.3 Feeding and nutrition

Spiny lobsters are omnivorous scavengers that feed on a wide variety of benthic invertebrates such as bivalves, small crustaceans, polychaetes, echinoderms, gastropods and sometimes algae (Mayfield et al. 2000; Goni et al. 2001; Nelson et al. 2006). Their diet is generally high in protein, moderate in carbohydrate and low in lipid for energy and growth (Williams 2007a). Spiny lobsters require feed that contains ten essential amino acids (arginine, methionine, valine, threonine, isoleucine, lysine, histidine, phenylalanine and tryptophan), five essential polyunsaturated fatty acids (linolenic acid, eicosapentaenoic acid, linoleic acid, docosahexaenoic acid and arachidonic acid) and seven essential minerals (calcium, copper, phosphorus, potassium, magnesium, selenium and zinc) for growth (Nelson et al. 2006). Crustaceans, such as spiny lobsters, rely extensively on chemoreception to detect and locate their food (Derby et al. 2001; Grasso and Basil 2002). They are attracted to foods with characteristic release of compounds with low molecular weight, and that are water and ethanol soluble, as well as amphoteric compounds (Williams 2007b). Spiny lobsters are described as “nibblers” where they manipulate their food externally into smaller particles with their mouthparts by tearing, pulling and grinding (Guillaume and Ceccaldi 2001). This kind of feeding habit can often be very messy, with the potential to release waste food particles.

The digestive system of spiny lobster consists of a long alimentary tract that is divided into three main regions (i.e. the foregut, midgut and hindgut) (Perera and Simon 2014). The digestion process in spiny lobsters begins almost immediately after ingestion. The ingested food particles are crushed and ground into smaller pieces in the foregut by the gastric mill (Icely and Nott 1992). During digestion, the digestive gland or hepatopancreas secretes digestive enzymes (i.e., trypsin, α-amylase, chymotrypsin, α-glucosidase, chitinase, lipase, etc.) to aid with the digestion process (Ceccaldi 1997; Perera and Simon 2014). The activity of the various digestive enzymes changes according to the ontogeny of the spiny lobster and
the type of diet being consumed by the spiny lobsters (Johnston 2003; Simon 2009a). The digestive gland also plays a major role in nutrient absorption and storage in spiny lobsters (Nelson et al. 2006). The larger non-digestive food particles (usually more than 1 μm in diameter) are passed directly into the hindgut for excretion (Ceccaldi 1997).

In most existing sea-cage aquaculture systems, spiny lobsters are predominantly fed with “trash fish”, which usually consists of a mixture of locally harvested low-value fish (70 %) and shellfish (30 %) which are chopped into manageable pieces for the lobsters (Arcenal 2004; Tuan and Mao 2004). In recent years, considerable research effort has been directed into developing artificial pelleted feed for spiny lobsters and currently only one such product is available commercially from Lucky Star Brand (Taiwan Hung Kuo Industrial Co., Ltd, Taiwan). Unfortunately, the artificial pelleted feed produced currently is still unable to meet the growth rates achieved with “trash fish” (Williams 2007b). It is probable that spiny lobsters find it difficult to fully digest artificial pelleted feed compared to “trash fish” (Simon and Jeffs 2008). As feed technology advances, it is very likely that artificial pelleted feed will replace the use of “trash fish” for on-growing of spiny lobsters because of improved growth and survival of lobsters and the convenience for feeding.

1.3 Environmental impacts from aquaculture

The aquaculture industry has been effective in increasing seafood production, during a time when the production from wild seafood resources is constrained. Despite the increased aquaculture production, such activities have been criticised for degrading the surrounding environment (Tovar 2000; Pearson and Black 2001). Environmental effects from aquaculture include nutrient enrichment in both the water column and seafloor sediments, often leading to habitat alteration, changes in benthic macroinvertebrate assemblages, and sometimes also leading to damage of wild populations of the cultured species (Wu et al. 1994; Beveridge 2004; Hartstein and Rowden 2004) (Figure 1.2). The extent of the spatial and temporal scale of the impacts of discharges from an aquaculture operation depends very much on the species being aquacultured, the husbandry and culturing methods in use, as well as the hydrography of the aquaculture site, including water residence times (Paez-Osuna 2001; Vita and Marin 2007). For example, sea-cage aquaculture areas that are deep with strong currents generally
would have less of an environmental impact compared to shallow areas with weak flushing (Wu et al. 1994). The sources of discharges from an aquaculture operation are feed wastage, together with excretion, faecal production, and carbon dioxide from the respiration of the aquaculture animals (Wu 1995). These are discharged into the receiving environment as dissolved nutrients (e.g. nitrogen, ammonia and phosphorus), dissolved gases (e.g., carbon dioxide) and solid waste (i.e., uneaten feed and faecal particles). Key factors often affecting the amount or concentration of the aquaculture discharges of both soluble and solid forms, include animal size, temperature, salinity, the type of diet being fed to cultured animals, and the stocking density (Chen and Lai 1993; Handy and Poxton 1993; Corea et al. 1995; Paez-Osuna 2001). Generally, the more intensive the aquaculture activities are (i.e., large aquaculture operation with very high stocking density) the greater the extent of the waste discharges and the higher the risk of resulting environmental impacts.

Figure 1.2. Overview of potential environmental effects from a typical sea-cage aquaculture operation using fish as a model species. (from Forrest et al. 2007, with permission).
1.3.1 Effects on water column

Dissolved oxygen (DO)

Depletion of dissolved oxygen (DO) can occur within and around sea-cage aquaculture operations due to respiration by the cultured animals and the aerobic microbial degradation of waste materials (Forrest et al. 2007). Low DO values (i.e. 2.0 – 3.5 mg L\(^{-1}\)) have been reported inside a fish aquaculture area in some previous studies which seem to increase rapidly with increasing distance from the sea-cages (Tovar et al. 2000; Wu et al. 1994). Excessive DO depletion in the water column (i.e., anoxic conditions) could potentially cause stress or kill the cultured animals and other animals (e.g., epibiota) (Forrest et al. 2007). However, significant depletion of DO in the water column usually only occurs when aquaculture cages are heavily stocked or when the cages are located at shallow sites with weak flushing (La Rosa et al. 2002).

In spiny lobster aquaculture the sea-cages are not usually heavily stocked, with stocking densities typically of between 8-10 individuals m\(^{-3}\) for lobsters ranging in size from 100 g to a marketable size of 500-800 g, i.e., a total biomass of 0.8 – 8 kg m\(^{-3}\) (Priyambodo and Sarifin 2008; Arcenal 2004). Lobsters of smaller sizes (i.e., <100 g) tend to be stocked at much higher densities, but lower overall biomass. Oxygen consumption by spiny lobsters is influenced by the lobster’s weight and temperature (Crear and Forteath 2000). Oxygen consumption in spiny lobsters increases with activity and feeding (Radford et al. 2004). For example, recently fed *Jasus edwardsii* has maximum oxygen consumption between 0.114 ± 0.006 mg O\(_2\) g\(^{-1}\) h\(^{-1}\) and 0.121 ± 0.008 mg O\(_2\) g\(^{-1}\) h\(^{-1}\), which is almost double the oxygen consumption of a starved lobster (0.064 ± 0.001 mg O\(_2\) g\(^{-1}\) h\(^{-1}\)) (Radford et al. 2004). Lobsters are nocturnal animals, hence they are more active during the night compared to daytime (Jernakoff et al. 1987). For example, the oxygen consumption of juvenile *Jasus edwardsii* increased from 0.066 ± 0.004 mg O\(_2\) g\(^{-1}\) h\(^{-1}\) during daytime to 0.074 ± 0.003 mg O\(_2\) g\(^{-1}\) h\(^{-1}\) at night when they were more active and feeding (Radford et al. 2004). However, cultured lobsters often lose their diurnal pattern of activity over time in aquaculture systems (James et al. 2001). At the scale of a typical spiny lobster sea-cage farm (i.e., a total biomass 8 kg m\(^{-3}\) of water) the biological oxygen demand from the lobsters can elevate as high as 23.2 g O\(_2\) day\(^{-1}\) in 1 m\(^3\) of water, which is relatively low compared to other aquaculture species. In contrast, salmon aquaculture at 17.7 kg m\(^{-3}\) can generate oxygen demands of 48 gO\(_2\) day\(^{-1}\) m\(^{-3}\) of water.
(Wildish et al. 1993). Therefore, it is assumed that extremely low dissolved oxygen should not be a significant issue within or near the vicinity of lobster farms. Caution would still have to be taken if sea-cages are situated in a relatively shallow area without sufficient flushing or in waters with high natural biological oxygen demand.

**Dissolved nutrients**

Nutrient excretion from aquacultured animals is a result of the digestion and catabolism of feed and typically takes the form of nitrogenous compounds, especially dissolved ammonia and urea. In addition, there is also the release of dissolved nitrogen due to leaching from faecal material and uneaten feed into the environment (Handy and Poxton 1993; Beveridge 2004). As a result, nitrogen concentrations are usually elevated to some extent in the water surrounding sea-cage aquaculture sites (Tovar et al. 2000; Islam 2005). The type of diet used in an aquaculture system can affect the production of nutrient excretion. Cultured fish fed with trash fish have resulted in higher nitrogen excretion compared with those fed with formulated pelleted diet because of the poor FCR associated with trash fish (Leung 1996). This is also the case for nutrient leaching from uneaten feed, where the production of nutrient is higher for trash fish than formulated pelleted diet (Hasan 2012). A study on sea-cage aquaculture of areolate grouper in Hong Kong found total nitrogen discharge was 17 times higher when using trash fish compared with formulated pelleted diet (Chu 2000). When the stocking density of sea-cages is low, the type of diet tends to have a less influence on the amount of nitrogen excreted from cultured species (Hasan 2012). Excessive input of nitrogenous compounds into the aquatic environment can cause hyper-nutrification of the water column promoting rapid eutrophication and triggering excessive primary production. Ammonia is the most important nitrogenous nutrient excreted by most aquacultured species and usually takes the form of ammonium ions (\(\text{NH}_4^+\)) and un-ionised ammonia (\(\text{NH}_3\)). The ratio of \(\text{NH}_3\) to \(\text{NH}_4^+\) in the environment is affected by temperature, pH, and to a lesser extent, by dissolved oxygen, salinity and pressure (Wu 1995; Oliver 2008). Unionised ammonia is acutely toxic to aquatic organisms and at a range of 0.1 – 3.35 mg L\(^{-1}\), and it is highly toxic to marine species, capable of inhibiting growth in animals as well as causing mortality (Chen et al. 1990; Chen and Lin 1992; Handy and Poxton 1993). Oxidisation of ammonia in the environment by microbes produces nitrite and nitrate. Nitrite at
high levels can also be toxic to marine species (Handy and Poxton 1993; Jensen 2003). For example, exposure to concentrations of nitrite between 12.9 mg L\(^{-1}\) and 87.2 mg L\(^{-1}\) can cause formation of methaemoglobin in fish which can lead to hypoxia in marine species (Crawford and Allen 1977; Scarano et al. 1984). Nitrate is generally not as toxic as both ammonia and nitrite, but elevated concentrations may lead to reduced immune response which can then lead to infection with disease (Tucker 1998). The toxicity level of these dissolved nitrogen compounds depends on the species, exposure period and a range of environmental parameters (i.e., DO, temperature, salinity, pH) (Chen et al. 1990; Young-Lai et al. 1991; Chen and Lin 1992; Handy and Poxton 1993; Wu 1995). The release and elevation of concentrations of nitrogenous compounds (i.e., ammonia, nitrite and nitrate) will promote primary production in aquatic environments which may potentially trigger algal blooms (Pillay 1992).

Faecal particles and uneaten feed containing phosphorus are discharged from aquaculture operations into the surrounding water as dissolved inorganic phosphorus (Brooks et al. 2002). Phosphorus itself is not toxic in seawater, but can be a key contributor to the formation of algal blooms (Phillips 1993; Islam 2005). Some algal blooms can be toxic or greatly elevate biological oxygen demand which has the potential to kill cultured stock resulting in substantial economic losses. For example, in 1989 aquacultured salmon and trout in Norway experienced mass die off of 750 t of fish due to the bloom of the toxic phytoplankton species *Prymnesium parvum* (Kaartvedt et al. 1991). This particular toxic algal bloom incident was subsequently directly linked to the nutrient loadings from the finfish aquaculture. Besides this single reported incident, there have been no other studies confirming a significant connection between nutrient eutrophication from marine aquaculture and the triggering of harmful algal bloom events (Wu 1995; Forrest et al. 2007).

Spiny lobsters are ammonotelic, whereby their main excretory product is ammonia from the metabolism of protein (Radford et al. 2004). Lobsters release ammonia as well as amino-compounds, such as free amino acids, through diffusion from the gill epithelium, as well as directly through the antennal and maxillary glands (Regnault 1987). In addition, urea and amino-compounds, such as free amino acids, are also released from their gill epithelium (Binns and Peterson 1969). The ammonia production of spiny lobsters increases as the temperature and body weight increases (Crear and Forteath 2002). Total ammonia nitrogen (TAN) excretion of *Jasus edwardsii* and *Panulirus cygnus* increases exponentially with increasing temperatures between 5 and 30 °C, and increases linearly with increasing body
weight (Crear and Forteath 2002). Furthermore, ammonia production also increases after the lobsters have been fed or are active (Crear and Forteath 2002; Kemp et al. 2009). Maximum TAN excretion rates of *J. edwardsii*, *P. cygnus* and *P. homarus rubellus* at 7-8 h after feeding are 7.5, 8 and 20 µg TAN g⁻¹ hr⁻¹ respectively, which are approximately 6-7 times higher than pre-feeding lobsters (Crear and Forteath 2002; Kemp et al. 2009). Based on the average stocking density of 30 individuals m⁻² for 500 g spiny lobsters (Petersen and Phuong 2010), one hectare production of lobsters in sea-cages has the potential to excrete 1.1 to 3.0 kg TAN hr⁻¹. The maximum recommended concentration of ammonia in water used for culturing lobsters is below 0.5 mg L⁻¹ and ideally less than 0.1 mg L⁻¹ (Jeffs and Hooker 2000). Ammonia levels more than 10 mg L⁻¹ have been shown to inhibit growth in crustaceans (Chen and Lin 1992) while levels of 83 mg L⁻¹ are lethal to spiny lobsters (Battaglene and Cobcroft 2003). Waste nitrogen produced from spiny lobster sea-cage aquaculture has only been estimated using simple mass balance methods by combining knowledge of the total nitrogen in the feed provided to spiny lobsters in comparison to the estimated quantities that was retained in the resulting biomass of lobsters. A spiny lobster farm with production of 1800 t of lobsters was estimated to release an estimated 800 t of waste nitrogen per year into the coastal environment (Tuan and Hung 2008). Thus, there are still gaps in the knowledge of environmental impacts from the release of this nitrogenous waste from spiny lobster aquaculture.

Dissolved phosphorus is mainly leached from solid farm wastes (faeces and uneaten feed) from the lobster farm. Such dissolved nutrient is unlikely to be harmful to the cultured lobsters and wild marine species but is a key contributor to algal blooms in the water column. Some algal blooms can be toxic to marine organisms, hence dissolved phosphorus is another important nutrient output from lobster farms to understand. To date, no studies on phosphorus output from spiny lobster sea-cage aquaculture have been published.

### 1.3.2 Effects on benthic sediment

Deposition of uneaten feed and faeces on the seabed enriches benthic sediments with organic matter which includes key nutrients of carbon, nitrogen and phosphorus. Feed wastage from sea-cage aquaculture may range from 1% to 41% depending on the type of feed,
feeding practices, aquaculture method and the aquaculture species (Thorpe et al. 1990; Leung et al. 1999; Brooks et al. 2002). Typical feed wastage for aquacultured salmon that are fed with a pelleted diet is around 3-5 % (Brooks et al. 2002; Beveridge 2004). In contrast, up to 40 % of feed wastage has been recorded from sea-cage aquaculture of fin-fish that are fed with trash fish (Wu et al. 1994; Leung et al. 1999). This indicates that aquaculture production using trash fish would produce higher amounts of feed waste compared with pelleted diets. In a European salmon aquaculture operation that was using artificial diet, uneaten feed contributed around 80-84 % of carbon, 52-95 % of nitrogen and 82 % of phosphorus released into the environment from the farm operation (Wu 1995). Faeces are also an important source of nutrients released into the environment, with an estimated 4 % of feed ingested by aquacultured fish being released as labile organic material in the faeces (Brooks et al. 2002). In sea-cage aquaculture of the grouper (Epinephelus areolatus) fed with trash fish the faecal production was measured at 2730.9 ± 385 mg of dry faeces kg⁻¹ body wt d⁻¹ and waste nitrogen at 47.2 ± 7 mg N kg⁻¹ body wt d⁻¹ (Leung et al. 1999). In sea-cage aquaculture of gilthead seabream, deposited faecal material on the seabed was made up 10 % of the total nitrogen and 44 % of the total phosphorus of the total feed input (Lupatsch and Kissil 1998).

Organic waste material from sea-cage aquaculture is degraded by bacteria which increases the oxygen demand in the benthic sediments (Davenport et al. 2003). When the oxygen demand exceeds the oxygen diffusion rate from the overlying water, anaerobic processes become dominant, thus encouraging the formation of anoxic conditions and the proliferation of anaerobic bacteria (Davenport et al. 2003). Extreme anaerobic conditions in the benthic sediments will give rise to the production of hydrogen sulphide and methane from the seabed (Forrest et al. 2007). Hydrogen sulphide is known to be toxic to fish and other marine organisms even at low concentrations (approximately 7 µg L⁻¹) (Reynolds and Haines 1980). Elevated deposition of organic matter on the seabed is known to affect both the farm and the surrounding environment, with alteration in benthic sediment quality and the community structure of associated benthic organisms (Islam 2005; Forrest et al. 2007). For example, nutrient enriched sediment with limited dissolved oxygen makes the impacted area inviting to opportunistic organisms, such as the marine polychaete worm, Capitella sp. (Forrest et al. 2007). A reduced diversity of benthic organisms are typically found beneath aquaculture sea-cages and within farming areas where benthic deposition of organic matter has been elevated (Wu et al. 1994). The spatial extent of reduced species diversity of benthic
organisms can be from 25 m up to 1 km from the sea-cage, depending on the hydrodynamics of the aquaculture site and the amount and type of organic matter being released from the sea-cage (Brown et al. 1987; Weston 1990; Wu et al. 1994). In contrast, there seems to be a negative correlation between the abundance of opportunistic macrobenthos and the distance from the farm cage or impacted site (Figure 1.3) (Beveridge 2004).

![Diagram of generalised changes in benthic infauna](from Forrest et al. 2007, with permission)

**Figure 1.3.** Schematic diagram of generalised changes in benthic infauna as a result of organic enrichment associated with scale of proximity to a sea-cage fish aquaculture operation. (from Forrest et al. 2007, with permission).

Deposition of uneaten feed and lobster faeces will play an important part in organic loading on the seabed for the sea-cage aquaculture of spiny lobsters. To date studies on the potential environmental impact from lobster aquaculture have only estimated the overall total nitrogen discharge which included both dissolved and particulate matter of organic and inorganic waste. However, the fate of nitrogen is critical to determining the potential environmental impacts of any aquaculture activity. For example, if greater quantities of nitrogen end up on the seabed than being released as DIN then it is more likely to alter the sediment quality and the benthos within the vicinity of the aquaculture site. In addition, only
one study has documented the potential carbon loading from the impoundment operation for the American lobster, where approximately 0.23 kg m\(^{-2}\) yr\(^{-1}\) of organic waste was produced for lobster enclosures with an average stocking density of 3.79 kg m\(^{-2}\) (Thlusty and Preisner 2005). To date, no studies have quantified the seabed carbon footprint as a result of spiny lobster sea-cage aquaculture. Thus, there is a large gap in the knowledge of spiny lobster aquaculture and its environmental management despite such activities being in operation for almost 40 years.

### 1.3.3 Effects on wild population

In some sea-cage aquaculture operations, there will be interactions between the operation and the wild population of cultured organisms that can potentially result in negative effects. The wild population can be at risk of being infected with diseases from cultured animals and vice versa. For example, infectious diseases such as pancreatic necrosis (IPN) virus could be transferred from a rainbow trout farm to native brown trout (Munro et al. 1976). Wild population of salmonids in Scotland have reportedly been infected with sea lice that originated from aquacultured fish, and which may have been responsible for a subsequent decline in the wild population (Pearson and Black 2001). Also, the escape of cultured animals to the wild is inevitable. Aquaculture cages can be vulnerable to vandalism (Beveridge 2004) and aquacultured animals can also escape as a result of predator or storm damage to sea-cages (Phillips 1985; Beveridge 2004). Aquacultured animals can also escape from sea-cages during routine husbandry processes such as animal grading and harvesting (Phillips 1985). It is estimated that up to 2 million salmon escape from sea-cages in the North Atlantic annually (Naylor et al. 2005). Aquaculture farms in Scotland have recorded more than 1.7 million salmon escapees since 1998 (Aitken 2002) and in Chile between 9 – 18.6 million salmon have escaped since the 1980s (Soto et al. 2001). In 2005, a storm event at the Faroe Islands resulted in the escape of 600,000 salmon from sea-cages (Seafoodnews 2002). Animals that have escaped from aquaculture can potentially interbreed with the wild population which can alter the genetic makeup of the wild population and can be detrimental because traits selected in aquaculture breeding programmes can differ significantly from those required for survival in the wild (Forrest et al. 2007; Naylor and Burke 2005). Recent studies of the effect of escaped Atlantic salmon have shown that they can reduce the genetic diversity of the wild salmon population (McGinnity et al. 1997; McGinnity et al. 2003). The offspring produced from
escaped aquaculture salmon breeding with wild salmon are thought to have much lower fitness level with reduced survival and recruitment rate (McGinnity et al. 1997; McGinnity et al. 2003). Besides interbreeding with the wild population, escaped aquaculture animals that share similar diets to the wild population may also compete with wild fish for food resources, and they may also prey on the wild population. Either of those scenarios can cause the wild population to decline (Phillips et al. 1985; Beveridge 2004; Forrest et al. 2007).

Currently there are no published accounts of spiny lobsters escaping from an aquaculture operation, although escapes have been observed in experimental sea-cage operations (A. Jeffs, pers. comm.). In the current aquaculture production of spiny lobster, the seed for stocking sea-cages are harvested from the wild as puerulus and juvenile lobsters (Arcenal 2004; Tuan and Mao 2004; Priyambodo and Sarifin 2008). Therefore, concern over genetic alterations of lobsters in the natural environment due to interbreeding with escapees is negligible. Continuous collection of large numbers of lobster seed from the wild will not be environmentally sustainable in the longer term as wild stocks of lobsters will be depleted (Williams et al. 2001). In the Philippines, lobster farmers have adopted the practice of releasing 10% of farmed lobsters that have been grown to maturity (>1.5 kg) to a designated lobster sanctuary with the aim of helping to replenish seed stocks (Arcenal 2004). Extensive research has been dedicated to rearing lobsters from egg to juvenile in order to reduce the pressure of harvesting seed lobsters from the wild population (Kittaka and Booth 1994; Phillips and Matsuda 2011).

Sub-standard aquaculture conditions, poor quality feed and high stocking densities in a farming system can facilitate disease outbreaks that could potentially spread to the wild population (Hung and Tuan 2009). There have been reports of black gill and milky disease outbreaks in lobster farms in Vietnam (Williams et al. 2001). In 2006, the presence of milky disease in many of the lobster culture regions in Vietnam resulted in 80-90% mortality in aquacultured lobsters (Ly 2009). Other potential infectious diseases that lobsters are susceptible to are gaffkemia, shell disease, vibriosis and fungal diseases (Evans and Brock 1994). Disease outbreaks are usually opportunistic infections caused by microbial pathogens that are already widely distributed in the surrounding marine environment (Evans and Brock 1994). Prevention can be made by monitoring water quality and diet in the lobster aquaculture systems (Evans and Brock 1994). At present, extensive knowledge of disease in lobster is still lacking and is in need of further research.
1.3.4 Effects of chemical residue

Various chemicals are used in aquaculture practices as feed supplements, vaccines and treatment for diseases in aquacultured animals (Forrest et al. 2007; Gowen 1991). Chemicals are also used to control biofouling of aquaculture structures (Davenport et al. 2003). Aquaculture structures like fish cages create ideal habitats for a wide range of fouling species to settle and establish. In order to control biofouling, farmers sometimes apply antifoulant coatings on their nets, mooring lines and floats. Antifoulant used in aquaculture frequently contains copper compounds (Forrest et al. 2007). In addition, copper is also included as a micronutrient in most fish feeds and can therefore be released to the environment via waste fish feed and faeces (Dean et al. 2007). Hence, the environment around sea-cages used for culturing fin-fish can have elevated copper concentrations (Brooks et al. 2002). For example, an assessment of a salmon farm in Marlborough Sounds, New Zealand, revealed 70 mg kg\(^{-1}\) to 265 mg kg\(^{-1}\) of copper in the seabed sediment directly beneath the farm. Such levels exceed ANZECC (2000) sediment quality guidelines for possible ecological effects (65 mg kg\(^{-1}\)) (Forrest et al. 2007). Copper even at low levels can be toxic to marine organisms, particularly larval stages of invertebrates (Brooks et al. 2002). Moreover, copper residue from antifoulants could accumulate in aquacultured animals. The liver and tissue from Chinook salmon grown in pens treated with copper-based antifoulant have been found to contain traces of copper (Peterson et al. 1991). Copper residues could potentially be transferred to humans who consume cultured animals containing copper (Philips 1993; Sapkota et al. 2008). High concentration of copper in humans can be harmful to their health (Ma and Rao 1997; Sapkota et al. 2008).

Antibiotics and other therapeutants are sometimes used to treat diseases in aquacultured animals. These chemicals are either administered through feed or in the form of “bath” treatments (Pillay 1992; Beveridge 2004). During “bath” treatment, aquacultured animals are corralled into a solid enclosure within the sea-cage which is then dosed with the therapeutant, and then the chemical and the animals are generally released back into the sea-cage, with the therapeutant being dispersed into surrounding waters (Beveridge 2004). Bacterial pathogens of cultured species could potentially develop resistance to antibiotics if these drugs were used regularly (Davenport et al. 2003; Wu 1995). There is also a concern over the possibility of transferring antibiotic resistant bacterial strains to humans (Pillay 1992). In addition, antibiotics can affect sedimentary biogeochemical processes by interfering
with the ecology and composition of natural bacterial communities (Davenport et al. 2003). Application of antibiotics containing oxytetracycline, oxolinic acid or flumequine to sediments in experimental tanks has shown a 10 % decrease in sulphate reduction rate (Davenport et al. 2003). Furthermore, oxytetracycline has also been shown to inhibit bacterial nitrification, which could lead to build up of ammonia and nitrite in sediments (Davenport et al. 2003).

Feed supplements containing the mineral zinc are usually fed to aquacultured animals (Burridge et al. 2010). Zinc is a micronutrient that is critical to the growth of many aquaculture species and is known to prevent cataract formation and other health problems in fin-fish (Forrest et al. 2007). Zinc can accumulate in sediments beneath fish farms and can be toxic at high concentrations (Forrest et al. 2007; Burridge et al. 2010). An assessment of the sediments beneath salmon farms in the Marlborough Sounds in 2005 found zinc concentrations to be between 420 and 560 mg kg$^{-1}$. Such levels of zinc exceeded the ANZECC (2000) sediment quality guideline for ‘probable’ ecological effects (410 mg kg$^{-1}$)(Hopkins et al. 2006).

The situations and conditions of chemical residues associated with fish farming aquaculture practices that have been mentioned above are expected to be no different for spiny lobster aquaculture. An array of chemicals and therapeutants are currently used in spiny lobster aquaculture including formalin, vitamins and doxycylin antibiotics (Ly 2009). However, there is little information available on how widespread the use of chemicals and therapeutics in sea-cage lobster farming are currently.

1.3.5 Effects on wildlife (Predators)

Predators and scavengers such as seabirds, some marine mammals, as well as some carnivorous fish species can be attracted by aquaculture facilities or can encounter them in the course of their routine movements (Beveridge 2004). Aquaculture areas can provide a source of food for marine predators and scavengers. In Scotland in the 1970s and 1980s, up to 90 % of the fish farms had predation problems caused by piscivorous birds, while 80 % of fish farms suffered predation from seals (Mills 1979; Ross 1988). One of the environmental issues from the interaction of sea-cage aquaculture and these animals is sea-cage net entanglement.
For example, bottlenose and common dolphins have been entangled in sea-cages holding southern blue-fin tuna in South Australia (Kemper and Gibbs 2001). Seabirds and marine mammals can also damage nets and thereby increasing the chance of cultured organisms escaping (Pemberton and Shaughnessy 1993; Forrest et al. 2007). Predation from wild animals can cause production and financial losses to the aquaculture operators (Pillay 1992). In the 1980s, the loss through predation in salmon farms in British Columbia was around 1.5% (Rueggeberg and Booth 1989), whilst approximately 0.8% of all fish stocked in Norwegian aquaculture farms were lost due to predation by wild animals for the period of 1994 - 1999 (Beveridge 2004). Estimated losses in Scottish salmon aquaculture industry due to predation by wild animals were £1.4–4.8 million in 1987 (Ross 1988). Salmon farmers in the United States have estimated USD5 million of their annual farm gate sales value is lost via predation by seals (Davenport et al. 2003). To minimize predation from seabirds and marine mammals, farmers sometimes harass them with noise-making devices and which can result in their death (Pillay 1992; Wiirsig and Gailey 2002).

To date, there have been no published reports on predation and net entanglement issues in spiny lobster aquaculture facilities. Generally, lobsters are not desirable food to seabirds and marine mammals. Hence, it is assumed that negative impacts on these organisms from lobster aquaculture activities are likely to be minimal. However, lobsters are highly attractive to some sharks (Wetherbee et al. 1990; Smith and Herrkind 1992), which could be a potential issue in spiny lobster aquaculture.

1.4 Objectives of the present thesis research

Globally, the development of sea-cage aquaculture for spiny lobsters has been important in developing alternative supplies of spiny lobsters when production from wild fisheries are constrained. The emergence of sea-cage aquaculture of spiny lobsters also offers the potential to increase the production of this highly valuable seafood product. Despite the rapid expansion of lobster aquaculture activity, the environmental impacts from this activity are not as well studied as some other aquaculture species, such as fin-fish, prawns or mussels (Briggs and Funge-Smith 1994; Wu 1995; Ruiz 2001; La Rosa et al. 2002; Hartstein and Rowden 2004; Asche et al. 2012). Full comprehension on the environmental impacts from
spiny lobster aquaculture is crucial for maintaining sustainable spiny lobster aquaculture as well as helping authorities in decision and policy making. Therefore, the aim of the research presented in this thesis is to undertake the first comprehensive study of the potential environmental impacts from spiny lobster aquaculture. The research will attempt to characterise the major biological outputs (i.e., carbon deposition on the seabed and elevation of dissolved inorganic nitrogen (DIN)) from spiny lobster sea-cage aquaculture into the surrounding environment. As part of the research project, idealised lobster farms will be examined with aid of computer modelling (using numerical modelling software) to investigate the effects of both deposition of carbon on the seabed and of DIN into the surrounding water column from sea-cage spiny lobster aquaculture.

The computer modelling work is conducted using DHI Mike 21 and Ecolab software. The modelled spiny lobster farms in this study are hypothetical, but the modelled location is an existing location in Malaysia that has hydrodynamic conditions which are typical of a commercial spiny lobster farm in Asia or New Zealand. A hypothetical farm is used in the study because of logistical reasons (i.e., difficulty in gaining access into actual lobster farms at the time of this study). In addition, the difficulty in collecting accurate field measurements such as faecal settling velocity and highly labile dissolved nitrogenous compounds in the marine environment restricts the experiments to be conducted under controlled laboratory conditions. The modelling software has the flexibility of changing the temperature parameter within the model set up to suit the farming location, whether it be in a tropical or temperate location. Therefore, a single modelled location is used in the study for both spiny lobster species, so that the modelled results are comparable. The data required for the modelling component is either measured in-situ, by experiments or from past published literature. Current measurements and bathymetry data for the modelling work are measured in-situ with an acoustic doppler current profiler (ADCP) and eco-sounder respectively. Primary model input data such as faecal settling velocity as well as faecal carbon content for the carbon deposition model and DIN excretion for DIN model as measured in the laboratory research are the main drivers for reliable modelled results and have not been previously measured in other studies. Secondary model input data such as percentage of feed converted to faeces and percentage of feed waste have not been measured for spiny lobsters before, therefore, it is based on past studies on salmon farming as spiny lobsters and salmon are likely to be
comparable species for these parameters. Modelling scenarios of different stocking density and FCR are existing values used in current commercial spiny lobster aquaculture production.

To derive reliable measures of lobster faecal and nutrient output parameters experimental methods suitable for field conditions with limited research facilities had to be first developed and tested in New Zealand with a temperate lobster species (*Jasus edwardsii*) before transferring the research methods to a rural area in Malaysia for a tropical lobster species (*Panulirus ornatus*) which is the main targeted aquaculture species in Asia. Hence, the research work for this thesis is structured and presented as follows:

Chapter 2:

- Examining the physical aspects (i.e., settling velocity, carbon and nitrogen content) of faecal material produced by *J. edwardsii*.
- Predicting potential carbon footprint on the seabed from particulate waste (faecal material and waste feed) as a result of *J. edwardsii* being aquacultured in sea-cage system using hydrodynamic computer modelling.
- Comparing different parameters (i.e., stocking density, feed conversion ratio (FCR)) that could influence the resulting carbon footprint from spiny lobster sea-cage aquaculture.

The contents of this chapter have been published as:

Chapter 3:

- Measuring dissolved inorganic nitrogen (DIN) excretion from *J. edwardsii* from different dietary treatments (seafood diet, artificial diet and unfed).
- Predicting potential DIN elevation from sea-cage aquaculture of *J. edwardsii* using hydrodynamic computer modelling.
• Comparing different parameters (i.e., stocking density, feed conversion ratio (FCR), diet) that could influence the resulting elevated DIN within the vicinity of spiny lobster sea-cage aquaculture operation.

The contents of this chapter have been published as:

Chapter 4:

• Characterising the various farm waste (i.e., faecal material, waste feed, DIN output) produced by *P. ornatus*, including the settling velocity of faecal strands, carbon and nitrogen content of faeces, and nutrient leaching rate of faecal material, and how these characteristics may vary with diets used in spiny lobster farms.

The contents of this chapter have been published as:

Chapter 5:

• Using hydrodynamic computer modelling to predict the potential carbon footprint on the seabed and DIN elevation from sea-cage aquaculture of *P. ornatus*.
• Examining the effects of farming parameters such as stocking density, FCR and diets on the predicted carbon and DIN loading from sea-cage aquaculture of *P. ornatus*.

The contents of this chapter have been published as:
Chapter 2:

Assessment of the Production and Dispersal of Faecal Waste from the Sea-cage Aquaculture of Spiny Lobsters, *Jasus edwardsii*

2.1 Introduction

Spiny lobsters are one of the world’s most valuable seafood species and have a high market demand in more than 90 countries (Williams et al. 2001; Hart 2009). Currently, a variety of spiny lobster species are being commercially cultured mostly in sea-cages in Asian-Pacific countries, such as Vietnam, Philippines, Indonesia, Malaysia and Thailand while experimental sea-cage cultures are carried out in New Zealand (Jeffs and Hooker 2000; Arcenal 2004; Priyambodo and Sarifin 2008; Hung and Tuan 2009; Jones 2010; Phillips and Matsuda 2011). At present, Vietnam has the most extensive sea-cage aquaculture production of spiny lobsters with an estimated 49,000 sea-cages located in coastal waters (Jones 2010). In 2008/09, cultured lobster production in Vietnam was estimated to be approximately 1,500 t at a value of more than US$60 million (Jones 2010). A wide variety of commercially important spiny lobster species are being used in sea-cage aquaculture, including *Panulirus ornatus*, *P. homarus*, *P. stimpsoni*, *P. polyphagus*, *P. longipes*, *P. penicillatus*, *P. versicolor* and *Jasus edwardsii* (Jeffs and James 2001; Phillips and Matsuda 2011).

Lobster sea-cages typically have been adapted from those used to culture tropical fish in Asian countries (Tacon and Halwart 2007). They usually consist of a wooden rectangular frame (3 to 6 m sided square), held just above the surface of the sea by a series of floats, and used to suspend a cuboid-shaped mesh net which can extend up to 6 m below the surface. The mesh net usually consists of knotted polypropylene netting, as frequently used by trawling fishing vessels, with a variety of mesh sizes used to retain lobsters of different sizes. Lobsters move freely around the netting in the same manner as a spider would on a web, and are known to derive a small proportion of their diet from removing fouling organisms off the netting (Simon and James 2007). The lobster sea-cages are commonly located in shallow bays with average depth of 10 – 20 m and stocked with juvenile lobsters caught from the wild, as commercial scale hatchery production of spiny lobsters is yet to be fully developed (Arcenal
2004; Hung & Tuan 2009). These lobsters are then harvested when they reach a marketable size of 800 – 1000 g (Arcenal 2004; Hart 2009). Captive lobsters are mostly fed with freshly caught low value fin-fish which is harvested locally (70 %) and is commonly known by the misnomer “trash fish”, and shellfish (30 %), which is usually chopped and fed out onto fine mesh feeding trays placed on the floor of the sea-cage (Hung and Tuan 2009). Formulated pelleted feed for the aquaculture of spiny lobster has recently become commercially available and is likely to increasingly replace the use of natural feeds (Tuan & Hung 2008).

Despite the extent of this emerging aquaculture activity very little is known about the potential environmental impact of sea-cage aquaculture of spiny lobsters. There has been some evidence that feeding of low-value fish and shellfish to lobsters in Vietnam results in a decrease in water and benthic sediment quality both beneath, and in areas adjacent to the farms (Tuan 2005). Dissolved inorganic nitrogen levels at some sites in Vietnam where lobsters are cultured have been found to exceed the level of 0.5 mg L⁻¹ recommended for aquaculture, however, the source of the elevated nitrogen was not determined so it may not have been derived directly from the lobster farming activity (Tuan 2005). A subsequent study found elevated release of soluble inorganic nitrogen associated with the feeding of low-value fish and shellfish diet (Tuan and Hung 2008). It is well known that the deposition of solid waste (uneaten feed and faecal material) from aquaculture sea-cages contributes to the nutrient loading on the seabed (Brooks et al. 2002). Elevated nutrient levels on the seabed are known to commonly alter sediment quality as well as benthic community structure in and around aquaculture areas (Hartstein and Rowden 2004; Cromey et al. 2012). For example, sea-cage aquaculture of fin-fish fed with trash fish has been found to degrade the seabed due to deposition of uneaten feed and faeces, but is usually confined within 1 km from the sea-cages (Pearson and Black 2001; Wu 1995; Wu et al. 1994). The spatial and temporal extent of solid waste deposition is highly dependent on the hydrography of the aquaculture site, including current regimes, as well as the amount and settling velocities of the solid waste produced by the aquacultured organisms (Hartstein 2003; Magill et al. 2006; Vita and Marin 2007). A number of studies have investigated the settling velocities of faecal particles from Atlantic salmon (Salmo salar), gilthead sea bream (Sparus aurata), sea bass (Dicentrarchus labrax) and cod (Gadus morhua) (Chen et al. 1999b; Chen et al. 2003; Cromey et al. 2009; Magill et al. 2006). To date, there have been no published studies on the quantification and settling velocities of faecal materials produced by spiny lobsters. This information is essential
Chapter 2

for beginning to determine the potential benthic depositional impact from faecal material produced from lobster sea-cage aquaculture.

Deposition modelling is a useful predictive tool to determine the potential effects from solid waste accumulation on the seabed from sea-cage aquaculture (Magill et al. 2006). Such models are increasingly used by regulatory authorities to help regulate and manage aquaculture developments (Cromey et al. 2009). The majority of the published deposition modelling work undertaken on fin-fish aquaculture, has used various hydrological modelling programmes (e.g., DEPOMOD (Cromey et al. 2002a; Magill et al. 2006), CODMOD (Cromey et al. 2009), AWATS (Dudley et al. 2000), MERAMOD (Cromey et al. 2012), MOM (Hansen et al. 1997)).

This study aims to predict the intensity of deposition from solid waste (faecal material and feed waste) accumulation on the seabed from a hypothetical commercial spiny lobster aquaculture operation by combining laboratory measures of spiny lobster faecal settling rates with an advanced hydrological model, a modified Mike21 FM sediment transport module (Kurian et al. 2009) coupled with a two dimensional calibrated, hydrodynamic model.

2.2 Materials and methods

2.2.1 Husbandry methods

A total of seven spiny lobsters, *Jasus edwardsii*, of a range of sizes, from 100 to 800 g were collected from rocky reefs near Leigh, in northern New Zealand and transported to the Leigh Marine Laboratory in November 2011. This size range of spiny lobsters is representative of typical lobster size that would be aquacultured from small juveniles to a target market size of 800 to 1000 g (Arcenal 2004; Hart 2009). Spiny lobsters were kept in aerated polyethylene tanks (13, 41 and 68 L depending on the size of lobster) with a flow through seawater system. The tanks were supplied with water of ambient temperature (16 ± 1 °C) and salinity of 34 ± 1 ppt. Light was maintained at 12L/12D photoperiod. Freshly opened green-lipped mussels (*Perna canaliculus*) were fed to the spiny lobsters three times a week, at a ratio of approximately 3 % of the lobster’s body weight during each feeding time. Before starting the experiment, spiny lobsters were acclimatized in the tanks for at least 1 week.
2.2.2 Faecal strand settling velocity

Spiny lobsters are generally nocturnal feeders and observations showed that lobsters that were fed the night before produced faeces early in the morning. Thus, to ensure the faecal material was fresh, faecal strands were collected early each morning. Faecal strands (n = 59) were carefully collected from tanks with a 3 ml Pasteur pipette to avoid strand breakage. After carefully blotting dry, individual faecal strands were weighed wet (m), and then measured for length (L) and diameter (D) using digital callipers. A theoretical cylinder shape was assumed for the faecal strands. Volume (V) and density (ρ) of each faecal strand was calculated as follows:

\[ V = \pi * \left( \frac{1}{2} D \right)^2 * L \]

\[ \rho = \frac{m}{V} \]

A settling column made of transparent acrylic plastic tube (height: 2 m, inner diameter: 100 mm) was used to determine the settling velocity of spiny lobster faecal strands as per the methods outlined by Moccia et al. (2007b). Faecal strands were gently introduced at the top of the settling column to avoid strand breakage and the duration taken for the individual faecal strand to settle to the bottom (T) of the column was timed with a stop watch. Water in the settling column was maintained at 16 ± 1 °C with salinity of 34 ± 1 ppt. The settling velocities (V) of spiny lobster faecal strands were calculated as follows:

\[ V = \text{height of settling column} / T \]

A separate experiment was also conducted to determine if there were differences in settling velocity of the faecal strands produced by lobsters of different sizes. A total of six lobsters, one for each of the following sizes, 100, 200, 400, 500, 600, and 800 g, were used in the experiment. Faecal strands were collected fresh early each morning and measured for weight, length and diameter. The settling velocities of faecal strands were determined as previously outlined with sample sizes of 28, 21, 38, 11, 22 and 20 faecal strands for lobsters from the sizes of 100 – 800 g respectively. In addition, samples of collected faecal material from spiny lobsters of 100, 200, 300, 400, and 600 g were also analysed for carbon and nitrogen content after rinsing in freshwater to remove salt, freeze drying, and then processing with an elemental analyser (Exeter Analytical CE-440).
2.2.3 Hydrodynamic modelling of depositional effects

A modified Mike21 FM particle mass transport module (Kurian et al. 2009) was used to simulate the dispersion of spiny lobster faeces in the water column and settling on the seabed (McCowan et al. 2001). The model treats lobster faeces as cohesive particles that sink in the water column until they are deposited on the seafloor according to the hydrodynamic conditions. This is likely given that from observations of more than 300 faecal strands in the velocity chamber none were observed to disintegrate. Therefore, the faecal settling data obtained from the settling experiment were used as an input for the hydrodynamic modelling. To adequately represent the natural variability in the settling velocity of faeces for the modelling, faecal strand settling velocity was distributed into three fractions for each of which, dispersal was modelled separately, and then the results combined for an overall estimate of benthic accumulation of faecal material (Table 2.1). Instead of using a homogenous current condition, the Mike21 model takes into account the changes in current conditions due to the bathymetry, wind and wave forcing using environmental data collected from the modelled location. Furthermore, the model also includes sedimentary erosion, transport and deposition processes under the action of currents and wave conditions to stimulate as close to the natural ocean processes as possible.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Proportion of Faecal Output (%)</th>
<th>Faeces Settling Velocity (cm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1</td>
<td>43</td>
<td>0.85</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>32</td>
<td>0.90</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>25</td>
<td>0.95</td>
</tr>
</tbody>
</table>
2.2.4 Model input and sources

The hypothetical lobster farming site used in this study was selected as a potential site to conduct sea-cage spiny lobster aquaculture, i.e., 10 – 30 m water depth, 1 km offshore. The bathymetry of the site for use in the modelling was taken from British Admiralty Sea Charts, based on UTM-50 projection. An eco-sounder was also used to collect additional higher resolution bathymetric information. Current measurements, both current speed and direction were collected in-situ using an Acoustic Doppler Current Profiler (ADCP) deployed over a period of 14 days. Current measurements were recorded at intervals of 10 min with a depth resolution of 50 cm. Wind data used for modelling at this site were sourced from NOAA (National Oceanic and Atmospheric Administration, USA), by extraction of the data from their global forecasting system meteorological model (GFS).

The layout of spiny lobster sea-cages was based on the layout typically used in the commercial spiny lobster sea-cage aquaculture in many parts of the world (Figure 2.1). A square block of 16 floating sea-cages was used as the base unit with each square sea-cage within the block measuring 5 × 5 m, with a suspended cuboid net extending 5 m below the sea surface and enclosing a total of 125 m³ of water. A more extensive farming arrangement was also examined where five square blocks, each of 16 sea-cages (i.e., total of 80 sea-cages) were set out in a continuous straight line. The ‘rafting up’ of groups of sea-cages is common practice as it provides for more efficient anchoring and operations above sea level (De Silva and Phillips 2007).
Figure 2.1. A block of 16 sea-cages, each measuring $5 \times 5$ m, as is typically used in commercial spiny lobster aquaculture in Malaysia. The net sea-cages extend up to 5 m depth.

Scenarios of two typical lobster stocking densities were used in the model, a low and high stocking density of 3 and 5 kg m$^{-3}$ respectively (Arcenal 2004; Priyambodo and Sariffin 2008). The corresponding production values for low and high stocking density were 0.375 and 0.625 t of lobsters per year respectively, based on typical growth projections of lobsters (Du et al. 2004; Hoang et al. 2009).

The quantity of faeces released into the environment by the lobsters held in the sea-cage depended on feed conversion ratio (FCR). Three previously reported FCR values for spiny lobsters were used in the present model, FCR = 1.28 (Crear et al. 2000), FCR = 3 (Petersen et al. 2009), and a worst case scenario of FCR = 5. For the hypothetical lobster farm, a single feeding event per day was assumed. A time series of faecal release was created in the model to simulate the daily feeding schedule and to estimate the amount of feed converted to faecal material and discharged to the seabed (Table 2.2). An estimate of the total carbon content of the faeces of 20 % was used for the modelling as this analyses was
conducted prior to the results from the elemental analyses of lobster faeces becoming available. This estimate was a mid-value from published data for carbon content of the faeces previously reported for a range of shrimp and fish species (Brigolin et al. 2009; Landrum and Montoya 2009; Beardsley et al. 2011). In addition, 5% of feed waste was assumed to accumulate on the seabed.

Table 2.2. Faeces deposition values used in the hydrodynamic model for low (3 kg m\(^{-3}\)) and high (5 kg m\(^{-3}\)) stocking densities in a hypothetical spiny lobster farm with production of 0.375 t yr\(^{-1}\) and 0.625 t yr\(^{-1}\) respectively.

<table>
<thead>
<tr>
<th>Production</th>
<th>Low Stocking Density 0.375 t yr(^{-1})</th>
<th>High Stocking Density 0.625 t yr(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR</td>
<td>1.28</td>
<td>1.28</td>
</tr>
<tr>
<td>Overall faeces output</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>kg faeces yr(^{-1}) cage(^{-1})</td>
<td>95</td>
<td>161</td>
</tr>
<tr>
<td>kg faeces day(^{-1}) cage(^{-1})</td>
<td>0.26</td>
<td>0.44</td>
</tr>
<tr>
<td>Faeces output at feeding time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg faeces feeding hr(^{-1}) cage(^{-1})</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>Faeces output at non-feeding time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg faeces feeding hr(^{-1}) cage(^{-1})</td>
<td>0.0026</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

The near-bed current speed used in the model was 4.5 cm s\(^{-1}\) (Cromey et al. 2002a). Since this is a calibration variable and no published data is available for spiny lobster species, a standard critical shear stress for deposited layer material of 0.004 N m\(^{-2}\) was set based on similar values used for salmon and mussel faeces. The model also assumed that re-suspension of material previously deposited to the seafloor would only take place when bottom flow conditions exceeded the critical shear stress threshold.

To estimate the spatial pattern of spiny lobster faecal deposition to the seabed the hydrodynamic dispersal model was run for the various combinations of stocking density, sea-cage arrangement, FCR value, for a simulated 1 year period. Model output was calculated as the spatial accumulated mass of organic carbon deposited on the seabed over the 1 year
modelling period in kilogrammes of carbon per year. It was assumed that all carbon deposited to the seabed was accumulated (not broken down through microbial processes or by deposit feeding organisms) over time, i.e., period of 1 year. In addition, a separate hypothetical 4 ha spiny lobster aquaculture operation was also modelled to calculate the amount of faecal material that would leave the model domain during the stimulation. Results from that simulation indicated less than 1% of spiny lobster faecal material left the model domain. It is evident that spiny lobster faecal material were sufficiently cohesive not to re-suspend to a great extent after their deposition on the seabed even with dynamic bottom current speed such as 10 cm s\(^{-1}\).

An organic carbon deposition rate of 0.73 kg C m\(^{-2}\) yr\(^{-1}\) was set as a threshold level to identify those seabed areas that would potentially be impacted by faeces deposition. This value was derived from findings of previous studies of aquaculture waste deposition and was considered conservative for sedimentary benthic habitats over which sea-cage operations are normally placed (Hargrave et al. 1994; Sowles et al. 1994; Cromey et al. 2002b; Gilibrand et al. 2002).

### 2.2.5 Hydrodynamic modelling calibration

Calibration is the process by which model parameters are adjusted within reasonable limits in order for the model predictions to be more closely align with the real time measurements taken from the field. In this study, the hydrodynamic model was successfully calibrated to ADCP current measurements for the site prior to running the faeces deposition modelling. The calibration parameters in the Mike21 FM model included bed friction, eddy viscosity, Coriolis forcing and predicted versus measured tidal levels.

### 2.2.6 Data analyses

SigmaPlot version 11.0 was used for all statistical analyses. The settling velocity data for faecal strands did not fulfil normal distribution assumptions required for the use of parametric statistical methods, even after transformation. Thus, the non-parametric
Spearman’s rank correlation test was used to assess the relationships between faecal strand weight and settling velocity, faecal strand length and settling velocity, the volume of faecal strand and settling velocity, as well as between faecal strand density and settling velocity. The settling velocity of faecal strands, as well as the carbon, and nitrogen contents of lobster faeces were each compared for samples taken from lobsters of different sizes with a Kruskal-Wallis one way analysis of variance followed by a pairwise multiple comparison analysis using Dunn’s method.

2.3 Results

2.3.1 Faecal settling velocity

Settling velocities of faecal strands from the spiny lobster, *Jasus edwardsii*, ranged from 0.4 to 2.0 cm s\(^{-1}\) with an overall average settling velocity of 0.9 ± 0.1 cm s\(^{-1}\) (mean ± SE). There was no significant difference between the settling velocity of faecal strands from lobsters of different sizes except between 100 and 200 g lobsters (P < 0.05). The median settling velocity of faecal strands from 100 g lobsters was on average 0.1 cm s\(^{-1}\) slower than for 200 g lobsters. Given that the overall faecal settlement velocities of lobsters of a range of sizes showed minimal overall differences among size classes, the settling data for the faeces produced by lobsters over the full range of sizes were pooled and used for modelling faecal output of sea-cages which would contain lobsters covering a range of sizes.

The settling velocity of faecal strands was weakly correlated with faeces weight (P < 0.05, \(\rho = 0.36\)) with heavier faeces sinking considerably faster than lighter faeces (Figure 2.2). Similarly, the settling velocity of faecal strands was also positively, but weakly correlated with the faeces density (P < 0.05, \(\rho = 0.29\)) (Figure 2.3). Neither the length nor the volume of faecal strand was correlated with settling velocity (P > 0.05, \(\rho = -0.11\) and -0.08 respectively) (Figure 2.4 and 2.5).
Figure 2.2. The weight (g) of spiny lobster faecal strands plotted against their measured settling (cm s\(^{-1}\)), \(n = 59\).

Figure 2.3. The density (g mm\(^{-3}\)) of spiny lobster faeces plotted against their measured settling velocity (cm s\(^{-1}\)), \(n = 59\).
Figure 2.4. The length (mm) of spiny lobster faeces plotted against their measured settling velocity (cm s\(^{-1}\)), \(n = 59\).

Figure 2.5. The volume (mm\(^3\)) of spiny lobster faeces plotted against their measured settling velocity (cm s\(^{-1}\)), \(n = 59\).
Elemental analysis showed that faecal material (in dry matter) produced by spiny lobster fed solely on mussels contained on average 35 ± 1 % (mean ± SE) and 5 ± 0.3 % of carbon and nitrogen respectively. Carbon content in spiny lobster faecal material was not significantly different among the various lobster sizes (P > 0.05). By contrast, there was a significant difference in the nitrogen content of the faecal material of lobsters over the five size classes (P < 0.05). The mean nitrogen content of faeces produced by 100 g spiny lobsters were approximately 2 % higher than for all other lobster sizes.

### 2.3.2 Current conditions and hydrodynamic calibration

Analysis of the ADCP current measurements shows that the current flow at the site was dominated by wind driven currents, mostly flowing towards the northeast during the ADCP deployment period. In-situ current conditions were divided into three layers; surface (0.5 – 11 m), mid water (12 m) and near bed (0.5 m above the seabed) (Table 2.3). Current speed at the top layer could reach up to 60 cm s⁻¹ with an average of 24 cm s⁻¹ over the 14 days deployment of the ADCP. In the bottom layer, the current speed varied around an overall average of 10 cm s⁻¹. The depth averaged current speed was in the order of 18 cm s⁻¹ on average over the measurement period. The maximum depth averaged current speed measured at the site was 33 cm s⁻¹ while the minimum was 9 cm s⁻¹.

**Table 2.3. Current speed for surface, middle and bottom layer of the water column at a lobster aquaculture site in Malaysia used for the hypothetical lobster farm for modelling purposes.**

<table>
<thead>
<tr>
<th>Layer of water column</th>
<th>Mean current speed (cm s⁻¹)</th>
<th>Max. current speed (cm s⁻¹)</th>
<th>Min. current speed (cm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top layer (0.5 – 11 m)</td>
<td>24</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>Middle layer (12 m)</td>
<td>20</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>Bottom layer (0.5 m above seabed)</td>
<td>10</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>
The hydrodynamic calibration exercise showed that the simulated current speed was 16 cm s\(^{-1}\), which was 2 cm s\(^{-1}\) less than the average current speed measured in-situ in real time. The overall trend of the measured current speed and direction was followed by the model predictions with only minor discrepancies. The overall level of agreement between measured and modelled flows were determined to be sufficient for their subsequent use in faeces depositional modelling.

### 2.3.3 Hydrodynamic modelling of faecal deposition

Overall, the hydrodynamic models showed that the carbon deposition rate contributed by the spiny lobster faeces ranged from 0.25 to 0.80 kg C m\(^{-2}\) yr\(^{-1}\) directly beneath the sea-cage and gradually decreasing to between 0.05 and 0.10 kg C m\(^{-2}\) yr\(^{-1}\) as it was dispersed away from the sea-cages. The spatial extent and mass of carbon deposited to the seabed was found to very much depend on the stocking density of the spiny lobsters, the FCR, as well as the layout of the sea-cages. In the smaller farm layout (16 cages) the deposition of faecal material for all three modelled FCR’s resulted in deposition being concentrated directly beneath the sea-cages with some dispersal around the sea-cages (Figure 2.6). Hence, the mass of carbon was at its highest directly beneath the sea-cages, reaching a maximum of 0.60 kg C m\(^{-2}\) yr\(^{-1}\) and over 0.80 kg C m\(^{-2}\) yr\(^{-1}\) for low and high stocking densities respectively. The spatial extent of the lowest modelled level (i.e., 0.05 kg C m\(^{-2}\) yr\(^{-1}\)) of faeces deposition for the smaller farm layout at low stocking density were up to 12, 25 and 33 m from the perimeter of the sea-cages for FCR values of 1.28, 3 and 5 respectively. By contrast for high stocking density in the smaller farm layout, the lowest level (i.e., 0.05 kg C m\(^{-2}\) yr\(^{-1}\)) of faeces deposition on the seabed extended up to 20, 33 and 46 m from the perimeter of the sea-cages for the same range of FCR values.

The model for the larger farm of 80 sea-cages arranged in a line, showed the deposition of faecal material covered a more extensive area than for the smaller farm layout (Figure 2.7). The hydrodynamic model illustrated that the faeces deposition on the seabed could potentially extend up to 30 and 40 m from the outer margin of the sea-cages for low stocking density with FCR values of 3 and 5 respectively, and as far as 40 and 50 m from the sea-cages for the high stocking density with FCR values of 3 and 5 respectively.
hydrodynamic modelling indicated that the faeces deposition for the larger farm layout could potentially reach up to 0.5 kg C m\(^{-2}\) yr\(^{-1}\) directly beneath the farm for the low stocking density and 0.7 kg C m\(^{-2}\) yr\(^{-1}\) for the high stocking density. Overall, these carbon deposition estimates were about 0.1 kg C m\(^{-2}\) yr\(^{-1}\) lower than those for the same sets of parameters for the smaller farm layout due to the wider overall dispersal of faecal material from this larger farm design.

Regardless of the stocking density, FCR values and layout of cages, the faeces deposition extended further from the northern side of the sea-cages for both small and large farm layouts, which corresponds to the predominant current direction of the study site. Overall, the deposition of faecal material only exceeded the organic carbon deposition threshold of 0.73 kg C m\(^{-2}\) yr\(^{-1}\) for the most extreme combination of model inputs, i.e., high stocking rate (5 kg m\(^{-3}\)), poor FCR (5), for 16 sea-cages. In this extreme scenario the threshold of organic carbon loading was only exceeded in the area directly below the sea-cage.
Figure 2.6. Benthic carbon mass deposition extrapolated to 1 year for layout of 16 spiny lobster sea-cages at three food conversion ratios, 1.28, 3 and 5. (a), (c), and (e) low stocking density (3 kg m\(^{-3}\)); (b), (d), and (f) high stocking density (5 kg m\(^{-3}\)).
Figure 2.7. Benthic carbon mass deposition extrapolated to 1 year for layout of 80 spiny lobster sea-cages at two food conversion ratios, 3 and 5. (a) and (c) low stocking density (3 kg m$^{-3}$); (b) and (d) high stocking density (5 kg m$^{-3}$).

2.4 Discussion

This study indicated that the weight and density of spiny lobster faecal strands were factors influencing their settling velocity while their length and volume had no effect on their settling velocity. Past research on faecal material of salmon also shows that settling velocity of faeces were not related to either their weight, diameter or length (Chen et al. 1999b; Chen et al. 2003). In contrast, faecal material from cod settles faster with increasing volume, diameter and length of faecal strands (Cromey et al. 2009).

The overall mean settling velocity of lobster faeces measured in this study, (i.e., $0.90 \pm 0.05$ cm s$^{-1}$) was much lower than the mean settling velocity of faeces of Atlantic salmon (range from 2.0 to 6.6 cm s$^{-1}$; with a majority of studies reporting an average of 3.2 cm s$^{-1}$) and cod (*Gadus morhua* L.) (mean velocity = 3.7 cm s$^{-1}$) (Findlay and Watling 1994; Panchang et al. 1997; Elberizon and Kelly 1998; Chen et al. 1999b; Dudley et al. 2000; Cromey et al. 2002a; Chen et al. 2003; Cromey et al. 2009). The faster settling velocity in
both salmon and cod is due to heavier faeces and lower faecal volume resulting in denser faecal material lobster (Chen et al. 1999b; Chen et al. 2003; Cromey et al. 2009). In comparison, the mean settling velocity of spiny lobster (*J. edwardsii*) faeces was higher than those measured in sea bream (*Sparus aurata* L.) and sea bass (*Dicentrarchus labrax* L.), with mean sinking velocities of 0.5 and 0.7 cm s\(^{-1}\) respectively (Magill et al. 2006). The reported particle size of faeces from sea bream and sea bass were much smaller than spiny lobster faeces, hence their correspondingly lower settling velocity (Magill et al. 2006). The differences in faecal material settling velocity of different aquaculture species will have significant implications for the dispersion of faecal material on the seabed, with settling velocities being directly proportional to horizontal dispersal distances (Gowen et al. 1989). The increase in the dispersion of faeces, reduces the localised deposition of faecal material, and thereby reduces the potential for localised impact on benthic biota (Reid et al. 2009).

Variation of faecal settling velocity among different species of aquacultured fin-fish is thought to be related to variations in the digestive system from species to species, producing faecal material of varying density (Magill et al. 2006). But in the case of spiny lobsters, the digestive tract and digestive physiology is similar among spiny lobster species (Mikami and Takashima 2008). Therefore, it is likely that the faeces density and settling velocity would also be similar among spiny lobster species. However, digestibility of different diets is likely to change the density of faecal material produced (Reid et al. 2009), with higher digestibility decreasing the faecal density and hence reducing the faecal material settling velocity (Amirkolaie et al. 2005; Ogunkoya et al. 2006). Currently available formulated feed pellets are known to yield poorer digestibility compared to mussels in spiny lobster (C. Simon pers. comm.). Therefore, it could be expected that a typical spiny lobster aquaculture operation using low-value fish as feed would potentially have lesser impact on the seabed compared to formulated feeds, but this may not be the case, since feeding low-value fish is known to result in very poor FCR in lobster aquaculture (an average of 28 – 32 on a fresh weight basis) (Edwards et al. 2004; Hung and Tuan 2009; Ly 2009; Hung et al. 2010). Furthermore, part of the potential impact on the seabed is contributed by feed wastage (Beveridge 2004; Wu 1995). Aquaculture species fed with low-value fish (seafood diet) can have up to 45 % feed wastage, which is 3 to 9 times higher than aquaculture systems using artificial feed (Wu 1995). Thus, spiny lobster aquaculture systems using seafood diet would potentially have much higher benthic impact compared to those fed with formulated feeds.
Chapter 2

The carbon content in faecal material of spiny lobster fed solely on mussels (average of 35%) was within the range of those fed with formulated feed in a previous study (20% - 40%) (Simon 2009a), but was substantially higher than the estimated carbon content used in our modelling (20%). Therefore, higher levels of carbon deposition could be anticipated than predicted by our modelling, although even doubling the carbon deposition rates predicted by the model does not exceed the carbon deposition threshold (0.75 kg C m\(^{-2}\) yr\(^{-1}\)) unless spiny lobsters are stocked with the high density and have poor FCR value (5 kg m\(^{-3}\) and 5 respectively). Ultimately, it is likely that commercially available formulated feeds for spiny lobsters will match the digestibility reported for shrimp and fish diets, and used in our modelling, i.e., 20% carbon.

Over all of the modelled scenarios, the deposition of faecal waste from the spiny lobster sea-cage aquaculture was concentrated directly beneath the sea-cages and was spread out between 12 and 50 m from the perimeter of the sea-cages depending on the farm layout, stocking density and FCR values. The depositional pattern from this hypothetical spiny lobster sea-cage aquaculture is consistent with fin-fish aquaculture, where faecal material is mainly deposited directly beneath the sea-cages (Cromey et al. 2002a; Cromey et al. 2009; Doglioli et al. 2004; Magill et al. 2006; Panchang et al. 1997). However, fin-fish aquaculture can sometimes have more widespread deposition of solid waste, of up to 320 m from the farm site (Cromey et al. 2002a). A combination of factors can explain this wider dispersal, including greater total faecal output from fish farms, stronger current regimes, and deeper farm sites. For example, higher current speeds facilitates the re-suspension and wider dispersion of faecal material previously deposited on the seabed (Dudley et al. 2000). Due to the requirements of spiny lobsters to hold onto sea-cage netting it is unlikely that the aquaculture of spiny lobsters will be conducted in deeper, more exposed locations, where water currents and wave movements are likely to be greater. Therefore, it is likely that faecal dispersion from lobster sea-cages will tend to be more localised with much less re-suspension of previously settled faecal material than the general pattern previously observed for fin-fish farming.

In this study the total faecal production was found to increase in direct proportion to increasing FCRs, and stocking densities used for modelling (Table 2.2). Consequently, the hydrodynamic models indicated that the higher FCR values and the higher stocking density resulted in a greater carbon footprint of faecal material on the seabed. Reported FCR for the
aquaculture of salmon was 1.19 (Aksu et al. 2010), sea bass and sea bream were both 1.3 (Doglioli et al. 2004) and are similar to our lowest FCR value for our hypothetical spiny lobster farm (FCR = 1.28). However, compared to spiny lobster aquaculture, fin-fish aquaculture usually has much higher stocking densities, which results in greater overall production of faecal material. Stocking density in a fin-fish aquaculture system can range from 10 - 50 kg m\(^{-3}\) (Doglioli et al. 2004; Turnbull et al. 2005; Kongkeo et al. 2010; FAO 2012). Therefore, in a scenario where the FCR value was the same for both fin-fish and spiny lobster sea-cage aquaculture, a typical spiny lobster aquaculture system would produce up to 10 times less carbon as faeces than a fin-fish aquaculture system. It is unlikely in the future that spiny lobster sea-cage aquaculture will be able to greatly increase stocking densities of sea-cages due to the requirement of lobsters to use the net surface for holding, and because high density stocking of spiny lobsters tends to depress growth and increase cannibalism (Simon and James 2007).

Regardless of stocking density or FCRs, when comparing both layouts of cages, the larger farm had less impact under the sea-cages. The orientation and layout of the sea-cages appeared to play an important role in determining the accumulation of waste material on the seafloor beneath the sea-cages probably because of the influence of the sea-cage on current speed around the farming site (Plew 2011). It is possible that positioning sea-cages in a line in the larger farm layout reduced the cage hydrodynamic drag effects, thus increasing the current speed around and beneath the sea-cages, and thereby dispersing the waste material further away from the sea-cages. However, whether this was always the case would be dependent on the hydrodynamics of a specific aquaculture location.

The average current speed for the bottom layer at the study site was 10 cm s\(^{-1}\) with depth averaged currents speed in the order of 18 cm s\(^{-1}\) on average over the measurement period which was rather dynamic. A well-established spiny lobster aquaculture in Van Phong Bay, Vietnam had similar flow rates to our study site with average current speed of 17.7 cms\(^{-1}\) during the winter monsoon and 9.8 cm s\(^{-1}\) during the summer monsoon (Vinh et al. 2004). However, if a spiny lobster aquaculture site was to be at a shallower and more sheltered area that has lower current flow rates than those measured above, it is expected that the general principles of the interactions between faecal waste and hydrodynamic flow from this study would still apply. Accumulation of spiny lobster faeces would generally still be directly under the sea-cages but with a less tighter zone of impact around the sea-cages as faeces may not be
able to disperse as far with lower current speed (Hartstein and Stevens 2005). The potential environmental impact on the seabed from spiny lobster aquaculture with lower current speed scenarios will still be considered relatively low when compared to fin-fish aquaculture because of the relatively low stocking density.

The results presented in this study were based on a hypothetical spiny lobster aquaculture site. There is the potential to carry out further studies at spiny lobster farming sites to further validate the results generated from the current model. Nonetheless, the end result of faeces dispersion on the seabed is anticipated to differ slightly from site to site with varying hydrodynamic regime. Future environmental impact studies on spiny lobster sea-cage aquaculture should also focus on examining dissolved nutrient (e.g., ammonia) outputs from spiny lobster aquaculture and its effect in the surrounding water column.

2.5 Conclusion

Spiny lobster sea-cage aquaculture, like any other sea-cage aquaculture (e.g., fin-fish species) will result in deposition of some solid waste (faecal material and feed waste) onto the seabed. The hydrodynamic models in this current study indicated that a typical spiny lobster sea-cage aquaculture operation is unlikely to have widespread depositional impact on the seabed due to a combination of the relatively low stocking densities that are possible with spiny lobsters, and the low settling velocities which have been measured for lobster faeces, which facilitates their dispersal with water currents. In this study a conservative faecal carbon deposition threshold of 0.73 kg C m⁻² yr⁻¹ was only exceeded for a small area of the seafloor directly under a lobster farm operating with high stocking density and very poor FCR. Therefore, the development of highly digestible artificial feeds for spiny lobster aquaculture will be important for reducing the potential for environmental impacts from this activity, especially if higher stocking densities are to be used.
Chapter 3:

Characterising the Fate of Nitrogenous Waste from the Sea-cage Aquaculture of Spiny Lobsters, *Jasus edwardsii* using Numerical Modelling

3.1 Introduction

The strong global market demand and high prices for spiny lobsters has resulted in increasing pressure on wild fisheries and subsequently generated great interest in the development of spiny lobster aquaculture (Hart 2009; Jeffs 2010; Williams et al. 2001). Spiny lobster aquaculture was pioneered in Vietnam in the mid-1990’s using wild-caught juvenile lobsters as seed stock that are on-grown until they reach a marketable size in floating sea-cages located in shallow coastal waters (Arcenal 2004; Hart 2009). The cultured lobsters are fed low-value fish (70 %), often known as trash fish, including shellfish (30 %), although some growers have started using artificial pelleted feed (Williams 2007b; Hung and Tuan 2009). Lobster aquaculture has subsequently spread from Vietnam to other Asia-Pacific countries such as Indonesia, Malaysia, Philippines, Thailand, China and New Zealand (Arcenal 2004; Cox and Johnston 2003; Hung and Tuan 2009; Jeffs and Hooker 1999; Jones 2010; Phillips and Matsuda 2011; Priyambodo and Sarifin 2008; Rao et al. 2010). The species of spiny lobsters used for sea-cage aquaculture now includes *Panulirus ornatus*, *P. homarus*, *P. stimpsoni*, *P. polyphagus*, *P. longipes*, *P. penicillatus*, *P. versicolor* and *Jasus edwardsii* (Jeffs and James 2001; Ly 2009; Phillips and Matsuda 2011).

Sea-cage aquaculture activities result in the release of nitrogen compounds into the environment, mostly in the form of ammonia, resulting from proteolysis by the cultured organisms (Wu 1995). Elevated ammonia concentrations between 0.09 and 3.35 mg L⁻¹ are known to be toxic to some marine fish (Handy and Poxton 1993), and ammonia levels of more than 10 mg L⁻¹ have been shown to inhibit growth in crustaceans (Chen and Lin 1992). Ammonia can be transformed to nitrite and nitrate through oxidation, and high levels of nitrite
and nitrate can also be toxic to aquatic organisms and can also contribute to eutrophication in the water column (Beveridge 2004; Tucker 1998). Spiny lobsters are ammonotelic, where 60–100 % of total excreted nitrogen is made up of ammonia (Kemp et al. 2009). The ammonia is mostly excreted via the gill epithelium (Crear and Forteath 2002). In sea-cage aquaculture where spiny lobsters are grouped together in a sea-cage, it is expected that there will be an elevation of inorganic nitrogen compounds in the water column in the vicinity of the sea-cages.

The primary input of nitrogen (N) into a sea-cage aquaculture systems comes from the feed given to the cultured animals (Beveridge 2004). Nitrogen content within an aquaculture feed depends on the protein content of the feed which can be easily calculated by dividing the percentage of protein in the feed by a factor of 6.25 (Ai et al. 2004). Cultured spiny lobsters are predominantly fed with a mixture of by-catch or trash fish (5.6 – 7.3 % N) (Djunaidad et al. 2003; Xu et al. 2007; Azmat et al. 2008; Hasan 2012) and shellfish (8.8 % N) (Simon and Jeffs 2008), but some lobster farmers have started using artificial diet which contains approximately 8.5 % nitrogen (Lucky Star brand proximal composition, www.luckystarfeed.net). Nitrogen from the feed is then released into the surrounding environment through several pathways. Some of the nitrogen from the feed is digested and anabolised by the spiny lobsters for building body mass, while some is catabolised for energy and the nitrogenous waste that is generated is excreted as dissolved inorganic nitrogen (DIN) (Kemp et al. 2009; Radford et al. 2004). Nitrogen is also discharged within faecal particles produced from the cultured animals as well as from waste feed that sinks to the seabed (Brooks et al. 2002). Thus, to assess the DIN output from a spiny lobster sea-cage aquaculture system, it is necessary to know how much DIN spiny lobsters excrete in relation to the feed and feeding habits of the animal.

Temperature, body weight, feeding activity, time of day and type of diet all have marked effects on the excretion of dissolved nitrogen by lobsters, and this also varies among lobster species (Crear and Forteath 2002; Kemp et al. 2009; Radford et al. 2004). For example, previous studies have shown that post-prandial total ammonical nitrogen (TAN) excretion rate of J. edwardsii, P. cygnus, and P. homarus rubellus can be up to 7.5, 8, and 20 μg TAN g⁻¹ hr⁻¹, respectively (Crear and Forteath 2002; Kemp et al. 2009). In contrast, there is limited knowledge on dissolved nitrogen (i.e., all soluble nitrogen products such as nitrate and nitrite) excretion rates from spiny lobsters fed with artificial diets. The only published
study on nitrogen output from a lobster farm found that *P. ornatus* held in sea-cages were estimated to generate nearly twice as much total waste nitrogen (i.e., including both particulate and dissolved organic and inorganic nitrogen) when fed with trash fish versus an artificial diet, i.e., 402 g waste N kg\(^{-1}\) versus between 269 to 297 g waste N kg\(^{-1}\) lobster (Tuan and Hung 2008). Further knowledge on the rates of excretion of dissolved nitrogen from spiny lobster fed with artificial diets is important because continuing improvements in the formulation of artificial lobster feeds is likely to lead to their increasing use in commercial aquaculture.

Therefore, the aim of this study was to quantify the dissolved nitrogenous output from spiny lobsters in sea-cage aquaculture when fed with either trash fish or artificial diet. Due to the difficulty in conducting accurate field measurements of highly labile dissolved nitrogenous compounds in the marine environment, experiments were conducted under controlled laboratory conditions to measure the DIN discharge from captive spiny lobsters, *J. edwardsii*. These measured data were then incorporated into a well-developed numerical modelling program (Mike21-ECO Lab) developed by the Danish Hydraulic Institute (DHI) to trace the subsequent spatial dispersal of DIN around a hypothetical spiny lobster aquaculture site.

### 3.2 Materials and methods

#### 3.2.1 Husbandry methods

Spiny lobsters, *J. edwardsii*, of a range of body mass (n = 6; 100 - 700 g wet weight) were collected from rocky reefs near Leigh, in northeastern New Zealand and transported to the Leigh Marine Laboratory in March 2012. This body mass range of spiny lobsters is representative of typical lobster body masses that would be grown out in sea-cages (Arcenal 2004; Hart 2009). The lobsters were housed individually in aerated polyethylene tanks within a flow-through seawater system. The tanks were fitted with a graduated depth scale so that the volume of seawater they contained could be precisely determined. The temperature and salinity of water in tanks were kept constant at 18 ± 0.5 °C and salinity of 34 ± 1 ppt. Light was maintained at 12L/12D photoperiod. Spiny lobsters were acclimatized in these tanks for
at least 1 week before being used in experiments and fed with a variety of feeds, including those used in the later experimentation.

### 3.2.2 Daily DIN production

Spiny lobsters were not fed for 3 days and were then fed in the late evening (1800 h) at a ration of approximately 3% of their body weight (in wet weight) with freshly opened green-lipped mussels (*Perna canaliculus*). The lobsters were allowed 2 h to consume all of the feed. Seawater flow to and from each tank was shut off and then a 120 ml seawater sample was collected from each tank with a plastic syringe and filter (Whatman GF/F 0.7 μm). Seawater samples were subsequently taken at 2 h intervals for a period of 24 h after the first sample was taken. Seawater samples were immediately analysed for ammonia, nitrate and nitrite concentrations (μg N L⁻¹ - Hach colorimeter DR890). Prior to water analyses, the colorimeter was calibrated with standard solutions of ammonia, nitrite and nitrate in order to yield the most precise subsequent measurements. The DIN output was calculated as the sum of the quantities of ammonia, nitrite and nitrate after being adjusted for the total seawater volume of the tank that was holding the lobster. The experimental methods were repeated with the same set of spiny lobsters for two other treatments: one where they remained unfed after 3 days and the other where lobsters were fed and consumed 3% of their body weight (in wet weight) with artificial diet. The artificial diet used in this study was moist artificial diet made up of ground shrimp pellets from Grobest Feeds Corporation, India, Ltd. and bound with 2% alginate and cut into 2 cm³ cubes (Johnston et al. 2003; Simon 2009a). The lobsters were given a weaning period from fresh mussels to artificial diet of at least 2 weeks.

DIN excretion rates (μg N hr⁻¹) from individual lobsters were estimated from the serial sampling of increasing DIN at 2 hourly intervals over the 24-h sampling period (a total of 12 measurements of DIN excretion rates per lobster, μg N hr⁻¹). This allowed the calculation of a mean hourly rate of DIN excretion (μg N hr⁻¹) for the entire 24 h sampling period for each lobster, and these mean hourly DIN excretion rates were compared among lobsters of different body mass. To compare DIN excretion between the three different dietary regimes (i.e., mussels, artificial feed and unfed), the measurements of DIN excretion were standardised by lobster body mass (μg N g⁻¹ hr⁻¹). Values from the 24 h DIN measurements
were also used to calculate the mean percentage of feed in both wet and dry weight (based on moisture content in the diet) that was released as mass of DIN by lobsters from the two diet treatments. The moisture content in mussels was 79.8 % (Simon and James 2007) while the moist artificial diet had 42.2 % moisture based on laboratory measurements where the artificial diet was oven-dried at 60 °C for 24 h to obtain their dry weight and calculated for the mean percentage difference between dry and wet weight of seafood.

3.2.3 DIN modelling

Numerical modelling software (ECO Lab) coupled with a two-dimensional hydrodynamic model (Mike 21) developed by DHI was used to assess the dispersion of dissolved inorganic nitrogenous output from a hypothetical spiny lobster sea-cage aquaculture system (Tasmania Department of Primary Industries et al. 2011; DHI 2013). ECO Lab is an open-source generic modelling tool for simulating aquatic ecosystem processes such as nutrient cycling, the fate of released toxic chemicals and heavy metals that are driven by the in situ hydrodynamic conditions, and the output is reported as average values for the water column at points on the modelled spatial grid (DHI 2009). This modelling module simulates natural biochemical processes and ecological interactions and provides accurate spatial predictions of the fate of the released materials. For this study, a customised ECO Lab template for describing the influence of the nitrogen cycle on water quality (including nitrogen pathways through phytoplankton, zooplankton, and detritus, nitrification process of oxidising ammonia into nitrate, as well as the denitrification process of converting nitrate into nitrogen or nitrous oxide resulting in the removal of nitrogen from both the water column and seabed by being released as gas into the atmosphere), was developed based on the extensive study of the fate of nitrogen in the marine environment by Fennel et al. (2006). This ECO Lab template has been previously used to accurately quantify the spatial dispersal of nitrogenous waste from salmon sea-cage aquaculture operations in Australia (Tasmania Department of Primary Industries et al. 2011).

The hypothetical lobster aquaculture site used in this study was selected as a site that contained the characteristics that were typical for conducting commercial sea-cage spiny lobster aquaculture in Asia (6° 23´ 0.9´´ N, 116° 18´ 25.6´´ E), i.e., 10 – 30 m water depth, 1
km offshore, mean current speed of 20 cm s\(^{-1}\). Layouts of spiny lobster sea-cages were typical of that used in an extensive commercial spiny lobster sea-cage aquaculture. A total of 80 floating sea-cages arranged in four parallel rows of 20 sea-cages in a (10 × 2 arrangement) spaced 100 m apart (Figure 3.1). Each sea-cage measured 16 × 16 m, with a suspended cuboid net extending 5 m below the sea surface and enclosing a total of 1280 m\(^3\) of water. The background DIN concentration (initial ambient DIN concentration at the site) in the model domain was set at 5 μg N L\(^{-1}\) based on measurements taken at site.

![Figure 3.1. Layout of 80 sea-cages used in the dissolved inorganic nitrogen (DIN) model simulation. Each square represents a sea-cage with dimension of 16 × 16 m extending down to 5 m below the sea surface.](image)

A combination of different stocking densities and feed conversion ratios (FCR) were used in the model simulation. Two typical stocking densities were considered for this study, a low and high stocking density of 3 and 5 kg m\(^{-3}\), respectively (Arcenal 2004; Priyambodo and Sarifin 2008). Three previously reported FCR values for spiny lobsters were also used in the model, FCR = 3 (Petersen et al. 2009), FCR = 5 (Hung et al. 2010) and a worst case FCR
scenario of 28 (Edwards et al. 2004). For each modelled scenario, the quantity of DIN discharged (mg N L\(^{-1}\) s\(^{-1}\)) from each sea-cage within the lobster farm was derived from the total amount of feed (in wet weight) fed out to each sea-cage that has been converted into DIN (Table 3.1). The total amount of feed used per cage was calculated by multiplying the volume of each sea-cage (1280 m\(^3\)) with the stocking density (low density – 3 kg m\(^{-3}\) or high density – 5 kg m\(^{-3}\)) and FCR values of either 3, 5 or 28 and the amount of feed converted to DIN was based on the mean percentage of feed (in wet weight) converted to DIN that was previously measured during the experimental portion of the study for mussels and artificial diets (0.58 % and 0.16 % respectively).

Table 3.1. Quantity of DIN discharged (mg N L\(^{-1}\) s\(^{-1}\)) from each sea-cage for different combinations of stocking density (3 and 5 kg m\(^{-3}\)), FCRs (3, 5 and 28) with *J. edwardsii* fed on two different diets, mussels and artificial used in DIN model.

<table>
<thead>
<tr>
<th>FCR</th>
<th>Stocking Density (kg m(^{-3}))</th>
<th>Mussels (mg N L(^{-1}) s(^{-1}))</th>
<th>Artificial Diet (mg N L(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>2.119</td>
<td>0.584</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.531</td>
<td>0.974</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3.531</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.885</td>
<td>1.624</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>19.775</td>
<td>5.455</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32.958</td>
<td>9.092</td>
</tr>
</tbody>
</table>

The DIN model simulations were run for a period of one month of farm operation by which time the interaction of the released DIN with the environment was stable and provided useful spatial concentrations of the fate of the DIN. Any DIN concentration generated by modeling that was higher than the initial measured background DIN concentration of 5 µg N L\(^{-1}\) was considered to be elevated. The modelled DIN output consisted of predictions of the spatial arrangement of 16 successive concentration ranges of DIN between <4 and 116 µg N L\(^{-1}\). The model generated two sets of results: the mean elevated DIN which would indicate the most likely elevation of DIN in the surrounding environment contributed by the lobster farm after 1 month of production, and maximum elevated DIN concentration that showed the highest possible DIN elevation caused by lobster farming after 1 month of production.
acceptable dissolved nitrogen level for aquaculture was set at 0.5 mg L\(^{-1}\) (equivalent to 500 µg N L\(^{-1}\)) which has previously been recommended by government regulators in the Asian region specifically for aquaculture operations (Tuan 2005).

### 3.2.4 Data analyses

The comparison of DIN output (standardised for lobster body mass) for each two hourly sampling interval over the 24 h period (total of 12 sampling intervals) for each of the three dietary regimes for the lobsters were analysed with a non-parametric Friedman repeated-measures analysis of variance on ranks test as the data were not normally distributed even after transformation. When there were no differences found in the DIN output among the 12 sampling intervals, the DIN output data was pooled to test the overall DIN output from lobsters treated with the three different dietary regimes (i.e., unfed, mussel and artificial feed) using Mann-Whitney test. Linear regression was used to examine the relationship between lobster body mass and their average hourly DIN output (µg N hr\(^{-1}\)) over the 24 h period for the three dietary regimes (i.e., unfed, mussel and artificial diet). All statistical analyses were carried out using SigmaPlot version 11.

### 3.3 Results

#### 3.3.1 Effect of different dietary regimes on DIN production

The mean DIN excretion rate during 24 hours for unfed spiny lobsters (marked as the baseline level of DIN excretion from lobsters) was consistently low over the entire period with an overall mean (± SE) of 1.10 ± 0.12 µg N g\(^{-1}\) hr\(^{-1}\) (Figure 3.2). After feeding, the mean excretion rate of DIN tended to increase immediately and after a period of 8 – 10 h, it gradually decreased. Lobsters fed with the artificial diet took 10 h to return to baseline DIN excretion levels, but those fed with mussels tended to take longer. However, there were no significant differences among the mean DIN outputs as measured at two hourly intervals over the 24 h period within each of the three dietary regimes (mussels: Q=9.2, P=0.6; artificial diet: Q=8.3, P=0.7; unfed: Q=19.7, P=0.053) (Figure 3.2). Lobsters fed with mussels had
significantly higher overall mean DIN output than unfed lobsters (Z=8.4, P<0.001). The mussel diet increased the mean baseline DIN excretion rate by more than eightfold with a mean DIN excretion rate of 9.07 ± 0.90 μg N g⁻¹ hr⁻¹. Likewise, lobsters fed with mussel diet also had a mean output that was more than four times higher than those fed with the artificial diet (Z=7.2, P<0.001). However, even though the artificial diet had nearly double (2.11 ± 0.41 μg N g⁻¹ hr⁻¹) the mean DIN excretion rate of unfed lobsters, they were not significantly different (Z=0.4, P=0.7).

Figure 3.2. Mean dissolved inorganic nitrogen (DIN) excretion rate (μg N g⁻¹ hr⁻¹ ± S.E.) of spiny lobsters over a period of 24 hours after being given three different dietary treatments, mussel, artificial diet and unfed.

3.3.2 Effect of lobster body mass on DIN production

For both unfed lobsters and those fed with mussels, the mean hourly DIN excretion rate over 24 h (μg N L⁻¹) increased with the lobster body mass (unfed: F=47.8, P<0.05, r²=0.92; mussels: F=8.2, P<0.05, r² =0.67) (Figure 3.3a and c). In contrast, the mean hourly
DIN excretion rate over 24 h from spiny lobsters fed with artificial diet were not significantly different among the lobsters of different body mass (F=0.4, P=0.58, r²=0.08) (Figure 3.3b).

Regardless of lobster body mass and diet, on average 95.5 ± 1.0 % of the DIN excreted from the spiny lobsters was composed of ammonia, 3.0 ± 0.7 % by nitrate and 1.5 ± 0.4 % by nitrite. The average percentage of the amount of feed that was converted into DIN over a 24 h period when lobsters were fed with mussels was 0.58 % of wet weight (2.88 % of dry weight) of the feed while for lobsters fed with artificial diet the equivalent values were 0.16 % and 0.28 %, respectively.

### 3.3.3 DIN modelling

The highest elevated DIN concentration for all modelled scenarios was always found to be concentrated directly below the sea-cages, while those sea-cages located towards the northwestern side of the lobster farm generally had elevated levels of DIN being dispersed for distances of more than 200 m away from the perimeter of sea-cages which was further than other areas of the sea-cages. This pattern of dispersal coincided with the prevailing water current direction as measured at the site with ADCP. Thus, the dispersal of the same mean DIN concentration was found to cover a greater distance from the northern perimeter of the sea-cages as compared to the southern perimeter of the sea-cages for all of the modelled scenarios.

The highest elevated mean DIN concentrations for scenarios using mussels for lobster feed and stocked at low density (3 kg m⁻³) were 6, 6.4 and 15.0 µg N L⁻¹ for FCRs of 3, 5 and 28 respectively, while at high stocking density (5 kg m⁻³) the highest elevated mean DIN concentrations increased to 6.4, 7.4 and 20.0 µg N L⁻¹ for FCRs of 3, 5 and 28 respectively (Figure 3.4, 3.5 and 3.8). Where lobsters were fed with artificial diet, the highest elevated mean DIN concentrations for lobster farms that were stocked with low density of lobsters were 5.4 µg N L⁻¹ for both FCRs of 3 and 5, and 7.4 µg N L⁻¹ when the FCR was 28. For the lobster farm scenario that used artificial diet, the high stocking density scenario had the highest elevated mean DIN concentrations of 5.4 µg N L⁻¹ for FCR of 3, and 5.8 µg N L⁻¹ and 8.0 µg N L⁻¹ for FCRs of 5 and 28 respectively.
Figure 3.3. Hourly mean DIN excretion rate (µg N hr⁻¹) of lobsters in relation to their body mass (g). a) Spiny lobsters fed with mussels; b) fed with artificial diet; c) unfed spiny lobsters.
Figure 3.4. Spatial distribution of mean DIN concentration (µg N L⁻¹) generated around lobster aquaculture sea-cages for modelled scenarios with FCR of 3 and 5. (a-d) lobsters fed with mussels; (e-h) lobsters fed with artificial diet.
Figure 3.5. Spatial distribution of mean DIN concentration (µg N L\(^{-1}\)) generated around lobster aquaculture sea-cages for modelled scenarios with FCR of 28. (a-b) lobsters fed with mussels; (c-d) lobsters fed with artificial diet.

The maximum elevated DIN concentrations resulted with DIN elevation of above 11.2, 15.2 and 68 µg N L\(^{-1}\) for scenarios where lobsters were fed with mussels and stocked at low density for FCRs of 3, 5 and 28 respectively (Figure 3.6, 3.7 and 3.8). For scenarios using the same diet (mussels) but with high stocking density, the highest maximum DIN concentrations were above 15.2, 28 and 108 µg N L\(^{-1}\) for FCRs of 3, 5 and 28 respectively. For modelled scenarios feeding the artificial diet, at low stocking density for FCRs of 3, 5 and 28, the corresponding highest elevated maximum DIN concentrations were 6.8, 8 and 28 µg N L\(^{-1}\) while at high stocking density with the same FCRs, the respective highest elevated maximum DIN concentrations were 8, 9.2 and 36 µg N L\(^{-1}\).

The spatial extent of the elevated mean and maximum DIN concentration for all modelled scenarios using mussels as lobster feed were generally dispersed beyond 200 m away from the perimeter of the sea-cages with the highest DIN concentrations located directly beneath the sea-cages. For scenarios with lobsters fed on the artificial diet, at low stocking
density with FCR of 3, 5 and 28, the mean elevated DIN concentration dispersed up to a distance of 10, 110 and more than 200 m away from the perimeter of the sea-cages respectively, while at high stocking density, the mean elevated DIN extended from the sea-cages as far as 110 m for FCR of 3, and more than 200 m for both FCR of 5 and 28. The maximum elevated DIN in all modelled scenarios with lobsters fed with artificial diet, regardless of stocking density and FCRs, showed dispersion of elevated DIN to be more than 200 m away from the perimeter of the sea-cages. Overall, the spatial distribution patterns for both mean and maximum DIN concentrations did not differ much among the modelled scenarios using different stocking densities and FCRs; however, scenarios using mussel diet consistently produced a more extensive plume of elevated DIN for the same concentration of elevated DIN compared to the artificial diet. For example, at low stocking density, FCR of 3, and feeding artificial diet, the mean elevated DIN concentration of 5.4 µg N L$^{-1}$ dispersed to only 10 m away from the perimeter of sea-cages, whilst for the same scenario but feeding mussels it extended to over 200 m. Overall, the spatial distribution of mean DIN concentration was always more extensive with higher stocking density and higher FCR. For example, in the modelled scenario where the spiny lobsters were fed with the artificial diet at FCR of 3, the mean DIN concentration of 5.4 µg N L$^{-1}$ at a farm with low stocking density would spread approximately 10 m away from the perimeter of the sea-cages compared to 110 m for a high stocking density scenario. Likewise, for low stocking density and lobsters fed with artificial diet, the mean DIN concentration of 5.4 µg N L$^{-1}$ would extend up to 20 m away at FCR of 3, but extend to 110 m when modelled for an FCR of 5.

Finally, all the modelled scenarios indicated that the lobster farms demonstrated some degree of DIN elevation in the surrounding environment regardless of the stocking density, FCR or the type of diet used in the lobster farm. The intensity of elevated DIN concentration and the extent of DIN dispersal for both elevated mean and maximum DIN depended on the combinations of stocking density of the sea-cages (3 or 5 kg m$^{-3}$), FCRs (3, 5 or 28) as well as the diets being fed to the spiny lobsters (mussels or artificial diets). None of the combinations of stocking densities, FCRs and diet used in the DIN modelling scenarios resulted in either the highest mean or maximum elevated DIN concentrations exceeding the recommended threshold of 500 µg L$^{-1}$.
Figure 3.6. Spatial distribution of maximum DIN concentration (µg N L\(^{-1}\)) generated around lobster aquaculture sea-cages for modelled scenarios with FCR of 3 and 5. (a-d) lobsters fed with mussels; (e-h) lobsters fed with artificial diet.
Figure 3.7. Spatial distribution of maximum DIN concentration (µg N L⁻¹) generated around lobster aquaculture sea-cages for modelled scenarios with FCR of 28. (a-b) lobsters fed with mussels; (c-d) lobsters fed with artificial diet.
Figure 3.8. Sensitivity analysis of the highest mean (a) and maximum (b) DIN concentration (µg N L$^{-1}$) generated around the modelled lobster aquaculture sea-cages in relations to stocking density and FCR.
3.4 Discussion

3.4.1 DIN production

Feeding clearly had an effect on the DIN excretion rate of spiny lobsters with the nitrogen excretion rate tending to increase immediately after *Jasus edwardsii* had been fed a meal, as has been observed previously (Crear and Forteath 2002; Radford et al. 2004) (Table 3.2). Different types of diets used as feed for the spiny lobsters appear to influence the DIN excretion rate. For example, the quantity of nitrogen excretion of juvenile *Panulirus interruptus* fed with squid was eight times more than those fed with mussels (Diaz-Iglesias et al. 2011). In this current study, natural feed (mussels) resulted in higher DIN excretion rate from lobsters than when they were fed the artificial diet by 4.3 times. This is most likely due to mussels (55.2 % protein) having higher protein content than the artificial diet (40 % protein). Previously researches have shown that total ammonia excretion increased with the protein levels in diets (Ballestrazzi et al. 1994; Yang et al. 2002). Thus, a potential way to reduce DIN excretion in spiny lobster is to substitute protein content in the feed with either carbohydrate or lipid to potentially reduce the reliance on protein as a source of dietary energy, the metabolism of which is otherwise responsible for nitrogen excretion (McGoogan and Gatlin 1999).

The current study illustrated that the DIN excretion per hour (µg N h⁻¹) increased as the body mass of spiny lobster increased (Figure 3.3). Similar trends have been observed in a previous study where the TAN excretion (mg TAN h⁻¹) was positively correlated to the weight in two spiny lobster species *J. edwardsii* and *P. cygnus* (Crear and Forteath 2002). Higher DIN excretion per hour as body mass increased is to be expected as metabolic maintenance needs to increase with body mass. It is also possible that the digestibility of feeds by spiny lobster of smaller body mass differs to those of larger body mass when fed with the same diet. A study has shown that there was significant ontogenetic shifts in the digestive gland activity of *J. edwardsii* (Johnston 2003), which presumably could affect the hourly DIN excretion in spiny lobsters of different body mass range. In addition, smaller juvenile lobsters seem to make greater use of carbohydrate for energy which can result in differences of feed digestibility between smaller juvenile and adult lobsters (Smith et al. 2005). Carbohydrates and lipid were also found to be more extensively used in post-larvae and juveniles of the Malaysian river prawn, *Macrobrachium rosenbergii* (Diaz-Herrera et al. 1992). Hence
increased dietary inclusions of carbohydrates and lipids in artificial diets for spiny lobsters may help to increase the efficiency of diets whilst also reducing DIN outputs.

Table 3.2. Previous studies on pre-feeding and post-feeding nitrogen excretion rate of various lobster species

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet</th>
<th>Pre-feeding nitrogen rate (µg TAN g⁻¹ h⁻¹)</th>
<th>Post-feeding nitrogen rate</th>
<th>Recovery time (h)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Jasus edwardsii</em> (600 – 900 g)</td>
<td>Squid – <em>Nototodarus gouldii</em> (3 % of lobster body weight)</td>
<td>&lt; 2</td>
<td>6.28 time pre-feeding rate</td>
<td>26</td>
<td>Crear and Forteath (2002)</td>
</tr>
<tr>
<td><em>Jasus edwardsii</em> (0.7 – 2.8 g)</td>
<td>Squid (3 % of lobster body weight)</td>
<td>1.82 ± 0.21 (day)</td>
<td>12 times pre-feeding rate</td>
<td>18 (day)</td>
<td>Radford et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.96 ± 0.39 (night)</td>
<td>16 times pre-feeding rate</td>
<td>36 (night)</td>
<td></td>
</tr>
<tr>
<td><em>Homarus gammarus</em> (300 g)</td>
<td>Shrimp – <em>Crangon crangon</em> (L.)</td>
<td>3.1</td>
<td>4 times pre-feeding rate</td>
<td>18</td>
<td>Wickins (1985)</td>
</tr>
<tr>
<td><em>Jasus lalandii</em> (110 – 130 mm carapace length)</td>
<td>Mussel – <em>Choromytilus meridionalis</em></td>
<td>1.63</td>
<td>7.7 times pre-feeding rate</td>
<td>10</td>
<td>Zoutendyk (1987)</td>
</tr>
<tr>
<td><em>Panulius cygnus</em> (380 – 520 g)</td>
<td>Squid – <em>Nototodarus gouldii</em> (3 % of lobster body weight)</td>
<td>&lt; 2</td>
<td>5.6 times pre-feeding rate</td>
<td>30</td>
<td>Crear and Forteath (2002)</td>
</tr>
<tr>
<td><em>Panulirus homarus rubellus</em> (170.15 ± 23.79 g)</td>
<td>Squid – <em>Loligo vulgaris reynaudi</em> (3 % of lobster body weight)</td>
<td>Approx. 3</td>
<td>7.58 times pre-feeding rate</td>
<td>42</td>
<td>Kemp et al. (2009)</td>
</tr>
</tbody>
</table>
Temperature is another factor that could alter the DIN excretion rate of spiny lobsters. It has generally been found that higher temperature increases the ammonia excretion in crustaceans (Regnault 1987). TAN excretion in both *J. edwardsii* and *P. cygnus* increased significantly and exponentially with temperature (Crear and Forteath 2002). The cultured temperature used in this study was 18 °C which was within the documented optimum temperature for growth in *J. edwardsii* (i.e., between 18 °C and 21 °C) (Booth and Kittaka 1994; Thomas et al. 2000). An increment of 4 °C in water temperature would increase TAN excretion of *J. edwardsii* by 25 % (Crear and Forteath 2002). Based on the low mean DIN production at the optimum growth temperature for lobsters demonstrated in this study (i.e., unfed – 1.10 ± 0.12 μg N g⁻¹ hr⁻¹; mussels – 9.07 ± 0.90 μg N g⁻¹ hr⁻¹, artificial diet – 2.11 ± 0.41 μg N g⁻¹ hr⁻¹), increase in cultured temperature above the optimum range will not cause a major issue in regards to DIN output from lobster aquaculture on the environment. Nevertheless, temperature would still be a crucial water parameter to monitor during the course of lobster aquaculture activities, more so for farms located in temperate regions with greater seasonal variation. This is to ensure cultured temperatures are optimum for growth while ensuring minimal DIN excretion.

### 3.4.2 DIN modelling

Numerical modelling is a useful tool that can be used to forecast the potential effect of water quality over time from sea-cage aquaculture activities and is becoming a popular tool for aquaculture management and regulating aquaculture activities (Tasmania Department of Primary Industries et al. 2011; Wu et al. 1999). The majority of the published studies using numerical models to simulate aquaculture environmental impacts have been on fin-fish aquaculture (Doglioli et al. 2004; Gillibrand and Turrell 1997; Piedrahita 1988). This is the first study to examine DIN output from spiny lobster aquaculture in sea-cages and its potential impact on the surrounding water column using numerical modelling methods. Results from the DIN models above showed that the overall mean elevated DIN within the vicinity of the spiny lobster sea-cages was between 5.4 and 20 μg N L⁻¹ with increasing stocking density and FCRs, regardless of the type of diet used to feed the lobsters. In this study, when the lobster farm was at high stocking density (5 kg m⁻³) and poor FCR of 28, where lobsters were fed with mussels, the resulting elevated DIN was at 20 μg L⁻¹. A fish farm with similar stocking
density (4.43 kg m$^{-3}$) and production (490 t) and feeding the cultured fish with trash fish as for the lobster farm used in this study (5 kg m$^{-3}$, 512 t) also showed similar dissolved nitrogen (18 μg L$^{-1}$) in the water column in the vicinity of the farming site (Wu et al. 1999). Such dissolved nitrogen elevation was still within the recommended level of 500 μg N L$^{-1}$. In addition, all the modelled scenarios showing the maximum elevated DIN regardless of stocking density, FCRs and the type of diets used were also within the recommended level (ranging from 6.8 to 108 μg N L$^{-1}$). Therefore, DIN elevation can be expected to not be an issue in lobster farming operations similar to the modelled farm in this study, even when the sea-cages are stocked at high density with poor FCR.

The overall modelling results of scenarios of various stocking density (low and high stocking density), FCRs (3, 5 and 28) and the two types of diets used were maintained close to the ambient DIN conditions of 5 μg N L$^{-1}$, with modelled results showing limited increases of mean DIN of only 0.4 up to 3 μg N L$^{-1}$. The exception to this was when the lobsters were fed with mussels and the FCR was 28 for both low and high stocking density. Overall, such results indicated that the release of DIN from the lobster farm was generally minimal. Therefore, phytoplankton and zooplankton communities were able to quickly assimilate the DIN that has been released into the water column to maintain ambient DIN conditions. Moreover, the mean current speed of 18 cm s$^{-1}$ in the hypothetical farming site used was sufficient to dilute and disperse the DIN loading from spiny lobster aquaculture even at high stocking density. A study on a bluefin tuna farm with production of 1000 t yr$^{-1}$ showed no significant difference in the dissolved nutrient between the water column of the farm site and control site due to strong currents in the farming site (Aksu et al. 2010). Evidently, hydrodynamic conditions of the farm site is an important factor to consider in order to maintain low levels of dissolved nutrients especially when the farm has high stocking density.

When comparing DIN elevation between the two dietary treatments, scenarios where lobsters were fed with artificial diet demonstrated substantially lower DIN loading than when lobsters were fed with mussels. Artificial diet was able to reduce the mean DIN elevation from the modelled lobster farm by 16 – 23 % for FCRs of 3 and 5 (Figure 3.4), and up to 60 % for FCR of 28 (Figure 3.5). In the case of maximum DIN elevation, the use of artificial diet was able to achieve between 39 % and 67 % elevated DIN reduction for all three FCRs (Figure 3.6 and 3.7). Previous research has also estimated that the total nitrogen loading (both particulate and dissolved nitrogen) from a lobster farm reduced by 26 - 33 % when switching
from feeding lobsters with natural seafood to artificial diet (Tuan and Hung 2008). This was because artificial diet usually contained less protein than natural seafood as previously discussed. Also, feeding cultured animals with natural seafood usually results in higher FCR values compared to artificial diet (Hung et al. 2010; Wu 1995). In the case of spiny lobster aquaculture, feeding trash fish usually generates FCRs of 26 - 28 (Edwards et al. 2004; Hung et al. 2010) while artificial diet would typically have an FCR of 3 - 5 (Hung et al. 2010; Tuan and Hung 2008). As a result, the initial input of nitrogen as lobster feed into the aquaculture system is about four to eight times higher for natural seafood than for an artificial diet. Nitrogen leaching from feed waste can also contribute to the overall DIN loading (Beveridge 2004; Davenport et al. 2003). Previous research has estimated that feed waste from a fin-fish farm using trash fish was markedly higher than artificial diet (trash fish: 45 % feed waste; artificial diet: 3-5 % feed waste) (Brooks et al. 2002; Wu 1995). Thus, the overall quantity of DIN leached from waste feed can also expected to be higher when using trash fish diet compared to using artificial diet in an aquaculture operation. Therefore, substituting trash fish diet with artificial diet can lower the quantity of DIN released from lobster aquaculture into the environment.

3.5 Conclusion

The wide range of modelled scenarios in this study resulted in the highest mean and maximum elevated DIN concentrations within the recommended aquaculture level of less than 0.5 mg L\(^{-1}\) even when the lobster farm was stocked with high density and poor FCR of 28. Hence, a typical spiny lobster sea-cage aquaculture operation is unlikely to cause adverse effects on the water quality due to release of DIN. Regardless, feeding spiny lobsters with artificial diet reduced the DIN output considerably (up to 60 % in mean DIN excretion). Spiny lobster fed with diets containing higher protein content, such as natural seafood (e.g., mussels) increases the DIN excretion. Therefore, the use of lower protein artificial diets is highly recommended for further reducing DIN excretion from lobster farming production. This could be achieved through using alternative energy sources to protein, such as lipid or carbohydrate, in artificial diets. In addition, lobsters of different body mass appear to have varying digestive and metabolic abilities for the utilisation of the proximate components of diets (i.e., protein vs lipid vs carbohydrate), thus affecting the hourly excretion of DIN.
Therefore, there might be a need to develop an artificial diet with specific ratios of various
nutrient components (protein, lipid, carbohydrate) to suit the nutrient and energy requirements
of lobsters of different body mass to maintain a relatively low DIN excretion. Moreover, this
study showed that numerical modeling of nutrient dispersal is a useful tool to examine the
water quality within the vicinity of lobster farm sites and can be used as part of management
operations for sustainable aquaculture of spiny lobster.
Chapter 4:

Characteristics of Faecal and Dissolved Nitrogen Production from Tropical Spiny Lobster, Panulirus ornatus

4.1 Introduction

Sea-cage aquaculture can cause nutrient enrichment which can often negatively impact both the seabed and the water column (Hartstein and Rowden 2004). Nutrient loading from sea-cage aquaculture systems comes in two dominant forms; solid organic material and dissolved inorganic compounds (Beveridge 2004). The primary sources of solid organic materials are faecal matter and waste feed that are released directly into the environment (Brooks et al. 2002). These discharged solids will eventually settle to the seabed, generally in the vicinity of the source sea-cage, and will promote organic nutrient enrichment of the sediment which in turn can alter the benthos (Hartstein and Rowden 2004). For example, sea-cage aquaculture of fin-fish has been found to degrade the seabed for up to 1 km from the sea-cages due to the deposition of waste feed and faeces (Pearson and Black 2001; Wu 1995;). Besides the release of solids, most aquaculture species excrete nitrogen in a soluble form, most often as ammonia (Binns and Peterson 1969).

Ammonia can be transformed to nitrite and then subsequently to nitrate through oxidation. Besides being toxic to aquatic organisms, high levels of nitrite and nitrate, as well as ammonia, can also cause eutrophication in the water column (Beveridge 2004). Waste feed and faecal material can also leach nitrogen into the water column while sinking to the seabed or subsequently whilst being broken down by microbes (Brooks et al. 2002).

Hydrodynamic dispersal modelling is frequently being used as a predictive tool to determine the potential effects from solid waste accumulation on the seabed or nutrient enrichment in the water column from sea-cage aquaculture (Cromey et al. 2009; Doglioli et al. 2004; Magill et al. 2006). Such predictive tools are cost-effective and are increasingly used by regulatory authorities to help understand and manage the environmental effects of
aquaculture developments (Cromey et al. 2009). To enable reliable modelling of aquaculture waste, accurate measures of the relevant parameters, such as the settling velocity of faecal material, nutrient excretion from cultured animals, as well as the nutrient leaching from farm waste, are essential input data for the models (Reid et al. 2009). These parameters are most often specific to species, animal sizes and diets (Pérez et al. 2014).

Asian countries such as Vietnam, Philippines, Indonesia, Malaysia, India and Thailand have the fastest growing production of sea-cage aquaculture of spiny lobsters globally (Phillips and Matsuda 2011). Among the spiny lobster species cultured in Asia, *Panulirus ornatus*, the ornate tropical lobster, is the main targeted species as it is the most valuable due to its colourful appearance and relatively short grow-out duration of around 18 months (Hung and Tuan 2009). These spiny lobsters are usually cultured in shallow coastal areas using traditional wooden floating frames with suspended cages of netting holding low stocking densities of 8 – 10 lobsters m$^{-3}$ (Arcenal 2004; Priyambodo and Sarifin 2008). The vast majority of spiny lobster aquaculture operations feed their lobsters exclusively on low-value seafood species, commonly known as “trash fish”, although a small number of lobster farmers have begun using commercial pelleted diets (Hung and Tuan 2009). At present, the knowledge on the potential environmental impacts from lobster aquaculture is very limited. There have been no published studies on the quantification of the settling velocities of faecal material produced by the tropical spiny lobster. Although the dissolved nutrients excreted from spiny lobsters have been studied in several lobster species, most of the studies only quantified the total ammonical nitrogen (TAN) without including nitrate and nitrite which are also important dissolved inorganic nitrogenous wastes from aquaculture operations (Crear and Forteath 2002; Kemp et al. 2009; Radford et al. 2004; Zoutendyk 1987). Furthermore, it is not clear how any of these outputs may change with different diets. This biological information is essential for beginning to determine the potential environmental impacts from benthic depositional and dissolved inorganic nutrient enrichment from tropical lobster sea-cage aquaculture.

The aim of the present study is to quantify and characterise the settling velocity of faeces and the rate of dissolved inorganic nitrogen (DIN) production from *P. ornatus*. Elemental composition of carbon and nitrogen in faeces, as well as the dissolved inorganic nitrogen leaching from spiny lobster aquaculture waste (faeces and waste feed) was also
determined. In addition, the effect of different diets on these parameters was also quantified and compared.

4.2 Materials and methods

4.2.1 Husbandry methods

Two sets of eight spiny lobsters, *P. ornatus* (n = 16) were kept in two separate aerated concrete tanks from May to July 2013. The lobsters were acclimatized for at least one week before conducting feeding experiments. Each set of spiny lobsters consisted of one animal from each of the following sizes, 100, 200, 300, 400, 500, 600, 700 and 800 g. The concrete tanks were set up with flow-through sea water systems. The temperature and salinity of seawater in tanks were kept at ambient, 28 ± 1 °C and salinity of 32 ± 1 ppt. Light was maintained at 12L/12D photoperiod. One set of spiny lobsters was fed with seafood diet made up of mixture of chopped fresh seafood (mixed fin-fish and squid), while the other set was fed with an artificial spiny lobster diet (Lucky Star brand, Taiwan Hung Kuo Industrial Co., Ltd, Taiwan). Two different-sized artificial feed pellets with the same nutrient composition were used in the experiment; smaller pellets (2.5 mm diameter) were fed to 100–300 g lobsters while bigger pellets (3 mm diameter) were fed to lobsters between 400 and 800 g.

4.2.2 Faecal strand settling velocity and elemental analyses

Both sets of spiny lobster were fed twice daily (once each in the morning and evening) to excess with their respective diets (seafood or artificial diet). Faecal material was collected from each lobster the following morning from faecal collectors based on methods described by Irvin and Tabrett (2005) and Simon (2009a). Fifteen individual faecal strands were isolated from the faecal collectors for lobsters of each size and weighed wet (*m*), and then measured for length (*L*) and diameter (*D*) using digital callipers. A theoretical cylindrical shape was assumed for the faecal strand, therefore both volume (*V*) and density (*ρ*) of each faecal strand could be calculated as follows:
\[ V = \pi \left( \frac{1}{2} D \right)^2 L \]

\[ \rho = \frac{m}{V} \]

The settling velocity of the faecal strand was determined using a settling column made of transparent acrylic plastic tube (height: 2 m, inner diameter: 100 mm) and filled with seawater at 28 °C and salinity of 32 ppt (Moccia et al. 2007b). Individual faecal strands were gently introduced at the top of the settling column with tweezers, and the duration taken for the strand to settle to the bottom \( T \) of the column was timed with a stopwatch. The following equation was used to calculate the settling velocity \( V \) of each faecal strand:

\[ V = \frac{\text{height of settling column}}{T} \]

Collected spiny lobster faecal material was also analysed for carbon and nitrogen content using an elemental analyser (TrueSpec CHNS) with three replicates analysed for each lobster size (100 – 800 g) for both dietary treatments.

### 4.2.3 Dissolved inorganic nitrogen (DIN) production

The lobsters from the two sets were each transferred into an individual aerated polyethylene tank with flow-through seawater. The spiny lobsters were starved for 3 days before being fed at 1800 h with their previously assigned diets (in wet weight) at a ration of 3 % of the lobster body weight, which provided sufficient amount of feed for the lobsters to reach satiation (Simon 2009b). The lobsters were allowed 2 h to consume all their feed. After 2 h the in-flow and out-flow of the tank was turned off before commencing the serial water sampling. Water samples of 120 ml were collected at 2 h intervals for a period of 24 h with a plastic syringe with an attached filter (Whatman glass microfiber filter GF/F 0.7 μm). Seawater samples were immediately analysed for ammonia, nitrite and nitrate concentrations (μg N L\(^{-1}\) - Hach colorimeter DR890). The colorimeters were calibrated with standard solutions of ammonia, nitrite and nitrate. The experimental methods were also repeated with one set of spiny lobsters with a treatment where they were unfed.

The sum of ammonia, nitrite and nitrate measurements for each water sample were combined and the total quantity of released DIN was calculated from the total seawater
volume of the experimental lobster tank minus the DIN measured in the water at the outset. Changes in the DIN output from lobsters over the 24 h sampling period were estimated from the increasing DIN measured in the tanks based on the serial sampling at 2 h intervals. An average hourly rate of DIN output was calculated from the 24 h of DIN measurements and was standardised by lobster body mass to compare DIN output among lobsters from different sizes for the dietary treatments (i.e., seafood, artificial diet and unfed).

Values from the 24 h DIN measurements were also used to calculate the mean percentage of feed (in wet weight) that was released as mass of DIN by lobsters from the two diet treatments. These calculated mean percentages were then adjusted to the dry weight of the feed based on moisture content in the diet. Moisture content of seafood was 74 % which was based on laboratory measurements where fish and squid samples were oven dried at 60 °C for 24 h to obtain their dry weight and calculated for the mean percentage difference between dry and wet weight of seafood. The artificial feed used in this study contained 8 % of moisture (Lucky Star brand proximal composition, www.luckystarfeed.net).

4.2.4 Nutrient leaching experiment

Two replicate portions (0.5-1 g) of the two lobster diets (seafood or artificial diet) and faecal material from spiny lobsters fed with either of the diets were weighed (wet weight) and put into 500 ml beakers filled with artificial seawater (Red Sea – Coral Pro Salt Brand, Red Sea Fish Pharm Ltd, Israel) (28 ± 1° C, 32 ± 1 ppt). The beakers were then placed on an automated shaker (Laboshaker D600) at a speed of 10 rpm to stimulate the movement of food or faecal material slowly settling through the water column (Moccia et al. 2007b). A 60 ml water sample was collected from each treatment after 0, 5, 10, 20, 40 and 80 min and analysed for ammonia, nitrite, and nitrate as described previously. The sum of ammonia, nitrite and nitrate measurements for each sample was combined and the average hourly DIN leaching rate was calculated. To compare DIN output among the diets and faecal samples, DIN output was standardised by the wet weight of the diets and faecal samples. The DIN excretion rate for the different diets (seafood and artificial diet) based on the samples wet weight was then adjusted for their dry weight according to the moisture content of the diets.
4.2.5 Statistical analyses

The effect of the combination of lobster size and diet (seafood and artificial diet) on the settling velocity of the faeces that they produced was determined with a two-way repeated measure ANOVA followed with a post-hoc Holm-Sidak pairwise multiple comparison. A logarithmic transformation was used to normalise the data. A priori it was found that lobster size did not greatly influence the settling velocity of faeces of individual diets allowing these data to be pooled from lobsters of all sizes for each diet so they could be used in linear regression analyses for determining if length, weight, volume and density of faecal strands influenced the corresponding settling velocity.

The effect of lobster size and diet (seafood and artificial) on the carbon content in the faeces was determined with two-way repeated-measures ANOVA and where the overall analysis was significant pairwise multiple comparisons of means were undertaken with Holm-Sidak tests. Before analysis, the data for the carbon content of faeces was normalised with an arcsine square root transformation. The data for the nitrogen content of the faeces were not normally distributed even after transformation. Therefore, a non-parametric Friedman repeated-measures analysis of variance on ranks test was performed to determine the effect of lobster size on the nitrogen content in faeces produced from feeding each of the two diets. When there were no differences found in the nitrogen content among lobsters of different sizes, the nitrogen content results were pooled to test the overall nitrogen content in faeces between the two diets using Mann-Whitney test.

A repeated-measures two-way ANOVA followed by post-hoc Holm-Sidak pairwise comparisons to determine whether there were differences in the DIN output at the two hourly intervals over the 24 h period for the three dietary regimes, i.e., unfed, seafood and artificial feed. A square root transformation was used to normalise the data which was confirmed with a Shapiro-Wilk test.

The DIN leaching rate data for the two different diets (seafood and artificial) standardised for their dry weights, as well as the leaching rate data for faecal material produced by lobsters fed on these two different diets standardised for their wet weights, were not normally distributed even after transformation. Therefore, non-parametric Friedman repeated measures analyses of variance were used to make comparisons of the individual sets.
of data for DIN leaching rates for the five sampling intervals spread over 80 min for individual diets or faecal material. Where Friedman repeated-measures analysis of variance did not find a difference in DIN leaching rates resulting from the five sampling intervals for each individual diet and faecal material, then the results were pooled and used for a comparison of the overall DIN leaching rates between the two diets as well as for the faecal material resulting from the different dietary treatments using a Mann-Whitney test.

All statistical analyses were performed using SigmaPlot version 11.0 and SPSS Statistics version 22.

4.3 Results

4.3.1 Effect of lobster size and diet on the faecal settling velocity

Overall there was no difference in the sinking velocity of faeces produced by *Panulirus ornatus* over a range of different sizes (F=1.9, P=0.07), however, there was a difference in the sinking velocity of faeces resulting from feeding lobsters the two diets (F=28.7, P<0.001) (Figure 4.1). Lobsters fed with a seafood diet produced faecal strands with a settling velocity ranging from 0.23 to 0.44 cm s\(^{-1}\) and a mean of 0.30 ± 0.01 cm s\(^{-1}\) (± SE), which was significantly faster than those fed on the artificial diet with settling velocity ranging between 0.16 and 0.28 cm s\(^{-1}\) and a mean of 0.22 ± 0.01 cm s\(^{-1}\). However, there were significant differences in the sinking velocity of faeces produced as a result of the interaction between the diet and lobster size (F=4.7, P<0.001). Pairwise comparisons of individual means showed that the differences in sinking velocity of the faeces produced by the two diets tended to be more pronounced among larger lobsters than for smaller lobsters.
4.3.2 Effect of length, weight, volume and density on the faecal settling velocity

The settling velocity of faecal strands from both dietary treatments did not vary in relation to the length of faecal strands for either diet (seafood - F=0.7, P=0.41; artificial - F=0.7, P=0.41) (Figure 4.2). However, the settling velocity of faeces did vary in relation to both the weight and volume of the faecal strands for both diets (weight: seafood - F=49.8, P<0.001, r²=0.35, artificial - F=11.6, P<0.001, r²=0.09; volume: seafood - F=45.6, P<0.001, r²=0.32, artificial - F=5.0, P<0.05, r²=0.04). The spiny lobsters fed with artificial diet produced faeces with settling velocity that increased with the corresponding density of the faecal strands (F=18.7, P<0.001, r²=0.21), whereas faeces produced from the seafood diet did not vary in this manner (F=0.7, P>0.42).
Figure 4.2. Spiny lobster faecal strand length (mm), weight (g), volume (mm$^3$) and density (g mm$^{-3}$) plotted against their measured settling velocity (cm s$^{-1}$) for both dietary treatments, natural seafood and artificial. Seafood diet (n = 120): figures a, c, e, and g. Artificial diet (n=120): figures b, d, f, h.
4.3.3 Elemental analyses of faecal material

Overall the mean percentage of carbon in the spiny lobster faeces was not significantly different between the two diets (F=10.3, P=0.08). However, there was a significant difference in the percentage of carbon in faeces among lobsters of different sizes (F=3.4, P<0.05) and there was a significant difference in carbon content in faeces resulting from the interaction between lobster size and diet (F=4.4, P<0.05) (Figure 4.3). Pairwise comparisons of individual means of faecal carbon content showed that faeces produced from the artificial diet had higher carbon content than those produced from the seafood diet only for lobsters of 500, 700 and 800 g. Lobsters of 800 and 700 g fed on the artificial diet produced faeces with higher carbon content than lobsters of 400 g. Overall there was a tendency for lobsters of a larger size (>400 g) to produce faeces with higher carbon content when feed on artificial diet.

Mean carbon content in faeces produced in lobsters of all sizes fed with the seafood diet ranged from 24.8 ± 2.1 % to 37.1 ± 5.7 % while faeces produced from feeding the artificial diet had carbon content ranging from 34.8 ± 3.5 % to 49.2 ± 1.4 %. Regardless of lobster size and diet treatments, the overall mean carbon content in faecal material was 36.2 ± 1.3 %.

There was no significant difference in the nitrogen content in faeces produced by lobsters of different sizes for both diet treatments (seafood- Q=5.7, P=0.58; artificial- Q=12.4, P=0.09) (Figure 4.4). The mean nitrogen content in faeces was also not significantly different between the seafood and artificial diets (Z=1.7, P=0.09). The overall mean nitrogen content in faecal material was 5.0 ± 0.2 %.
Figure 4.3. Mean percentage (± S.E.) of carbon of the faecal material produced by lobsters over a range of sizes fed with either seafood diet or artificial diet.

Figure 4.4. Mean percentage (± S.E.) of nitrogen of the faecal material produced by lobsters over a range of sizes fed with either seafood diet or artificial diet.
4.3.4 Effect of diets on DIN production

Overall, there were no significant differences in the mean DIN output as measured at two hourly intervals over the 24 h period ($F=1.5$, $P=0.15$). Likewise, the mean DIN output was not different for different diets at different times over the 24 h period (i.e., interactive term, $F=0.8$, $P=0.73$). However, the three dietary regimes, (i.e., unfed, seafood and artificial diet) did significantly affect the overall mean DIN output ($F=23.5$, $P<0.001$). Pairwise comparison determined that the mean DIN output from both fed diets was significantly higher than that for unfed lobsters (seafood vs not fed, $t=6.5$, $P<0.001$; artificial vs not fed, $t=5.2$, $P<0.001$). However, there was no difference in the mean DIN output for different diets at different times over the 24 h period (i.e., interactive term, $F=0.8$, $P=0.73$). However, there was no difference in the mean DIN output between lobsters on the two diets (seafood vs artificial, $t=1.3$, $P=0.2$). The mean DIN excretion rate over 24 h for unfed lobsters was $5.84 \pm 1.59 \, \mu g \, N \, g^{-1} \, hr^{-1}$ with individual measurements ranging from 4.47 to 8.78 $\mu g \, N \, g^{-1} \, hr^{-1}$ (Figure 4.5). When lobsters were fed with seafood the mean DIN excretion rate over 24 h was $12.66 \pm 1.98 \, \mu g \, N \, g^{-1} \, hr^{-1}$ and for lobsters fed artificial feed, it was $11.39 \pm 2.36 \, \mu g \, N \, g^{-1} \, hr^{-1}$, this represented DIN output that was 54 % and 49 % higher than for unfed lobsters respectively. Overall, regardless of diet treatment, excretion of ammonia made up $81.5 \pm 7.0$ % of the total DIN output, while nitrate and nitrite contributed $12.5 \pm 5.5$ % and $6.0 \pm 2.5$ % respectively to the total DIN output.

The excretion rate of DIN for both fed lobster treatments tended to increase immediately after feeding and remained elevated for at least the 24 h recording period compared to the unfed lobsters for which DIN remained relatively stable over the 24 h period. Two distinct peaks were recorded in the DIN excretion from both fed lobster treatments over the 24 h, first one immediately after feeding and the second one after 14 h for lobsters fed with seafood and after 18 h for lobsters fed with artificial diet. However, these apparent increases were not detected in statistical comparisons of the mean DIN output at the two hourly intervals over the 24 h period.

When lobsters were fed with seafood diet, on average 0.92 % of wet weight (3.53 % of dry weight) of the feed was converted into DIN output over 24 h by the lobsters while for artificial diet the equivalent values were 0.55 % and 0.60 % respectively.
Figure 4.5. Mean dissolved inorganic nitrogen (DIN) excretion rate (µg N g\(^{-1}\) hr\(^{-1}\) ± S.E.) of spiny lobsters over a period of 24 hours after being given three different dietary treatments, seafood, artificial diet and unfed.

### 4.3.5 Nutrient leaching rate from faeces and feed

The measured DIN leaching rate from all diet and faecal samples was highly variable both among individual samples and among the sampling time intervals (i.e., 5, 10, 10, 20, 40 and 80 min). The DIN leaching rate from both sets of diet samples standardised for their dry weights (i.e., seafood and artificial) did not vary over the five sampling intervals extending out to 80 min from immersion for the two different diets (seafood - \(Q=1.2, P=0.88\); artificial – \(Q=6.9, P=0.14\)). The mean DIN leaching rate for the seafood diet was 720.0 ± 656.1 µg N g\(^{-1}\) hr\(^{-1}\) and for the artificial diet was 389.3 ± 178.4 µg N g\(^{-1}\) hr\(^{-1}\) and were not significantly different (\(Z=0.75, P=0.45\)).

The DIN leaching rate from both sets of faecal samples standardised for their wet weights (i.e., resulting from seafood and artificial diets) did not vary over the five sampling intervals extending out to 80 min from immersion (seafood - \(Q=5.2, P=0.27\); artificial –
Q=3.1, P=0.54). The mean DIN leaching rate for faeces produced on the seafood diet was 645.2 ± 313.8 µg N g⁻¹ hr⁻¹, and for faeces resulting from the artificial diet was 123.0 ± 81.3 µg N g⁻¹ hr⁻¹ and were not significantly different between the two types of faeces sources from feeding lobsters different diets (Z= 0.76, P=0.45).

4.4 Discussion

This study has characterized the faecal material produced by *P. ornatus* in relation to its settling velocity as well as in relation to its length, weight, volume and density. Faeces length was found to be a poor predictor of settling velocity of faecal strands produced by spiny lobster as it has been in other species, such as salmon (Chen et al. 1999b; Chen et al. 2003). In contrast faeces weight and density were more intuitive and reliable predictors of faecal settling velocity in spiny lobsters of a range of sizes and on two different diets. Faeces weight and density have also been found to be reliable predictors of faecal settling velocity in other seafood species, such as aquacultured fin-fish (Moccia et al. 2007b; Ogunkoya et al. 2006). The settling velocity of faecal material produced by *P. ornatus* over a range of sizes (100 – 800 g) showed no differences for either the seafood or artificial diet. These results are consistent with those found for the spiny lobster, *J. edwardsii* (Lee et al. 2014). Evidently, small and large lobsters produced faecal strands of similar weight and density.

The mean settling velocity of faecal material produced by *P. ornatus* fed with seafood was 36 % higher than those produced by lobsters fed with artificial diet largely as a result of the greater weight, volume and density of this faecal material compared with the artificial diet. Rainbow trout (*Onchorhyncus mykiss*) fed with different types of artificial diets produced faeces with differences in settling velocity (Moccia et al. 2007b). These differences are thought to be due to differences in the digestible component of the diet which can alter the faecal characteristics such as density (Reid et al. 2009). Faecal material with higher settling velocity will be deposited to the seabed closer to sea-cages that are used for culturing spiny lobsters (Gowen et al. 1989). Thus, it is very likely that faecal material produced by lobsters fed with artificial feed would have a wider depositional footprint on the seabed than those fed with seafood diet due to their respective differences in faecal sinking velocity. The temperate spiny lobster species, *Jasus edwardsii* produced faeces with higher settling velocity than those recorded in this study, with a mean settling velocity of 0.9 ± 1cm s⁻¹ (Lee et al. 2014).
Similarly, fin-fish species generally have been found to have higher recorded faeces settling velocity than those of tropical spiny lobster, ranging from 0.5 to 8.0 cm s\(^{-1}\) depending on the species of fish (Chen et al. 2003; Cromey et al. 2002a; Magill et al. 2006; Piedecausa et al. 2009). Therefore, with the same farm specifications (i.e., stocking density, FCR, diet and hydrodynamic), culturing both temperate spiny lobsters and fin-fish would have a less extensive zone of faecal deposition to the seafloor than those of tropical spiny lobsters.

The carbon content in faeces produced by lobsters fed with artificial diet increased with lobster size. There is a possibility that the digestibility of the artificial diet used in this study differed between smaller and larger spiny lobsters as has been observed in other lobster species (Smith et al. 2005). It has been suggested that there may be significant ontogenetic shifts in the digestive gland activity of spiny lobster (Johnston 2003), which presumably could affect the carbon content in the lobster faeces in spiny lobsters of different size range. Alternatively, the larger pellet size (2.5 vs 3 mm diameter) of the same composition fed to lobsters >400 g may have been more difficult for the lobsters to digest because of the physical size difference or differences in pellet manufacturing.

The DIN excretion in *P. ornatus* increased by about two fold following feeding with either seafood or artificial diets. The post-feeding increase in nitrogen excretion has also been demonstrated in other spiny lobster species such as *J. edwardsii, J. lalandii, P. cygnus* as well as *P. homarus rubellus* (Crear and Forteath 2002; Kemp et al. 2009; Radford et al. 2004; Zoutendyk 1987). Total ammonical nitrogen excretion after feeding was 4 to 6 times higher than pre-prandial excretion in *J. edwardsii* (Crear and Forteath 2002; Radford et al. 2004), 7 times higher in both *J. lalandii* and *P. homarus rubellus* (Kemp et al. 2009; Zoutendyk 1987) and 3 times higher in *P. cygnus* (Crear and Forteath 2002). In addition, the double peak in nitrogen excretion at around 2 and 14 h reported in this study has also been observed in other studies on various lobsters species (Crear and Forteath 2002; Hawkins et al. 1986; Kemp et al. 2009; Zoutendyk 1987). It has been suggested that this increase is the result of elevated metabolism due to increased physical activity, digestive processes, hormonal secretions or a combination of these factors (Hawkins et al. 1986). Another study highlighted that the first peaks represented total ammonia nitrogen (TAN) from metabolizing protein followed by production of TAN through excretory losses in the second peak (Crear and Forteath 2002). According to the daily DIN excretion results, it would take *P. ornatus* more than 24 h for DIN excretion rate to return back to the unfed pre-feeding state. Studies on other species of spiny
lobsters have showed that post-feeding recovery can take from between 10 and 42 h (Crear and Forteath 2002; Kemp et al. 2009; Radford et al. 2004; Zoutendyk 1987). The duration taken for post-feeding DIN excretion to return to baseline levels is important for fully understanding the potential build-up of dissolved nitrogen waste around sea-cage aquaculture system especially if the lobsters were fed the next meal before the DIN excretion from the previous meal has returned to baseline levels.

The estimated daily DIN excretion rate of *P. ornatus* measured in this study did not differ significantly between the seafood diet and artificial pelleted diet. In contrast, a similar feeding trial conducted with *J. edwardsii* showed that the mean DIN excretion rate from mussels (9.07 ± 0.93 μg N g⁻¹ hr⁻¹) increased by four times of the excretion rate generated by lobsters fed on artificial diet (2.11 ± 0.32 μg N g⁻¹ hr⁻¹)(Lee et al. 2015b). In that study, it was speculated that the differences in DIN production was due to the differences in protein content between mussels and the artificial diet used (protein content of 55.2 % and 40 % respectively). In fin-fish total ammonia excretion has been shown to increase in response to increasing dietary protein (Ballestrazzi et al. 1994)

Regardless of the diet treatments used, the mean DIN output from spiny lobster after being fed in this study was 12 μg N g⁻¹ hr⁻¹ (2.88 × 10⁻⁴ mg N kg⁻¹ day⁻¹) where the majority (81.5 %) of it was ammoniacal nitrogen (9.78 μg N g⁻¹ hr⁻¹ or 2.35 × 10⁻⁴ mg N kg⁻¹ day⁻¹). A study on sub-tropical fin-fish species documented ammoniacal nitrogen excretion rate in adult mangrove snapper, *Lutjanus argentimaculatus*, and areolate grouper, *Epinephelus areolatus*, to be 558 mg N kg⁻¹ day⁻¹ and 375 mg N kg⁻¹ day⁻¹, respectively (Leung 1996). These recorded DIN excretion rates of fin-fish were markedly higher compared to the excretion rate of lobsters in this current study, indicating that the potential impact of elevated DIN from a lobster farm is likely to be minimal and much lower than a typical fin-fish farm.

Based on recorded DIN measurements in this study, the DIN leaching rate from faeces and diet samples (mean leaching rate between 123.0 ± 81.3 and 720.0 ± 656.1 μg N g⁻¹ hr⁻¹) tended to be markedly higher than the DIN excretion rate by the lobsters (mean DIN excretion rate between 5.84 ± 1.59 and 12.66 ± 1.98 μg N g⁻¹ hr⁻¹). The leaching of nitrogen from both faecal material and waste feed from spiny lobster aquaculture will contribute to DIN input into the water column in addition to the direct DIN excretion by the lobsters. This study found that the measured DIN leaching rates of the samples of faeces and diets were highly variable
making it difficult to detect any differences among them, although past studies have shown that nutrient leaching from faecal material generally has faster/greater loss of nitrogen than feed pellets (Burford and Williams 2001; Piedecausa et al. 2009). It has been suggested that the digestive enzyme activity, as well as microbial activity within the faecal material facilitates the breakdown of faeces and the release of nutrients (Tlusty et al. 2000). Moreover, solubility or friability of feed is lower than faecal material with seafood being less soluble than artificial diet when immersed in water for long periods (Simon and Jeffs 2008; Tlusty et al. 2000).

A study on nutrient leaching from shrimp faeces and pellets illustrated that the leaching nutrients were mainly in the form of organic compounds instead of inorganic compound (Burford and Williams 2001). Therefore, the measured nutrient leaching from this study may have underestimated the total nitrogen leaching from excreted faeces and waste feed. Further research on nutrient leaching should focus on the leaching rate of dissolved organic nitrogen as well as DIN. Regardless, for spiny lobster farms, attention to minimising feed wastage appears to be of potential significance in reducing nitrogenous eutrophication. The widespread use of feeding trays to deliver feed to benthic feeding spiny lobsters in sea-cages may also be of importance in reducing feed losses and nutrient input to the surrounding environment.

4.5 Conclusion

Lobster aquaculture can potentially cause environmental impacts such as faecal deposition and DIN elevation. However, the intensity of those impacts will generally be less extensive than those of fin-fish farming especially for the environmental elevation of DIN. The biological parameters measured in this study will have implications in the prediction of potential environmental impacts from lobster aquaculture. In particular, the different diets used in spiny lobster sea-cage aquaculture have been shown to be important in dictating the extent of deposition and dispersal of faecal material on the seabed. Using a seafood diet in a lobster farm will result in a more localised and intense faecal depositional footprint compared to an artificial diet because of the faster settling velocity of the resulting faecal material. Dissolved inorganic nitrogen is excreted by lobsters assimilating protein and therefore,
increasing protein composition of the diet is likely to elevate the DIN excretion from lobsters. Moreover, faecal material as well as waste feed will also contribute to the overall lobster aquaculture DIN output as they have been shown to be an important source of inorganic nitrogen which leaches into the water column.
Chapter 5:

Modelling Carbon Deposition and Dissolved Nitrogen Discharge from Sea-cage Aquaculture of Tropical Spiny Lobster, *Panulirus ornatus*

5.1 Introduction

Sea-cage aquaculture of spiny lobsters (Palinuridae) is an important economic activity in many Asian countries including Vietnam, Philippines, Indonesia, Malaysia, India and Thailand (Jones 2010; Phillips and Matsuda 2011). A wide variety of commercially important spiny lobster species are being used in sea-cage aquaculture, including *Panulirus ornatus*, *P. homarus*, *P. stimpsoni*, *P. polyphagus*, *P. longipes*, *P. penicillatus* and *P. versicolor* (Arcenal 2004; Ly 2009; Tuan and Mao 2004). Among these lobster species, the ornate tropical lobster, *P. ornatus* is the most valuable species. Wild caught juvenile lobsters are held in sea-cages and grown to a marketable size of between 800 – 1000 g (Arcenal 2004; Hart 2009). Cultured lobsters are either fed with manufactured artificial diet or more commonly with “trash fish” (a mixture of locally caught low-value fish and shellfish). At present, Vietnam has the greatest annual production of spiny lobsters from sea-cage aquaculture of approximately 1,900 t, valued at more than US$65 million in 2006 (Jones 2010; Petersen and Phuong 2010; Petersen and Phuong 2011).

Culturing marine organisms, such as fin-fish, in sea-cages, can cause nutrient enrichment in the surrounding environment that is capable of negatively impact the seabed and the water column (Hartstein and Rowden 2004; Wu et al. 1994). Sources of nutrient enrichment from sea-cage aquaculture include faecal material from undigested feed and waste feed that will eventually sink to the seabed as particulate organic material (i.e., organic carbon), whereas excretion from aquacultured animals results in dissolved inorganic nutrients being directly released into the seawater (Beveridge 2004; Tovar et al. 2000). Excess organic carbon on the seabed can promote anoxic conditions in sediments which can greatly reduce
the diversity of the benthos and in extreme cases can lead to the formation of bacterial mats and the outgassing of methane and hydrogen sulphide from the seabed (Forrest et al. 2007). One of the main excretory products from protein metabolism by cultured species is dissolved inorganic nitrogen (DIN; i.e. ammonia, nitrite and nitrate) (Handy and Poxton 1993). The increase of DIN in seawater not only promotes microalgal production, but at high concentrations can also be toxic to marine species (Beveridge 2004; Islam 2005).

Although the sea-cage aquaculture of *P. ornatus* has been rapidly expanding across parts of Asia, the available information on the environmental impacts of this activity are limited. Previous studies estimated the production of *P. ornatus* in sea-cages resulted in the release of an estimated total of 269 - 402 g N kg$^{-1}$ into the environment depending on the type of diet being fed to the spiny lobsters, i.e., trash fish or artificial feed, respectively (Tuan and Hung 2008; Ly 2009). These data do not distinguish between organic nitrogen (i.e., such as bound in faecal material and waste feed) and dissolved inorganic nitrogen (DIN) (i.e., from excreted metabolic waste) and therefore makes it difficult to begin to determine the fate and impact of these nutrients released into the environment in different ways. Furthermore, the organic carbon deposition on the seabed from a *P. ornatus* sea-cage operation has not been determined, despite being an important parameter for determining the likely environmental impact of this activity on sediments. Therefore, the aim of this research is to undertake a more detailed assessment of the potential environmental impacts from the sea-cage aquaculture of *P. ornatus* by using hydrodynamic modelling combined with previous laboratory measures of nutrient outputs to predict the likely environmental consequences from a hypothetical spiny lobster sea-cage operation using both artificial diet and seafood diet, and for a variety of stocking densities and FCR. The hypothetical spiny lobster sea-cage aquaculture operations that were modelled incorporated environmental data from a field location suitable for establishing this activity, and realistic spiny lobster farming scenarios based on variations on the emerging current commercial practice for this activity in parts of Asia.
5.2 Materials and methods

5.2.1 Spiny lobster farm layout for modelling

For the purposes of this study, several hypothetical spiny lobster aquaculture farm layouts were used that were consistent with realistic lobster sea-cage farm operations that are used in parts of Asia. The lobster farms were located in coastal water at depths of 10 – 30 m with sea-cage layouts based on designs from typical spiny lobster aquaculture operations used in parts of Asia (Table 5.1; Figure 5.1 – 5.3). A range of farming scenarios for the hypothetical lobster aquaculture operations were modelled consisting of combinations of different stocking densities, varying feed conversion ratios (FCR), (Edwards et al. 2004; Hung et al. 2010; Petersen et al. 2009), and diet type (natural seafood or artificial diet) (Table 5.1). Two lobster farm layouts were used in the depositional model: a) a small farm layout which used a square block of 16 floating sea-cages as the base unit with each sea-cage within the block measuring 5 × 5 m, with a suspended cuboid net extending 5 m below the sea surface and enclosing a total of 125 m$^3$ of water (Figure 5.1); b) a large farm layout which was a more extensive farming arrangement where five square blocks, each square block representing 16 sea-cages (i.e., total of 80 sea-cages) were set out in four continuous straight lines (Figure 5.2). A much larger farm layout (i.e., 80 larger sea-cages) was used for the DIN modelling because the preliminary DIN modelling results indicated that the smaller farm layouts used for carbon deposition modelling (i.e., 16 and 80 smaller sea-cages) was of an insufficient scale to result in environmental DIN elevation due to the lobster farming operations. Since it is predicted that in the future lobster farms may produce much higher tonnage than at present, the larger farm layout was used for DIN modelling and the smaller model for the carbon deposition modelling. The much larger farm layout was set up with four sets of sea-cages aligned in a continuous straight line, with each set of sea-cages consisting of 20 sea-cages strung together (i.e., total of 80 large sea-cages), each sea-cage measured 16 × 16 m, extending to 5 m below the sea surface and enclosing a total of 1280 m$^3$ of water (Figure 5.3).
Table 5.1. Cage layout, stocking density and FCR of hypothetical spiny lobster sea-cage aquaculture farms used for both carbon deposition and DIN modelling.

<table>
<thead>
<tr>
<th></th>
<th>Total no. of cages</th>
<th>Dimension of each cage (m)</th>
<th>Volume of each cage (m$^3$)</th>
<th>Stocking density (kg m$^{-3}$)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depositional Model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Small farm)</td>
<td>16</td>
<td>$5 \times 5 \times 5$</td>
<td>125</td>
<td>3 &amp; 5</td>
<td>1.28, 3, 5</td>
</tr>
<tr>
<td>(Large farm)</td>
<td>80</td>
<td>$5 \times 5 \times 5$</td>
<td>125</td>
<td>3 &amp; 5</td>
<td>1.28, 3, 5</td>
</tr>
<tr>
<td>DIN Model</td>
<td>80</td>
<td>$16 \times 16 \times 5$</td>
<td>1280</td>
<td>3 &amp; 5</td>
<td>3, 5, 28</td>
</tr>
</tbody>
</table>

Figure 5.1. Small farm layout of 16 cages used in carbon deposition model simulation. Each sea-cage measuring $5 \times 5$ m extending down to 5 m below the sea surface.
Figure 5.2. Large lobster farm layout showing the 80 cages used in the carbon deposition model. Each square block is made of 16 individual sea-cages with each sea-cage measuring $5 \times 5$ m extending down to 5 m below the sea surface.

Figure 5.3. Layout of lobster farm consisting of 80 sea-cages used in the dissolved inorganic nitrogen (DIN) modelling. Each square represents a sea-cage with dimension of $16 \times 16$ m extending down to 5 m below the sea surface.
5.2.2 Model inputs and sources

The fundamental basis for running both depositional and DIN models is hydrodynamic information for the lobster farm site. Therefore, data on the bathymetry, current and wind for a typical lobster farm site in Asia were gathered. The bathymetry of a site that was typical of one used for lobster aquaculture in Southeast Asia was taken from British Admiralty Sea Charts, based on UTM-50 projection, and improved with higher resolution data collected over an area of 3 km$^2$ surrounding the farm site using an echosounder deployed from a small research vessel. Water current measurements, both current speed and direction, were collected in-situ by deploying an acoustic Doppler current profiler (ADCP) for 14 days at the farm site. The ADCP was set to record at intervals of 10 min with a depth resolution of 50 cm. Wind data used for modelling at this site were sourced from NOAA’s (National Oceanic and Atmospheric Administration, USA), global forecasting system meteorological model (GFS).

5.2.3 Deposition model

The potential environmental impact on the seabed from faecal deposition from the hypothetical lobster farm was predicted using depositional modelling software, Mike21 FM particle mass transport module (Kurian et al. 2009). This model treats spiny lobster faeces as cohesive particles that will sink in the water column until they are deposited on the seabed according to the hydrodynamic conditions of the modelled location and calibrated by the ADCP measurements (Table 5.2). To adequately represent the natural variability of the settling velocity of faeces of *P. ornatus* for the model, previously measured faecal settling velocity (Unpublished data from Chapter 4) were divided into three convenient fractions for each of which, dispersal was modelled separately, and then the results combined for an overall estimate of benthic accumulation of faecal material (Table 5.3). The Mike21 model also takes into account the changes in current conditions due to the bathymetry, wind and wave forcing using the environmental data collected from the farm site. Furthermore, sedimentary erosion, transport and deposition processes under the action of currents and wave conditions were included in the model to simulate the natural ocean processes.
Table 5.2. Current speed for surface, middle and bottom layer of the water column at a hypothetical lobster aquaculture site in Southeast Asia.

<table>
<thead>
<tr>
<th>Layer of water column</th>
<th>Mean current speed (cm s(^{-1}))</th>
<th>Max. current speed (cm s(^{-1}))</th>
<th>Min. current speed (cm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top layer (0.5 – 11 m)</td>
<td>24</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>Middle layer (12 m)</td>
<td>20</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>Bottom layer (0.5 m above seabed)</td>
<td>10</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5.3. Frequency distribution of settling velocity (cm s\(^{-1}\)) of faecal strands (n = 120) from spiny lobster, *P. ornatus* fed on two different diets, seafood and artificial derived from laboratory measurements and used in depositional model.

<table>
<thead>
<tr>
<th>Proportion of Faecal Output (%)</th>
<th>Faecal Settling Velocity (cm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seafood Diet</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>43</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>32</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>25</td>
</tr>
</tbody>
</table>

The bed re-suspension speed used in the model was 4.5 cm s\(^{-1}\) with a standard critical shear stress of 0.004 N m\(^{-2}\) for lobster faecal material deposited on the seabed (Cromey et al. 2002a) which at this site was composed of homogenous consolidated course sediment that was unlikely to be resuspended. These values were calibration variables and were based on similar values used for salmon and mussel faeces, which have similar organic content to lobster faecal material, as there are no published data for the critical shear stress for lobster faeces. Therefore, resuspension of lobster faecal material previously deposited to the seabed would only take place when the bottom flow conditions exceeded the critical shear stress threshold.

A single feeding event per day was assumed to occur at the hypothetical spiny lobster farm. Currently, there is no information on how much feed is converted to faeces by spiny
lobsters nor how much feed goes to waste in a typical spiny lobster farm, so values based on salmon were used. The amount of feed converted to faeces was estimated at 22% (Chen et al. 1999b) which is based on well-studied commercial aquaculture species, and while the comparable values for spiny lobster aquaculture are yet to be determined, adjusting these assumed values will deliver a direct proportional response in the modelled carbon loading from the hypothetical lobster farm. The model assumed 5% of feed was wasted and released to the environment from the farm (Brooks et al. 2002). Again this value was based on other well-studied aquaculture species; however, spiny lobster farming operations typically use feed trays to deliver, contain, and recover uneaten feed, so perhaps this value is an overestimated of feed wastage. The carbon content of faecal material used in the model was 31% and 40% for scenarios where spiny lobsters were fed with natural and artificial diets, respectively, based on the results of elemental analyses of the faeces of lobsters fed these two diets (Lee et al. 2015a). The depositional model was run for the various combinations of lobster stocking density (3 and 5 kg m\(^{-3}\)), arrangement of sea-cages (16 and 80 sea-cages), and FCR values (1.28, 3 and 5) (Table 5.1 for parameter combinations) for a simulated one year period. The Mike21 model output was the spatial distribution of accumulated mass of organic carbon from faeces and waste feed deposited on the seabed over a one year period (measured as kg C m\(^{-2}\) yr\(^{-1}\)). It was conservatively assumed that all carbon deposited to the seabed was accumulated with no microbial processes or deposit feeding organisms breaking down the deposited carbon over time.

An acceptable threshold rate of organic carbon deposition was set at 0.73 kg C m\(^{-2}\) yr\(^{-1}\) to identify those areas of seabed that would potentially be impacted by the deposition of solids derived from the lobster farm. This value was derived from findings of previous studies on aquaculture waste deposition and was considered conservative for sedimentary benthic habitats over which sea-cage operations are normally placed (Cromey et al. 2002b; Gilibrand et al. 2002; Hargrave et al. 1994; Sowles et al. 1994).

5.2.4 DIN model

Ecological modelling software (ECO Lab) developed by the Danish Hydraulic Institute (DHI) was used to run a two-dimensional model to assess the dispersion of DIN
output from a hypothetical spiny lobster sea-cage operation (DHI 2013; Tasmania Department of Primary Industries et al. 2011). ECO Lab is an open-source generic tool for implementing aquatic ecosystem models driven by the in-situ hydrodynamic conditions to simulate the fate of environmentally active materials, such as nutrients, toxic chemicals, and heavy metals, that are released into aquatic environments (DHI 2009). This model aims to simulate natural biochemical processes to provide accurate spatial predictions of the fate of discharges to the marine environment. For example, the model includes regeneration of DIN from previously deposited organic material and subsequent transport and dispersion into the water column.

For this particular study, a customised ECO Lab model template was used which had previously been developed for describing the influence of the release of DIN into the marine environment on subsequent water quality based on research by Fennel et al. (2006). The ecological variables included in this model template incorporated the interactions of DIN with the water column, (i.e., nitrogen pathways through phytoplankton, zooplankton, and detritus) and the seabed, where nitrogen was modelled as being broken down and mineralised. The model template also encompassed the nitrification process of oxidising ammonia into nitrate, as well as the denitrification process of converting nitrate into nitrogen or nitrous oxide, ultimately resulting in the removal of nitrogen from both the water column and seabed by being released as gas into the atmosphere (Fennel et al. 2006).

The quantity of DIN discharge (mg N L\(^{-1}\) s\(^{-1}\)) for each sea-cage from the lobster farm used in the DIN model in each modelling scenario was based on the total amount of feed (in wet weight) fed out to each sea-cage and that was calculated to have been partially converted to DIN (Table 5.4). The total amount of feed used per cage was calculated by multiplying the volume of each sea-cage (1280 m\(^3\)) with the stocking density (low density – 3 kg m\(^{-3}\) or high density – 5 kg m\(^{-3}\)) and FCR values of either 3, 5 or 28 and the amount of feed converted to DIN was based on the mean percentage of feed (in wet weight) converted to DIN that was previously measured for seafood and artificial diets (0.92 % and 0.55 % respectively) (Lee et al. 2015a). The DIN model simulations were run for a period of one month by which time the interaction of the released DIN with the environment had become stable, and provided useful spatial concentrations of the fate of the DIN.
Table 5.4. Rate of DIN discharge (mg N L\(^{-1}\) s\(^{-1}\)) from each sea-cage for different combinations of stocking density (3 and 5 kg m\(^{-3}\)), FCRs (3, 5 and 28) with *P. ornatus* fed on two different diets, seafood and artificial calculated DIN measurements from laboratory experiments and used in DIN model.

<table>
<thead>
<tr>
<th>FCR</th>
<th>Stocking Density (kg m(^{-3}))</th>
<th>Seafood Diet (mg N L(^{-1}) s(^{-1}))</th>
<th>Artificial Diet (mg N L(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>3.361</td>
<td>2.009</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.601</td>
<td>3.347</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>5.601</td>
<td>3.349</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.335</td>
<td>5.581</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>31.267</td>
<td>18.752</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>52.278</td>
<td>31.253</td>
</tr>
</tbody>
</table>

The results from modelling DIN dispersion from hypothetical lobster farm scenarios were generated as the mean and maximum elevated DIN concentration in waters surrounding the spiny lobster farm. The mean elevated DIN concentration was predicted by the model as the average concentration of DIN at any location around the farm over a month period, whereas the maximum elevated DIN was the highest concentration of DIN at any location around the farm over a month period. The mean elevated DIN concentration was used to illustrate the typical distribution of DIN that could be expected around the lobster farm, whereas the maximum elevated DIN concentration was indicative of the worst case situation. For the purposes of the modeling, any DIN concentration that was higher than the initial DIN concentration of 5 µg N L\(^{-1}\) was considered to be elevated. An acceptable dissolved nitrogen level for aquaculture was set at 0.5 mg N L\(^{-1}\) (equivalent to 500 µg N L\(^{-1}\)) which was recommended by governmental regulations specifically for aquaculture operations (Tuan 2005).
Chapter 5

5.3 Results

5.3.1 Depositional model

All modelled scenarios of small farms (i.e., 16 sea-cages) with spiny lobsters being fed with either seafood diet or artificial diet indicated that the highest mass of accumulated carbon deposition on the seabed was directly beneath the sea-cages, which then gradually decreased with increasing distance from the sea-cages until reaching 0.10 kg C m$^{-2}$ yr$^{-1}$ (the lowest mass of carbon deposition recorded in the modelled results; Figure 5.4 – 5.6). Small farms with low stocking density (3 kg m$^{-3}$) that used seafood diet produced the highest carbon deposition of 0.20 kg C m$^{-2}$ yr$^{-1}$ for the FCR of 1.28, compared to 0.35 kg C m$^{-2}$ yr$^{-1}$ for the moderate FCR of 3, and 0.65 kg C m$^{-2}$ yr$^{-1}$ for the poorest FCR of 5 (Figure 5.4). In comparison, when lobsters were fed with artificial diet, the highest rates of carbon deposition were 0.15, 0.30 and 0.60 kg C m$^{-2}$ yr$^{-1}$ for FCRs of 1.28, 3 and 5 respectively (Figure 5.5). In contrast, small farms with high stocking density (5 kg m$^{-3}$) with lobsters fed with seafood diet, the highest accumulated carbon mass ranged from 0.30 kg C m$^{-2}$ yr$^{-1}$ for the best FCR (1.28) to more than 0.80 kg C m$^{-2}$ yr$^{-1}$ for the poorest FCR (5). When artificial diet was used in small farms with high stocking density, the deposited carbon mass was 0.25, 0.60 and more than 0.80 kg C m$^{-2}$ yr$^{-1}$ for FCRs of 1.28, 3 and 5 respectively (Figure 5.5). The accumulated carbon on the seabed that emanated from the small farm extended mainly further from the northern side of the sea-cages which corresponded to the predominant current direction of the study site (Figure 5.4 and 5.5). The spatial extent of the lowest mass of deposited carbon to the seabed (i.e., 0.10 kg C m$^{-2}$ yr$^{-1}$) at small farms were similar between the two diets when farms were stocked with the same stocking density and with the same FCRs. At low stocking density, the spatial extent of the lowest mass of carbon deposited to the seabed was as close as 8 m and up to 34 m away from the perimeter of the sea-cages with increasing FCRs (1.28, 3 and 5). In comparison, the extent of the coverage of the lowest accumulated carbon mass that extended out from the perimeter of the sea-cages of the small farms with high stocking density increased from 14 m up to 50 m with the increasing FCRs.
Figure 5.4. Estimated cumulative organic carbon deposition (kg C m$^{-2}$ yr$^{-1}$) to the seabed for layout of lobster farm consisting of 16 sea-cages stocked at either low (3 kg m$^{-3}$) or high density (5 kg m$^{-3}$) of lobsters fed with natural seafood diet at three different FCRs - 1.28, 3 and 5.
Figure 5.5. Estimated cumulative organic carbon deposition (kg C m\(^{-2}\) yr\(^{-1}\)) to the seabed for layout of lobster farm consisting of 16 sea-cages stocked at either low (3 kg m\(^{-3}\)) or high density (5 kg m\(^{-3}\)) of lobsters fed with artificial diet at three different FCRs - 1.28, 3 and 5.
Figure 5.6. Sensitivity analysis of maximum carbon mass (kg C m\(^{-2}\) yr\(^{-1}\)) generated around the modelled lobster farm in relations to stocking density, farm size, and dietary regime

At the large farms (i.e., 80 sea-cages), the highest carbon mass deposited on the seabed was also accumulated directly beneath the sea-cages for all modelled scenarios for both diets (seafood and artificial diet; Figure 5.6 – 5.8). The deposited carbon mass then gradually decreased as the faecal material and waste feed dispersed further away from sea-cages until reaching the lowest deposited carbon mass of 0.10 kg C m\(^{-2}\) yr\(^{-1}\). When lobsters are stocked at low density (3 kg m\(^{-3}\)) at large farms, the deposition of carbon mass was consistent for both diets at 0.35 kg C m\(^{-2}\) yr\(^{-1}\) for a FCR of 3, and 0.50 kg C m\(^{-2}\) yr\(^{-1}\) for a FCR of 5 (Figure 5.7 and 5.8). In contrast, at the high stocking density (5 kg m\(^{-3}\)) in the large farms the carbon mass deposition at FCR of 3 was 0.50 kg C m\(^{-2}\) yr\(^{-1}\) for both diets whereas the deposited carbon mass with FCR of 5 was more than 0.80 kg C m\(^{-2}\) yr\(^{-1}\) for both diets. As observed for the small lobster farm scenario, the accumulated carbon mass from operating the large farm also dispersed mainly to the northern side of the sea-cages which corresponded to the predominant current direction of the study site (Figure 5.7 and 5.8). The modelled scenarios of large farms with low stocking density for FCR of 3 fed with seafood or artificial diet illustrated that the dispersal of the lowest level of modelled carbon mass (0.10 kg C m\(^{-2}\)}
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yr\(^{-1}\)) was 33 m away from the perimeter of the sea-cages, while the spatial extent of the lowest level of deposited carbon mass from lobsters stocked at high density with the same FCR (3) was 50 and 56 m away from the perimeter of the sea-cages for farms using seafood diet and artificial diet, respectively. An identical pattern of spatial coverage of the lowest carbon mass deposition was generated by low stocking density at FCR of 5 using seafood diet and artificial diet, respectively (Figure 5.7 and 5.8). With high stocking density at FCR of 5 in these large farms, the spatial distribution of the lowest carbon mass deposition was 89 and 100 m away from the perimeter of sea-cages for seafood and artificial diets respectively (Figure 5.7 and 5.8).

The results of all modelled scenarios from both small and large lobster farms (i.e., 80 vs 20 sea-cages) were within the proposed threshold (0.73 kg C m\(^{-2}\) yr\(^{-1}\)) for accumulative organic carbon deposition except when the sea-cages were stocked at high density (5 kg m\(^{-3}\)) and had the worst FCR (FCR = 5). In these instances, the threshold of accumulated carbon deposition was only exceeded in a localised area immediately below the sea-cages, and within a 5 m radius of the perimeter of the sea-cages.

Figure 5.7. Estimated cumulative organic carbon deposition (kg C m\(^{-2}\) yr\(^{-1}\)) to the seabed for layout of lobster farm consisting of 80 sea-cages stocked at either low (3 kg m\(^{-3}\)) or high density (5 kg m\(^{-3}\)) of lobsters fed with natural seafood diet at FCRs – 3 and 5.
Figure 5.8. Estimated cumulative organic carbon deposition (kg C m\(^{-2}\) yr\(^{-1}\)) to the seabed for layout of lobster farm consisting of 80 sea-cages stocked at either low (3 kg m\(^{-3}\)) or high density (5 kg m\(^{-3}\)) of lobsters fed with artificial diet at FCRs – 3 and 5.

5.3.2 DIN modelling

All the modelled scenarios of DIN dispersion from hypothetical lobster farm scenarios showed some degree of DIN elevation within and around the sea-cages up to distances of more than 200 m from the perimeter of sea-cages for both mean and maximum DIN elevation. The highest elevated mean and maximum DIN concentration for all modelled scenarios were found to be directly underneath the sea-cages, especially within the two rows of sea-cages located closest towards the northwestern side of the farm. The elevated concentration of DIN decreased gradually to background ambient levels as it dispersed further away from the sea-cages. For the elevated mean DIN, the dispersal of DIN for all modelled scenarios seemed to be predominantly towards the northern part of the farm which coincided with the prevailing current direction. Thus, the dispersal of the same mean DIN concentrations were found to cover a wider distance from the northern perimeter of the sea-cages when compared with the southern perimeter of the sea-cages for most of the modelled scenarios. In contrast, the maximum elevated DIN for all modelled scenarios were dispersed predominantly towards the
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southern part of the sea-cages and had a more extensive spatial distribution to the southern perimeter of the sea-cages than from the northern perimeter of the sea-cages.

The highest elevated mean DIN concentrations for lobster farm scenarios using seafood diet and stocking at low density (3 kg m$^{-3}$) with FCRs of 3, 5 and 28 were 6.4, 7 and 20 µg N L$^{-1}$, respectively, while at high stocking density (5 kg m$^{-3}$), the highest elevated mean DIN concentrations were 7, more than 7.8 and 25 µg N L$^{-1}$ for FCRs of 3, 5 and 28 respectively (Figure 5.9 – 5.11a). Where lobsters were fed with artificial diet, the modelled scenarios at low stocking density with FCRs of 3, 5 and 28 had the highest elevated mean DIN concentrations of 5.8, 6.2 and 15 µg N L$^{-1}$, respectively, while at high stocking density the highest elevated mean DIN concentrations were 6.2, 7 and 20 µg N L$^{-1}$ for FCRs of 3, 5 and 28 respectively.

For the maximum DIN concentrations, the results for modelled scenarios with low stocking density using seafood diet showed the highest concentrations were 15.2, 21 and 100 µg N L$^{-1}$ for FCRs of 3, 5 and 28 respectively, whereas at high stocking density the highest elevated maximum DIN concentrations were 21, 28 and 165 µg N L$^{-1}$ for the respective FCRs (Figure 5.11b - 13). Results for modelled scenarios using artificial diets and stocked at low density, the highest elevated maximum DIN concentrations were 10.8, 14.4 and 60 µg N L$^{-1}$ for FCRs of 3, 5 and 28, respectively, while at high stocking density with the same FCRs, the respective highest elevated maximum DIN concentrations were 14.4, 28 and 100 µg N L$^{-1}$. 
Figure 5.9. Estimated mean dissolved inorganic nitrogen (µg N L⁻¹) for a lobster farm layout consisting of 80 sea-cages stocked at either low (3 kg m⁻³) or high density (5 kg m⁻³) at FCRs – 3 and 5. (a-d) spiny lobsters fed with seafood diet; (e-h) spiny lobsters fed with artificial diet.
Figure 5.10. Estimated mean dissolved inorganic nitrogen (µg N L⁻¹) for a lobster farm layout consisting of 80 sea-cages stocked at either low (3 kg m⁻³) or high density (5 kg m⁻³) at FCR of 28. (a and b) spiny lobsters fed with seafood diet; (c and d) spiny lobsters fed with artificial diet.
Figure 5.11. Sensitivity analysis of DIN (µg N L\(^{-1}\)) generated around the modelled lobster farm in relations to stocking density, FCR, and dietary regime. (a) highest mean DIN; (b) highest maximum DIN.
Figure 5.12. Estimated maximum dissolved inorganic nitrogen (µg N L\(^{-1}\)) for a lobster farm layout consisting of 80 sea-cages stocked at either low (3 kg m\(^{-3}\)) or high density (5 kg m\(^{-3}\)) at FCRs – 3 and 5. (a-d) spiny lobsters fed with seafood diet; (e-h) spiny lobsters fed with artificial diet.
Figure 5.13. Estimated maximum dissolved inorganic nitrogen (µg N L$^{-1}$) for lobster farm layout consisting of 80 sea-cages stocked at either low (3 kg m$^{-3}$) or high density (5 kg m$^{-3}$) at FCR of 28. (a and b) spiny lobsters fed with seafood diet; (c and d) spiny lobsters fed with artificial diet.

Overall, both concentration and spatial distribution of elevated mean and maximum DIN concentrations depended on the combinations of stocking density of the sea-cages (3 or 5 kg m$^{-3}$), FCRs (3, 5 or 28), as well as the diets being fed to the spiny lobsters (seafood or artificial diets). The mean and maximum elevated DIN concentrations were within the recommended threshold of 500 µg N L$^{-1}$ for all results derived from scenarios that used all possible combinations of stocking densities, FCRs and diet used in the DIN modelling.

The spatial distribution of elevated mean and maximum DIN concentration for all modelled scenarios were generally dispersed beyond 200 m of the perimeter of the sea-cages with the highest DIN concentrations directly beneath the sea-cages. Overall, the spatial distribution patterns for the DIN concentrations did not differ much between the stocking densities and FCRs, however, scenarios using seafood diet illustrated a more extensive spatial distribution for both elevated mean and maximum DIN concentration when compared with using artificial diet. Nevertheless, when comparing the dispersal of the same DIN
concentration whether it was mean or maximum DIN concentration, the spatial distribution was always more extensive with higher stocking density and higher FCR. For example, in the scenario where the spiny lobsters were fed with the seafood diet at FCR of 3, the mean DIN concentration of 6.2 µg N L$^{-1}$ at a low stocking density farm would spread approximately 20 m away from the northern perimeter of the sea-cages compared with 130 m for a high stocking density scenario. In the same scenario at low stocking density with spiny lobster fed seafood diet, the mean DIN concentration of 6.2 µg N L$^{-1}$ at FCR of 3 would disperse 20 m away from the northern perimeter of the sea-cages whereas at a FCR of 5, it would extend out to 100 m.

5.4 Discussion

5.4.1 Depositional model

The highest deposition of carbon mass from the aquaculture of *P. ornatus* was concentrated directly beneath the sea-cages and gradually decreased as it was dispersed away from the sea-cages. This demonstrated that the majority of the impact from carbon deposition to the seabed from spiny lobster farms was localised directly below the sea-cages. Such a pattern of deposition is also common to fin-fish and shellfish aquaculture operations (Hartstein and Stevens 2005; Magill et al. 2006; Panchang et al. 1997). The overall deposition of accumulated carbon mass in this study ranged from 0.15 kg C m$^{-2}$ yr$^{-1}$ up to more than 0.80 kg C m$^{-2}$ yr$^{-1}$ depending on the stocking density, FCR, and diet fed to spiny lobsters and the farm layout. These rates of benthic accumulation are similar to those estimated for the Australasian red spiny lobster, *Jasus edwardsii*, from temperate waters, suggesting that there may be little difference in the likely impacts from the many different species of spiny lobsters that are cultured in sea-cages (Lee et al. 2014). In comparison, the organic waste production when American lobsters were held at an average stocking density of 3.79 kg m$^{-2}$ in an impoundment in Maine was higher at approximately 0.23 kg m$^{-2}$ yr$^{-1}$ (Tlusty and Preisner 2005). The results of the modelling revealed that lobster aquaculture resulted in a much lower concentration of organic waste accumulating on the seabed than is typical for fin-fish aquaculture (2.5 – 15 kg m$^{-2}$ yr$^{-1}$), even when the FCR is higher in lobster aquaculture (Cromey et al. 2002a; Cromey et al. 2009; Dudley et al. 2000; Magill et al. 2006). The FCR in
this study was up to 5, while the FCR in a typical fin-fish aquaculture is usually <2 (Brooks et al. 2002; Doglioli et al. 2004). Therefore, a spiny lobster farm would have higher organic matter loading derived from the feed being released into the environment compared with fin-fish aquaculture systems with the same overall production. Despite the higher FCR in lobsters vs. fish, for typical sea-cage aquaculture operations, the organic waste deposition will be much lower for lobsters due to the much lower stocking density used in lobster aquaculture, which is the result of lobsters only being able to utilize the floor area of the sea-cage, whereas fin-fish use the entire water column enclosed by the sea-cage and can be stocked at densities of up to 50 kg m\(^{-3}\) (Doglioli et al. 2004; Kongkeo et al. 2010; Tlusty and Preisner 2005).

Overall, the depositional modelling results show there are only slight variations in the carbon loading between the seafood and artificial diets. In the small lobster farm at both low stocking density (3 kg m\(^{-3}\)) and high stocking density (5 kg m\(^{-3}\)), the artificial diet generated only 0.05 kg C m\(^{-2}\) yr\(^{-1}\) less carbon loading than the seafood diet directly beneath the sea-cages for all modelled FCRs (1.28, 3 and 5). In addition, the pattern of carbon loading beyond the perimeter of the farm was similar for both types of feed for the equivalent stocking densities and FCRs (Figure 5.4 and 5.5). In contrast, for the large lobster farm layout, the accumulated carbon immediately beneath the sea-cages was similar for both natural and artificial diets for scenarios with the same stocking densities and FCRs (0.35 – 80 kg C m\(^{-2}\) yr\(^{-1}\) depending on FCR and stocking combinations), however, the pattern of carbon loading beyond the farm was less extensive for seafood diet compared to the artificial diet. This indicates that the different diets fed to cultured spiny lobsters will have implications for the resulting deposition and dispersion of waste to the seabed. Regardless of stocking density and FCRs, in small lobster farms, artificial diet tended to consistently produce a lower carbon loading on the seabed without changing the spatial extent distribution of the deposited carbon. In contrast, in the large farm, artificial diet resulted in the same amount of carbon deposition on the seabed as seafood diet, but with a wider spatial dispersion of carbon mass.

The key factor influencing the modelled differences in the benthic carbon loading from lobster farms using seafood versus artificial food was differences in the settling velocity of \textit{P. ornatus} faeces on these diets. Faeces produced by lobsters fed with artificial diet had slower settling velocity than those produced by lobsters fed with the natural diet (0.22 ± 0.01 cm s\(^{-1}\) versus 0.30 ± 0.01 cm s\(^{-1}\) respectively) (Lee et al. 2015a). Settling velocity of faeces is inversely proportional to horizontal dispersal distances (Gowen et al. 1989) and consequently
the slower settling velocity of faeces produced by artificial diet resulted in their greater dispersal and the corresponding greater spatial extent of the carbon loading around the sea-cages. As a result, using artificial diet in lobster farms will lower the benthic carbon loading directly beneath the sea-cages but will cause a more extensive coverage of carbon mass on the seabed. An increased dispersion of faeces can be expected to reduce the localised effects on the benthic community (Reid et al. 2009).

In the large hypothetical lobster farm, the highest carbon mass deposited on the seabed was generally slightly lower (by 0.05 kg C m$^{-2}$ yr$^{-1}$) but with a more extensive spatial distribution (increased by approximately 16 m up to 50 m) than those of the smaller farm layout with the same stocking density and FCR. Such alteration in the overall carbon mass deposition illustrated that the layout of the sea-cages in the same farming area would influence the accumulation of waste material on the seabed. It is probable that positioning the sea-cages for the small farm in a straight line, in a manner similar to the larger farm layout would reduce the cage hydrodynamic drag effects, thus increasing the current speed around and beneath the sea-cages, thereby dispersing the waste material further away from the sea-cages (Plew 2011).

The percentage of feed converted to faeces (22%) (Chen et al. 1999b) used in the model was based on studies on salmon because the amount of feed converted to faeces for spiny lobster has not been quantified. It is likely that the depositional model may have underestimated or overestimated the deposited carbon mass depending if the actual conversion percentage is higher or lower than 22 % for spiny lobster. Thus, it is crucial to quantify how much feed is converted to faecal material for spiny lobster to improve the accuracy of the modelling results. Moreover, in aquaculture operations where cultured animals are fed with seafood, past studies have shown that the FCR can be as high as 28 (Edwards et al. 2004) and the feed wastage up to 45 % (Wu 1995). These values were much higher than the ones used in the model set up (FCR 1.28 – 5; feed wastage of 5 %). Therefore, if these higher FCR and feed wastage figures for seafood diets are in operation, then the model simulation would have greatly underestimated the carbon deposition on the seabed. FCR and feed wastage data based on the specific diet used in a spiny lobster aquaculture operation would be essential to improve the accuracy of the outputs from the simulated depositional model.
5.4.2 DIN modelling

Modelled scenarios demonstrated that there was potential for the elevation of DIN near the spiny lobster farms, the extent of which was influenced by the combinations of stocking density, FCRs and type of diet used. Modelling results indicated that the mean DIN concentrations for all the various scenarios of low and high stocking density (3 and 5 kg m\(^{-3}\)) for both diets used (seafood and artificial diet) remained close to the initial condition (5 µg N L\(^{-1}\)) of the hypothetical farm except for when the FCR was at its highest setting of 28 resulting in mean DIN elevation of 15 – 25 µg N L\(^{-1}\). An FCR of 28 at an aquaculture operation would only be likely to occur when lobsters are fed with “trash fish” (Edwards et al. 2004). In addition, regardless of stocking density and FCR, the mean elevated DIN was generally lower at lobster farms using artificial feed than seafood diet (reduces mean DIN concentrations by 9 - 25 %). Therefore, switching spiny lobster farms from using a seafood diet to an artificial diet can help lower the FCR and reduce environmental elevation of DIN.

The maximum DIN concentration (showing the potential worst case scenarios) in all simulated scenarios for both types of diets were all at least double and sometimes can be up to 33 times above the initial conditions with increasing stocking density (3 and 5 kg m\(^{-3}\)) and FCR (3, 5 and 28). Nevertheless, the resulting elevated maximum DIN concentrations for all modelled scenarios regardless of stocking density, FCR and the type of diets were still within the recommended acceptable dissolved nitrogen level for aquaculture of 0.5 mg N L\(^{-1}\) (equivalent to 500 µg N L\(^{-1}\)) (Tuan 2005). This showed that DIN elevation would not be an issue for tropical spiny lobster aquaculture in a well flushed farm site like the one used in this study, even when it is stocked at high density with very poor FCR.

A study on a tuna farm with production of 1000 t yr\(^{-1}\) observed that although the dissolved nitrogen tended to be higher at the farm site, there was no significant difference in the dissolved nitrogen between farm and control sites (Aksu et al. 2010). It was concluded that the placement of the sea-cages in deep water with strong currents helped to avoid elevation of dissolved nitrogen next to the farm (Aksu et al. 2010). Rapid dispersal of released dissolved nitrogen was also observed on a fin-fish farm in the Ligurian Sea with a stocking density of 20 kg m\(^{-3}\) which rarely exceeded 4 µg L\(^{-1}\) of total dissolved nitrogen because of rapid dispersal due to persistent wind-driven surface currents (Doglioli et al. 2004). The low environmental DIN concentrations resulting from all of the modelled scenarios used in this study.
study indicate that the mean current of 20 cm s\(^{-1}\) at the study site provided for sufficient dispersal and dilution of DIN (Table 5.2). Therefore, the placement of lobster farms in areas with a sufficient current regime is important for maintaining low environmental DIN concentrations, especially for lobster farms with high stocking density and poor FCR.

The sea-cages used for the DIN modelling were much larger than those used in the carbon depositional model (sea-cage measured at 16 × 16 m versus 5 × 5 m). Thus, the overall production from the 80 sea-cage farm layout used for the DIN model was substantially higher than the large 80 sea-cage farm layout used for the carbon deposition model when both farms have the same stocking density (e.g., 512 versus 50 t yr\(^{-1}\) at stocking density of 5 kg m\(^{-3}\)). However, even when these substantially larger farms were modelled with high stocking density (5 kg m\(^{-3}\)) and poor FCR, the resulting elevation of DIN in the surrounding environment was still within the recommended level (0.5 mg N L\(^{-1}\)). In contrast, when the high stocking density and poor FCR were used for the carbon deposition modelling of the large lobster farm, the carbon loading on the seabed exceeded the threshold of 0.73 kg C m\(^{-2}\) yr\(^{-1}\). This indicates that benthic carbon deposition should be of greater concern than the elevation of DIN when managing the potential environmental impacts generated by sea-cage lobster farming.

5.5 Conclusion

This study indicated that the potential environmental impacts from particulate organic deposition to the seabed and DIN release from spiny lobster aquaculture is much lower compared to the aquaculture of other species, such as fin-fish species. Nevertheless, sea-cage aquaculture of spiny lobsters may still affect sensitive benthic habitats like seagrass and coral reefs in the immediate vicinity of the farming operation, mainly directly below the sea-cages up to a distance of 100 m but at times further than 200 m (only for DIN elevation). The environmental effects from spiny lobster aquaculture in sea-cages were generally localised direct below the sea-cages with benthic and pelagic eutrophication extending further from sea-cages where lobsters are fed using natural seafood with very poor FCR. Thus, substituting natural diets with artificial diets with good FCR can reduce the overall nutrient enrichment of both seabed and water column. Locating spiny lobster farms in relatively hydrodynamically
active coastal areas where lobsters are fed using highly digestible artificial diets with low FCR will help to minimize environmental sequelae. A lobster farm with low stocking density and good FCR situated in a well flushed environment is still at risk of benthic carbon loading but not DIN elevation. Therefore, monitoring regimes should focus on the deposition of particulate waste from lobster farms to ensure that farming operations maintain sustainable practices.
Chapter 6: General Discussion

The present study represents the first attempt at quantifying and determining the likely fate of the two key nutrients (i.e., carbon and DIN) released by spiny lobsters when aquacultured in sea-cages that have the greatest potential to contribute to the overall environmental impact of this activity. This research included measuring important parameters such as faecal strand settling velocity, nitrogen leaching from faeces and uneaten feeds, and determining how different lobster diets influenced DIN and faecal carbon.

6.1 Characterising spiny lobster faecal material

6.1.1 Faecal settling velocity

The settling velocity of faecal material is one of the key factors in determining how far the organic carbon in faeces that is discharged from a lobster farm will disperse and finally deposit on the seabed. The faster the settling velocity, the closer to the farm the organic carbon deposition will occur (Gowen et al. 1989). In this study, the overall settling velocities of faecal material produced by both of the spiny lobsters species that were examined were highly variable. *Jasus edwardsii* fed with mussels produced faeces with higher mean settling velocity of $0.90 \pm 1.00$ cm s$^{-1}$ (Lee et al. 2014) than the faeces produced by *Panulirus ornatus* fed with either seafood (mixture of fish and squid) or artificial diet, i.e., mean settling velocities of $0.30 \pm 0.01$ cm s$^{-1}$ and $0.22 \pm 0.01$ cm s$^{-1}$ respectively (Lee et al. 2015a). The higher settling velocity of the faeces from *J. edwardsii* compared to those of *P. ornatus* appeared to be due to differences in faecal density. The mean density of faecal material from *J. edwardsii* fed with mussels was $4.0 \times 10^{-4}$ g mm$^{-3}$ while the mean density of faecal material produced by *P. ornatus* fed with either a mixture of fish and squid, or an artificial diet were $2.0 \times 10^{-4}$ g mm$^{-3}$ and $1.0 \times 10^{-4}$ g mm$^{-3}$ respectively (Figure 2.3 in Chapter 2 and Figure 4.2g and 4.2h in Chapter 4). These differences in faecal density may be directly due to the composition of the three different feeds provided to the lobsters. Different diets (i.e., different seafood diets and artificial diet) can have different digestible components which can alter the
resulting faecal characteristics such as density (Reid et al. 2009). Although the digestive tract and digestive physiology is broadly similar among spiny lobster species (Mikami and Takashima 2008), general differences in the digestive processing of food have been noticed between tropical and temperate spiny lobster species which may also explain the observed differences in faecal density between these two species examined in this study (Williams 2007a; Williams 2007b). Cooler water temperatures in temperate regions could have resulted in slower gut evacuation but higher nutrient absorption in J. edwardsii, thus producing denser faeces with higher settling rate than the tropical lobster in warmer water. Generally, nutrient absorption declines with a more rapid gut evacuation rate which can be due to increasing temperature (Johnston and Mathias 1996). For example, a study on walleye (Stizostedion vitreum) larvae indicated that their gut evacuation rate was higher at 20 °C than 15 °C (Johnston and Mathias 1996). Similarly, a study on spotted seatrout (Cynoscion nebulosus) found that the gut evacuation rate declined with increasing temperature (Wuenschel and Werner 2004).

Temperature and salinity can alter the density of seawater which can also alter the settling velocity of faecal material (Piedecausa et al. 2009; Reid et al. 2009). The faeces settling velocity experiments for the two different species of lobsters were conducted using different water temperatures and salinities, 16 ± 1 °C and 34 ± 1 ppt for J. edwardsii and 28 ± 1 °C and 32 ± 1 ppt for P. ornatus. However, these differences in temperature and salinity are relatively minor in terms of seawater density and viscosity and are unlikely to markedly affect faecal sinking rates.

6.1.2 Elemental analyses

Spiny lobsters are able to exploit a wide variety of diets due to the diverse range of enzymes in their digestive system (Johnston 2003). However, different diets will contain components with different digestibility that will ultimately affect the carbon and nitrogen content in their faeces. The resulting carbon and nitrogen content in faecal material will most sink to the seabed where it will enhance organic nutrients in the sediment. Australasian red spiny lobsters (J. edwardsii) fed solely on mussels produced faeces containing an average of 35 ± 1 % (mean ± SE) carbon and 5 ± 0.3 % of nitrogen (Lee et al. 2014). Whereas when a

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mixture of fish and squid was fed to ornate spiny lobsters (*P. ornatus*) they produced faeces with 31 ± 0.5 % carbon and 5 ± 0.1 % of nitrogen. When ornate spiny lobsters were fed artificial diet (Lucky Star Brand, Taiwan Hung Kuo Industrial Co., Ltd, Taiwan) the faeces tended to have elevated carbon content (41 ± 0.6 %) and similar nitrogen content compared with the natural seafood diet, i.e., 4.6 ± 0.1 % (Lee et al. 2015a). Overall, the carbon and nitrogen content of spiny lobster faeces were broadly consistent to those reported for the faeces of other aquaculture species. For example, faecal material from Atlantic salmon contained 27 – 40 % carbon and 2.3 – 4.9 % nitrogen depending on the composition of their diets (Chen et al. 1999a; Chen et al. 2003; Crawford et al. 2002). Similar carbon and nitrogen content has also been found in the faeces of cultured rainbow trout (*Oncorhynchus mykiss*), i.e., 30.0 – 45.7 % carbon 2.8 – 5.2 % nitrogen (Moccia et al. 2007a; Pillay 1992). Further development of more easily digestible artificial diets for spiny lobsters has the potential to lower the nutrient content in lobster faecal material that would otherwise contribute to the organic loading on the seabed.

Elemental analyses of faecal material produced by *J. edwardsii* when fed mussels showed that lobster of a 100 g produced faeces that had around 2 % more nitrogen than lobsters of larger sizes. This is likely to be due to a less well developed ability of the digestive tract of juvenile lobsters to process the protein in mussels compared with larger lobsters. In contrast, the carbon content in faeces produced by *P. ornatus* fed with artificial diet increased with lobster size, indicating that the digestibility of the artificial diet used in this study also differed between smaller and larger spiny lobsters. This difference was likely to be due to the different sized pellets made from the same formulation used for the lobsters of small versus larger sizes (i.e., 2.5 vs 3 mm diameter pellet) which may have been more difficult for the larger lobsters to digest. Hence, attention to digestive capabilities of different size lobsters and developing specialised diets to cater to their specific needs, as well as improving formulation, will be important for reducing the nutrient discharge derived from faecal material released from lobster farming.
6.2 DIN production

6.2.1 DIN excretion from spiny lobsters

Feeding activity at least doubled the baseline DIN excretion of unfed spiny lobsters of both species (i.e., *J. edwardsii* and *P. ornatus*) in this study (Lee et al. 2015a; Lee et al. 2015b). Similar results have been found in studies of other spiny lobster species (Crear and Forteath 2002; Kemp et al. 2009; Zoutendyk 1987). The level of DIN output from lobsters also depended on the protein content of the diets being consumed by the lobsters. Since the main form of DIN output from spiny lobsters is excreted ammonia from catabolizing protein sourced from the feed, it could be expected that the DIN output would increase as protein content in the feed increases (Ballestrazzi et al. 1994). This was clearly demonstrated in the 24 h DIN output measurements for *J. edwardsii* after being fed with two diets (seafood and moist artificial diet); seafood (mussel) contained 55.2 % protein (8.8 % nitrogen) while the moist artificial diet (from Grobest Feeds Corporation, India, Ltd. and bound with 2 % alginate) contained 40 % protein (6.4 % nitrogen) (Lee et al. 2015b). Mean DIN excretion from lobsters fed with mussel was 3.3 times higher than for lobsters fed the artificial diet. Unfortunately, protein and nitrogen measurements were not made for the seafood diet used for *P. ornatus*, which would have allowed a parallel comparison with the artificial diet used in this second spiny lobster species (Lucky Star Brand: 45 % protein; 7.2 % nitrogen).

The mean DIN output from *J. edwardsii* and *P. ornatus* after being fed in this study was 5.6 μg N g⁻¹ hr⁻¹ (1.3 × 10⁻⁴ mg N kg⁻¹ day⁻¹) and 12 μg N g⁻¹ hr⁻¹ (2.9 × 10⁻⁴ mg N kg⁻¹ day⁻¹) respectively where the majority (about 88.5 %) of it was ammoniacal nitrogen. Studies on a variety of fin-fish species have documented ammoniacal nitrogen excretion rate to be much higher than those measured in this study for spiny lobsters. For example, adult mangrove snapper, *Lutjanus argentimaculatus*, and areolate grouper, *Epinephelus areolatus*, have been found to excrete 558 and 375 mg N kg⁻¹ day⁻¹ respectively (Leung 1996). In addition, ammonia excretion from Atlantic salmon has been measured as ranging from 53.3 to 187.2 mg N kg⁻¹ day⁻¹ (Bergheim et al. 1991). Such comparisons suggest that the potential impact of elevated DIN from a spiny lobster farm is likely to be much lower than for a fin-fish farm, especially as stocking densities are typically much lower than for commercial finfish sea-cage aquaculture operations.
It is possible that the incorporation of suitable lipid and carbohydrate ingredients in diets may provide alternative energy sources for spiny lobsters, thereby reducing DIN excretion from the catabolism of protein for energy. This may also assist in reducing the cost of lobster feed because protein sources are generally more expensive ingredients included in formulated aquaculture feeds. Another factor that can affect the DIN excretion of lobsters is water temperature whereby DIN excretion is positively correlated with increasing temperature (Regnault 1987; Thomas et al. 2000). Temperature effects on DIN excretion were not examined in this study, however, previous research has shown that total ammoniacal nitrogen (TAN) excretion in both *J. edwardsii* and *P. cygnus* increased exponentially with temperature (Crear and Forteath 2002). An increment of 4 °C in water temperature increased TAN excretion of *J. edwardsii* and *P. cygnus* by approximately 25 % and 100 % respectively (Crear and Forteath 2002). Therefore, having ambient water temperature around the sea-cages that is as close to the optimum temperature for the species is of importance for not only maintaining high growth rates and food conversion but also low DIN excretion. Preferable farming sites will be in areas with optimum water temperature for lobster growth, i.e., optimum water temperature for *J. edwardsii* ranges from 18 – 21 °C; *P. ornatus* ranges from 25 – 28 °C (Booth and Kittaka 1994; Jones et al. 2001; Thomas et al. 2000). It can be expected that during periods when water temperature exceeds the optima for the lobster species, higher levels of DIN will be released. However, based on the low DIN production from both studied lobster species in this study (*J. edwardsii* was kept in 18 °C water; *P. ornatus* was kept in 28 °C water), the increment of DIN excretion due to increased temperature should not be of great concern for spiny lobster aquaculture.

Spiny lobsters are known to excrete DIN in the form of urea (1.2 % of total nitrogen excreted) as well as amino compounds (0.6 % of total nitrogen excreted) (Binns and Peterson 1969). These two forms of DIN were not measured during the DIN production experiments in this study due to difficulties in applying reliable assay techniques. However, both urea and amino compounds are only minor contributors to the overall DIN excretion by lobsters. Hence, the inclusion of urea and amino compounds will not dramatically alter the DIN production results obtained from this study, although it may provide a more complete comprehension of DIN excretion by spiny lobsters.
6.2.2 Nutrient leaching from waste feed and faecal material

Both waste feed and faecal material are known to leach DIN into the water column as they sink down and deposit on the seabed. Waste feed and faeces deposited to the seafloor will continue to be broken down by bacteria and release further DIN and thereby contributing further to the overall DIN output from spiny lobster aquaculture systems. This is the first study to quantify the leaching of DIN from waste feed and faecal material from spiny lobster aquaculture. DIN leaching from lobster faeces and waste feed were not significantly different, with mean DIN leaching ranging from 123.0 ± 81.3 and 720.0 ± 656.1 µg N g⁻¹ hr⁻¹ (Lee et al. 2015a). In comparison, previous studies have shown that nutrient leaching from faecal material is generally faster and has greater loss of nitrogen than for feed pellets because the pellets are designed to be more water stable (Burford and Williams 2001; Piedecausa et al. 2009). Moreover, it is also expected that dissolved nutrient release will be greater for smaller sized particles due to the larger surface area to volume ratio compared with larger sized particles (Chen et al. 1999b). Other factors such as elevated water temperature and lower pH have also been shown to increase the leaching of dissolved nutrients from waste feed and faecal material from fish (Kibria et al. 1997). Future studies could focus on better defining the different effects (i.e., temperature, pH, particle size) on nutrient leaching from waste feed and faeces generated from lobster farms.

6.2.3 Nitrogen budget for lobster farm production

Nitrogen budgets in regards to lobster aquaculture can be calculated using the results from the elemental analyses on lobster faecal material and DIN production for the two types of diets used in this study (i.e., seafood and artificial diet). A diet of mussel will be used as an example for the seafood diet while the Lucky Star brand of artificial lobster diet will be used as example for artificial diet for the nitrogen budget. Excreted urea and amino compounds were not measured in this study, therefore published data on those two forms of DIN are used for the nitrogen budget. It is assumed that 1.2 % of nitrogen output is in the form of urea and 0.6 % of nitrogen output is in the form of amino compounds (Binns and Peterson 1969). A nitrogen budget for the production of 1 kg of lobster with an FCR of 28 for the seafood diet and FCR of 3 for the artificial diet can be calculated using the parameters derived in the
current study. For this purpose, the amount of feed converted to faeces was estimated at 22% for both diets (Lee et al. 2014).

For the seafood diet the mussel used as feed contains 8.8% nitrogen (Simon and Jeffs 2008) and therefore the production of 1 kg of lobsters will require 28 kg of mussel feed, which contains a total of 2.5 kg of nitrogen. This nitrogen is then divided between, faeces (deposit on the seabed), DIN (into the water column) and nitrogen stored in the tissue of the lobster. Observations indicate the following breakdown: faeces produced by lobsters fed with solely mussels contained 5% of nitrogen while 2.88% of feed in dry weight is converted to DIN (i.e., a sum of ammonia, nitrate and nitrite) (Lee et al. 2015b). Therefore, a total of 0.313 kg of nitrogen (12.5% of total nitrogen) is released in faecal material and a total of 0.806 kg of nitrogen (32.2% of total nitrogen) is excreted as DIN (i.e., a sum of ammonia, nitrate and nitrite). In addition, a total of 0.030 and 0.015 kg of nitrogen is released as urea and amino compounds respectively. The remaining 1.336 kg of nitrogen (53.4% of total nitrogen) is most likely stored in the tissues of lobsters (i.e., used for growth).

For the artificial diet the production of 1 kg of lobster will need 3 kg of artificial diet which contains 7.2% of nitrogen, meaning that a total of 0.22 kg of nitrogen is required. Lobsters fed with artificial diet produced faecal material that contained 4.6% of nitrogen (Lee et al. 2015a), indicating that 0.031 kg of input nitrogen (14.1% of total nitrogen) is released as faeces. The resulting nitrogen release into the water column from this study showed that 0.6% of feed in dry weight is converted to DIN (i.e., a sum of ammonia, nitrate and nitrite). This indicates that 0.018 kg of nitrogen (8.1% of total nitrogen) is excreted as DIN (i.e., a sum of ammonia, nitrate and nitrite). A total of 0.003 kg is excreted as urea while 0.001 kg is excreted as amino compounds. The remaining 0.167 kg of nitrogen (75.9% of total nitrogen) is most likely stored in the tissues of lobsters (i.e., used for growth).

6.3 Numerical models

6.3.1 Carbon deposition and DIN modelling

Numerical modelling can be used to predict the potential impact of carbon deposition and DIN elevation from a sea-cage farm on the surrounding environment according to the
hydrodynamic conditions at the farming site. Consequently, numerical modelling is becoming an important tool among aquaculture management agencies and regulators (Tasmania Department of Primary Industries et al. 2011; Wu et al. 1999). There is a range of modelling software available for examining the release of waste materials from sea-cage aquaculture operations, such as DEPOMOD (Cromey et al. 2002a; Magill et al. 2006), CODMOD (Cromey et al. 2009), AWATS (Dudley et al. 2000), MERAMOD (Cromey et al. 2012), MOM (Hansen et al. 1997). The majority of the published studies using numerical models to simulate aquaculture environmental impacts have been on fin-fish aquaculture (Cromey et al. 2009; Doglioli et al. 2004; Gillibrand and Turrell 1997; Magill et al. 2006; Piedrahita 1988). This is the first study to use numerical modelling methods to examine carbon and DIN loading from spiny lobster aquaculture in sea-cages and its potential impact on the seabed and surrounding water column. Hypothetical sea-cage farms based on typical lobster farm layouts using various combinations of low and high stocking densities (3 and 5 kg m\(^{-3}\)), FCRs (1.28, 3, 5 and 28) and diet treatments (seafood and artificial diet) were used as modelling scenarios in this current study so that the corresponding environmental outputs could be estimated using the modelling tool.

The results of the modelling showed that overall carbon deposition, as well as DIN elevation in the water column, were at their highest directly beneath the sea-cages and gradually decreased as the nutrient plume (carbon and DIN) dispersed away from the sea-cages. This general pattern of dispersal of particulate and dissolved nutrients into the environment away from the sea-cages was observed in all modelled scenarios regardless of the stocking density, FCRs and diet treatments, and is also consistent with the patterns of dispersal of nutrients released from fin-fish farms (Magill et al. 2006; Panchang et al. 1997). Overall, the carbon deposition modelling indicated that the depositional threshold of 0.73 kg C m\(^{-2}\) yr\(^{-1}\) was only exceeded when the hypothetical lobster farm had a high stocking density and poor FCR. Overall, the DIN modelling indicated that the recommended DIN level for aquaculture (500 μg N L\(^{-1}\)) was not exceeded. Therefore, it seems unlikely that sea-cage lobster aquaculture will have an adverse effect on the surrounding environment provided the stocking density and FCR are kept relatively low. However, sensitive habitats, such as seagrass beds and coral reef, may be impacted if lobster farming operations are sited over these habitats as these habitats have lower tolerance to enrichment with nutrients (both carbon and DIN). Tolerance limits for carbon deposition and DIN elevation on these more sensitive
habitats have previously been recommended at 0.55 kg C m\(^{-2}\) yr\(^{-1}\) and 14 µg N L\(^{-1}\) respectively (Díaz-Almela et al. 2008; Lapointe 1997). Based on those limits and the resulting carbon and DIN modelling results from this study, current production of spiny lobsters will only pose a threat if seagrass beds were within a 100 m radius from sea-cages for scenarios of high stocking density with FCRs of 3 and 5, or if coral reefs were within the 200 m radius from the sea-cages for scenarios of low and high density with FCR of 28. One way for regulators to mitigate nutrient loadings on these more sensitive habitats is to apply a buffer zone between the lobster farm and the sensitive habitat, such as requiring lobster sea-cage farms to be sited at least 100 - 200 m away from sensitive habitats.

The combination of stocking density and FCR did affect the overall carbon deposition on the seabed at the lobster farm, with deposited carbon mass positively correlated with the increasing stocking density and FCR. Similarly, modelled results of elevated DIN output from the lobster farm also showed a positive correlation with increasing stocking density and FCR. Apart from these effects (i.e., stocking density and FCR), the type of diets being fed to lobsters had the most pronounced influence on the resulting carbon deposition on the seabed as well as DIN elevation in the water column. Both carbon deposition and DIN elevation models indicated that seafood diet (either solely mussel flesh or a mixture of fish and squid) resulted in higher levels of carbon and DIN output than artificial diet. For example, the deposited carbon mass from the hypothetical *P. ornatus* lobster farm feeding a mixture of fish and squid resulted in 0.05 kg C m\(^{-2}\) yr\(^{-1}\) more carbon deposition than those using either solely mussel or an artificial diet to feed the lobsters. In the case of DIN elevation, regardless of stocking density and FCR, the mean elevated DIN was generally lower (9 - 25 %) at modelled lobster farms feeding an artificial diet compared with feeding a seafood diet consisting of either solely mussel or a mixture of fish and squid. Furthermore, the FCR from artificial diets (FCR 3 – 5) (Hung et al. 2010; Tuan and Hung 2008) is considerably lower than for mixed seafood diets (FCR 26 – 28) (Edwards et al. 2004; Hung et al. 2010). Therefore, it is highly recommended that lobster farmers substitute seafood diet with artificial diet in sea-cage lobster aquaculture in order to minimise environmental impacts from lobster aquaculture. The future development of highly digestible artificial diets using carbohydrate and lipid as the dominant energy source is of importance to aid in maintaining low environmental impacts from lobster aquaculture. Moreover, reducing feed waste can also reduce the overall nutrient loading into environment from a lobster farm.

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Regardless of stocking density, FCRs and the type of diet, when comparing the different layouts of cages (small farm or large farm), the large farm had less carbon deposition under the sea-cages than the smaller farm layout. The orientation and layout of the sea-cages appeared to play a role in determining the accumulation of waste material on the seafloor beneath the sea-cages. This was probably because the orientation of the sea-cages influences the hydrodynamic flows around the farming site (Plew 2011). It is possible that positioning sea-cages in a line in the larger farm layout helped to reduce the cage hydrodynamic drag effects, thus increasing the water currents around and beneath the sea-cages, and thereby dispersing the waste material further away from the sea-cages. Whether this was always the case would greatly depend on the hydrodynamics of a specific aquaculture location and the layout of the sea-cages in relation to the hydrodynamic flow. This study did not simulate DIN outputs using different sea-cage layouts as the overall mean DIN output for all the modelled scenarios in the layout of 80 sea-cages were similar to the ambient background DIN level (5 μg N L⁻¹). Therefore it could be expected that a smaller farm layout will also maintain similar DIN output to ambient background levels and not be of any environmental concern.

6.3.2 Comparing to fin-fish sea-cage aquaculture

The carbon deposition from a lobster farm is markedly lower and has a small zone of benthic impact than for a typical fin-fish sea-cage farm even when the FCR of a lobster farm is at the high end of the reported range. Fin-fish farms generally have FCR of less than 2 (Brooks et al. 2002; Doglioli et al. 2004) while this study used FCRs of 1.28, 3 and 5. The carbon loading from a fin-fish farm (2.5 – 15 kg C m⁻² yr⁻¹) can be at least 18 times more than the modelled lobster farm (maximum deposited carbon mass 0.15 – more than 0.80 kg C m⁻² yr⁻¹) (Lee et al. 2014; Lee et al. 2015b). As for the spatial extent of the carbon deposition, fin-fish farms have been found to have deposited measurable carbon mass to the seabed at distances of up to 1 km away from the farm site (Cromey et al. 2002a; Wu et al. 1994). This zone of elevated carbon deposition is at least nine times further than estimated for a lobster farm operating with similar or lower FCR than for the fin-fish farm. A combination of factors can explain the higher and more extensive spatial distribution of the carbon footprint generated by fin-fish farms, including higher stocking density, greater total faecal output, the presence of stronger current regimes, and deeper farm sites that are often used for fin-fish
farms. It is unlikely in the future that spiny lobster sea-cage farming will be able to greatly increase stocking densities of sea-cages or be shifted to deeper waters with stronger current regimes because lobsters rely on holding onto the surface of the sea-cage netting, and because high density stocking of spiny lobsters tends to depress growth and increase cannibalism (Simon and James 2007). Therefore, the resulting spatial pattern of benthic carbon deposition from lobster farms will tend to be more localised with much less re-suspension of previously settled faecal material and waste feed than the general pattern observed for fin-fish farming.

The relatively minimal DIN elevation of the water column observed from modelling spiny lobster farms was consistent with the low DIN elevation that has been found in sea-cage fin-fish farming. Past studies on DIN output from fin-fish farms have demonstrated that the DIN level around the fish cages are not significantly different from the ambient level (Aksu et al. 2010; Doglioli et al. 2004; Wu et al. 1999). Likewise, this current study showed that the mean elevated DIN in all modelled scenarios at various stocking densities (low and high stocking density), FCRs (3, 5 and 28), and various types of diets (seafood and artificial diets) all resulted in only relatively minor elevation of DIN over and above the ambient background DIN conditions of 5 μg N L⁻¹. For example, only small elevations in the mean DIN (increment of 0.4 up to 3 μg N L⁻¹) from ambient background levels were observed except for when the FCR was at its highest (28) which resulted in a mean DIN elevation of 15 – 25 μg N L⁻¹. Moreover, all modelled lobster farm scenarios generated DIN outputs that were within the recommended threshold level of 500 μg N L⁻¹. Such results indicate that the release of DIN from lobster farms was most likely to be minimal, due to the modelled rapid assimilation of the released nitrogen by phytoplankton and zooplankton communities in the water column. However, it is important that the current conditions are sufficient to dilute and disperse the DIN loading from the spiny lobster sea-cages even at high stocking density. This study showed that a mean current speed of 20 cm s⁻¹ was sufficient to maintain low environmental DIN concentrations, even with high stocking density and poor FCR.

6.3.3 Limitation to numerical modelling

The accuracy of numerical modelling relies heavily on the quality of the data input used for the set-up of the model. For modelling carbon deposition, the values for the
percentage of feed converted to faeces and amount of waste feed released are particularly important parameters to be included in the model. In this study, it was assumed that 22 % of feed was converted to faeces based on previous studies on salmon because the amount of feed converted to faeces for spiny lobster has not been adequately quantified (Chen et al. 1999b). Therefore, it is likely that the depositional model may have underestimated or overestimated the deposited carbon mass for lobster sea-cage farms depending on whether the actual conversion percentage is higher or lower than the 22 % used in the modelling. In scenarios where lobsters were fed with seafood, the values of waste feed of 5 % and FCR of 1.28 – 5 were used and these are much lower than some previously published studies for spiny lobsters, which have shown that the FCR can be as high as 28 (Edwards et al. 2004) and the feed wastage reaching up to 45 % (Wu 1995). Thus, if these higher FCR and feed wastage figures for seafood diets are occurring on a lobster farm, then the model simulation would have greatly underestimated the carbon deposition on the seabed. FCR and feed wastage data based on the specific diet used in a spiny lobster aquaculture operation would be essential to improve the accuracy of the outputs from the simulated depositional model.

The carbon deposition model used in this study assumes that the deposited particulate waste from the hypothetical lobster farm accumulated on the seabed for a year without being broken down. However, in reality the deposited carbon on the seabed will be naturally remediated by microbial activity and sediment feeding by macrobenthos. Previous research has shown that after 5 to 8 months, 3 – 20 % of the deposited carbon will be mineralised with the carbon released into the water column mainly in the form of dissolved carbon dioxide (Hall et al. 1990; Holmer et al. 2002). Using these figures it can be estimated that for benthic deposits of faecal material from spiny lobster sea-cage farming that contained 31 - 41 % of carbon (Lee et al. 2014; Lee et al. 2015a) only 11 – 38 % of the deposited carbon is likely to remain on the seabed up to 8 months after arrival. Hence, the modelled results for accumulated carbon in this current study likely to be overestimates. Therefore, incorporating measures of natural benthic remediation of deposited carbon will greatly improve the accuracy of future model predictions of the benthic impacts of spiny lobster sea-cage farming.

The DIN models were simulated using conversion factors of the percentage of feed converted to DIN based on the wet weight of feed. Both seafood and moist artificial diets contain high moisture content (40 – 80 % moisture) compared to dry artificial diet (8 %) (Simon 2009a; Simon and James 2007; Lee et al. 2015a; Lee et al. 2015b). Therefore,
accounting for the moisture content could have been a confounding factor, which may have led to an underestimation of how much of the feed being fed out was actually converted to DIN after the cultured animal had assimilated its food. For example, the conversion factor for the percentage of feed converted to DIN for the seafood diet (mixture of fish and squid) used in Chapter 4 was 0.92 % based on wet weight of feed whereas if it had been based on the dry weight of feed, the conversion factor would have increased to 3.53 %. In addition, the differences in moisture content between the various types of diet (seafood, moist and dry artificial diets) make it more difficult to compare the DIN modelling results when using wet weight measurements. Thus, it is recommended that future studies use measurements based on the dry weight of feed.

The current study has only provided an initial insight into the relative importance of some key production parameters (e.g., type of diet, FCR, farm layout and stocking density) in relation to the release of key nutrients most likely to cause environmental impacts (i.e., carbon deposition and DIN elevation). The carbon deposition and DIN modelling work in this study were based on laboratory measurements of biological parameters (i.e., settling velocity of faeces and DIN excretion) coupled with hypothetical lobster aquaculture operations, and therefore an important next step is the validation of the modelled results with in situ measurements of benthic carbon accumulation and water column DIN at a range of locations around an operating sea-cage lobster farm. Furthermore, phosphorus in both particulate and dissolved form is also a potentially important driver of environmental change in both the benthic and water column components of the marine ecosystem (Buschmann et al. 2007). Both waste feed and faecal material are contributors to the release of phosphorus (dissolved and particulate) into the environment. Thus, future studies investigating phosphorus loading from waste feed and faecal material of spiny lobster sea-cage farming is required.

6.4 Implications of environmental impact studies on aquaculture

Environmental impact studies on aquaculture activities have been widely used to aid governmental organizations (e.g. Department of Fisheries) and aquaculture farmers in decision making on best practice methods to increase aquaculture production while protecting the integrity of the environment. Such studies can be applied in a variety of assessment
projects such as carrying capacity studies for aquaculture farm expansion, site selection for new aquaculture ventures, and designing cage layouts for maximising farming potential at any one farming site. For example, the Department of Fisheries in Western Australia has used an environmental impact assessment in support of their proposal for a designated zone specifically for the development of aquaculture in Cone Bay located in the Kimberley region of Western Australia (Government of Western Australia, Department of Fisheries 2013). Environment impact assessment was also used by the Tasmanian State Government in Australia for the development and expansion of aquaculture water space in Macquarie Harbour (Tasmania Department of Primary Industries et al. 2011).

Similarly, the current study has significant implications for authorities working to manage the sustainability of the development of spiny lobster sea-cage aquaculture. Results from this study have been included in an environmental impact assessment report for a commercial lobster farm development in Malaysia, that was submitted to the Environmental Protection Department in Malaysia in 2014 (DHI 2014). Within this environmental impact assessment the results of this study were also applied for determining the potential carrying capacities for lobster farm development in a range of potential farming sites. The modelling approach presented in this current research could also be a starting point for the development of a benthic and water column forecasting system to provide real-time information and predictions (e.g., carbon deposition) for lobster farmers wishing to improve the environmental performance resulting from their farm management practice.

6.5 Conclusion

Spiny lobster sea-cage aquaculture, like any other sea-cage aquaculture (e.g., fin-fish species) will result in carbon deposition from settled solid waste (i.e., faecal material and feed waste) on the seabed as well as DIN elevation in the water column from the release of dissolved nitrogen through lobster excretion. However, the results of this study found that the overall impact from both carbon deposition and DIN elevation were limited and lower than for a typical fin-fish farm. The potential environmental impacts from the sea-cage aquaculture of spiny lobsters, such as altering the benthos and eutrophication of the water column, will generally be localised directly below the sea-cages but has the potential to extend to a distance
of up to 200 m from the perimeter of the sea-cage. The type of diet being fed to cultured spiny lobsters plays an important role in determining the intensity of the environmental impacts. Lobsters fed with seafood with very poor FCR tend to have a more extensive impact than artificial diet with good FCR. Thus, lobster farmers should be encouraged to substitute seafood diets with artificial diets with good FCR to reduce the overall nutrient enrichment of both seabed as well as the water column. Based on the modelled scenarios in this study, it can be concluded that to minimise environmental impacts, a lobster sea-cage aquaculture operation needs to be located in hydrodynamically active site and to feed cultured lobsters with a highly digestible artificial diet, that preferably contains suitable lipid or carbohydrate as an alternative source of energy to protein. Moreover, feed waste escaping from the lobster sea-cages should be kept to a minimum.
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