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Advancing the nutrition of juvenile spiny lobster, *Jasus edwardsii*, in aquaculture

Cédric Johan Simon

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Abstract

The development of cost-effective culture systems such as sea-cages and nutritionally adequate formulated diets for juveniles is of major importance to the advance of commercial spiny lobster aquaculture. Formulated diets have the potential to improve production performance, but to date fresh mussels have consistently produced better growth than formulated diets in spiny lobsters. Reduced food consumption due to the poor attractiveness of formulated diets has been suggested as one of the reasons for the slow growth. Whether gut physiology, processing and digestion could also impair consumption on formulated diets remains to be investigated. Diet digestibility, in particular the digestion of carbohydrates included in formulated diets, is one particular area that needs further research. The objective of this thesis was to make some significant advances in addressing two major bottlenecks (i.e., rearing system and nutrition) currently constraining the commercial ongrowing of J. edwardsii juveniles. Key findings were the following:

- Sea-cage culture systems of the right design can provide significant growth advantages over tank culture systems, especially on formulated diets, by addressing some of the dietary inadequacies via supplemental nutrition from biofouling.

- Food consumption on formulated diets is significantly constrained in J. edwardsii juveniles because gut throughput is reduced due to a small foregut capacity (2.5-3%), expansion of the baseline dry formulated diet after ingestion, and slow rates of foregut filling (1-2 h), foregut evacuation (10 h), faecal throughput (>34 h) and appetite revival on the formulated diet (>18 h).

- An intensified intracellular digestion of the formulated diet in the digestive gland may be responsible for the lengthy appetite revival.

- Improving the digestibility of formulated diets appears to be essential to maximise the amount of nutrient assimilated per meal in J. edwardsii.

- Diet digestibility can be improved significantly by decreasing the starch inclusion level and selecting particularly digestible carbohydrate sources for energy (i.e., dextrin, cooked starch, glycogen, and native wheat starch) and feed binder (i.e., carboxymethyl cellulose). Among the digestible carbohydrate sources identified, native wheat starch and carboxymethyl cellulose appears to be best utilised by J. edwardsii juveniles.
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1. Chapter One – General introduction

1.1. Introduction

1.1.1. Spiny lobsters

There are over 47 species of spiny lobsters (Crustacea: Decapoda: Palinuridae) of which 33 species support commercial fisheries. The most commercially important genera are *Panulirus*, *Jasus* and *Palinurus*. Members of the genus *Panulirus* are found in the tropical regions of the world, while the genera *Jasus* and *Palinurus* inhabit the temperate regions (Lipcius & Eggleston, 2000). Three species of spiny lobster are found in New Zealand, *Jasus edwardsii*, the southern or red rock lobster, *Sagmariasus verreauxi*, the packhorse or green rock lobster, and a deep-water species, *Projasus parkeri* (Booth, 2006). All three genera make up the broad evolutionary group known as the Silentes because they lack a stridulating organ at the base of the antennae unlike that found in other palinurids (Booth, 2006). Spiny lobsters have a strong calcareous exoskeleton which is shed at ecdysis to allow growth. The exoskeleton is reinforced to protect internal organs of the cephalothorax and the gill chamber, which opens near the base of the five pairs of walking legs. The abdomen is segmented and muscular with pleopods on the ventral surface, a pair of biramous uropods and a telson forming the tail fan at the posterior end.

In New Zealand, *J.edwardsii* is by far the most important lobster species in terms of commercial, recreational and customary fisheries. It occurs throughout the country from the Three Kings Islands in the north, to the Auckland Islands in the south, and to the Chatham Islands in the east. *S.verreauxi* is far less abundant and mainly found in northern New Zealand (Booth, 1986). These spiny lobsters inhabit shallow near-shore rocky or reef environments, but can also be found on deeper light foul ground. They are cryptic, hiding in rock crevices during the day and come out at night to feed (Booth, 2006). *J. edwardsii* grows discontinuously to the minimum harvestable size (around 95 mm carapace length) in six years. A mature female lobster (3 to 7-year-old) can carry between 100,000 and 500,000 fertilized eggs for 3-6 months which hatch into a short naupliosoma stage followed by a long (12-22 months) planktonic phyllosoma phase (Booth, 1986, 2006). The phyllosoma passes through 11 recognisable larval stages before metamorphosing into the puerulus stage, which
swim back to the coast often over great distances (>100 km) to settle in shallow water (Jeffs et al., 2005). The completely transparent puerulus (25 mm) moults into a juvenile spiny lobster 1-3 weeks after settlement (Booth, 2006).

1.1.2. Current status of spiny lobster fisheries

Spiny lobsters are one of the most highly esteemed and valuable seafood products and form the basis of major commercial fisheries throughout the world (Lipcius & Eggleston, 2000). The world catch of spiny lobsters (i.e., palinurids) fluctuates between 70,000 and 85,000 tonnes (t) per annum for the last 40 years, with 71,729 tonnes harvested in 2008 (FAO Fishstat database, 2008). The genera Panulirus, Jasus and Palinurus account for 82.2%, 12.2% and 5.6% of the total palinurid landings respectively (Lipcius & Eggleston, 2000).

The New Zealand fishery production of all spiny lobsters remains static at around 2,500-3,000 t per annum (i.e. commercial and recreational catch) (Jeffs, 2003a). The once large Chatham Islands fishery with landings of up to 6,000 t per year now has annual landings of about 300 t (Booth, 2006). In 2008, 2,638 t of J. edwardsii worth NZD 180M were harvested, which made up 13.2% of the total New Zealand fisheries export returns (NZ Seafood Industry Council, 2008). The bulk of New Zealand’s spiny lobster exports are composed of live J. edwardsii (S. verreauxi accounting for less than 1% of exports) which are mostly destined to Asian markets such as Hong Kong, China, Japan and Taiwan, while a much smaller quantity of frozen tails are sold mainly in the USA (Booth, 2000).

1.1.3. Rationale for spiny lobster aquaculture

In recent years there have been major concerns regarding the over-exploitation of wild stocks of spiny lobsters, stimulated by the intense international demand and high market prices. Until the 1990s spiny lobster populations in New Zealand were declining steadily, but the implementation of individual transferable quota with set total allowable commercial catches, as well as other management strategies, have been highly successful in slowing this trend (Lipcius & Eggleston, 2000). In contrast, some fisheries (e.g. J. lalandii in Namibia) have severely depleted stocks and have suffered virtual collapse of the fisheries due to poor
management (Lipcius & Eggleston, 2000). The huge demand for spiny lobsters worldwide has forced well-managed fisheries to their maximum sustainable capacity, leaving reduced opportunity for further increases in production (Van Barneveld, 2001; Bryars & Geddes, 2005). Even so, global demand for spiny lobsters is undersupplied, resulting in high market prices (NZD 68.5 kg\(^{-1}\) of live \textit{J. edwardsii}) (NZ Seafood Industry Council, 2008). In addition, an undersupplied niche market for plate-sized lobsters (100-300 g) exists in Japan (Jeffs, 2003a). As this product is under the minimum legal catch size of most commercial fisheries, there is a good opportunity for aquaculture to target this lucrative market and attract premium prices with a product of constant quality and size, that is available all year round (Booth & Kittaka, 2000). These high prices and market opportunities have created a great amount of commercial and research interest in increasing yield via some form of aquaculture (Van Barneveld, 2001). The spiny lobster \textit{J. edwardsii} has therefore been selected as one of five high-value potential candidates for New Zealand aquaculture in an attempt to diversify the local aquaculture industry from the three currently grown species: Greenshell™ mussel, Chinook salmon and Pacific oyster (Lee, 2004).

1.1.4. Development of spiny lobster aquaculture

1.1.4.1. Adult holding and ongrowing

Holding and ongrowing wild-caught adult spiny lobster through a moult to increase weight yield could be seen as a relatively simple form of aquaculture used for value-adding. Higher price from a given catch can be obtained through not only increasing weight, but also allowing re-growth of missing limbs, ameliorating exoskeleton colour (i.e., speckled/white lobsters changed to a redder coloration using carotenoid pigments in the diet) and targeting markets at times of peak demand and price (Van Barneveld, 2001). It was shown in a pilot study that adult \textit{J. edwardsii} can be held alive for an extended period of time (16 weeks) in sea-cages and with excellent survival (96%) if fed mussels regularly (Lorkin et al., 1999). Bryars and Geddes (2005) also demonstrated the product enhancement potential of live-holding adult lobsters using formulated diets. Live-holding and fattening adult spiny lobsters is now performed by a few commercial groups in South Australia (Van Barneveld, 2001; Bryars & Geddes, 2005) and New Zealand (Jeffs, 2003a) as well as in India (James & Marian,
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2003), Brazil and Japan (Booth & Kittaka, 2000). Other forms of aquaculture are based on the ongrowing of early juveniles.

1.1.4.2. Culture of hatchery produced early juveniles

There are two potential sources of early settled juvenile lobsters (i.e., pueruli and first instar juveniles); larval culture and the collection of early juveniles from the wild (Jeffs & Hooker, 2000; Geddes et al., 2001). The larval culture of *J. edwardsii* has been the subject of intense research in Japan (Kittaka, 1994, 1997), New Zealand (Tong et al., 1997, 2000) and Australia (Thomas et al., 1998a; Ritar, 2001). However, only a very small number of pueruli have been raised from eggs in the three countries and hatchery technology is still a long way from becoming commercially viable (Kittaka et al., 2005). This holds true for all palinurid species (Kittaka, 2000; Kittaka et al., 2005). With breeding and maturation having been achieved quite successfully with all species examined under laboratory conditions (MacDiarmid & Kittaka, 2000; Smith & Ritar, 2005), the major issue remains the high mortalities reported during the long and problematic phyllosoma phase (Kittaka, 1997). In culture, the planktonic phyllosoma of *J. edwardsii* passes through 11 distinct larval stages (17 instars) before reaching the postlarval puerulus stage some 9 to 12 months later (Kittaka, 1997; Kittaka et al., 2005). Larval mortalities are thought to be the consequence of poor nutrition (Kittaka, 1997; Matsuda, 2009), as well as disease and tank design issues (Kittaka, 1997, 2000; Ritar, 2001; Matsuda & Takenouchi, 2005).

1.1.4.3. Ongrowing wild-caught early juveniles

Until successful hatchery technology is developed, the only practical way of increasing the volume of lobster production is to capture wild early juvenile lobsters and to culture them to market size, thereby circumventing the high natural mortality (75-97%) that otherwise occurs for settling juveniles in the wild (Van Barneveld, 2001). In New Zealand and Australia, the harvest of early juveniles was allowed legally under compensatory mechanisms aimed at achieving “biological neutrality” by counteracting the potential increased fishing mortality (Gardner et al., 2006). The harvest of commercial quantities of early juveniles for ongrowing was made possible in New Zealand in 1996. The Ministry of
Fisheries approved the issue of special permits to harvest 40,000 early juveniles (i.e., 30 kg green weight) for aquaculture in exchange for one tonne of term transferable quota (Jeffs, 2003a). A high abundance of early settled juveniles has been found around Gisborne, but extreme inter-annual variations in the supply of lobsters have created difficulties for aquaculture operations in gathering sufficient early juvenile lobsters to stock aquaculture ongrowing operations (Booth & Tarring, 1986; Jeffs, 2003a). In addition, high collection cost, mortalities during ongrowing and elevated costs of land-based systems have severely constrained the commercial aquaculture of lobsters from wild-caught juvenile in New Zealand (Booth, 2006). No further special permits have been issued in recent years making the collection of early juveniles for culture currently illegal in New Zealand (Phil James, pers. comm., 2008). Nevertheless, the technique of collecting and ongrowing pueruli and early juveniles is now widely applied in countries such as Indonesia, Phillipines, Malaysia, Taiwan, Singapore, India (Booth & Kittaka, 2000) and notably Vietnam, where sea-cage production was estimated to exceed 3,000 t (USD 90M) in 2006 (Williams, 2007). However, this aquaculture activity is based entirely on feeding fresh by-catch from coastal fisheries (mostly trash fish), which has serious environmental impacts. In 2007, environmental degradation from inappropriate feeding practices and unmanaged industry development led to mass mortalities in Vietnam through the occurrence of the “milky lobster disease”, reducing production to 1,400 tonnes (Williams, 2009). These environmental impacts together with higher labour costs would not allow this approach to proceed in New Zealand (Williams, 2007).

1.1.5. Aquaculture systems for ongrowing early juveniles

1.1.5.1. Land-based tank culture

Most studies to date have reared lobsters in land-based recirculating or flow-through tanks (Hooker et al., 1997; Crear et al., 2000), but there has been relatively little research on optimising tank design (Geddes et al., 2001). Providing artificial shelters in culture tanks, such as small plastic tables, pipes, oyster mesh and bricks have shown to improve survival but not the growth rate of juvenile lobsters (Crear et al., 2000; James et al., 2001). In brief, optimal water parameters for *J. edwardsii* are; temperature at 18-22 °C (Thomas et al., 2000),

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dissolved oxygen above 80% saturation, nitrogenous waste (NH$_3$ <0.5 mg l$^{-1}$; NO$_2$ <1 mg l$^{-1}$; NO$_3$<100-140 mg l$^{-1}$), pH between 7.8 and 8.4, and salinity between 33 and 35 ppm (Booth & Kittaka, 2000). Photoperiod has been shown to affect moult increment, with growth being greatest when a period of darkness is provided because it stimulates foraging and feeding. However, the dark period should be of limited duration because nocturnal activity can result in unnecessary energy expenditure (Geddes et al., 2001). The gregarious behaviour of spiny lobsters compared to homarid, or clawed, lobsters is a major advantage for their aquaculture because spiny lobsters can be stocked at high density with low levels of cannibalism, provided the lobsters are fed to satiety. Early juveniles (<30 mm carapace length, CL) can be stocked at densities up to 100 ind m$^{-2}$ of tank floor area without detrimental effect on growth and survival (James et al., 2001). The best growth and survival of adults (75 mm CL) can be attained at stocking densities of 15-30 ind m$^{-2}$ (Rayns, 1991), although such densities are unlikely to be economically viable (Geddes et al., 2001).

The major bottleneck with commercial production of J. edwardsii is the slow growth rate of this species. Juvenile lobsters held in Tasmania took three years to reach 300 g when fed fresh mussels and held at an optimum water temperature of 18 °C (Kittaka and Booth, 2000). Most other studies have only extrapolated early juvenile growth rates to estimate the growth period required to reach market size rather than experimentally culturing through to market size (Manuel, 1991; Hooker et al. 1997). Growth rates in captivity have been generally equivalent to those measured in the wild at similar temperatures, which suggests no cumulative negative effect of captivity (Geddes et al., 2001). Nevertheless, the high infrastructure and running costs associated with land-based facilities make the culture of J. edwardsii in tanks not commercially viable. The economic prospect for commercial ongrowing of juvenile lobsters would improve with the development of sea-cage technologies and species-specific formulated diets (Jeffs & Hooker, 2000).

1.1.5.2. Sea-based cage culture

Sea-cage culture appears as the most economical approach to grow spiny lobster in commercial quantities but published research work remains scarce. The potential of sea-cages for the culture of early juveniles of J. edwardsii was assessed at three locations in New
Zealand (i.e., Hauraki Gulf, Waitemata Harbour and Wellington Harbour) by Jeffs and James (2001). Growth rates were similar, if not higher than those recorded in tanks, possibly due to the presence of additional natural food material fouling the cages. However, mass mortalities occurred at the Hauraki Gulf site due to water temperatures rising above 24 °C in summer, highlighting the importance of site selection. The lower water temperatures of the Wellington Harbour led to reduced mortality, and despite the slower growth rate than achieved at the more northerly sites, it might be better suited for year round commercial production.

Commercial trials have also been performed by various aquaculture groups in the Coromandel and Marlborough Sounds (Jeffs, 2003a). The commercial sea-cages that have been used to date are made of a sturdy stainless steel frame, are suspended beneath mussel long-lines, and require heavy lifting equipment for manoeuvring and feeding (James, 2002a, b). Progress in sea-cage design and rearing practices should reduce the associated labour and boat costs. Factors that need to be taken into consideration when selecting suitable sites for sea-cage farming of J. edwardsii would include (Jeffs, 2003a):

- Sheltered to semi-sheltered waters with low to medium water current
- Waters of low natural turbidity and pollution
- Salinity of around 33 ppm and temperature <24 °C with little fluctuation
- Depth of between 5 and 30 m preferably over soft sediments
- Close to boat and barge access as well as mooring point for servicing
- Potential for integration with mussel farming
- Clear of navigation routes

1.1.6. Nutrition of spiny lobsters

1.1.6.1. Feeding behaviour

Spiny lobsters, like all other decapod crustaceans, rely heavily on chemical signals for food detection, localisation and ingestion (Derby et al., 2001; Grasso & Basil, 2002).
Leaching of low molecular weight compounds such as amino acids, amines, nucleotides, and organic acids from the food forms the basis of the chemical signal (Derby, 1984; Williams et al., 2005). Among the plethora of chemoattractants and stimulants identified, glycine, taurine and betaine are the most potent compounds for *J. edwardsii* (Tolomei et al., 2003).

In the wild, *J. edwardsii* is active mainly at night, mostly foraging and feeding just after dusk until about midnight (McKoy & Wilson, 1980). Higher oxygen consumption and ammonia excretion related to activity and feeding have been measured in tanks at night (Radford et al., 2004). Nevertheless, captive lobsters are often found to lose their diurnal pattern of activity in culture systems through time (James et al., 2001). Feeding also slows down and ultimately ceases a few days before and after ecdysis (Geddes et al., 2001). Spiny lobsters have a messy feeding habit, even more so than penaeid shrimps, slowly grinding up potential food with their mouthparts (Fielder, 1965; Cox et al., 2008). The mouthparts involved during feeding include the pereiopods to seize the food, and maxillipeds, maxillae and mandibles to fragment and orient the food towards the mouth (Sheppard et al., 2002). The mandible, with its calcified molar and incisor, is functionally specialised for grinding large calcified material (Geddes et al., 2001). The poor feed handling efficiency of lobsters represents a key issue for the development of formulated diets for aquaculture.

1.1.6.2. Natural diets

Spiny lobsters consume a wide range of marine food including slow moving benthic invertebrates such as molluscs, echinoderms, polychaete worms and crustaceans as well as occasional amounts, possibly incidental, of macroalgae (Fielder, 1965; Juanes, 1992; Diáz-Arredondo & Guzmán-Del-Proo, 1995; Lipcius & Eggleston, 2000; Nelson et al., 2006). Fresh marine food is preferred to decomposing or terrestrial food (Fielder, 1965). This diet selection characterises spiny lobsters as opportunistic carnivores, which have evolved to efficiently utilise food that is high in protein, low in lipid, and contains moderate to high levels of carbohydrate (i.e., glycogen) (Williams, 2007). Ontogenetic dietary shifts between early and late juveniles have been reported in some species (Jernakoff et al., 1993; Johnston, 2003). In *J. edwardsii* carbohydrates such as laminarin appear to be more important in the diet of small juveniles (Johnston, 2003). The ability to exploit a variety of diets throughout life
history implies a high degree of flexibility in the digestive physiology of *J. edwardsii*
(Johnston, 2003).

1.1.6.3. Feeding fresh food in culture

Previous nutritional studies on juvenile *J. edwardsii* have used a variety of fresh natural foods such as cockle, squid, mussel and shrimp (Kensler, 1967; James & Tong, 1998; Bryars & Geddes, 2005). Fresh food has been used to ongrow lobsters in all commercial ventures to date (Williams, 2007). Mussels (i.e., *Perna canaliculus, Mytilus galloprovincialis* and *M. edulis*) in particular have been shown to be excellent feed, supporting high survival and the best growth rates reported to date in temperate spiny lobster species (Hooker et al., 1997; James & Tong, 1997; James, 1998; James & Tong, 1998; Geddes et al., 2001; Dubber et al., 2004; Williams, 2007; Johnston et al., 2007). Therefore, they have been used extensively as a control diet to assess growth performance of lobsters fed newly developed formulated diets (Williams, 2007). In New Zealand the large quantity of waste mussels (i.e., >500 t per annum) produced by the Greenshell™ mussel industry makes their use on a commercial scale feasible, particularly if the lobster farms are integrated with mussel farms (James & Tong, 1998; Jeffs & Hooker, 2000; Geddes et al., 2001). Nevertheless, there are major drawbacks associated with using fresh mussels and fresh food in general for the aquaculture of spiny lobsters, which makes the practice not cost-effective:

- High purchase cost (Jeffs & Hooker, 2000)
- High storage cost and the inability to store fresh mussels for extended period. Early juvenile fed frozen or aged mussels grew significantly slower than those fed fresh mussels (James & Tong, 1997)
- High labour cost associated with opening mussels (i.e., for small juveniles), feeding and cleaning tanks or sea-cages (Jeffs & Hooker, 2000; Jeffs & James, 2001)
- Seasonal variation in availability of natural foods is a major problem in most countries (Geddes et al., 2001). However, in New Zealand the well-developed mussel aquaculture industry (32,000 t in 2008) would allow growers to have access to large quantities of suitable natural food throughout the year (NZ Seafood Industry Council, 2008)
1.1.6.4. Formulated diets

The success of intensive spiny lobster aquaculture worldwide will invariably depend on providing a cost-effective and nutritionally adequate formulated diet (Williams, 2007). In the past decade, considerable research effort has been dedicated to the development of formulated diets for spiny lobster but with relatively slow progress. As shrimp and lobsters share some similarities in their feeding strategies, the characteristics of shrimp diets, such as high attractiveness, palatability and water stability have been considered to suit the feeding behaviour of lobsters (Crear et al., 2000). External mastication by lobster mouthparts tends to disintegrate pellets quickly, requiring stable diets of the correct physical size to optimise food intake and reduce wastage during manipulation (Sheppard et al., 2002). *J. edwardsii* have been found to readily ingest commercial shrimp diets, but grow slower when fed these diets than when fed a fresh mussel diet (Crear et al., 2002). Most studies on lobster nutrition have closely followed the approach used for penaeid nutrition research, focusing on developing dry pellets with fishmeal as the main protein source (Glencross et al., 2001; Ward et al., 2003; Smith et al., 2003, 2005a; Johnston et al., 2007). With the ornate lobster, *Panulirus ornatus*, and the western spiny lobster, *P. cygnus*, growth improved progressively as dietary protein content increased, suggesting a high protein (>56% of dry matter, DM) requirement (Glencross et al., 2001; Williams, 2007). A lower protein requirement (42-47% DM) was determined for small *J. edwardsii* juveniles (Ward et al., 2003). In another study, *J. edwardsii* juveniles grew best on diets containing 10-12% DM lipid, with no negative effect of lipid up to 18.5%, and 17.5-29% DM gelatinised starch (Johnston et al., 2003). Nevertheless, the slow growth of the temperate lobster species (i.e., *P. cygnus* and *J. edwardsii*) on these formulated diets in comparison to a commercial kuruma prawn, *Marsupenaeus japonicus*, diet and fresh mussel, makes the outcomes of the latter nutritional experiments questionable (Williams, 2007). Other protein sources, such as mussel meal and krill meal, were identified to produce better growth rates but their high cost for inclusion in a formulated diet restrict their inclusion to low levels to produce cost-effective diets (Fordyce, 2004; Williams, 2007). Several reasons for the poor growth rates of spiny lobster on formulated diets have been suggested as follows:
• Inadequate amino acid profile of fishmeal as the main dietary protein source (Fordyce, 2004)

• Deficiency in particular essential lipid classes such as cholesterol (Williams, 2007)

• Poor digestibility and utilisation of dietary carbohydrates (Johnston et al., 2003; Ward et al., 2003)

• Incorrect pellet size and physical form restricting consumption (Geddes et al., 2001; Sheppard et al., 2002).

• Poor water stability resulting in excessively rapid leaching of chemoattractants and feeding stimulants from the feed, in turn reducing consumption (Williams et al., 2005)

• Lack of growth stimulators from krill, mussel, or squid meals (Cruz-Ricque et al., 1987; Williams, 2007)

• Inadequate frequency of feeding of test diets (Smith et al., 2005a; Williams, 2007; Johnston et al., 2008)

• Reduced food intake caused by poor attractability and palatability (Glencross et al., 2001; Williams et al., 2005; Nelson et al., 2006; Johnston et al., 2007)

Issues involved around the gut physiology and digestive ability of spiny lobsters to process formulated diets have been largely overlooked by nutritionists. This represents a key unexplored research area which has the potential to provide explanations regarding the poor food consumption and associated slow growth rates of spiny lobsters on current formulated diets. Investigating key aspects of the digestive processing and digestion of formulated diets in comparison with fresh food could lead to a better understanding of the feeding and nutritional requirements of the studied species, which are critical factors for its aquaculture. Studying these aspects in cultured J. edwardsii juveniles was one of the major aims of the present thesis.
1.1.7. Digestive physiology of spiny lobsters

1.1.7.1. Gut structure and function

The gut of crustaceans is divided into three distinct regions: fore-, mid-, and hindgut. Foregut and hindgut\(^1\) are ectodermal in origin and have a chitinous lining while the midgut is devoid of any cuticle and composed of different organs such as the digestive gland\(^2\) and diverticula.

The foregut is divided into an oesophagus and a stomach with anterior and posterior chambers. The foregut chambers are specialised for the treatment of ingested food and include a gastric mill for crushing particles and mixing with digestive fluid, several longitudinal grooves composed of setae for directing digesta, and a filter-press to prevent particles above 1 µm in diameter from entering the digestive gland (Brunet, 1994). The gastric mill is lacking in larval lobsters (Factor, 1995) and early phyllosoma stages (I-II) have a poorly developed filter-press that allows large particles to enter the digestive gland (Smith et al., 2009). The foregut is the site of primary digestion by enzymes that flow from the digestive gland. Nutrient absorption at the level of the foregut is negligible due to the chitinous lining of the epithelium. The ingested food enters the anterior chamber, passes through the gastric mill into the pyloric chamber, and returns anteriorly to pass through the system again several times until the food particles are small enough to flow through the filter-press into the digestive gland. The larger non-digestible particles are passed directly into the hindgut for excretion (Fig. 1.1.) (Ceccaldi, 1997).

The digestive gland plays the major digestive role in decapods which includes the secretion of digestive enzymes, intracellular digestion and absorption of nutrients, storage of reserves (i.e., lipid, glycogen, minerals), distribution of reserves during the intermolt cycle, and disposal of waste products (Ceccaldi, 1997). It represents 2 to 6% of the total body wet weight of decapods and is composed of two glandular lobes flanking the foregut and anterior midgut. The digestive gland opens from the digestive tract by a pair of primary ducts, one leading to each half of the gland, which branches into secondary ductules and extensively subdivides to form a complex of blind-ending tubules that constitute the glandular mass.

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\(^{1}\) Also called intestine; the term hindgut was used throughout the present study.  
\(^{2}\) Also called hepatopancreas; the term digestive gland was used throughout the present study.
Circular and longitudinal fibres around each tubule are responsible for the movement of liquid, nutrients and cells through the organ and the emptying of secretions and wastes into the foregut and hindgut respectively. The diverse functions of the digestive gland are achieved by several types of specialised epithelial cells (see section 1.1.7.2.) (Ceccaldi, 1997).

Fig 1.1. Hypothetical generalised diagram of circulation of digestive gland fluid and ingested food in the decapod foregut. Dotted lines represent the path of solid food; solid lines represent the path of fluid. AC, anterior chamber (cardiac stomach); AD, anterior diverticulum of midgut; DG, digestive gland opening; FP, filter-press; HG, hindgut; LG, lateral grooves; MG, midgut; O, ossicles of gastric mill; OES, oesophagus; PC, posterior chamber (pyloric stomach); PCG, dorsolateral grooves; VG, ventral grooves (from Dall & Moriarty, 2003).

Diverticula are found in several decapod crustaceans but vary in number and length depending on the species. Lobsters have a short posterior diverticulum extending from the midgut (Dall & Moriarty, 1983). The diverticulum and hindgut may have a role in the absorption of food, ions, and water (osmotic balance) but the contributions to the processes of digestion and absorption are probably minor because of the small surface area in comparison to the tubules of the digestive gland (Factor, 1995). The diverticulum may also contribute components which can activate enzymes or promote changes in pH (Dall & Moriarty, 1983).
The anterior epithelium of the midgut is composed of secretory cells lining the lumen that produce a peritrophic membrane which envelop non-digested material to facilitate excretion. The synthesis of the peritrophic membrane has nutritional and energetic costs (Ceccaldi, 1997).

The hindgut is a simple cuticle-lined tube which extends the length of the abdomen and ends at the anus located at the ventral surface at the base of the tail. Epithelium cells containing numerous mitochondria occur along the hindgut which may have an important role in water absorption and ion transport (Ceccaldi, 1997). The posterior end of the hindgut is composed of a sphincter and a rectum that intake seawater to assist in defecation and possibly the removal of electrolytes excreted in the gut (Dall & Moriarty, 1983).

1.1.7.2. Digestive cells of the digestive gland

In Decapoda, the tubules of the digestive gland are lined by epithelial cells, which are differentiated into four distinct types: E- (embryonic), F- (fibrillar), B- (blister-like) and R- (resorptive) cells (Brunet et al., 1994). The origin and function of each line of cell-type are contentious but most recent reviews tend to agree that E-cells differentiate into F- and R-cells, and B-cells arise from F-cells (Ceccaldi, 1997). E-cells are found at the apex (distal tip) of the tubules, are small with a large nucleus, and are the only cell-type to display mitosis. F-cells have a highly basophilic cytoplasm (i.e., stain blue to dark purple in H&E stain preparation), contain enzymes that are secreted during the extracellular digestive phase in the foregut, and might uptake nutrients and fluids via pinocytosis. B-cells have an immense vacuolar apparatus that is likely to play a role in the intracellular digestive phase and the excretory elimination of residual substances. R-cells have long microvilli and assimilate nutrients via contact digestion and molecular transport into the cell where lipids and glycogen are stored (Fig 1.2.) (Brunet et al., 1994).
Fig. 1.2. Schematic drawings of the ultrastructural details of R-, F- and B-cell types of the digestive gland tubules. ac, apical complex; bi, basal invaginations; bl, basal lamina; cm, circular muscle fibre; cv, clear vesicle; dv, dense-core vesicle; Fe, iron granule in supranuclear vacuole; gly, glycogen; gol, Golgi body; HEM, blood surrounding tubule; ld, lipid droplet; lm, longitudinal muscle fibre; Lu, lumen of tubule; myo, myoepithelial network; np?, possible neurosecretory process; pin, pinocytic channels and vesicles; sec, surface enteric coat; vac, vacuole. Transition zone (between B-cell zone and distal tip) and B-cell zone (between proximal region and transition zone) correspond to locations over the length of the tubule (from Dall & Moriarty, 2003).

1.1.7.3. The digestion process

Digestion begins almost immediately after feeding. The ingested food is physically processed by the gastric mill, and chemically digested by enzymes already present in the
foregut and freshly secreted by the F-cells of the digestive gland during feeding (Dall & Moriarty, 1983). The digestion is performed at slightly acidic pH (5-7) which corresponds to the optimum activity range for most digestive enzymes (Gibson & Barker 1979). This primary extracellular phase of digestion differs in duration among decapods, foregut retention time ranging from 2 h in penaeid shrimps (Nunes & Parsons, 2000) to 12 h in larger decapods such as lobsters (Barker & Gibson, 1977; Sarda & Valladares 1990) and crabs (Hill, 1976). The later intracellular digestive and absorptive phase begins with the migration of the dissolved nutrients to the digestive gland, and finishes 12 to 48 h after feeding with the excretory phase of waste products (i.e., holocrine extrusion of B-cells) (Dall & Moriarty, 1983; Brunet et al., 1994). The regeneration of hepatopancreatic cells via mitosis of the E-cells starts around 24 h after feeding and marks the end of the digestive cycle in most adult decapods (Al-Mohanna & Nott, 1986).

Decapod crustaceans have a wide range of digestive capabilities mediated by the production of various enzymes including proteases (e.g., trypsin, chymotrypsin, carboxypeptidases A and B), esterases, lipases and carbohydrases (e.g., α-amylase, α-glucosidase, α-maltase, α-saccharase, galactosidase, chitinase, chitobiase and cellulase) (Ceccaldi, 1997). A detailed review of the activity and type of digestive enzymes present in Decapoda can be found in Gibson and Barker (1979). The activity of digestive enzymes is known to vary according to the water temperature, the time of day and night (i.e., circadian rhythm) (Rodriguez et al., 1976), the moulting stage (Perera et al., 2008a), the nutritional condition (Johnston et al., 2004a), through ontogenic development (Johnston, 2003), and during vitellogenesis (Ceccaldi, 1997). Food intake and diet composition also induce adaptive changes in enzyme activities in Crustacea (Barker & Gibson, 1977; Van Wormhoudt et al., 1980; Le Moullac et al., 1994). Quantitative changes in digestive enzyme activities related to ingestion of formulated diets have not been determined in spiny lobsters. Enzymes present in the digestive tract of spiny lobsters have been identified but studies have mainly covered the biochemical aspects of enzymes and their activity changes during ontogenic development and moulting (Johnston, 2003; Perera et al., 2008a). Although much may be assumed about the digestive system of spiny lobsters based on studies of other decapod crustaceans, the development of species-specific diets and feeding practices for spiny lobster aquaculture requires further studies (Geddes et al., 2001).
1.2. Objectives of the present thesis

The main objective of this thesis is to make some significant advances in addressing the two major bottlenecks (i.e., rearing system and nutrition) currently constraining the commercial ongrowing of *J. edwardsii* juveniles. The research has strong commercial application and follows on from previous research in New Zealand and Australia on the nutrition and aquaculture of *J. edwardsii*. This includes investigating critical aspects of the gut physiology, processing and digestion of formulated diets in relation to fresh mussels in an attempt to comprehend the reasons for the poor performance of current formulated diets for spiny lobsters. Diet digestibility, in particular the digestion of carbohydrates included in formulated diets, both as binders and as an energy source, is one particular area that is currently poorly understood. Therefore, there is a need for the research to identify the most digestible carbohydrate source for future use in formulated diets for *J. edwardsii*. The specific aims of the thesis (for each chapter) are as follows:

**Chapter 2:**
- develop and test a novel sea-cage rearing system that would be cost-effective for culturing juvenile *J. edwardsii* in New Zealand;
- compare the performance of the prototype sea-cage with an experimental land-based tank and a commercial sea-cage; and
- compare the performance of fresh mussel, a dry formulated diet, a moist formulated diet, and natural biofouling alone for the ongrowing of juvenile *J. edwardsii* in the prototype sea-cage.

**Chapter 3:**
- measure the rate of feed intake on the dry formulated diet and fresh mussel flesh for different sizes of *J. edwardsii* juveniles; and
- measure the physical capacity of the foregut and investigate the potential relation between foregut volume and maximum feed intake.

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3 The dry formulated diet tested in this chapter was used as a baseline diet in the subsequent chapters for measuring key parameters of feeding and digestive processing in comparison with a diet of fresh mussel flesh in *J. edwardsii* juveniles (chapters 3, 4 and 5) and for comparison with other experimental diets (chapters 6 and 7).
Chapter 4:

- measure the food consumption and performance of *J. edwardsii* on the dry formulated diet at various feeding frequencies;
- characterise appetite revival on the formulated diet and fresh mussel flesh; and
- measure the foregut evacuation, gut throughput rate and apparent digestibility on the two diets for three different sizes of *J. edwardsii* juveniles.

Chapter 5:

- investigate the digestive enzyme activity changes in response to feeding on the formulated diet and fresh mussel flesh within the foregut and digestive gland; and
- measure the changes in digestive fluid pH and digestive gland structure in response to feeding on the two diets in *J. edwardsii* juveniles.

Chapter 6:

- estimate the digestibility of various carbohydrate sources by measuring the rates of hydrolysis *in vitro* using enzyme homogenates; and
- estimate the digestibility and utilisation of various carbohydrate sources by measuring post-prandial haemolymph glucose concentrations following ingestion of semi-purified diets containing different carbohydrate inclusion levels and sources.

Chapter 7:

- measure the apparent digestibility (i.e., dry matter, total starch, nitrogen and carbon) of fishmeal-based formulated diets containing different carbohydrate sources, inclusion levels, binder types and ingredient particle sizes in large *J. edwardsii* juveniles;
- compare these apparent digestibility estimates with those obtained from the two different methods used previously (chapter 6); and
- identify the best carbohydrate source and binder for use in future formulated diets for *J. edwardsii*. 
1.3. Notes on the thesis structure

The present thesis is structured with a general introduction, six research chapters, and concludes with a general discussion. The research chapters are referenced throughout the thesis as their corresponding published journal articles rather than by their chapter number. The status and reference of the corresponding publications are given below for clarification:


Plate 2. Sea-cage culture of *Jasus edwardsii* juveniles. A) A prototype sea-cage with reduced biofouling at locations accessible to the cultured lobsters (Photo: Phil James, 2007); B) Measuring carapace length of experimental lobsters during a census; C) Dry formulated diet; D) Moist formulated diet; E) Differences in exoskeleton colours between juvenile lobsters reared in tanks on the formulated diet (left) and in sea-cages on fresh mussel (right); F) Final size of juveniles reared in the prototype sea-cages on four dietary treatments (from left to right: unfed, moist formulated diet, dry formulated diet, and fresh mussel), note the darker exoskeleton of sea-cage reared lobsters compared to tank-reared lobster on the dry formulated diet (E, left).
Chapter 2

2. Chapter two – Performance assessment of formulated diets in a novel sea-cage design

Published as:


2.1. Introduction

Sea-cage culture (Jeffs & Hooker, 2000; Jeffs & James, 2001) and the development of a cost-effective nutritionally adequate formulated diet (Williams, 2007) have appeared as crucial elements to improving the economic prospects for commercial spiny lobster farming (Jeffs & Hooker, 2000). Research on sea-cage farming of spiny lobster remains scarce (Jeffs & Hooker, 2000; Jeffs & James, 2001). Adult spiny lobsters have been held alive and fattened in sea-cages in New Zealand, Australia, India, Brazil and Japan (Booth & Kittaka, 2000; Van Barneveld, 2001; James & Marian, 2003; Bryars & Geddes, 2005), large *Panulirus argus* juveniles were successfully (first 45 days) ongrown in experimental sea-cages in Bahia de la Ascension (Lozano-Álvarez, 1996) and only a single published study by Jeffs and James (2001) has shown the potential of sea-cages to culture *Jasus edwardsii* from puerulus. In addition, sea-cage commercial trials have been performed by various aquaculture groups in the Coromandel and Marlborough Sounds. Lobsters grown on fresh mussels in those sea-cages exhibited faster growth rates than lobsters held in previous commercial land-based tanks in New Zealand. However, there were several issues identified with the structural design of the sea-cage including a loss of integrity when exposed to adverse weather conditions, escapees, difficulty in feeding, cleaning and maintenance. The cost of fresh food, boat travel, equipment and labour rendered the commercial sea-based culture attempts uneconomic (Phil James, pers. comm.).

Fresh mussels (i.e., *Perna canaliculus*, *Mytilus galloprovincialis* and *M. edulis*) have been shown to be excellent feeds for temperate spiny lobster species, supporting good growth rates and high survival (Hooker et al., 1997; James & Tong, 1997, 1998; Geddes et al., 2001;
Glencross et al., 2001; Dubber et al., 2004; Williams, 2007). However, the unreliability of the food source and the high cost associated with storage, opening and feeding fresh mussels have remained a major issue affecting the economic viability of commercial lobster ongrowing (Jeffs & Hooker, 2000). Consequently, the development of a cost-effective nutritionally adequate formulated diet has appeared essential for the commercial culture of spiny lobsters (Williams, 2007). In the past decade, considerable research effort has been dedicated to the development of formulated diets for spiny lobster juveniles. However, growth on all experimental diets to date has remained slower than on fresh mussel flesh. Although Barclay et al. (2006) developed an experimental diet for *Panulirus ornatus* juvenile which resulted in faster growth rates than green and blue mussels, the mussels used in that study had been frozen and commercially processed. James and Tong (2007), working with *J. edwardsii* juveniles, found poorer growth rates when feeding frozen green-lipped mussel compared to fresh. This clearly indicates that the growth results of Barclay et al. (2006) are not directly comparable to the true benchmark diet of fresh mussel. The slow growth rate of *J. edwardsii* and other lobster species (Nelson et al., 2006) on formulated diets could be the consequence of various factors including an incorrect pellet size, physical form and texture (Geddes et al., 2001; Sheppard et al., 2002; Thomas et al., 2003), reduced water stability (Sheppard, et al., 2002; Devey, 2004; Williams et al., 2005), and excessively rapid leaching of potent chemoattractants and feeding stimulants (Lee & Meyers, 1996; Smith et al., 2001; Tolomei et al., 2003; Williams et al., 2005). The formulated diets of the present study were made in two different formats (i.e., moist diet and dry pellet) that were highly water stable in an attempt to address these issues and to better suit the feeding strategy performed in this experiment (diets fed three times a week).

This study has two principal aims; 1) to compare the performance of three holding systems for juvenile lobsters: a prototype sea-cage, a commercial sea-cage, and indoor flow-through tanks, and, 2) to compare the performance of four dietary regimes for juveniles lobsters when cultured in a prototype sea-cage: a dry formulated diet, a moist formulated diet, fresh mussel, and unfed (feeding only on the natural biofouling of the sea-cage).
2.2. Materials and methods

2.2.1. Culture systems

2.2.1.1. Sea-cages

Two rafts anchored in 12 m water depth 50-100 m offshore of the Mahanga Bay aquaculture facility (National Institute of Water and Atmospheric Research Ltd.) in Wellington, New Zealand, were used as floating structures to suspend 12 prototype sea-cages and a single large commercial sea-cage divided into three replicate compartments (Fig. 2.1.).

![Diagram of sea-cage system](image)

Fig. 2.1. One of the two experimental rafts with four prototype sea-cages and the commercial sea-cage. The other raft was located in proximity and consisted of eight prototype sea-cages.

Temperature and light intensity loggers were deployed at 2.5 m depth to monitor these parameters every hour throughout the experiment. The prototype sea-cage consisted of four perforated plastic trays strapped together and surrounded by a sock-net attached to a supporting bar on a raft (Fig. 2.2.). The perforated matrix gave juvenile lobsters a high surface area to utilise (sea-cage internal surface area $\approx 2.86$ m$^2$). Vertical and horizontal surfaces were measured to calculate the internal surface area, as both surfaces have been found to be utilised by lobsters during sea-cage culture (James, 2002b). Diets were supplied via a feeding tube.
running through the sea-cage, with the lower tray lined with fine plastic mesh to serve as a feeding station (Fig. 2.2.). The prototype sea-cages were surrounded by an outer net preventing any predation on lobster limbs and antennae protruding through the mesh (Fig. 2.1.).

![Prototype sea-cage used in the experiment.](image1)

The commercial sea-cage consisted of three compartments (surface area of each compartment $\approx 4 \, \text{m}^2$) made from a stainless steel frame and hard plastic mesh and fitted with feeding tubes for ease of feeding (Fig. 2.3.). The sea-cage was held at the same depth (2.5 m) as the prototype sea-cages but was not surrounded by a predator net (Fig. 2.1.).

![Commercial sea-cage used in the experiment.](image2)
2.3.1.2. Indoor flow-through tanks

Three 27 l black polyethylene flow-through tanks $0.47 \times 0.35 \times 0.21$ m (depth) (surface area including 0.10 m immersed side-walls $\approx 0.33$ m$^2$) were used for comparison with the sea-cages. The tanks were fitted with an airstone and supplied with flow-through (3 l min$^{-1}$) 70 µm filtered seawater (i.e., no biofouling organisms) at the same ambient temperature as the sea-cages (the water intake being in close proximity and at the same depth as the sea-cages). The tanks contained no shelters, and were cleaned and monitored regularly to ensure lobsters were fed to satiety. Photoperiod was maintained at L8:D16 with a light intensity of 2-5 µmol photons s$^{-1}$ m$^{-2}$. Dissolved oxygen (>94% saturation, 8.80-9.25 mg l$^{-1}$), pH (8.02-8.27), ammonia (<0.03 mg l$^{-1}$), nitrite (<0.01 mg l$^{-1}$) and nitrate (<0.1 mg l$^{-1}$) were measured monthly.

2.2.2. Experimental animals

Early juvenile lobsters were caught in crevice collectors deployed in Gisborne, New Zealand (lat 38°39’S, long 178°05’E), during June-July 2005 (Booth & Tarring, 1986). The lobsters were transported and ongrown for three to four months on fresh mussel, *P. canaliculus*, in large flow-through tanks. 1,653 juvenile lobsters (mean carapace length ± S.D. 18.35 ± 1.62 mm; mean wet weight 2.85 ± 0.81 g) were randomly stocked in the three holding systems at a similar optimum initial density of 35 ind m$^{-2}$ (i.e., 100 lobsters in each prototype sea-cage, 140 lobsters in each compartment of the commercial sea-cage and 11 lobsters in each indoor flow-through tank) (James et al., 2001). A constant initial stocking density across the culture systems was deemed necessary to avoid differential effect on lobster performance (James et al., 2001; Johnston et al., 2006). As a higher biomass was required to achieve a similar stocking density in the sea-cages compared to the small experimental indoor tanks, it was expected that any behavioural differences between the culture systems would tend to favour the performance of lobsters in the tanks. The experiment lasted eight months from November 2005 to June 2006 and during this time any lobster mortalities were not replaced. To compare the performance of lobster juveniles in the three different holding systems, all lobsters were fed fresh *P. canaliculus* mussels three times a week. The mussels (Table 2.1) were purchased in 25 kg bags, held alive in a large flow-through tank for a maximum of three
weeks, shucked and fed shell-less. The feeding ration was equivalent to 5% body weight (BW) day\(^{-1}\) in the first four months and 2% BW day\(^{-1}\) afterward. The ration weight was adjusted after every census in order to ensure that lobsters were fed above satiety level throughout the experiment.

### 2.2.3. Dietary treatments

To investigate the effect of diet on lobster performance in the prototype sea-cages, four dietary treatments were each allocated haphazardly to three replicate prototype sea-cages; it was ensured that both rafts received all dietary treatments. The formulation of the dry formulated diet was based on nutrient levels giving the best results in previous nutritional studies (Johnston et al., 2003; Ward et al., 2003) (Table 2.1.). Fishmeal was used as the main protein source. The dry formulated pellets were steam extruded to ensure high water stability, and were slightly flattened and 5 × 10 mm in size to achieve optimal handling efficiency by juvenile lobsters (Sheppard et al., 2002). The moist formulated diet (Table 2.1.) was based on inexpensive and readily available local ingredients such as fish skin, fresh mussels (\textit{P. canaliculus}) and dried algae. The dietary components were bound using transglutaminase to achieve high water stability (Phil James, pers. comm.). The diet was stored frozen, a sample thawed each week, and cut into 2 cm\(^3\) cubes before feeding. The two formulated diets were fed three times a week at 5% body weight day\(^{-1}\), the ration size adjusted after every census.

Pilot studies and previous experiments have shown that natural biofouling can be a substantial food source for lobsters (Jeffs & James, 2001). Therefore, an unfed treatment, consisting of lobsters feeding only on the biofouling of the sea-cages, was also included to see whether natural food supply from the biofouling would be sufficient to maintain growth, survival and the nutritional condition of lobsters over the eight month period. The unfed treatment consisted of three prototype sea-cages that received one pre-conditioned fouled tray at the beginning of the experiment to avoid initial starvation.
Table 2.1. Nutrient composition of the three experimental diets. Values are based on dry weight. Refer to Table 3.1. for the ingredient composition of the dry formulated diet.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Moist formulated diet</th>
<th>Dry formulated diet</th>
<th>Fresh mussel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>42.4</td>
<td>42.1</td>
<td>55.2</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>13.9</td>
<td>12.2</td>
<td>11.5</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>16.5</td>
<td>36.7</td>
<td>21.5</td>
</tr>
<tr>
<td>Gross Energy (KJ g(^{-1})) (^2)</td>
<td>17.8</td>
<td>21.5</td>
<td>21.0</td>
</tr>
<tr>
<td>P/E ratio (gCP MJ(^{-1})) (^3)</td>
<td>23.8</td>
<td>19.5</td>
<td>26.3</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>27.2</td>
<td>9.0</td>
<td>11.8</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>60.5</td>
<td>7.3</td>
<td>79.8</td>
</tr>
</tbody>
</table>

\(^1\) Proximate analyses from the Nutritional Laboratory, Institute of Food, Nutrition and Human Health, Massey University: crude protein (Leco, total combustion method Association of Official Analytical Chemists AOAC 968.06), crude lipid (Soxtec extraction AOAC 991.36), moisture (convection oven 105 °C AOAC 930.15, 925.10), ash (furnace 550 °C AOAC 942.05), gross energy (bomb calorimetry) and carbohydrate (by difference).

\(^2\) KJ g\(^{-1}\) = kilojoules per gram

\(^3\) g CP MJ\(^{-1}\) = gram crude protein per millijoules

2.2.4. Experimental procedure and calculations

Lobster carapace length (CL; mm) was measured on day 1, 64, 112, 174 and 245. CL was taken along the dorsal line from the rostrum to the dorsal margin of the carapace using electronic calipers (Sylvac) and the Allegro field PC (Jupiter systems\(^\text{TM}\)). At every census, prototype and commercial sea-cages were checked for damage, repairs were made where necessary and any potential predators removed from the sea-cages. The penultimate census (day 174) could not be performed on the commercial sea-cage due to severe weather. Fifty lobsters per sea-cage were measured and the remaining lobsters were counted to calculate survival. Carapace length and wet weight of all lobsters were measured in the first and last sea-cage censuses and at every tank censuses. On day 82, the third compartment of the commercial cage ruptured in a storm and most lobsters were lost. Only data from the remaining two compartments were used to calculate growth and survival thereafter. Specific growth rates (SGR; % body weight increase per day) were calculated to account for the exponential increase in wet weight (Crear et al., 2000). Biomass was calculated to compare
lobster yield on the four dietary treatments, as initial lobster numbers (100) and wet weight (3 g) were the same.

\[
\text{S.G.R. (\% BW day}^{-1} \text{)} = (\ln \text{Final weight} - \ln \text{Initial weight}) \times 100 / \text{Number of days}
\]

\[
\text{Biomass (g) = (Final weight – Initial weight) } \times \text{number of lobster surviving}
\]

As for other crustaceans (Vogt et al., 1985; Vogt, 1987), digestive tubule histology has been found to be an adequate indicator of nutritional condition in *J. edwardsii* (Johnston et al., 2003). Therefore, a similar approach was used in this study. The digestive gland of five intermoult lobsters from each dietary treatment was dissected, fixed in 4% buffered formalin, embedded in paraffin wax, sectioned at 5 µm on a microtome and stained with hematoxylin and eosin for histological analysis (Diggles, 1999). Sections were examined on an Axiovert 200 microscope and images taken using the integrated Axiocam HRc camera (Zeiss). Structural illustrations and terminology were taken from Mantel (1983), Vogt et al. (1985) and Johnston et al. (2003). The diameter of digestive tubules (µm), B- and F-cell numbers were obtained using Axiovision software v4.5. Tubules were haphazardly selected (only tubules sectioned transversally were randomly measured).

### 2.2.5. Statistical analyses

Increase in mean carapace length for each prototype sea-cage and for the four dietary treatments were compared through time by means of a repeated measures ANOVA. Two-way nested ANOVAs were performed to compare mean carapace length at each census between dietary treatments (sea-cages nested within treatment). One-way ANOVAs were performed on averaged tank and sea-cage values for growth rate (CL increase per day), overall carapace length gain, biomass gain, wet weight gain, SGR and survival. Survival data were arcsine square-root transformed to normalise percentages. The mean diameters of digestive tubules (log transformed) and the mean number of B- and F-cells were compared between dietary treatments using two-way nested ANOVAs (individual lobsters nested within their respective dietary treatments). Significant differences (*P*<0.05) between the means were determined by post hoc comparison using Tukey-Kramer test (Zar, 1999). When *P*>0.05, non-significant results were stated as NS. The ANOVA assumptions of normality of residuals and
homogeneity of variances were satisfied (Shapiro-Wilk and Levene tests respectively, NS). All analyses were performed using the Statistical & Power Analysis Software NCSS 2004 (Utah, USA).

2.3. Results

2.3.1. Environmental parameters

Seawater temperatures followed the normal seasonal pattern, increasing from 14.6 °C (November 2005) at the beginning of the experiment to a maximum of 18.4 °C (February 2006) and decreasing to a minimum of 9.7 °C (June 2006) by the completion of the experiment. Daily ambient seawater temperatures averaged 15.2 ± 0.2 °C over the course of the experiment. Water temperature in the indoor flow-through tanks remained similar to the sea-based holding systems.

Light intensity at the depth of 2.5 m averaged 1.58 ± 0.10 µmol s⁻¹ m⁻² during the day (from 1000 to 1600 h) throughout the experiment. Daily maximum light intensity averaged 3.18 ± 0.22 µmol s⁻¹ m⁻². However, the light level inside the sea-cage is likely to have been much lower due to the extensive sheltering and coverage. Photoperiod ranged from L15:D9 in summer (December 2005) to L10:D14 in winter (June 2006).

2.3.2. Common biofouling and mobile species found in sea-cages

Biofouling species collected from the unfed sea-cage sock-net and trays consisted principally of mussels (10-30 mm green-lipped mussel, P. canaliculus, 5-45 mm blue mussel, M. edulis, 5-10 mm little black mussel, Xenostrobus pulex), serpulid worm (Galeolaria hystrix), barnacles (Elminius plicatus and Epopella sp.), ascidians (Corella eumyota and Aplidium benhami), sponges (Polymastia fusca), bryozoans (Bugula flabellata and Celleporella sp.) and seaweeds (Hymenocladia sanguinea, Hymenema palmata, Ulva lactuca). A substantial amount of small mussel shells (<10 mm), dead barnacles (<6 mm diameter) and serpulid worm tubes were collected from the feeding station (i.e., retained by
the small plastic mesh) of the prototype sea-cages and appeared to have been fed on by the lobsters (i.e., shell damage consistent with lobster feeding) (James & Tong, 1998). The mesh of the sea-cages remained free of most biofouling in areas accessible by foraging lobsters.

Other organisms commonly found in the sea-cages were brachyuran crabs and fishes (blennies and the smooth leatherjacket, *Meuschenia scaber*). Large numbers (>50) of small leatherjackets (5-10 cm) were collected from the commercial sea-cage on the last census and are likely to have competed for food and possibly preyed on lobsters.

### 2.3.3. Effect of dietary treatment and system design on survival

Survival of lobsters feeding on biofouling alone over the eight month period (66.3 ± 4.9%) was significantly lower than lobsters fed fresh mussels (83.3 ± 2.3%) but did not differ significantly from lobsters fed the dry formulated diet (79.3 ± 1.2%) (one-way ANOVA, $F=7.88$, $df=2.6$, $P<0.05$). Survival on the moist formulated diet (59.0 ± 17.8%) did not differ significantly from the other dietary treatments (one-way ANOVA, $F=1.58$, $df=3.8$, NS) (Table 2.2). The high variation in survival within the moist formulated diet treatment could have been the consequence of escapes as some lobsters were seen on the outside of the sea-cages.

Table 2.2. Comparison of the performance of juvenile *Jasus edwardsii* on four experimental dietary regimes in prototype sea-cages over an eight month growth experiment. Significant differences between means within the same row are marked by different letters ($P<0.05$).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unfed$^1$</th>
<th>Moist formulated diet</th>
<th>Dry formulated diet</th>
<th>Fresh mussel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight gain (g)</td>
<td>4.36 ± 0.75$^a$</td>
<td>9.76 ± 1.16$^b$</td>
<td>16.06 ± 0.82$^c$</td>
<td>28.05 ± 0.69$^d$</td>
</tr>
<tr>
<td>Carapace length gain (mm)</td>
<td>6.02 ± 0.83$^a$</td>
<td>11.30 ± 0.87$^b$</td>
<td>15.26 ± 0.60$^c$</td>
<td>21.00 ± 0.34$^d$</td>
</tr>
<tr>
<td>Biomass (g)</td>
<td>283 ± 30$^a$</td>
<td>567 ± 191$^a$</td>
<td>1272 ± 45$^b$</td>
<td>2338 ± 95$^c$</td>
</tr>
<tr>
<td>S.G.R. (% BW day$^{-1}$)</td>
<td>0.38 ± 0.05$^a$</td>
<td>0.60 ± 0.04$^b$</td>
<td>0.78 ± 0.02$^c$</td>
<td>0.96 ± 0.01$^d$</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>66.3 ± 4.9$^a$</td>
<td>59.0 ± 17.8$^{ab}$</td>
<td>79.3 ± 1.2$^{ab}$</td>
<td>83.3 ± 2.3$^b$</td>
</tr>
</tbody>
</table>

$^1$ Feeding only on biofouling
Survival of lobsters in the indoor tanks over the eight month experiment (87.9 ± 8.0%) and the equivalent prototype sea-cages (fed fresh mussel) (83.3 ± 2.3%) did not differ significantly (one-way ANOVA, \( F=0.64, df=1,4, NS \)) and were significantly higher than in the commercial sea-cage (55.4 ± 1.8%) (one-way ANOVA, \( F=8.26, df=2,5, P<0.05 \)) (Table 2.3).

Table 2.3. Comparison of the performance of juvenile *Jasus edwardsii* in three different holding systems fed fresh mussel over an eight month growth experiment. Significant differences between means within the same row are marked by different letters \((P<0.05)\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Commercial sea-cage</th>
<th>Tank</th>
<th>Prototype sea-cage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight gain (g)</td>
<td>15.62 ± 0.01(^a)</td>
<td>22.95 ± 1.87(^b)</td>
<td>28.05 ± 0.69(^b)</td>
</tr>
<tr>
<td>Carapace length gain (mm)</td>
<td>14.94 ± 0.20(^a)</td>
<td>18.75 ± 0.77(^b)</td>
<td>21.00 ± 0.34(^b)</td>
</tr>
<tr>
<td>S.G.R. (% BW day(^{-1}))</td>
<td>0.77 ± 0.00(^a)</td>
<td>0.86 ± 0.03(^b)</td>
<td>0.96 ± 0.01(^c)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>55.4 ± 1.8(^a)</td>
<td>87.9 ± 8.0(^b)</td>
<td>83.3 ± 2.3(^b)</td>
</tr>
</tbody>
</table>

### 2.3.4. Effect of dietary treatment and system design on growth and performance

#### 2.3.4.1. Carapace length increase through time in the prototype sea-cages

The CL of lobsters increased significantly between censuses for all fed treatments (repeated measures ANOVA, \( F=1518; 659.37; 94.44, df=4,8, P<0.001 \)). Lobsters feeding only on biofouling showed significant CL increase only in the first two months (repeated measures ANOVA, \( F=31.84, df=4,8, P<0.01 \)), achieving moderate growth rates (0.05 mm CL day\(^{-1}\)) similar to lobsters fed the moist formulated diet (one-way ANOVA, \( F=27.54, df=3,8, P<0.001; Tukey, NS \)). Carapace length increase on the fresh mussel diet was significantly faster than all other dietary treatments for every census throughout the experiment (repeated measures ANOVA, \( F=230.49, df=3,12, P<0.001 \)), except for the last census where no significant difference was found between fed treatments (0.05-0.06 mm CL day\(^{-1}\)) (one-way
ANOVA, $F=0.71, df=2.6, NS$). Lobsters fed the dry formulated diet grew faster than the unfed and moist formulated diet fed lobsters over the course of the experiment (repeated measures ANOVA, $F=230.49, df=3.12, P<0.001$) (Fig. 2.4).

Fig. 2.4. Mean carapace length (CL) increase (mm) ± S.E. of juvenile *Jasus edwardsii* left unfed (feeding only on biofouling) or fed three diets (mussel flesh, dry and moist formulated diets) in the prototype sea-cages over an eight months growth experiment. Numbers represent growth rate in daily CL increase (mm day$^{-1}$) between censuses. Significant differences between dietary treatments are marked by differing letters (growth rates: letters x to z; mean CL at census: letters a to d). The caret (^) indicates no significant difference in mean CL between two consecutive censuses within the same dietary treatment.

### 2.3.4.2. Specific growth rate

Over the full eight month duration of the experiment, the lobsters in the prototype sea-cages fed fresh mussel grew significantly faster (mean SGR of 0.96 ± 0.01% BW day$^{-1}$) than those fed the dry formulated diet (0.78 ± 0.02% BW day$^{-1}$), the moist formulated diet (0.60 ±
% BW day\(^{-1}\)) and those feeding on biofouling alone (0.38 ± 0.05% BW day\(^{-1}\)) (one-way ANOVA, \(F=50.28, df=3,8, P<0.001\)) (Table 2.2.). Furthermore, the mean SGR of lobsters fed fresh mussel in the prototype sea-cages was significantly higher than in the flow-through tanks (0.86 ± 0.03% BW day\(^{-1}\)) and the commercial sea-cage (0.77 ± 0.00% BW day\(^{-1}\)) (one-way ANOVA, \(F=25.87, df=2,5, P<0.01\)) (Table 2.3.).

### 2.3.4.3. Carapace length, wet weight and biomass gains

Overall, the carapace length and wet weight gains were significantly larger for lobsters fed fresh mussel in the prototype sea-cages (21.00 ± 0.34 mm; 28.05 ± 0.69 g) than for those fed the dry formulated diet (15.26 ± 0.60 mm; 16.06 ± 0.82 g), moist formulated diet (11.30 ± 0.87 mm; 9.76 ± 1.16 g) or feeding on biofouling alone (6.02 ± 0.83 mm; 4.36 ± 0.75 g) (one-way ANOVA, \(F=83.58; 135.92, df=3,8, P<0.001\)). The biomass gain followed a similar pattern (one-way ANOVA, \(F=69.26, df=3,8, P<0.001\)), although no significant difference was found between the biomass gain of lobsters fed the moist formulated diet and feeding on biofouling alone (Tukey test, \(NS\)) (Table 2.2.).

Overall, the mean gains in carapace length and wet weight of lobsters cultured in the prototype sea-cage (21.00 ± 0.34 mm; 28.05 ± 0.69 g) and flow-through tank (18.75 ± 0.77 mm; 22.95 ± 1.87 g) did not differ significantly but were significantly greater than in the commercial sea-cage (14.94 ± 0.20 mm; 15.62 ± 0.01 g) (one-way ANOVA, \(F=25.28; 19.43, df=2,5, P<0.01\)) (Table 2.3.).

### 2.3.5. Effect of dietary treatment on digestive gland histology

The dietary treatment had a significant effect on tubule diameter (two-way nested ANOVA, \(F=9.12, df=3,16, P<0.001\)). The mean (±S.E.) digestive tubule diameter was 343 ± 7 µm for lobsters fed fresh mussel in prototype sea-cages, 315 ± 7 µm for lobsters fed the dry formulated diet, 257 ± 6 µm for lobsters fed the moist formulated diet and 243 ± 6 µm for lobsters feeding on biofouling alone. Tubule diameter paralleled the trend in growth obtained from the different dietary treatments with larger tubule diameter found in lobsters.
Chapter 2

with faster mean growth. Dietary treatment had no significant effect on the number of B- and F-cells in the digestive gland tubules (two-way ANOVAs, $F=2.03; 3.12$, $df=3.16$, NS), however, there was a significant amount of variability between individual lobsters within the same dietary treatment (two-way ANOVAs, $F=11.23; 5.49$, $df=16.380$, $P<0.001$). R-cells were highly vacuolated (high lipid accumulation), uniform and columnar in shape in all fed treatments (Fig. 2.5., A, B, C). The digestive gland tubules of fed lobsters showed sound connective tissue ultrastructure, although, in some cases, those fed the moist formulated diet showed signs of tubule atrophy. The digestive gland tubules of lobsters left unfed were atrophied and the R-cells devoid of lipid vacuoles (Fig. 2.5., D).

Fig. 2.5. Transverse section (TS) of digestive gland tubules of juvenile lobsters left unfed (feeding only on biofouling) or fed three diets in the prototype sea-cages over an eight month growth experiment. (A) Fresh mussel, *Perna canaliculus*, diet. (B) Dry formulated diet. (C) Moist formulated diet. (D) Unfed. Lu, tubule lumen; Ct, connective tissue; Hs, haemolymph space; Hc, haemocyte; Nu, nucleus; Lv, lipid vacuole; F, F-cell; R, R-cell; B, B-cell. Atrophied tubules are not shown.
2.4. Discussion

Juvenile lobsters fed fresh mussels performed well in the prototype sea-cage, achieving high growth rate and survival. SGR and gains in carapace length, wet weight and biomass were significantly better than when fed the two formulated diets or when feeding on biofouling alone. As in previous studies (Crear et al., 2000; Thomas et al., 2003; Ward et al., 2003), fresh mussel outperformed formulated diets giving further indication of its high nutritional value. Digestive gland histology of fresh mussel fed lobsters showed high lipid vacuolation in R-cells (Gibson & Barker 1979; Johnston et al., 2003), sound connective tissue ultrastructure (Pascual et al., 1983; Catacutan & De la Cruz, 1989; Kumaraguru Vasagam et al., 2007) and large tubule diameter. The histology was indicative of very good nutritional condition at the end of the growth experiment (Vogt et al., 1985; Vogt, 1987; Johnston et al., 2003). Feeding fresh shucked mussels three times a week appeared as an adequate feeding protocol and removed the need for cleaning the cages. Lobsters can open live mussels of sizes up to their carapace lengths without compromising their growth rate (James, 1998), but the shells would have to be removed from the cages regularly. Previous commercial trials showed that this is a labour-intensive task (A. Jeffs, pers. comm.). Live mussels were also found to cluster in sea-cages and the lobsters were unable to detach and open the tightly bound mussels (Jeffs & James, 2001). Feeding shucked mussels daily could have arguably resulted in better growth (James & Tong, 1997), but would have been very time-consuming and likely to be not as cost-effective on a commercial scale.

Lobster fed the dry formulated diet in the prototype sea-cages grew slower than on fresh mussels but the overall performance was still encouraging. Survival remained high and was not significantly different from the fresh mussel treatment. Digestive tubules of lobsters fed formulated pellets resembled those of lobsters fed fresh mussel. The slower growth might in part have been simply a consequence of the lower protein level in the formulated diet in comparison to fresh mussel (Table 2.1.). In a previous study by Crear et al. (2000), the growth of *J. edwardsii* juveniles on a dry formulated diet (42.9% protein) was found to be higher than on a moist formulated diet (32.3% protein) at ambient temperature despite some evidence of feed intake compensation. A direct linear relationship between protein level and growth rate was also found for the spiny lobsters *P. cygnus* (Glencross et al., 2001) and *P. ornatus* (Smith et al., 2005a) fed experimental formulated diets. The growth response only flattened out in
large *J. edwardsii* (Williams, 2007) and juvenile *P. ornatus* (Smith et al., 2003) when dietary protein was greater than 50-55%. With the exception of the study by Ward et al. (2003), which found that *J. edwardsii* juveniles grew best at an intermediate level of dietary digestible protein (29-31%), spiny lobsters appear to require high dietary protein level to achieve good growth on formulated diets (Williams, 2007). Another possibility is that the dry formulated diet of this study was highly energetic, with high levels of lipid and carbohydrate compared to diets by Ward et al. (2003). The protein to energy (P/E) ratio may have been sub-optimal for *J. edwardsii* (19.53 < 29 g digestible crude protein per MJ of digestible energy), although American lobster grew best at a similar P/E ratio (Capuzzo & Lancaster, 1979). The high carbohydrate inclusion level (36.7%) of the dry formulated diet is considered the upper limit in most crustacean diets (Cuzon et al., 1994), particularly for carnivorous marine crustacean such as lobsters (Johnston et al., 2003). The high carbohydrate level could have reduced the digestibility of the dry formulated diet, although subsequent research suggests otherwise at least for the starch component of the carbohydrate fraction (see Simon, 2009d). Nevertheless, the growth rate recorded in this study on the dry formulated diet in the first 112 days (0.07 mm CL day\(^{-1}\) ≈ SGR=0.91 ± 0.04% BW day\(^{-1}\)) was relatively high in comparison to previous studies. Crear et al. (2000) obtained a similar SGR (0.96 ± 0.01%) and survival (73 ± 7%) in tanks with slightly smaller juvenile *J. edwardsii*. The lobsters were held at a similar ambient water temperature (16 ºC) and fed daily on a commercial giant tiger prawn, *Penaeus monodon*, diet. In contrast, the dry formulated diet in this current study was only fed three times a week in the sea-cages, which may not be often enough considering the high leachate rate of dry formulated diets (Williams et al., 2005). Increasing the feeding frequency to daily feed allotment could have improved growth rate as suggested by subsequent research (see Simon & Jeffs, 2008).

Biofouling as a supplementary source of nutrition is likely to have contributed to the high growth rates, the internal surfaces of the sea-cages remaining clean of most fouling due to lobster predation. Lobsters feeding on biofouling alone grew relatively well in the first two months and their survival was satisfactory, especially considering that some lobsters may have escaped from the sea-cages. Biofouling helped improve the biomass of unfed lobsters but only small fouling organisms appeared to have been eaten. Many small mussel shells (<10 mm), dead barnacles (<6 mm diameter) and serpulid worm tubes were collected from the feeding station of the sea-cages. Larger barnacles, mussels and ascidians were not eaten,
perhaps reflecting the small size of the lobsters or a dietary preference (James & Tong, 1998). Nevertheless, results showed that the nutritional condition of the unfed lobsters was poor, with the digestive gland tubules being atrophied and devoid of lipid vacuoles. Similar results were found for starved juvenile lobsters (Niles et al., 1993), or lobsters fed a suboptimal diet (Rosemark et al., 1980; Johnston et al., 2003), indicating that unfed lobsters utilised most of their lipid reserves during the growth period and were probably under intense nutritional stress by the end of the experiment. Nonetheless, biofouling alone could well have been sufficient to maintain the nutritional condition of lobsters for the first couple of months as suggested from the moderate growth rate (0.05 mm CL day$^{-1}$).

Lobsters fed the moist formulated diet performed poorly in comparison to the other dietary treatments, except during the last period (days 174-245) where growth rates did not differ significantly from other dietary treatments, probably due to the low seawater temperatures (12.8 °C). Interestingly, this shows that feeding a cheaper sub-optimal diet during the slow-growing winter months might be a more economic option for potential sea-cage lobster aquaculture operations in southern New Zealand. Nevertheless, in view of the overall growth, biomass gain and survival obtained, the moist formulated diet would not be suitable for the commercial aquaculture of juvenile spiny lobsters. The two formulated diets being isonitrogenous, lower protein level per se should not have accounted for the slow growth. Nutritional inadequacies such as high lipid and ash content, low gross energy, or perhaps the physical shape and texture of the moist formulated diet might have contributed to its poor performance.

Results from this study and a previous study by Jeffs and James (2001) indicate that sea-cage culture is a cost-effective and biologically feasible way of ongrowing juvenile *J. edwardsii* to market size. Similar survival and better growth rates can be obtained in the prototype sea-cages in comparison to indoor tanks even with a high biomass of lobsters. Factors as diverse as supplemental feed from biofouling, reduced stress, natural light levels and photoperiod, may all have contributed to the good performance of the sea-cages. Nevertheless, this study also shows that the effectiveness of a sea-cage for culturing juvenile spiny lobsters is highly dependent upon the structural design of the sea-cage. The growth and survival of small juvenile lobsters fed fresh mussels was highest in the prototype sea-cage but lowest in the commercial sea-cage. Several factors contributed to the poor performance of the
commercial sea-cage. Aspects of the structural design such as the large mesh size (12 mm), the absence of outer predator net and inner shelter would have allowed predation by fish and perhaps also cannibalism of lobsters undergoing ecdysis. The rigid structure of the sea-cage did not cope with the movement of the raft during higher sea swell conditions. During one storm several connectors snapped at the connection between mesh panels and the stainless steel frame, allowing the escape of lobsters from one compartment. Previous commercial trials using this type of sea-cage suspended under a mussel long-line achieved high growth rates (1.01% BW day\(^{-1}\)) similar to the prototype sea-cage of this study, although this appeared to have been due to warmer water temperature (19.5 ºC) and lower stocking density (12.5 ind. m\(^{-2}\)) (James & Jeffs, 2002). The survival of juvenile lobsters (60%) during these commercial trials was also comparatively lower to the survival recorded in this study in the prototype sea-cages (83.3%). In addition, at similar seawater temperatures (Wellington Harbour), Jeffs and James (2001) obtained a lower SGR (0.83% BW day\(^{-1}\), adapted growth data for lobsters of the same initial weight over 245 days) in their experimental sea-cages compared to the prototype sea-cages developed in this study.

The improved performance of the prototype sea-cage is likely to have been due to a more suitable design. Stacked perforated trays enabled high surface area to volume ratio resulting in high growth, good survival and homogenous lobster size. Furthermore, stocking densities in the prototype sea-cage can be maintained at similar levels as those performed in sheltered tanks (Johnston et al., 2006) without apparent detrimental effect on lobster performance. The complex structure minimises predation and cannibalism by providing shelter from fish and allowing lobsters undergoing ecdysis to move upward away from the cohort (pers. obs.). Lobsters seemed to tolerate the vertical movement of the sea-cages from wave action, which possibly improved water flow (James, 2006). It is estimated that around 50 lobsters per experimental sea-cage (at densities around 35 ind. m\(^{-2}\)) could be raised to market size (i.e., minimum legal size of 60 mm CL; 100 g lobster) from wild-caught early juveniles (i.e., post-pueruli) in 660 days at the relatively low water temperatures recorded in this study.

Supplemental feed from biofouling in sea-cages could be a major factor influencing the high growth rates and darker coloration of lobsters compared to those grown in tanks on *P. canaliculus*. The exoskeleton colour of lobsters grown in tanks on the dry formulated diet
in subsequent research (see Simon & Jeffs, 2008) was also whiter in comparison to lobsters reared in the prototype sea-cages on this diet (see Plate 2, E & F). The more natural exoskeleton pigmentation of lobsters cultured in the sea-cage would improve their market price and minimise the need for the inclusion of costly astaxanthin in dry formulated diets (Barclay et al., 2006). Growth of lobsters fed formulated diets in the prototype sea-cages was also encouraging in comparison to results obtained in tanks even at higher ambient temperature (see Simon & Jeffs, 2008). Nutrients or active constituents gained by feeding on fouling organisms, such as small barnacles and mussels, could have ameliorated any inadequacies in the nutritional quality of the diet. The growth on the dry formulated diet could be enhanced further via daily automated feeding (Simon & Jeffs, 2008), this feeding protocol being highly compatible with the current prototype sea-cage design and dry pelletised formulated diet format. The costs of boat running, labour and time required for servicing and feeding the sea-cage would be reduced in consequence, improving further the economic prospect of spiny lobster aquaculture.

In conclusion, this study demonstrates that good growth and survival of small *J. edwardsii* juveniles can be obtained in the novel prototype sea-cage even at suboptimal seawater temperatures. The performance recorded in this new sea-cage design was better than most previous results in tanks and sea-cages feeding fresh mussels or dry formulated diets to juvenile *J. edwardsii* of a similar size (refer to a summary table of previous growth rates in Thomas et al., 2003). Biofouling as a supplementary source of nutrition is likely to have contributed to the high growth rates, although feeding a nutritionally adequate diet remains necessary to achieve optimum performance. Natural biofouling as the sole source of nutrients is also not sufficient to maintain the growth and nutritional condition of a high biomass of juvenile lobsters for more than two months. The prototype sea-cage design would appear well suited for the commercial aquaculture of *J. edwardsii* and other species of spiny lobster around the world. Sea-cage rafts could be integrated to other culture operations such as mussel farms to benefit from the proximity and low cost of the high quality feed or fitted with cost-effective automated feeding arrangement of a nutritionally adequate dry formulated diet. Site selection is likely to play an important role in determining growth rate and survival, as temperature and biofouling will influence lobster performance. Subsequent feeding and digestion experiments in tanks with the current baseline dry formulated diet were performed to identify whether low food consumption or digestibility are at the origin of the slower growth rates compared to fresh mussel (see following chapters).
Plate 3. Feed intake rate and foregut filling in *Jasus edwardsii* juveniles fed mussel flesh and the dry formulated diet. A) Transparent plastic aquaria used in experiments of Chapters 3, 4, 5 and 6 to house lobster juveniles individually; B) A small (19 g) *J. edwardsii* juvenile fed mussel flesh; C) Microfiltration apparatus to measure dry formulated diet intake; D) Dissection of the digestive tract of a small juvenile (19 g) lobster 4 h after ingestion of formulated diet to satiation, note the complete filling of the foregut and the digesta being evacuated via the hindgut; E) and F) Dissections of intermediate size (42 g) juvenile lobsters showing foregut fullness and digesta volume, respectively, 2 h after ingestion of a satiation ration of formulated diet (left E and F) and mussel flesh (right E and F).
3. Chapter three – Feed intake rate and extent: relationship to foregut capacity

Published as:


3.1. Introduction

Poor food consumption has been identified as a possible reason for the slow growth in spiny lobsters raised on dry formulated diets (Glencross et al., 2001; Johnston et al., 2007; Williams, 2007). For example, no difference in food consumption was found among a range of formulated diets fed to juvenile spiny lobsters *Panulirus cygnus* (Glencross et al., 2001) and *Jasus edwardsii* (Ward et al., 2003). It was also observed that *P. ornatus* juveniles were less inclined to eat after dry formulated feed pellets had been immersed in water for 1-2 h, whereas shucked mussel remained attractive to the lobsters for 10 h or more after immersion (Williams et al., 2005). Poor chemoattraction and palatability of formulated diets compared to fresh mussel have been suggested as being the major causes for the low food consumption and consequently growth of *P. ornatus* (Williams et al., 2005). However, for the spiny lobster, *J. edwardsii*, from temperate waters, previous studies have reported very similar dry matter intake for a wide range of formulated diets and fresh mussel, varying from 2% BW day\(^{-1}\) in small juveniles (2-7 g wet weight) to 1% BW day\(^{-1}\) in larger juveniles (13-20 g wet weight) (Crear et al., 2000, 2002; Thomas et al., 2003; Tolomei et al., 2003; Ward et al., 2003). Tolomei et al. (2003) showed that the excitatory capacity of the extracts of a commercial shrimp diet and fresh mussel were similar, and the attractability of a formulated diet was greater than for mussel in *J. edwardsii* left unfed for 48 h. In addition, other studies have shown that feeding lobsters several meals a day does not improve feed intake or growth (Bordner & Conklin, 1981; Thomas et al., 2003; Cox & Davis, 2006; Jones, 2007; Johnston et al. 2008). This is unusual as theoretically more frequent delivery of feed should increase
attraction, palatability and reduce leaching from diets, thereby resulting in higher utilisation, feed intake and growth (Sedwick, 1979).

Nutritional studies have often relied on growth, food consumption, food conversion ratio and behavioural observations to determine the attractiveness and palatability of formulated diets for spiny lobsters (Tolomei et al., 2003; Williams et al., 2005). At present, data relating to food intake rate and maximum consumption in a single feeding event are lacking for spiny lobsters, despite their relevance to optimise formulated diet palatability and to develop effective strategies for delivering feed. Spiny lobsters are intermittent nocturnal feeders and differ fundamentally from penaeid shrimps which display virtually continuous ingestion throughout the day (Nunes & Parsons, 2000; Geddes et al., 2001). Shrimps achieve high levels of food consumption, despite the relatively small capacity of their foregut (2-3% BW), because of high rates of foregut filling (10 min) and emptying (2-4 h) (Nunes & Parsons, 2000). The contrary can be expected for lobsters which display much slower rates of gut clearance (Barker & Gibson, 1977; Kurmaly et al., 1990; Sarda & Valladares, 1990). Despite substantial research in diet development for spiny lobsters (Williams, 2007), relatively little is known about the digestive processes occurring after the ingestion of formulated diets and the potential for a negative effect on food consumption. There is a need to determine the relationship between body size and foregut capacity and its potential to limit food intake. It is generally assumed that the higher weight to volume ratio of dry formulated diets compared with fresh food will result in higher dry matter feed intake for a single feeding event (Ruohonen et al., 1997). Yet, cultured fish are known to be able to compensate for large differences in dietary water content so they can achieve similar dry matter intake (Ruohonen et al., 1997). Dry diets generally tend also to require moisturising before digestion can begin, with the moisture coming from internal secretion and ingestion, resulting in higher food volume in the fish stomach (Ruohonen et al., 1997).

This study measures feed intake on three size-classes of juvenile J. edwardsii, fed the flesh from freshly opened mussel (Perna canaliculus) and the dry formulated diet. The aims of the study are to determine; 1) the effect of body size and diet type on the rate of feed intake, and, 2) to establish if the maximum feed intake is limited by the volume of the foregut (i.e., foregut capacity).
3.2. Materials and methods

3.2.1. Experimental diets and design

The study used two diets, the dry formulated diet (see Table 3.1. for ingredient composition) and fresh mussel, *P. canaliculus*, which was held live in tanks of flowing seawater until required for the experiments. The proximate composition of the two diets was broadly similar when expressed on a dry weight basis (Table 2.1.) and previous research has demonstrated that both diets are nutritionally adequate (Simon & James, 2007). However, the formulated diet was more nutrient dense owing to a smaller moisture component compared with wet mussel flesh (Table 2.1.). For the feeding experiments, only the mussel mantle and gonad were used and hereafter referred to as mussel flesh (the same definition is used in the two following chapters as well). These mussel parts were removed from the valves of freshly opened mussels as a single piece of flesh and drained for five min on 250 µm sieves. This allowed minimising fluctuations in moisture level between meals. As a result, the moisture content of the mussel flesh used for experiments (77.0%) was slightly lower than that of the whole mussel (79.8%).

*J. edwardsii* juveniles caught yearly between 2004 and 2006 in crevice collectors deployed in Gisborne were grown communally on opened fresh mussel *P. canaliculus* at ambient seawater temperature (i.e., 9-18 °C). The spiny lobsters were fed a mixed diet of dry formulated diet and fresh mussel three times weekly to satiation for one month before the experiment. Three size-classes of juveniles were established arbitrarily according to their wet body weight: Size one, S1 (initial mean BW ± S.D. 18.8 ± 1.2 g); S2 (42.0 ± 2.6 g); and S3 (82.9 ± 4.9 g). Healthy intermoult lobsters with a complete set of functional feeding appendages were selected and stocked individually in tanks to avoid conspecific interactions. Lobsters were acclimated for 10 days to their respective experimental tank and diet (i.e., mussel flesh or formulated diet) before the start of the experiment. Feeding experiments were performed over three weeks for each size-class consecutively (i.e., a total of nine weeks) in transparent-plastic aquaria: 0.26 m × 0.15 m × 0.18 m (depth), 6 l. Aquaria received flow-through sea-water that had been cartridge-filtered (1 µm) at a constant flow rate of 0.6 l min⁻¹ and at a temperature of 18 ± 1 °C. The experimental tanks were illuminated with one 50 W incandescent light bulb (low light levels <0.5 µmol m⁻² s⁻¹) at a photoperiod of L12:D12 to
ensure the feed intake of lobsters would not be negatively affected by bright light levels (Bordner & Conklin 1981). Water quality was similar to previous *J. edwardsii* growth studies (Crear et al., 2000, 2003; Simon & James, 2007).

Table 3.1. Ingredient composition of the formulated diet. Values are based on dry weight.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Proportions (g kg(^{-1}) of diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal(^1)</td>
<td>260</td>
</tr>
<tr>
<td>Krill hydrolysate(^2)</td>
<td>100</td>
</tr>
<tr>
<td>Seaweed meal(^3)</td>
<td>90</td>
</tr>
<tr>
<td>Lupin meal(^4)</td>
<td>80</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>50</td>
</tr>
<tr>
<td>Yeast</td>
<td>70</td>
</tr>
<tr>
<td>Tapioca starch</td>
<td>250</td>
</tr>
<tr>
<td>Fish oil</td>
<td>70</td>
</tr>
<tr>
<td>Lecithin</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin mix(^5)</td>
<td>4</td>
</tr>
<tr>
<td>Stay-C (25%)</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mix(^6)</td>
<td>3</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>5</td>
</tr>
<tr>
<td>Betaine</td>
<td>2</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\) Peruvian fishmeal, AGRO & SEA CORP S.A. (68% CP)  
\(^2\) Spray dried krill hydrolysate, Speciality Marine Products, Canada (60-65% CP, 6-10% lipid, 12-16% ash, 2.5% fibre, 8% moisture)  
\(^3\) *Ascophyllum sp.*, Norway (5-10% CP, 2-4% lipid, 17-27% ash, <8% fibre, 12-15% moisture, 40-60% carbohydrate)  
\(^4\) Western Australian commercial feed manufacturer (42% CP, 13.5% lipid, 3.3% ash, 6.5% fibre, 34.8% Nitrogen Free Extract)  
\(^5\) Image Holdings, New Zealand (mg kg\(^{-1}\) vitamin mix): thiamine (B\(_1\)), 50; riboflavin (B\(_2\)), 40; Niacin (B\(_3\)), 200; pyridoxine (B\(_6\)), 60; cyanocobalamin (B\(_12\)), 0.1; Biotin, 2; retinol (A), 10000 IU; menadione (K\(_3\)), 20; Calcium Pantothenate, 200; Folic acid, 10; D/L α-tocopherol (E), 200; cholecalciferol (D\(_3\)), 5; and ascorbic acid (C), 500.  
\(^6\) Image Holdings, New Zealand (mg kg\(^{-1}\) mineral mix): Co (as CoCl\(_2\)·6H\(_2\)O), 0.05; Cu (as CuSO\(_4\)·5H\(_2\)O), 10.95; Fe (as FeSO\(_4\)·7H\(_2\)O), 20.1; Mg (as MgSO\(_4\)·7H\(_2\)O), 140; Mn (as MnSO\(_4\)·H\(_2\)O), 10.6; Se (as NaSeO\(_3\) and NaSeO\(_3\)), 0.2; K (as KI), 2.6; and Zn (as ZnSO\(_4\)·7H\(_2\)O), 150.
3.2.2. Feed intake

Feed intake was determined as the amount of dry matter ingested per lobster during a feeding event (i.e., meal). To calculate the rate and extent of feed intake, a total of 30 lobsters per size-class were allowed to feed on their respective diet for different randomly assigned meal durations (i.e., 0.5, 1, 2, 4, and 5 h). A non-feeding period of 48 h between meals was established to stabilise feed intake at each meal and to ensure lobsters returned to a pre-feeding basal metabolic state before the next meal (Crear & Forteath, 2000; Radford et al., 2004). Individual lobsters were re-used several times in the experiment after a four day recovery period during which they were fed twice every 48 h on their respective diet for 5 h. Re-used lobsters were randomly assigned a new experimental meal duration. As feeding declined or completely ceased around ecdysis, data obtained 96 h before and after ecdysis events were not used (14.5% of instances). A total of 366 feed intake measurements were analysed (Table 3.2).

Table 3.2. Number of replicate measurements of feed intake for three size-classes of juveniles *Jasus edwardsii*.

<table>
<thead>
<tr>
<th>Meal duration (h)</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>Formulated diet</td>
<td>Mussel flesh</td>
</tr>
<tr>
<td>0.5</td>
<td>13</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>1.0</td>
<td>11</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2.0</td>
<td>12</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>4.0</td>
<td>15</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>5.0</td>
<td>13</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>47</td>
<td>61</td>
</tr>
</tbody>
</table>

1^S1: 18.8 ± 1.2 g
2^S2: 42.0 ± 2.6 g
3^S3: 82.9 ± 4.9 g

Weight measurements were determined to the nearest milligram using a precision AG204 Mettler Toledo balance. To calculate feed intake, a known quantity of food was offered above satiety levels (3% BW). Uneaten formulated diet was recovered by micro-filtering the water using 1.2 µm pre-weighed glass microfibre filter papers (GM/C, 47 mm circles). Micro-filtration allowed complete collection of the finest fragments of uneaten food.
arising from manipulative losses while lobsters were feeding on the dry formulated diet. Filter papers were gently rinsed with distilled water to remove salts (Ward et al. 2003). Uneaten fresh mussel flesh was siphoned directly on allocated 250 µm sieves as no manipulative losses were apparent for this diet. Uneaten feed samples were individually dried in small plastic trays at 75 °C in a forced-fan oven for at least 20 h to achieve constant weight (Crear et al., 2002; Sheppard et al., 2002). The dry weight of the food provided at the start of a feeding event was back-calculated by accounting for the diet dry matter content and losses during immersion and the recovery procedure (i.e., water stability). Water stability was calculated by introducing a known amount of food in tanks (formulated diet, \( n = 58 \); mussel flesh, \( n = 45 \)) without lobsters and measuring recovered food after different immersion periods (i.e., 1-5 h) in a similar manner, and with similar water condition, as for feed intake calculation.

Mean feed intake (FI), as gram of dry matter (DM) per lobster, was calculated as follows (Soares et al., 2005):

\[
FI (g \text{ DM lobster}^{-1}) = \left[ \sum_N (WWo \times DM / 100 \times S) - DW_i \right] / N
\]

Where \( N \) is the number of individuals analysed (Table 3.2.), \( WWo \) is the wet weight of the food offered, DM is the dry matter content (%) of the food, S is the water stability (i.e., percentage of food remaining after immersion and recovery), \( DW_i \) is the dry weight of uneaten food recovered after the feeding event.

Mean specific feed intake (SFI), as a percentage of lobster BW, was calculated as:

\[
SFI (%) = \left[ \sum_N (FI / BW \times 100) / N \right]
\]

The volume (\( cm^3 \)) and moisture content (%) of both diets were measured after different immersion durations (dry formulated diet, \( n = 3 \) at 0.5, 1, 1.5, 2 h; mussel flesh, \( n = 6 \) at 2 h immersion) to calculate volumes corresponding to the measured FI. Volume was measured by displacement in a measuring cylinder. Lobsters from the three size-classes (S1, \( n = 28 \); S2, \( n = 12 \); S3, \( n = 12 \)) were allowed to feed for 2 h on similar amount (in DM) of either diets that corresponded to values close to the average satiation rations established during the feeding experiments, and euthanised via rapid chilling at \(-20\) °C. The wet weight of digesta
(i.e., food collected from foregut; WW\(_D\)), was measured and expressed as a ratio to the dry food intake (DW\(_{FI}\)) as follows:

\[
\text{DW}_{FI} / \text{WW}_D \times 100
\]

### 3.2.3. Foregut capacity

An estimated maximum volume of the foregut of juvenile *J. edwardsii* at various sizes was determined using a filling method described by Hill (1976). A total of 34 juvenile lobsters (9.6-106.6 g) starved for four days were euthanised via rapid chilling and dissected to expose their foreguts. The foregut wall was lined with chitin, relatively inflexible but soft with the anterior wall collapsing when empty. Foreguts were excised from the lobsters by cutting muscle attachments at the oesophagus and sectioning the midgut just posterior to the filter-press chamber (i.e., pyloric stomach) (see Fig. 1.1.). The sectioned end of the filter-press chamber was held closed using a small clip. The volumes of the foreguts were then measured by cleaning and filling to maximum capacity (i.e., without stretching foregut walls) with de-ionised water via the oesophagus. Foregut volumes did not include oesophagus volumes.

### 3.2.4. Statistical analyses

The effect of size-class, diet and meal duration on mean dry matter feed intake were compared using one-way ANOVA. Feed intake data were log\(_{10}\) transformed to satisfy ANOVA requirements for normality of residuals and homogeneity of variances (Shapiro-Wilk and Levene tests respectively, NS). If ANOVA detected a significant difference, Tukey-Kramer test was used for post hoc comparisons between individual pairs of means (\(P<0.05\)) (Zar, 1999). When \(P>0.05\), non-significant results were stated as NS. All analyses were performed using the Statistical & Power Analysis Software NCSS 2004 (Utah, USA). Mean dry matter feed intake in relation to meal duration was fitted with best-fit least square “exponential rise to maximum” regression curves (Sigmaplot 2004, v. 9.0, Systat software, Inc.) which best described the feed intake dynamics over the range of feeding durations (highest \(r^2\)).
3.3. Results

Mean FI for each meal duration on both diets increased significantly with lobster wet weight (one-way ANOVA, \(df=2, 336, F=282.38, P<0.001\)) (Fig. 3.1.) but decreased when expressed as a proportion of BW (one-way ANOVA, \(df=2, 336, F=64.59, P<0.001\)). No significant difference was found between FI obtained with mussel flesh and formulated diet for each meal duration and lobster size-class when analysed separately. However, the three combined size-classes of lobsters ingested greater dry matter quantities of formulated diet than mussel flesh in the first 30 min following diet introduction (one-way ANOVA, \(df=1, 54, F=6.72, P<0.05\)). Lobsters were still actively feeding on both diets 30 min after diet introduction, with FI of the combined size-classes being significantly lower for a meal of 30 min than for meals of 1 h or more (one-way ANOVA, \(df=4, 336, F=16.29, P<0.001\)) (Fig. 3.1.). However, allowing lobsters to feed for longer than 1 h on both diets did not result in further increases in mean FI (one-way ANOVA, \(df=3, 282, F=2.21, NS\)) (Fig. 3.1.). Although FI on mussel flesh in the largest size-class of lobsters (S3) appeared to increase beyond 1 h this trend was not statistically significant. The similar amount of food consumed when feeding for 1 h or more showed that lobsters reached a mean threshold intake of dry matter, or mean satiation ration, around 1 h after the introduction of food in the tanks (Fig. 3.1.). Mean satiation rations did not differ significantly between the two types of diet (one-way ANOVA, \(df=1, 282, F=0.55, NS\)). Regression curves provided a close fit to the data with maxima corresponding to satiation rations (Fig. 3.1.). When expressed as a proportion of lobster BW (SFI), satiation rations were equal to 1.20% BW, 1.11% BW and 0.77% BW for S1, S2, and S3 lobsters, respectively.
Fig. 3.1. Mean dry matter feed intake (g ± S.E.) for three size-classes of 48 h unfed juvenile *Jasus edwardsii* (S1, 18.8 ± 1.2 g; S2, 42.0 ± 2.6 g; and S3, 82.9 ± 4.9 g) allowed to feed on mussel flesh or the dry formulated diet for different meal durations. Means were fitted with best-fit curves, with FI, feed intake (g), and T, meal duration (h). First value in equation represents mean threshold feed intake value, or satiation ration, and exponent value represents the rate to reach threshold. The asterisk indicates a significant difference ($P<0.05$) in mean feed intake between 0.5 h and other meal durations for the combined treatments.

Both diets displayed high water stability, with dry matter retention being greater than 94% after 5 h of immersion (Fig. 3.2., A). Water immersion of the dry formulated diet resulted in considerable water intake and volume gain within 2 h (Fig. 3.2., B). There was no difference in dry matter content (mean ± S.E., 23.0 ± 0.3%) and dry weight to volume ratio (0.231 ± 0.004 g cm$^{-3}$) of mussel flesh after immersion. Wet weights of food in the foregut did not differ significantly when lobsters were fed similar dry matter quantities of the two diets (Table 3.3.), despite the higher moisture content of mussel flesh (77%) compared with the dry formulated diet even after 2 h of immersion (52%). The dry matter feed intake to wet weight
of digesta ratio was similar for the two diets (one-way ANOVA, \(df=1.46, F=1.74, \text{NS}\)) and this was consistent over the three size-classes (one-way ANOVA, \(df=2.46, F=2.43, \text{NS}\)) (Table 3.3.).

Fig. 3.2. A) Mean water stability (\(\% \pm \text{S.E.}\)) of mussel flesh and the dry formulated diet at various times over 5 h immersion. Water stability corresponds to remaining dry matter after losses following water immersion and the recovery procedure. B) Mean moisture (\(\%\)) and dry weight to volume ratio (g cm\(^{-3}\) \(\pm \text{S.E.}\)) of the dry formulated diet at various times over 2 h immersion. Note: 0 h on the x-axis corresponds to values prior immersion.
Table 3.3. Mean (± S.E.) dry matter feed intake to wet weight of digesta ratio (DWFI / WWD) for three size-classes of juveniles Jasus edwardsii. Significant differences between means within the same size-class are marked by different letters (P<0.05).

<table>
<thead>
<tr>
<th>Size-class</th>
<th>Formulated diet DWFI / WWD (%)</th>
<th>Mussel flesh DWFI / WWD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1^1</td>
<td>38.8 ± 1.8^a</td>
<td>33.3 ± 1.6^a</td>
</tr>
<tr>
<td>S2^2</td>
<td>37.3 ± 5.0^a</td>
<td>35.0 ± 3.5^a</td>
</tr>
<tr>
<td>S3^3</td>
<td>31.2 ± 3.4^a</td>
<td>30.1 ± 1.7^a</td>
</tr>
</tbody>
</table>

^1S1: 18.8 ± 1.2 g; n=28
^2S2: 42.0 ± 2.6 g; n=12
^3S3: 82.9 ± 4.9 g; n=12

Foregut capacity (FC; cm³) displayed a significant linear relationship to lobster BW (P<0.001, r²=0.97) (Fig. 3.3.). For the mean lobster BW for each of the three size-classes S1 (19 g), S2 (42 g) and S3 (83 g) the predicted foregut capacities were 0.58, 1.13 and 2.12 cm³ respectively based on this relationship (Table 3.4.). These volumes represented 3.05% (S1), 2.69% (S2) and 2.55% (S3) of the mean BW of the lobster from the different size-classes. Calculated foregut volume decreased slightly in proportion to body weight as for SFI.
Fig. 3.3. Relationship between lobster wet body weight (g) and measured foregut capacity (cm$^3$) in juvenile *Jasus edwardsii* (10-110 g). FC, foregut capacity (cm$^3$), and BW, lobster wet body weight (g).

Foregut filling for each size-class fed to satiation (Table 3.4.) was predicted from FI satiation rations (Fig. 3.1.) and their equivalent wet weight in the foregut after 2 h (Table 3.3.), in relation to the expected foregut volumes for the different lobster size-classes (Fig. 3.3.). Predicted foregut filling for the three size-classes ranged from 100% to 114% for lobsters fed the dry formulated diet and 105% to 129% for lobsters fed mussel flesh (Table 3.4.).
Table 3.4. Relationship between foregut capacity and mean satiation rations in *Jasus edwardsii*.

<table>
<thead>
<tr>
<th>Size-class</th>
<th>Diet</th>
<th>Predicted foregut capacity (cm$^3$)</th>
<th>Mean satiation rations (dry matter intake) (g)</th>
<th>Corresponding volume of food (cm$^3$)$^1$</th>
<th>Corresponding wet weight of food (g)$^1$</th>
<th>Corresponding wet weight of food in foregut (g)$^2$</th>
<th>Predicted foregut filling (%)$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (19 g)</td>
<td>Formulated diet</td>
<td>0.58</td>
<td>0.24</td>
<td>0.41</td>
<td>0.50</td>
<td>0.62</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>0.58</td>
<td>0.25</td>
<td>1.08</td>
<td>1.09</td>
<td>0.75</td>
<td>129</td>
</tr>
<tr>
<td>S2 (42 g)</td>
<td>Formulated diet</td>
<td>1.13</td>
<td>0.48</td>
<td>0.70</td>
<td>1.00</td>
<td>1.29</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>1.13</td>
<td>0.46</td>
<td>1.98</td>
<td>2.00</td>
<td>1.31</td>
<td>116</td>
</tr>
<tr>
<td>S3 (83 g)</td>
<td>Formulated diet</td>
<td>2.12</td>
<td>0.66</td>
<td>0.96</td>
<td>1.38</td>
<td>2.11</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>2.12</td>
<td>0.67</td>
<td>2.89</td>
<td>2.91</td>
<td>2.22</td>
<td>105</td>
</tr>
</tbody>
</table>

$^1$ Assuming a 2 h water immersion duration for the formulated diet
$^2$ Calculated from Table 3.3.
$^3$ Calculated as wet weight of food in foregut / volume of foregut × 100
3.4. Discussion

Results from this study indicate that *J. edwardsii* juveniles left without food for 48 h achieve similar dry matter feed intake on the dry formulated diet and mussel flesh in a single meal event lasting up to 5 h. This finding implies that the attractiveness and palatability of the dry formulated diet is adequate, with lobsters observed manipulating the food within 5 min of immersion, and higher dry matter intake on the formulated diet than on mussel flesh in the first 30 min. The formulated diet contains freeze-dried krill hydrolysate, the amino acid lysine, and the quaternary ammonium compound betaine, which are known to attract and stimulate feed intake in crustaceans (Smith et al., 2005b; Williams, 2007). However, there was no consumption advantage for dry matter intake in providing the higher nutrient density of the dry formulated diet compared with mussel flesh for 1-5 h. The difference in dry matter intake within 30 min on the two diets may also have been owing to the difficulty for lobsters to manipulate, cut and ingest large portions of mussel flesh in comparison to the relatively fast “grinding” behaviour exhibited on the dry formulated diet. The formulated diet contained more dry matter bite-for-bite than fresh mussel flesh.

Matching pellet size to lobster size has previously been found to reduce manipulative losses and wastage of formulated diet (Sheppard et al., 2002). However, in the current study, a single size of dry pellet was fed to different sizes of *J. edwardsii* juveniles (i.e., 20-80 g) without influencing the rate (1 h) to reach satiation. Manipulative losses when feeding on the dry formulated diet were not quantified in this study, but observations suggested that losses in relation to total food ingested were reduced (<10%) and not markedly different between the three lobster size-classes. Manipulative losses were reduced compared with losses (up to 50%) measured by Sheppard et al. (2002) possibly because the water stability of the dry formulated diet of this study (98.1% in 1 h) was better than their experimental diets (83.0-92.3% in 1 h).

Lobsters fed every 48 h were observed to be actively feeding only within the first 1-2 h of diet introduction and only occasionally returned to the food afterwards (i.e., 2-5 h). This behaviour was reflected quantitatively by similar mean dry matter feed intake for lobsters allowed to feed 1 h or more (i.e., satiation ration). As the response was similar for both diets, and lobsters are known to remain attracted to mussel for extended periods of time (e.g., 10 h)
(Williams, 2007), it is unlikely that the mean satiation rations that were reached were limited in extent by a lack of attraction to the feeds after a few hours of immersion. Mean satiation rations increased with the size-class of the lobster but decreased slightly in proportion to BW, as previously found for daily consumption in this species (James & Tong, 1998). It was observed that some lobsters that had fed on the formulated diet to satiation levels were still actively manipulating the pellets for a short time afterwards (10-20 min) but did not appear to ingest the food further. Those lobsters were also observed regurgitating some of the ingested food when hand-held around that time (i.e., 1-2 h after food introduction). These observations suggested that the mean satiation rations measured in this study (i.e., feeding lobsters for a short period every second day) were the result of the complete filling of the foregut to maximum capacity. In a previous study P. ornatus juveniles have been found to be less inclined to eat formulated diets after 1-2 h of immersion, which was attributed to the low attractiveness of the formulated diets in comparison to fresh mussel (Williams et al., 2005). However, it is possible that these lobsters displayed a reduced intake as a response to the complete filling of their foregut. In fact subjecting J. edwardsii to dietary extracts rather than feeding on diets resulted in a non-soaked commercial shrimp diet being more attractive than fresh mussel (Tolomei et al., 2003). The discrepancies between the two studies may also be due to differences in lobster species and feeding frequencies. Tolomei et al. (2003) allowed a 48 h period without food before measuring attractiveness by recording chemical sensing behaviour, whereas Williams et al. (2005) measured feed intake when lobsters were fed daily. Overall, the present results suggest that food attraction and consumption may be influenced by post-ingestive processes, as revealed in subsequent research (see Simon & Jeffs, 2008; Simon, 2009b).

Foregut capacity in J. edwardsii was found to be closely related to wet body weight within the range of sizes tested (i.e., 10-110 g). A similar finding was found for the mud crab, Scylla serrata (Hill, 1976). The foregut volume of J. edwardsii (2.5-3% BW) appears equally small to shrimps (Nunes & Parsons, 2000), and limits the amount of food lobsters consume when fed every 48 h. The similar mean satiation rations for the two diets were owing to similar wet weights of the two diets after ingestion when feeding for 2 h on a similar amount of dry matter. This result is unexpected given that after 2 h of immersion the dry formulated diet had lower moisture content (52%) than mussel flesh (77%). A similar phenomenon was observed in subsequent research (see Simon & Jeffs, 2008). The observed feeding habit of J.
edwardsii on the dry formulated diet is likely to have resulted in the ingestion of water in conjunction with the food. The formulated diet, as observed in the foregut, was ground up completely as a result of the “grinding action” of the mandibles during ingestion and the gastric-mill afterward, and had a paste-like consistency owing to the large amount of fluid present. In comparison, mussel flesh was present in large compacted pieces and little liquid was present in the foregut, apart from the distinctive dark brown chime discharged from the digestive gland (Pers. Obs., Plate 3). Digestive fluid secretions when feeding on the dry formulated diet may also have made up for the increase in moisture in foregut. Subsequent research has shown that digestive fluid volume accounts for around 24% of the capacity of the foregut prior feeding (see Simon, 2009b). The predicted foregut filling was higher than 100% for several possible reasons. Foregut evacuation and food digestion would have already started by 2 h as suggested by the results of subsequent research (Simon & Jeffs, 2008). In crustaceans, evacuation has been found to start prior to completion of feeding with no significant time-lag (Loya-Javellana et al., 1995; Nunes & Parsons, 2000). Early evacuation and digestion combined with the squeezing of mussel flesh pieces during ingestion, may explain the smaller volume of mussel flesh after ingestion compared to before ingestion. Lastly, in the present study the method used to measure foregut capacity may have slightly underestimated the actual maximum foregut volume achievable by lobsters while feeding, as oesophagus volume was not included. The mean satiation rations measured in this study were similar to satiation rations (i.e., 1.3-1.4% BW) measured for similar sizes of the Australian red claw crayfish, Cherax quadricarinatus, which were also observed to fill their foregut to maximum capacity (Loya-Javellana et al., 1995). Overall, the satiation rations measured in these decapods are lower than for penaeid shrimps (Soares et al., 2005), despite the similar foregut sizes in relation to BW, because of the slower rates of food intake and foregut evacuation in the larger decapods (Joll, 1982; Loya-Javellana et al., 1995).

This study shows that a similar dry matter feed intake can be achieved on the dry formulated diet compared to fresh mussel when juvenile *J. edwardsii* are fed for short durations every second day. Given the feed intake rates on both diets measured in this study, allowing the lobsters to feed for periods of 1-2 h should be sufficient to achieve maximum feed intake for at least 5 h. Juvenile *J. edwardsii* of similar sizes to the S1 lobsters held at 18 °C and fed daily have previously been found to have dry matter food consumption around 1% BW day⁻¹ (Crear et al., 2002; Thomas et al., 2003; Tolomei et al., 2003). Such a level of food consumption is
equivalent to just less than the capacity of one foregut per day. Together these results suggest that food intake can be maximised with feeding more often than every 48 h as previously performed in experimental sea-cages (Simon & James, 2007). The formulated diet also appears as attractive and palatable as fresh mussel. The lack of difference for the amount of both dry matter and wet weight of the two diets in the foregut of lobsters suggests that dry diets in their current form are not effective for increasing dry matter intake in *J. edwardsii* (Ruohonen et al., 1997). Improvements in formulated diet format (texture and moisture) may help to reduce the ingestion of water or compensatory digestive secretions and thereby allow an increase in dry matter intake. With feed intake restricted by the small foregut size in the lobsters, formulating diets of high digestibility will also be vitally important to maximise the amount of nutrient assimilated per meal.
Plate 4. Feeding frequency and gut evacuation in *Jasus edwardsii* juveniles fed mussel flesh and the dry formulated diet. A) Rearing tanks with automatic feeders used to test the effect of feeding frequency; B) Juvenile lobster raised from the tank bottom on a plastic-coated iron mesh grid after feeding to facilitate the collection of faecal strands by gravity; C) Faeces settled after feeding juvenile lobsters on the dry formulated diet; D) Micro-filtration of faeces on glass microfibre filter papers and washing with distilled water; E) Faecal collection from eleven individually housed lobsters 10 h after feeding on the formulated diet; F) Dissection of small (19 g) juvenile lobsters showing hindgut evacuation of digesta 18 h after feeding on dry formulated diet (left) and mussel flesh (right).
Chapter four – Appetite revival and food consumption under different feeding frequencies: relationship to gut evacuation

Published as:


4.1. Introduction

Despite more than 20 years of research in diet development for lobsters, relatively little is known about the digestive processes occurring after the ingestion of formulated diets and the potential for a negative effect on subsequent feed intake. Appetite revival has been extensively studied in fish but little research has been undertaken in crustaceans (Loya-Javellana et al., 1995). Several factors are known to affect appetite in fish including stomach fullness and rates of gastric evacuation (Colgan, 1973; Grove et al., 1978; Vahl, 1979), temperature (Elliot, 1975), animal size (Grove et al., 1978), respiratory rate (Muir & Niimi, 1972), dietary energy content (Bromley, 1987; Johansen et al., 2002), circulating metabolites and glucose metabolism (Le Bail & Boeuf, 1997). Lobsters are known to display longer rates of gut clearance (Barker & Gibson, 1977; Kurmaly et al., 1990; Sarda & Valladares 1990) compared to smaller decapods such as penaeid shrimp (Allan & Smith, 1998; Nunes & Parsons, 2000). Therefore daily throughput of food is likely to be proportionately smaller in lobsters when compared with smaller decapods (Kurmaly et al., 1990). Slower gut evacuation of formulated diets compared to fresh food would have the potential to reduce food consumption further (Simon, 2009a), but this is yet to be examined. Previous studies measuring the gut evacuation of crustaceans have used a single diet, either formulated (Loya-Javellana et al., 1995; Nunes & Parsons, 2000; Soares et al., 2005) or natural (Hill, 1976; Joll, 1982; Sarda & Valladares 1990; McGaw & Reiber, 2000). However, studies in cultured fishes indicate that diet texture and composition (e.g., moisture, energy, fibre and particle size) can greatly influence evacuation dynamics (Ruohonen et al., 1997; Hossain et al., 2000).
Chapter 4

The aim of the current study is to measure key parameters of feeding and digestive processing for fresh mussel flesh and the dry formulated diet for different sizes of the juvenile spiny lobster, *Jasus edwardsii*. The key parameters are, 1) food consumption at various feeding frequencies, 2) maximum feed intake and rate of appetite revival, and 3) rate of foregut evacuation and faecal production. Establishing differences in these key parameters has the potential to identify the cause of constraints in the performance of formulated diets in spiny lobster aquaculture.

4.2. **Material and methods**

4.2.1. **Effect of feeding frequency on food consumption and growth**

Sixty spiny lobster juveniles, *J. edwardsii*, (mean weight ± S.D., 9.97 ± 1.55 g) were fed a mixed diet of the dry formulated diet and fresh mussel for one month before the experiment began and were acclimated to their experimental tanks for one week. Twelve 27 l black polyethylene tanks identical to one used in Simon and James (2007) were fitted with airstones and supplied with constant 18 ºC flow-through 70 µm filtered seawater at a flow-rate of 0.8 l min⁻¹. Tanks remained free of shelters to facilitate food recovery for food consumption measurements. Water quality parameters and the photoperiod (L8:D16) were similar to a previous growth study (Simon & James, 2007).

Two tanks containing five lobsters each were used per experimental feeding treatment. The growth of lobsters was followed at the individual level by uniquely marking the lobsters via uropod clipping. The two tanks were the maximum amount of replication achievable given the restriction on the number of lobsters and automated feeders available for the growth experiment. The experiment lasted 13 weeks with lobster wet body weight measured on day 0, 52 and 90 by gently blotting lobsters dry and weighing on a Mettler PC 4400 balance (± 0.01 g accuracy). The experimental treatments consisted of dry formulated diet fed to satiety with a similar daily ration of 3% BW day⁻¹ but divided evenly into five feeding frequencies: 3 week⁻¹ (at 1700 h), 1 day⁻¹ (1700 h), 2 day⁻¹ (1700 and 0800 h), 5 day⁻¹ (1700, 2000, 0200, 0800 and 1100 h), and 5 night⁻¹ (1700, 2000, 2300, 0200 and 0500 h). The last two treatments
received the same number of meals per day but differed in the duration between two meals and the extent of feed allotment over the illuminated period. The sixth treatment consisted of a diet of fresh mussel fed as half-shell 3 week\(^{-1}\) (i.e. feeding ration around 3% BW day\(^{-1}\) in dry weight) as per Simon and James (2007). Uneaten food was collected by siphoning the tanks daily at 1600 h for lobsters fed 1 day\(^{-1}\) or more often, and at 1600 h the day of the feed allotment for lobsters fed 3 week\(^{-1}\). In order to be able to calculate feed intake the remaining feed from each tank was collected on a 250 µm sieve, washed gently with distilled water to remove salts and dried to constant weight at 75 °C for 20 h in a forced-fan oven. Consumption measurements were taken every second week (i.e., 7 weeks of consumption data), with four measurements from Monday to Friday for daily or multiple daily feeding and two measurements for the mussel and formulated diet treatments fed 3 week\(^{-1}\). Daily consumption during a particular week was calculated as the mean of all consumption measurements performed in that week. Water stability and dry matter loss from known amounts of the two diets (\(n=9\)) was calculated for fresh mussel at 48 h post-immersion and for the formulated diet at various immersion times to account for the differential leaching rate between the different feeding frequencies (Fig. 4.1.). The dry weight of the diets fed was back-calculated accounting for the diet dry matter content and losses due to leaching and the recovery procedure (i.e., water stability). For each fed ration in a particular feeding treatment, the time allowed to leach before food collection was calculated and the correct water stability coefficient applied to the ration accordingly. Mean food consumption (FC), in dry matter per lobster, was calculated as follows:

\[
FC (g \, DM \, lobster^{-1}) = [(WWo \times DM / 100 \times S) – DW_r] / N
\]

Where WWo is the wet weight of the feed offered, DM is the dry matter content (%) of the feed, S is the water stability (i.e., percentage of food remaining after leaching and recovery), DW\(_r\) is the dry weight of uneaten feed recovered after the feeding event and N is the number of individuals alive per tank at the time of the uneaten feed collection.

Mean specific food consumption (SFC), expressed as a percentage of lobster wet body weight, was calculated as follows:

\[
SFC \, (BW \, day^{-1}) = FC / BW \times 100
\]
Where BW is the mean lobster body weight for that tank, measured on days 0, 52 and 90. For consumption measurements obtained during weeks between censuses, BW was taken from the census closest to that week.

Weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival were calculated for each treatment \( (n=2 \) replicate tanks) as in previous studies on *J. edwardsii* (Crear et al., 2000; Ward et al., 2003; Simon & James, 2007). Weight gain and growth calculated for each tank corresponded to the mean of the values for each lobster in that tank.

\[
FCR = \frac{FC \times 90}{\text{weight gain}}
\]

Where FC is the averaged food consumption in dry matter per lobster over the entire growth experiment (90 days).

\[
PER = \frac{100}{(FCR \times CP)}
\]

Where CP is the crude protein content (in %DM) of the diets.

### 4.2.2. Appetite revival and gut evacuation

#### 4.2.2.1. Experimental setup

The same three size-classes of lobster juveniles established in Simon (2009a) were used in this study. Quantitative measurements were performed on each size-class consecutively in the same experimental tanks and conditions as per Simon (2009a), except for the photoperiod which was kept this time at L24:D0 to eliminate differences in diurnal and nocturnal feeding activities and metabolic rates (Crear & Forteath, 2000; Crear et al., 2003).

#### 4.2.2.2. Appetite revival

Appetite revival (AR) was determined as the dry matter of feed ingested per lobster
in a single 2 h feeding event (meal 2) after a satiation feeding event (meal 1), and expressed as a proportion of the average satiation meal (Loya-Javellana et al., 1995):

\[ \text{AR} = \frac{\text{meal 2}}{\text{meal 1}} \times 100 \]

Lobsters were left without food for 48 h prior to the delivery of a 2 h satiation meal (meal 1) of either fresh mussel flesh or the formulated diet. A meal duration of 2 h and ration corresponding to an SFI of 0.8-1.2% in dry matter (DM) of the body weight of the lobster was deemed sufficient to reach satiation, i.e., sufficient to fill the foregut of lobsters that were unfed for 48 h (Simon, 2009a). The introduction of food for meal 1 in water was designated as 0 h. Satiated lobsters were then fed meal 2 at different times following meal 1 (i.e., 6, 12, 18, 24, 30, 48, 72 h). The same diet was used for meal 1 and 2. Lobsters that did not reach satiation during meal 1 and/or fed less than 0.1% BW during meal 2 were not used for the experiment as those were considered not to display a normal appetite response. Individual lobsters were re-used several times in the experiment after a recovery period during which they had been fed at least twice on their respective diet. Re-used lobsters were randomly assigned a new experimental appetite revival duration between meal 1 and meal 2. A total of 560 appetite revival measurements were analysed, with at least five measurements performed for each diet at any single appetite revival time for each of the three size-classes of lobsters. Feed intake measurements (FI and SFI) were performed as per Simon (2009a).

4.2.2.3. Foregut evacuation

Foregut evacuation was measured for the S1 and S3 size-classes on the two diets using a serial sampling technique (Joll, 1982; Loya-Javellana et al., 1995; Nunes & Parsons, 2000). Lobsters left without food for 48 h were allowed to feed for 1 h and ingest the total of a pre-weighed meal corresponding to 50% of the satiety ration for that lobster size-class. Lobsters that did not completely ingest the meal were not sampled. Serial sampling involved sequentially euthanising replicate lobsters (S1, n=83; S3, n=43) at various time intervals (i.e., 1, 2, 4, 6, 8, 10, 14 h) following the initiation of the feeding event (i.e. 0 h). Serially sampled lobsters were dissected and the entire foregut contents removed (i.e., digesta), and then dried to constant weight at 75 °C for 20 h in a forced-fan oven to calculate remaining dry matter in
the foregut. Foregut evacuation was expressed as the proportion of dry matter remaining in the foregut of the initial intake of dry matter.

4.2.2.4. Faecal production

Faecal production was measured gravimetrically via serial faecal sampling events (i.e., 10, 14, 18, 34, and 42 h) for the three size-classes. A total of 12 lobsters per size-class that were unfed for 48 h to empty digestive tracts were fed a 1 h meal corresponding to 50% of the satiation ration of either formulated diet \( (n=6) \) or mussel flesh \( (n=6) \). Uneaten food was collected as outlined previously (Simon, 2009a) for feed intake determination and to clear the tanks of any non-faecal material. Lobsters were then raised from the tank bottom on a grid made of plastic-coated iron mesh to facilitate the separation and collection of faecal strands by gravity and prevent coprophagy (Plate 4, B). Faecal collection started at 10 h after the introduction of food and lasted up to 42 h to ensure the majority of faeces from the single meal event were sampled. Faeces were collected by micro-siphoning the tanks onto allocated pre-weighted 1.2 μm glass microfibre filter papers. Faecal production was expressed as the proportion of feed intake and as the proportion of total faeces produced (i.e., total faeces collected within 42 h post-feeding). A total of 180 samples were analysed. Dry matter apparent digestibility (\( AD_{DM} \)) was calculated gravimetrically as:

\[
AD_{DM} (\%) = \frac{(DM_{I} - DM_{F})}{DM_{I}} \times 100
\]

Where DM\(_{I}\) and DM\(_{F}\) are the dry matter of feed ingested (g) and dry matter of faeces produced within 42 h post-feeding (g), respectively.

4.2.3. Statistical analyses

One-way ANOVA was used to test for differences between the feeding frequency treatments at the completion of the experiment. Daily consumption means of the six dietary treatments \( (n=2 \) replicate tanks) were compared through time (i.e., 7 weeks of consumption measurements) by means of a repeated measures ANOVA.
The effect of diet and size-class on the feed intake of lobsters fed every 48 h was compared using a two-way ANOVA. The effect of diet, size-class and post-prandial time on the appetite revival of lobsters was compared by means of two-way ANOVAs followed by one-way ANOVAs when interactions between treatment found significant.

Linear least-square regression analyses were performed on the data (1-10 h) for foregut evacuation. Analyses of covariances (ANCOVA) were performed to compare the effect of lobster size and diet on the rates (slopes) of foregut evacuation. The effect of diet on the proportion of digesta remaining in the foregut for each sampling time was compared using one-way ANOVAs.

The effects of diet and size-class on the cumulative faecal production were compared through time by means of repeated measures ANOVA. The effects of diet and size-class on the dry matter digestibility were compared using one-way ANOVAs.

For all statistical analyses, the ANOVA assumptions of normality of residuals and homogeneity of variances were satisfied (Shapiro-Wilk and Levene tests respectively, NS). Percentage data were arcsine transformed. Significant differences ($P<0.05$) between the means were determined by post hoc comparison using Tukey-Kramer test (Zar, 1999). When $P>0.05$, non-significant results were stated as NS. All analyses were performed using the Statistical & Power Analysis Software NCSS 2004 (Utah, USA).

### 4.3. Results

#### 4.3.1. Effect of feeding frequency on food consumption and growth

Water stability of the dry formulated diet remained high (>90%) up to 20 h post-immersion. Stability decreased significantly to 68.5% when immersed for 48 h (one-way ANOVA, $df=8,69$, $F=165.8$, $P<0.001$). Mussel flesh was more stable (88.6%) than the formulated diet when immersed for 48 h ($df=1, 16$, $F=36.91$, $P<0.001$) (Fig. 4.1.).
Among lobsters fed the formulated diet, those fed once daily tended to show a better weight gain and higher specific growth rate compared to other feeding frequencies. Lobsters fed mussel grew significantly faster than those fed the formulated diet for all feeding frequencies (one-way ANOVA, $df=5,6$, $F=20.08$, $P<0.001$) except when fed daily (Tukey test, NS) (Table 4.1.). Apparent food consumption was significantly lower for lobsters fed several meals a day while not significantly different for lobsters fed mussel and formulated diet 3 week$^{-1}$ and 1 day$^{-1}$ (repeated measures ANOVA, $df=5,6$, $F=9.62$, $P<0.01$). Multiple daily feeding improved feed utilisation, with the FCR and PER of lobster fed 5 day$^{-1}$ being close to those obtained feeding fresh mussel flesh. Survival tended to be lower on the formulated diet than on mussel flesh (100%), but remained above 70% for all feeding frequency treatments (Table 4.1.).

Fig. 4.1. Mean water stability ($\% \pm $ S.E., $n=9$) of the dry formulated diet at various times over 48 h immersion. Water stability corresponds to remaining dry matter after losses owing to water immersion and the recovery procedure. Note: the water stability of fresh mussel ($\% \pm $ S.E., $n=9$) is shown after 48 h immersion only.
Table 4.1. Performance of juvenile *Jasus edwardsii* fed fresh mussel or the dry formulated diet under five different feeding frequencies. Significant differences between means within the same row are marked by different letters (*P*<0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulated diet feeding frequencies</th>
<th>Mussel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 night&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5 day&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>10.01 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.14 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>3.13 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.23 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>0.30 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FC (g DM lobster&lt;sup&gt;1&lt;/sup&gt; day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.10 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFC (% BW day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.80 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.78 ± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCR</td>
<td>2.62 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.92 ± 0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>PER (%)</td>
<td>90.84 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.66 ± 14.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>90 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
4.3.2. Appetite revival

Lobster feed intake on meal 1, corresponding to the dry matter consumed in a 2 h feeding event after 48 h without food, did not differ significantly between lobsters fed the formulated diet and fresh mussel for the three size-classes (two-way ANOVA, \( df=2,217, F=1.75, \text{NS} \)) (Table 4.2.). The average FI increased significantly with lobster size for the two diets (\( F=206.10, P<0.001 \)), but SFI was similar for S1 and S2 size-classes (1.13-1.27% BW) and lower (0.8-0.82% BW) for the S3 size-class (\( F=126.12, P<0.001 \)) (Table 4.2.).

Table 4.2. Mean feed intake ± S.E. during a 2 h feeding event (meal 1) on either mussel flesh or the dry formulated diet for three size-classes of juvenile *Jasus edwardsii* left without food for 48 h. Feed intake is expressed as FI, dry matter intake in grams (g), or SFI, percentage of lobster wet body weight (% BW). Feed intake values were assumed to correspond to 100% satiation for appetite revival calculation. Significant differences between means within the same column are marked by different letters (\( P<0.05 \)).

<table>
<thead>
<tr>
<th>Size-class</th>
<th>Diet</th>
<th>n</th>
<th>FI (g)</th>
<th>SFI (% BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (19 g)</td>
<td>Formulated diet</td>
<td>29</td>
<td>0.25 ± 0.01(^a)</td>
<td>1.22 ± 0.04(^a)</td>
</tr>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>40</td>
<td>0.25 ± 0.01(^a)</td>
<td>1.27 ± 0.05(^a)</td>
</tr>
<tr>
<td>S2 (42 g)</td>
<td>Formulated diet</td>
<td>36</td>
<td>0.51 ± 0.02(^b)</td>
<td>1.23 ± 0.04(^a)</td>
</tr>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>29</td>
<td>0.47 ± 0.02(^b)</td>
<td>1.13 ± 0.05(^a)</td>
</tr>
<tr>
<td>S3 (83 g)</td>
<td>Formulated diet</td>
<td>36</td>
<td>0.66 ± 0.03(^c)</td>
<td>0.80 ± 0.04(^b)</td>
</tr>
<tr>
<td></td>
<td>Mussel flesh</td>
<td>45</td>
<td>0.69 ± 0.03(^c)</td>
<td>0.82 ± 0.04(^b)</td>
</tr>
</tbody>
</table>

Appetite revival differed between diets, as well as between lobster size-classes but solely for lobsters fed the formulated diet. Appetite revival was significantly greater when feeding on mussel flesh compared to the formulated diet for up to 12 h post-feeding on a satiation meal for S1 lobsters (Fig. 4.2., A) and up to 24 h for the two larger size-classes (Fig. 4.2., B and C). Lobsters from the three size-classes displayed rates of appetite revival that did not differ significantly from 100% (i.e., fed every 48 h) when re-feeding on mussel flesh 12 h or more after a satiation meal. For the three size-classes, the only time at which a significantly
lower appetite than 100% was detected was when lobsters were re-fed mussel flesh 6 h after a satiation ration (two-way ANOVA, df=6,228, F=12.26, P<0.001) (Fig. 4.2.). In contrast, appetite revival on the formulated diet was significantly lower than 100% when allowed to feed again 18 h or less for S1 (one-way ANOVA, df=7,86, F=26.46, P<0.001) (Fig. 4.2., A), 24 h or less for S2 (df=6,132, F=60.68, P<0.001) (Fig. 4.2., B), and 30 h or less for S3 (df=6,60, F=21.08, P<0.001) (Fig. 4.2., C). For the three size-classes fed the formulated diet, mean appetite revival increased with time gradually but an initial lag in appetite revival was apparent. Appetite revival was less than 20% after 6 h and less than 50% after 12 h post-feeding on an initial satiation ration for all three size-classes. Mean appetite revival on both diets for the three size-classes did not improve significantly beyond 100% (i.e., appetite when fed every 48 h) if re-fed 72 h after a satiation meal confirming observations of foregut capacity (two-way ANOVA, df=1,217, F=1.50, NS).

4.3.3. Foregut evacuation

Foregut emptying time was approximately 10 h at 18 ºC for both size-classes and diets. However, the foregut of the lobsters did not empty completely in 10 h, with around 20% of the dry matter ingested remaining for a period longer than 14 h for both types of diet (Fig. 4.3.). Foregut evacuation rates over the first 10 h ranged from −6.26 ± 0.73% (S1 feeding on mussel flesh) (F=73.18, P<0.001) to −9.78 ± 0.87% (S3 on formulated diet) (F=127.78, P<0.001). The rate of evacuation of fresh mussel was faster for larger S3 lobsters than smaller S1 lobsters (ANCOVA, df=1,50, F=5.96, P<0.05), but no difference in evacuation rates were found between the two size-classes fed the formulated diet (ANCOVA, df=1,51, F=3.98, NS). Overall, foregut evacuation rates did not differ significantly between the two diets for both the S1 (ANCOVA, df=1,68, F=2.07, NS) and S3 size-classes (ANCOVA, df=1,33, F=1.25, NS). However, the proportion of meal remaining in the foregut of S1 lobsters at both 2 h and 6 h post-feeding was significantly higher for the formulated diet than for mussel flesh (one-way ANOVAs, F=7.96; 9.68, P<0.05) suggesting an initial delay in foregut emptying for S1 lobsters fed the formulated diet (Fig. 4.3.). Lobsters in the S3 size-class also tended to show a similar initial delay in evacuation when feeding on the formulated diet, with 85.6% of the formulated diet consumed still present 4 h post-feeding compared to 65.4% for mussel flesh (Fig. 4.3.).
Fig. 4.2. Mean appetite revival (%) ± S.E. on mussel flesh and the dry formulated diet for three size-classes of juvenile *Jasus edwardsii*, expressed as the relative dry matter intake during meal 2 in proportion of the average dry matter intake during an earlier satiation meal fed at 0 h (meal 1; see Table 4.2.), for various durations between the introduction of food for the two meals. A) S1 lobsters (19 g); B) S2 lobsters (42 g); C) S3 lobsters (83 g). Asterisks (* P<0.05; ** P<0.01; *** P<0.001) indicate significant differences in mean appetite revival between the two diets for each time.
Fig. 4.3. Mean proportion of dry matter intake (%) ± S.E. remaining in the foregut at various times post-feeding on mussel flesh and the dry formulated diet for two size-classes of juvenile *Jasus edwardsii*. A) S1 lobsters (19 g); B) S3 lobsters (83 g). P in the regression equation corresponds to the proportion of dry matter remaining in the foregut (%), and T the time (h) after the introduction of food. Regression slopes (S.E. of parameters in parentheses) indicate foregut evacuation rates. Asterisks (* P<0.05; ** P<0.01) indicate significant differences between the two diets for a particular time post-feeding.
4.3.4. Faecal production and apparent dry matter digestibility

Cumulative faecal production as a proportion of feed intake was significantly higher on the formulated diet than on mussel flesh through time (repeated measures ANOVA, $df=1,34$, $F=341.27$, $P<0.001$), resulting in better overall dry matter apparent digestibility ($AD_{DM}$) for fresh mussel flesh ($89.2 \pm 1.0\%$) than for the formulated diet ($62.1 \pm 1.2\%$) (one-way ANOVA, $df=1,34$, $F=278.55$, $P<0.001$) (Fig. 4.4.). There was a significant increase in $AD_{DM}$ with increasing lobster size (S1: $85.8 \pm 1.9\%$; S2: $88.9 \pm 1.1\%$; S3: $92.8 \pm 0.3\%$) when feeding on mussel flesh (one-way ANOVA, $df=2,15$, $F=8.84$, $P<0.01$), but not for the formulated diet (one-way ANOVA, $df=2,15$, $F=3.59$, NS).

Fig. 4.4. Mean cumulative proportion of feed intake (%) ± S.E. egested at various times post-feeding on mussel flesh and the dry formulated diet for three size-classes of juvenile *Jasus edwardsii*. 
Cumulative faecal production in the first 34 h post-feeding as a proportion of the total faeces produced was also found to be higher on the formulated diet (94%) than on mussel flesh (81%) (repeated measures ANOVA, $df=1,30$, $F=23.5$, $P<0.001$) and this difference was consistent between the three size-classes of lobster (repeated measures ANOVA, $df=6,90$, $F=0.27$, $P>0.25$). Therefore, the three size-classes were combined (Fig. 4.5.) (Winer et al., 1991). The first appearance of faecal strands varied from 5 h to 8 h post-feeding regardless of the lobster size and diet fed (pers. obs.). However, lobsters fed the formulated diet displayed a greater initial faecal production rate (32%) in the first 10 h post-feeding compared to lobsters fed mussel (16%) (one-way ANOVA, $df=1,30$, $F=34.69$, $P<0.001$) (Fig. 4.5.). Later in the sampling (34-42 h) significantly more faeces were produced in lobsters fed fresh mussel (19%) compared to the formulated diet (6%) (one-way ANOVA, $df=1,30$, $F=9.81$, $P<0.01$). Faeces produced after the last collection (42 h post-feeding) were rare and consisted of empty transparent peritrophic membrane.

![Fig. 4.5. Mean cumulative proportion of the total faecal production (%) for different time intervals post-feeding on mussel flesh and the dry formulated diet for the three pooled size-classes of juvenile Jasus edwardsii (n=18). Note: time intervals are not the same duration.](image-url)
4.4. Discussion

Juvenile *J. edwardsii* had a greater increase in body mass and tended to have higher survival when fed fresh mussel than the formulated diet as was previously found in experiments undertaken in sea-cages (Simon & James, 2007). For the formulated diet feeding treatments, weight gain and SGR tended to be higher, and food consumption was maximised, when lobsters were fed once daily. The generally slower growth rates of lobsters fed the same ration but divided into several meals may have been due to a reduction in apparent specific food consumption (0.74-0.8% BW day\(^{-1}\)) compared to lobsters fed one ration daily (1.15% BW day\(^{-1}\)). Cox & Davis (2006) also showed that growth was reduced when feeding early juvenile Caribbean spiny lobsters, *Panulirus argus*, with frozen seafood twice a day instead of once a day. Feeding one ration of *Artemia salina* once a day also produced better consumption rates in the European clawed lobster, *Homarus gammarus*, compared to lobsters fed smaller rations twice daily (D’Abramo & Conklin, 1985). In this current study, the food consumption of lobsters fed once daily (10-15 g lobster BW; FC= 0.14 g day\(^{-1}\)) did not differ significantly from lobsters fed three times a week (0.15 g day\(^{-1}\)) and lobsters fed fresh mussel flesh three times a week (0.14 g day\(^{-1}\)). The measured rates of food consumption were also similar to previous studies on this lobster species: 0.15 g day\(^{-1}\) (5-10 g BW) (Johnston et al., 2003), 0.14 g day\(^{-1}\) (5-15 g BW) (Thomas et al., 2003), 0.10-0.15 g day\(^{-1}\) (7.7-17.5 g BW) (Crear et al., 2002). These similarities in food consumption across studies suggest lobsters have a limited ability to vary the scale of their maximum feed intake. Food utilisation was highly variable with feeding frequency but also tended to be better when juvenile lobsters were fed once daily (FCR= 2.6; PER= 94%) compared to the other feeding frequencies. However, the best FCR and PER were for 5 day\(^{-1}\) fed lobsters, and lobsters feeding on fresh mussel. Similar feed conversion ratios have been obtained with daily feeding of commercial shrimp diets to juvenile lobster (Crear et al., 2002) suggesting that *J. edwardsii* juveniles were capable of efficiently utilising the experimental formulated diet for growth. Lobsters fed only three times a week were observed feeding on the diet even after 24 h of immersion. This was likely to have resulted in the similar diet consumption (1.27% BW day\(^{-1}\)), but lower utilisation (FCR=3.8; PER=63%) due to more extensive leaching of essential nutrients, compared to lobsters fed daily.
Despite the higher nutrient density of the dry formulated diet, on average lobsters consumed an equivalent amount of dry matter in 2 h every 48 h to lobsters feeding on mussel flesh which had a moisture content ten times higher. It was observed that the hydration and expansion of the formulated diet in the foregut of *J. edwardsii* may result in the diet losing its higher nutrient density advantage over fresh mussel flesh (Simon, 2009a). In addition, an initial delay in evacuation of the foregut when feeding on the formulated diet in comparison to fresh mussel was apparent in the present study, which could have also limited further intake of the formulated diet (Simon, 2009a). The lack of consumption advantage in terms of dry matter intake in providing the compact nutrient dense dry formulated diet to *J. edwardsii* could be due to the specific composition of the experimental diet, or a more general principle that would have significant implications for the effective use of formulated diets in lobster aquaculture. Whether lobsters self-compensate for the difference in moisture between diets or are unable to consume more dry food due to the volume gain is unclear. Clearly further consumption trials are needed to compare diets of similar quality but varying solely in moisture content.

Slow appetite revival on the formulated diet when compared to mussel flesh could be responsible for the reduced food consumption with increasing feeding frequency observed. From the appetite revival results and previous research on foregut capacity (Simon, 2009a), we would expect S1 lobsters (19 g) to fill their foregut with formulated diet to full capacity (i.e., ingest an average of 1.22% BW of dry matter) only if a minimum period of time of 24-30 h between meals at 18 °C is established, i.e. a specific food consumption of around 1-1.2% BW day\(^{-1}\). This is similar to the food consumption measured for the lobsters fed daily in this current study, as well as in previous growth studies (Crear et al., 2000; Johnston et al., 2003; Thomas et al., 2003). Given the slow appetite revival within 6 h of a satiation meal for formulated diet fed lobsters (11.4%), we would also expect lower food consumption when lobsters are allowed less time between meals. In contrast, lobsters of the three size-classes fed mussel flesh had an almost complete return of appetite within 12 h after a satiation meal suggesting the potential for a maximum dry matter intake of around 2.5% BW day\(^{-1}\). There is an indication that appetite revival in juvenile lobsters fed mussel was related to
the rates of foregut evacuation which remained relatively consistent across the range of juvenile sizes tested. This showed that further feed intake in *J. edwardsii* juveniles is possible while still processing a considerable proportion of a previous meal, however, this phenomenon appeared to be suppressed for the formulated diet. The ability to re-feed while digesting a meal is a feeding characteristic found in other crustaceans including the Australian red claw crayfish, *Cherax quadricarinatus* (Loya-Javellana et al., 1995), and various penaeid shrimp species (Hill & Wassenberg, 1987; Nunes & Parsons, 2000). Nevertheless, in conditions where food is continuously available to a cultured animal, appetite tends to be ultimately regulated by physiological factors other than foregut fullness, such as the level of nutrient load in the digestive gland and energetic requirements (Gibson & Barker 1979; Kurmaly et al., 1990). This would explain why lobsters allowed to feed continuously on fresh mussel displayed lower food consumption (1.05% BW day^{-1}) compared to what could be expected solely from appetite revival and foregut evacuation data.

Appetite revival on the formulated diet was slower than on fresh mussel and decreased with increasing lobster size. Lobsters of the size-classes S1 (19 g), S2 (42 g) and S3 (83 g) took 24, 30, and 48 h, respectively to achieve a mean feed intake similar in extent to their respective mean satiation ration. This suggested that, unlike for the lobsters fed fresh mussel, foregut fullness was not the primary factor regulating appetite revival. In contrast, *C. quadricarinatus* displayed faster rates of appetite revival when fed a dry formulated diet, which paralleled closely foregut evacuation and the secretion of fresh gastric fluid. The revival of appetite to a level above 50% occurred within 4 h post-feeding on a satiation ration (Loya-Javellana et al., 1995). In this current study, lobster appetite was less than 50% at 12 h post-feeding on formulated diet for a similar satiation ration size to that measured previously in *C. quadricarinatus*. Dietary energy was similar for the formulated diet and mussel flesh on a dry weight basis (see Table 2.1.), and the apparent dry matter digestibility of the formulated diet was lower than fresh mussel, therefore differences in energy assimilation were unlikely to have affected rates of appetite revival (Bromley, 1987). Differences in appetite revival maybe due to lower chemical attraction or palatability of the formulated diet compared to mussel (Williams et al., 2005). However, if this was the case it is much less likely that appetite revival on the formulated diet would vary with lobster size and time post-feeding as
seen in this study. Lobsters were observed to move immediately onto both formulated diet and fresh mussel to begin feeding. The time spent feeding on both diets was also similar suggesting that both diets were of similar attractiveness and palatability (Simon, 2009a). Post-ingestive feedbacks from the digestion of a former meal may play a role in suppressing appetite revival further. In *J. edwardsii*, gut fluid is acidic with the majority of carbohydrases having a pH optima between 4.5 and 5.5 and losing most of their activity at neutral pH (Johnston, 2003). Seawater ingestion with the food may therefore have the potential to influence digestion of carbohydrates in the foregut by buffering its acidic pH (see Simon, 2009b). The effect on digestive fluid pH measured in this latter study were reduced by the low levels of feed intake (lobsters fed three times a week) and it is possible that a greater effect would be observed under more continuous feeding conditions (Simon, 2009b). A reduced processing and digestion of the ingredients in the foregut could lead to an overload of the digestive gland with undigested fine particles (Al-Mohanna & Nott, 1987). This could negatively affect the production of fresh digestive enzymes for the next meal and lengthen the appetite revival on the formulated diet (Loya-Javellana et al., 1995). Studying the response of the digestive gland to feeding on the two diets provided some further evidence for this hypothesis (see Simon, 2009b).

Foregut evacuation was almost completed by 10 h after the introduction of food regardless of diet and lobster size (19-83 g). However, the evacuation rate of mussel flesh was faster for S3 lobsters than for S1 lobsters suggesting that larger lobsters were more efficient at processing a similar ration of mussel flesh than smaller ones. This was not the case for lobsters fed the formulated diet. There was no difference in the evacuation of formulated diet in relation to body size in the red claw crayfish, *C. quadricarinatus* (Loya-Javellana et al., 1995), or in the shrimps *Farfantepenaeus subtilis* (Nunes & Parsons, 2000) and *F. paulensis* (Soares et al., 2005). The results of this current study are in agreement with foregut evacuation times reported for a range of large decapods; 4-6 h in the spiny lobster, *Panulirus Cygnus*, at 25 ºC (Joll, 1982), 9 h for the crayfish, *C. quadricarinatus*, at 26 ºC (Loya-Javellana et al., 1995), 12 h for the crab, *Scylla serrata*, at 18-22 ºC (Hill, 1976) and the lobster, *Nephrops norvegicus*, at 14 ºC (Sarda & Valladares 1990). Hill (1976) also reported that half the crabs studied retained fish bone and shell in the foregut for 2-3 and 5-6 days respectively, those
evacuated probably via regurgitation. In this current study, the majority of the fraction remaining in the foregut after 10 h was made of suspended feed particles and mussel shell debris from the dried foregut fluid. A small delay in initial evacuation on the formulated diet was evident but this did not significantly affect the overall timing of evacuation due to faster subsequent foregut purging. No delay in foregut evacuation was found in *C. quadricarinatus* despite feeding on dry pellets (Loya-Javellana et al., 1995). However, a longer initial delay in foregut emptying was found in rainbow trout fed dry pellets compared to a natural diet of chopped herring (Ruohonen et al., 1997). The authors suggested that the water demand imposed by the dry pellets prolonged gastric processing, the trout having to drink and moisturize the food before gastric digestion and emptying could take place. Lobsters could show a similar passive or active water ingestion response to dry formulated diets, perhaps due to a similar requirement to moisturize the food for processing, digestion and evacuation. Alternatively, difficulties in feed processing due to the poor dry matter digestibility of the dry formulated diet may have caused the observed delay in foregut evacuation.

Cumulative faecal production differed between the two diets but was not influenced by lobster size (19-83 g). The faeces produced after 34 h post-feeding are likely to have come from the elimination of intracellular waste products (e.g., toxic mineral elements, digestive enzymes, undigested particles) from the digestive gland (Al-Mohanna & Nott, 1986). A similar timing (30-42 h post-feeding) was also found for the return of oxygen consumption to basal level after feeding (i.e., specific dynamic action) in *J. edwardsii* juveniles held at 13-15 ºC (Crear & Forteath, 2000; Radford et al., 2004) and therefore may mark the completion of digestion in those lobsters. Lobsters produced a greater proportion of faeces initially and a larger overall quantity when feeding on the formulated diet compared to fresh mussel. The difference in overall quantity was due to the lower apparent dry matter digestibility (*AD_{DM}* of the formulated diet (62.1%) compared to fresh mussel (89.2%). Low *AD_{DM}* (53-64%) was also found for previous experimental formulated diets fed to *J. edwardsii* (Ward et al., 2003). Given the similar dry matter consumption of the two diets, the lower digestibility and protein content may have played a part in the slower growth rates of lobsters on the formulated diet. In addition, differences in the timing of post-prandial foregut evacuation and faecal production between the two diets suggest that for the formulated
diet the digesta was initially slower to leave the foregut but appeared as a greater proportion in initial faecal production. This suggests that a greater proportion of the food material entered the hindgut directly and bypassed the digestive gland which is the major site for nutrient absorption in lobster digestion (Dall & Moriarty, 1983). In contrast, juvenile lobsters feeding on mussel flesh produced a low proportion of faeces in the first 10 h post-feeding, which is indicative that most of the evacuated foregut material was sufficiently digested to flow through the foregut filter-press into the digestive gland tubules for intraluminal digestion and assimilation (Dall & Moriarty, 1983). In fact, faster processing of mussel flesh in the foregut of larger S3 juvenile lobsters may have been responsible for the improved overall dry matter digestibility of mussel flesh compared to the smaller S1 lobsters. The low dry matter digestibility of the formulated diet could therefore be in part the consequence of difficulties in processing the food in the foregut into fine enough particles to flow into the digestive gland. The high water stability of the current formulated diet was achieved via the inclusion and gelatinisation of tapioca starch which may have resulted in the adhesion of the food particles after ingestion. In other studies large ingredient particle sizes (>124 µm) (Obaldo et al., 1998) and very water stable dry extruded diets (Obaldo et al., 1999) have been shown to negatively affect the growth rate of the whiteleg shrimp, Litopenaeus vannamei. However, the gelatinised tapioca starch of this diet has been found to be highly digestible (94%) in large 115 g J. edwardsii juveniles (see Simon, 2009d). Other ingredients (e.g., carrageenan binder, fishmeal, seaweed meal, yeast) are therefore more likely to have resulted in the poor digestibility (Simon, 2009d).

This study represents the first attempt at characterising the appetite and gross digestive processes occurring in juvenile spiny lobsters when fed on a natural and a formulated diet. Juveniles of the spiny lobster J. edwardsii have a small foregut capacity (2.5-3% BW) which restricts the food consumed per meal to a small amount relative to body size (Simon, 2009a). There also appears to be no advantage in terms of dry matter intake from feeding the nutrient dense dry formulated diet in comparison to fresh mussel. Furthermore, J. edwardsii cannot achieve high levels of food consumption on formulated diets in comparison to smaller decapods, such as penaeid shrimp, as foregut filling time (1-2 h) (Simon, 2009a), foregut clearance time (10 h) and gut throughput time (34-42 h) are longer. These values are similar to those measured in larger decapods.
such as *C. quadricarinatus* (Loya-Javellana et al., 1995). However, in contrast to *C. quadricarinatus*, juveniles of *J. edwardsii* display very slow rates of appetite revival on the present formulated diet. The low appetite revival was consistent with the trend toward lower levels of food consumption when feeding frequency was increased. The similar level of food consumption measured in previous studies suggests also that a poor appetite revival on experimental formulated diets may be a common phenomenon in cultured *J. edwardsii*. Until faster rates of appetite revival can be achieved, it is recommended to feed *J. edwardsii* juveniles a single ration daily in order to optimise consumption and growth on formulated diets.

Although diet attractability, feeding-stimulatory value and palatability are crucial to achieve high feed intake for spiny lobster aquaculture (Williams, 2007), this study suggests that appetite revival, possibly linked to digestive processes, may be another important factor influencing feed intake (see Simon, 2009b). Increasing appetite revival on formulated diets would assist in maximising feed intake by increasing meal frequency. From foregut filling and evacuation dynamics, small juvenile lobsters (10–20 g) would theoretically be able to consume up to approximately 2.5% BW of dry matter per day at 18 °C if fed twice daily at a 12 h interval. However, the consumption observed under continuous feeding conditions is also likely to be limited by the digestive capacity and nutrient load on the digestive gland.

Increasing the digestibility of formulated diets for *J. edwardsii* appears essential to improve the amount of nutrients assimilated per meal. A formulated diet would have to be highly digestible to sustain growth rates similar to those observed on a high-protein (on a dry matter basis), highly digestible food source like fresh mussel given the dry matter consumption and rates of appetite revival measured in this study. There are some indications that the reduced consumption and low digestibility of the dry formulated diet may have been due to difficulties in processing and digestion in the foregut into fine enough particles to flow into the digestive gland. Finer grinding of dietary ingredients and reducing carbohydrate inclusion might be beneficial to improve the digestibility of formulated diets for spiny lobsters. Still, it was shown later that reducing the fishmeal particle size of formulated diets does not improve apparent
digestibility, and that foregut processing and the dissolution of dietary ingredients may be more important factors affecting overall diet digestibility (see Simon, 2009b, d).

As a consequence of the results of the research presented in this chapter, further research was performed in the following chapter to better understand how the intracellular enzymatic digestion of diets within the digestive gland could influence consumption and appetite revival in spiny lobsters (Simon, 2009b).
Plate 5. Digestive enzymes in *Jasus edwardsii* juveniles fed mussel flesh and the dry formulated diet. A) Enzyme extraction workbench at NIWA Mahanga Bay, Wellington; B) Moulting stage identification in juvenile lobsters showing a pleopod from a lobster in premoult stage D2 characterised by epidermal retraction, epicuticle deposition and setal bifurcation; C) Digesta and digestive fluid collected from foregut for enzyme analyses; D) Digestive gland collected for enzyme analyses; E) Enzyme assay workbench at NIWA Greta Point, Wellington; F) Absorbance reading of a 96-well plate for trypsin activity determination.
5. Chapter five – Post-prandial changes in digestive enzyme activities and digestive gland structure in response to feeding on natural and formulated diets

Published as:


**5.1. Introduction**

For the culture of spiny lobsters where daily nutrient intake is restricted by the physical capacity of the digestive system (i.e., reduced volume and evacuation rates), growth may be limited by the ability of the digestive system to breakdown and assimilate specific nutrients from formulated diets (Simon & Jeffs, 2008). The capacity to obtain nutrients from a particular food source is largely determined by the physiological processes occurring in the digestive tract including the quantity and activity of the digestive enzymes that are present (Ceccaldi, 1997). An investigation into the digestive secretory response (i.e., timing of release and activity of digestive enzymes) of spiny lobsters upon feeding could help to establish a better understanding of their digestive abilities. Comparing the response between natural and formulated diets could also identify some of the shortcomings experienced with the performance of lobsters on formulated diets, and lead to their improvements as well as helping to establish appropriate feeding frequencies for commercial aquaculture (Uys et al., 1987).

Quantitative changes in digestive enzyme activities with feeding and type of food have not been determined in spiny lobsters. Enzymes present in the digestive tract of spiny lobsters have been studied in *Panulirus japonicus* (Galgani & Nagayama, 1987; Lida et al., 1991), *Jasus edwardsii* (Johnston, 2003; Johnston et al., 2004a, b), *P. interruptus* (Celis-Guerrero et al., 2004; Navarette del Toro et al., 2006) and *P. argus* (Perera et al., 2008a, b). Such studies have mainly covered the biochemical characteristics of enzymes, especially changes in their activity with increasing size and
with moulting. Previous research in other Crustacea has also demonstrated a wide range of digestive capabilities, including changes in the production of digestive enzymes to match long-term changes in diet (Hoyle, 1973; Van Wormhoudt et al., 1980; Lee et al., 1984; Lucien-Brun et al., 1985; Le Moullac et al., 1994; Guzman et al., 2001). However, the direct influence of feeding on enzyme secretion in Crustacea has only been investigated histologically in the crayfish, *Astacus leptodactylus* (see Barker & Gibson, 1977), the European lobster, *Homarus gammarus* (Barker & Gibson, 1977), the green tiger prawn, *Penaeus semisulcatus* (Al-mohanna et al., 1985), and more recently using quantitative biochemical methods in the giant tiger prawn, *P. monodon* (Ong & Johnston, 2006). None of these studies investigated the effect of diet type on the digestive response.

The specific aim of this study is to compare key features of the digestive processing found in cultured juveniles of the temperate spiny lobster *J. edwardsii* fed fresh mussel flesh and the dry formulated diet. This was achieved by identifying the post-prandial changes in; 1) soluble protein concentrations and the activities of four digestive enzymes (i.e., total protease, trypsin, α-amylase and α-glucosidase) sampled from the foregut and digestive gland; 2) the pH of the digestive fluid; and 3) the digestive gland structure. The long-term effects on digestive enzyme activities were also examined in juvenile spiny lobsters reared on the two diets.

### 5.2. Materials and methods

#### 5.2.1. Experimental animals and diets

Approximately 400 small (i.e., 20-60 g) juvenile lobsters, *J. edwardsii*, were held for six months communally in two 1000 l indoor tanks and conditioned by feeding either freshly opened and whole mussels, *Perna canaliculus* (M lobsters), or pellets of the dry formulated diet (F lobsters) prior to being randomly selected from each group for experimentation. Lobsters were held at ambient seawater temperatures that ranged between 10-18 °C over the experimental period and fed three times weekly to satiation (Simon & James, 2007).
5.2.2. Experimental design and feeding

Internoult lobsters were selected and stocked randomly in individual tanks to avoid conspecific interaction and formation of feeding hierarchies (Thomas et al., 2003). The same experimental tanks (6 l transparent-plastic aquaria) and experimental conditions (i.e., high water quality, photoperiod L24:D0, light intensity <0.5 µmol m$^{-2}$ s$^{-1}$) were used as in Simon and Jeffs (2008). The tanks were exposed to constant dim light to ensure that any measured response was a function of feeding and not influenced by endogenous circadian rhythms (Ong & Johnston, 2006). The lobsters were acclimated to the new conditions for 48 h and fed on either mussel flesh or dry formulated diet three times a week for three weeks. Mussel flesh consisted of mussel mantle and gonad tissues only, with the remainder of the mussel not used in experimental feeding to minimise the potential for enzyme contributions from the food (Simon, 2009a). Lobsters were then starved for 96 h to ensure they reached basal metabolic rate and that there would be no residual effect from prior feeding that would influence the results (Radford et al., 2004). Then in the following 30 min lobsters were allowed to ingest a similar amount in dry matter of their respective diets, corresponding to 50% of the satiation ration in a single meal for lobsters of this size (Simon, 2009a). As an experimental control for the disturbance associated with feeding, some lobsters which had been fed mussel flesh during the three week period of experimental feeding, were disturbed in a similar way (i.e., short illumination, water movement) to the fed lobsters but were not fed. The introduction of the food in the tank or disturbance event corresponded to 0 h. At the end of the feeding period (0.5 h), lobsters that did not completely ingest their allotted ration were not sampled in order to avoid differences in ingested food quantities that could affect the results. Five treatment groups were formed around combinations of prior rearing diet, as well as the subsequent diet and feeding for the experimental period (Fig. 5.1.):

- **MC** - lobsters reared on fresh mussel and then fed mussel flesh, except on the last meal where they were disturbed to simulate a feeding event
- **MM** - lobsters reared on fresh mussel and then fed mussel flesh
- **MF** - lobsters reared on fresh mussel and then fed the dry formulated diet
• FM - lobsters reared on the dry formulated diet and then fed mussel flesh

• FF - lobsters reared on and then fed the dry formulated diet

Fig. 5.1. Schematic illustration of the experimental plan. For simplicity, the individual tanks and randomisation of the treatments are not shown.

5.2.3. Experimental procedure

5.2.3.1. Post-prandial changes in soluble protein concentration and enzyme activities of lobsters reared on fresh mussel

A total of 120 M lobsters (mean ± S.D. wet body weight = 30.0 ± 3.9 g) were randomly selected from the pool of available lobsters, transferred to individual tanks and fed as in the procedure outlined previously (Section 5.2.2.) (Fig. 5.1.). Four lobsters per experimental dietary treatment (i.e., MC, MM and MF) were removed from their container and euthanised (−25 ºC) at each of ten post-prandial / post-disturbance times (i.e., 0.5, 1, 2, 4, 6, 8, 10, 12, 18, and 24 h) for digestive enzyme analyses (Section 5.2.4.) (Fig. 5.1).
5.2.3.2. Soluble protein concentration and enzyme activities of lobsters reared on the dry formulated diet

A total of 40 randomly selected F lobsters (30.6 ± 3.4 g) were transferred to individual tanks and fed as in the procedure outlined previously (Section 5.2.2.) (Fig. 5.1.). Four lobsters per experimental dietary treatment (i.e., FM and FF) were removed from their container and euthanised (− 25 ºC) at each of five post-prandial times (i.e., 1, 4, 6, 10, and 18 h) for digestive enzyme analyses (Section 5.2.4.).

5.2.3.3. Post-prandial changes in digestive gland structure and digestive fluid pH of lobsters reared on the dry formulated diet and fresh mussel

A total of 36 M lobsters (46.9 ± 5.7 g) and 24 F lobsters (42.5 ± 7.8 g) were transferred to individual tanks and fed as in the procedure outlined previously (Section 5.2.2.) (Fig. 5.1.). Three lobsters per experimental dietary treatment (i.e., MC, MM and FF) were euthanised (0 ºC) at each of eight post-prandial times (i.e., 1, 2, 4, 6, 8, 10, 18, 24 h) for MM and FF lobsters (i.e., n=24), and four post-disturbance times (i.e., 1, 4, 10, 24 h) for MC lobsters (i.e., n=12). Euthanised lobsters were brought to a chill-coma on ice and quickly processed for histology to avoid any potential damaging effect of long-term freezing on digestive gland histology (Ishine et al., 1999). The pH was measured directly in the foregut and digestive gland using a Euthec pH spear electrode (accuracy ± 0.01 pH) which had been calibrated at the temperature of the digestive fluid (i.e., 0-4 ºC). The pH of the diets was also measured in triplicate (0.1 g DM homogenised in 0.5 ml deionised water).

Digestive glands were dissected, processed for histology and examined as per Simon and James (2007). Haphazardly selected transverse sections of the digestive gland tubules were analysed for B- and F-cell numbers (n=10 tubules per slide), tubule surface area and tubule lumen surface area (n=5 tubules per slide). The ratio of tubule lumen surface area to the total surface area of the tubule was used to determine if the proportionate size of the tubule lumen changed in response to feeding over time on either diet (Ong & Johnston, 2006). R-cell numbers and lipid vacuolation could not be measured quantitatively but were visually assessed (Johnston et al., 2003).
5.2.4. Enzyme extraction and analyses

Whole lobsters sampled for digestive enzyme analyses (Sections 5.2.3.1. and 5.2.3.2.) were thawed on ice, weighed, and their digestive gland (DG) and foregut (FG) dissected out. The moult stages of the lobsters were determined as per Musgrove (2000). Each DG was weighed to the nearest milligram using a precision AG204 Mettler Toledo balance, homogenised in 1.5 ml of chilled 100 mM Tris, 20 mM NaCl buffer (pH 7.5) using a Ultra Turrax electric homogeniser (IKA-Werke) at 24,000 rpm (i.e., 6440 × g) for 30 s. Each FG containing digestive fluid and food was homogenised in 1.5 ml of the same buffer using a hand-held glass rod. The homogenates were centrifuged at 16110 × g for 5 min to pellet debris, and 200 µl aliquots of supernatant transferred to PCR tubes and stored at −20 ºC. FG fluid volume (µl) was determined after centrifugation by correcting for the volume of buffer added. The fed diets were also analysed for digestive enzymes to ensure that the digestive response would not be confounded by the potential for enzyme contributions from the food.

Trypsin and α-glucosidase assays were performed in duplicate at 37 ºC in 96 well, round-bottom clear microplates and absorbances read on a VersaMax™ microplate reader. Total protease and α-amylase assays were performed in duplicate at 20 ºC in 96 well round-bottom black UV microplates and fluorescence read in a FLUOstar™ OPTIMA UV-visible spectrophotometer. Appropriate enzyme standards and blanks were included with each analysis. Additional testing confirmed that enzyme activities were linear with incubation time and were measured at optimum pH. One enzyme unit was defined as the amount of enzyme that catalysed the release of 1 μmol of product per min for trypsin (p-nitroaniline) and α-glucosidase (p-nitrophenol) and was calculated using standard curves for the products. Total protease and α-amylase activities were determined using EnzChek® assay kits (Invitrogen, New Zealand) and enzyme units defined as per the EnzChek® protocols (Sections 5.2.4.1. and 5.2.4.3.). Specific activity was defined as enzyme activity per mg of soluble protein (SP) (Units mg⁻¹). The SP concentration (mg ml⁻¹) was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Total activity was defined as enzyme activity per millilitre of foregut digestive fluid (Units ml⁻¹) or gram of wet digestive gland tissue (Units g⁻¹).
5.2.4.1. Total protease

Total protease activity was measured using the EnzChek® Protease Assay kit (Invitrogen Cat. No. E6638). The assay quantified the release of BODIPY FL dye from the dye-labelled casein substrate by measuring directly the increase in fluorescence. One enzyme unit was defined as the increase of $10^3$ fluorescence units per min. The assay mixture contained a final substrate concentration of 5 µg ml$^{-1}$ BODIPY FL casein in 10 mM Tris-HCl (pH 7.8). Assays (200 µl) were initiated by the addition of substrate (100 µl) and the increase in fluorescence measured at an excitation / emission of 485 / 520 nm over 10 min (reading gain=1600). A positive control of 3 mg ml$^{-1}$ bovine pancreas trypsin (Sigma Cat. No. T4665) in 1 mM HCl was used.

5.2.4.2. Trypsin

Trypsin activity was assayed using N-α-benzoylarginine-p-nitroanilide hydrochloride (BAPNA) (Sigma Cat. No. B4875) dissolved in dimethyl sulfoxide (DMSO) as a substrate. The assay mixture contained a final concentration of 2 mM BAPNA in 100 mM ammonium bicarbonate, 100 mM NaCl and 0.2% (w/v) polyethylene glycol 6000 (PEG) (pH 8.0). Assays (100 µl) were initiated by the addition of substrate (50 µl) and the release of p-nitroaniline measured at 405 nm over 20 min using a p-nitroaniline (Sigma Cat. No. 185310) standard curve (0-0.5 mM). A positive control of 3 mg ml$^{-1}$ bovine pancreas trypsin (Sigma Cat. No. T4665) in 1 mM HCl was used.

5.2.4.3. α-amylase

α-amylase activity was measured using the EnzChek® Amylase Assay kit (Invitrogen Cat. No. E33651). The assay quantified the release of BODIPY FL dye from the dye-labelled starch substrate by measuring the increase in fluorescence. One enzyme unit was defined as the amount of enzyme required to liberate 1 mg of maltose from starch in 3 min at 20 °C, pH 7.9, using the α-amylase from Bacillus sp. (Sigma Cat. No. 6380) as an enzyme standard. A pH optimum of 5.0 for α-amylase in J. edwardsii was identified using a 100 mM citric acid – phosphate buffer system adjusted from pH 4.0 to 8.0. Therefore, the assay mixture contained a final substrate concentration of 100 µg ml$^{-1}$
BODIPY FL starch in either 50 mM MOPS buffer (pH 6.9) for the enzyme standard or 100 mM citric acid-phosphate buffer (pH 5.0) for samples. Assays (100 µl) were initiated by the addition of substrate (50 µl) and the increase in fluorescence measured at an excitation / emission of 485 / 520 nm over 10 min.

5.2.4.4. α-glucosidase

α-glucosidase activity was determined using p-nitrophenyl-α-D-glucopyranoside (Sigma Cat. No. N1377) as substrate. The assay mixture contained a final concentration of 4 mM substrate in 200 mM citric acid – phosphate, 50 mM NaCl and 0.2% (w/v) polyethylene glycol 6000 (PEG) (pH 7.0). A pH optimum of 7.0 for α-glucosidase from J. edwardsii was identified using a 100 mM citric acid – phosphate buffer system adjusted from pH 4.0 to 8.0. Assays (100 µl) were initiated by the addition of substrate (50 µl) and the release of p-nitrophenol measured at 400 nm over 60 min using a p-nitrophenol (Sigma Cat. No. 1048) standard curve (0-0.25 mM). A positive control of 3 mg ml\(^{-1}\) α-glucosidase from Saccharomyces cerevisiae (Sigma Cat. No. G5003) was used.

5.2.5. Statistical analyses

Only results from C4 stage intermoult lobsters were analysed as moult stage has been found to affect digestive enzyme activities of spiny lobster (Perera et al., 2008a). Post-prandial or disturbance (control) changes in SP concentration (Fig. 5.2.) and enzyme total activity (Fig. 5.3. and 5.4.) were analysed statistically by a three-way ANOVA which included all interactions between the following factors: dietary treatment (i.e., MC, MM, and MF), enzyme type (i.e., SP concentration included, total protease, trypsin, α-amylase and α-glucosidase) and time interval to sampling. This was performed on data from the DG and FG separately. Further one-way ANOVA analyses were performed for each level of factors showing significant interaction to determine their individual effect on enzyme production.

The effect of dietary treatment (i.e., MC, MM, MF, FM, and FF) was tested separately using one-way ANOVAs for the following variables: lobster weight; DG weight; DG index; FG fluid volume; SP concentration of DG and FG; total SP content
per lobster; proportion of total SP in FG; and the specific and total activities of digestive enzymes for both the DG and FG (Tables 5.1. and 5.2.). Data obtained for each time interval following feeding and simulated feeding were combined for these analyses.

The effects of dietary treatment (i.e., MM, FF), time post-prandial and organ (i.e., FG, DG) on the digestive fluid pH were tested using a three-way ANOVA (Fig. 5.5.). The effects of dietary treatment (i.e., MM, FF) and time post-prandial on the DG structure, as measured by B- and F-cell numbers per tubule, tubule surface area, and tubule lumen surface area to whole tubule surface area ratio, were tested using a two-way ANOVA (Table 5.3.). For these analyses MC data were omitted to obtain a balanced design because they were only sampled four times compared to the eight times for fed treatments (Section 5.2.3.3.). However, MC data were included when the effect of time post-prandial was not tested (i.e., effects of dietary treatment, organ type and the interaction tested using two-way ANOVA). Changes in pH through time post simulated feeding (MC) were tested using a two-way ANOVA (i.e., effect of time, organ and the interaction) or a one-way ANOVA for the histological derived data.

Before analyses, the ANOVA assumptions of normality of residuals and homogeneity of variances were tested using the Shapiro-Wilk test and Levene test respectively. Where necessary the data were transformed (log or square root) to satisfy the assumptions and all percentage data were arcsine square root transformed. Significant differences (\(P<0.05\)) between the means were determined by post-hoc comparisons of means using Tukey-Kramer test (Zar, 1999). When \(P>0.05\), non-significant results were stated as NS. All analyses were performed using the Statistical & Power Analysis Software NCSS 2007 (Utah, USA).

5.3. Results

5.3.1. Post-prandial changes in soluble protein concentration and enzyme activities of lobsters reared on fresh mussel

All of the enzymes tested were present in the FG and DG of both fed and unfed lobsters, although the measured SP concentration (Fig. 5.2.) and enzyme activities (Fig. 5.3. and 5.4.) were highly variable between individuals despite the careful selection of
lobsters from identical rearing and feeding conditions, intermoult stage, of similar weight and DG index (one-way ANOVA, NS) (Table 5.1.). Levels of natural enzyme activity present in the food sources ingested were undetectable and therefore their effect on enzyme activities can be safely assumed to be negligible.

For the FG data of M lobsters, the three-way orthogonal ANOVA revealed that the interaction between dietary treatment and time post-prandial or simulated feeding was significant (\(df=18, 385, F=4.13, P<0.001\)), and this was consistent across all the digestive enzymes (i.e., including SP concentration) (interaction dietary treatment \(\times\) enzyme type, \(df=36,385, F=0.69, NS\); interaction dietary treatment \(\times\) enzyme type \(\times\) time, \(df=72, 385, F=0.34, NS\)). Further ANOVA showed that there were two main peaks in SP concentration and enzyme activities of the FG of MM at 4 h and 18 h post-prandial (one-way ANOVA, \(df=9,115, F=6.25, P<0.001\)), but only one peak at 4 h for MF (\(df=9,145, F=3.91, P<0.001\)) (Fig. 5.2. and 5.3.). The FG of MC displayed higher SP concentration (Fig. 5.2.) and enzyme activity (Fig. 5.3.) just following the simulated feeding event (i.e., 0.5 h) compared to later in sampling (i.e., 8 h) (one-way ANOVA, \(df=9,125, F=2.83, P<0.01\)).

Changes through time in SP concentration (Fig. 5.2.) and enzyme activities in the DG of M lobsters (Fig. 5.4.) were not significant for the three dietary treatments (three-way ANOVA, all interactions NS, main factor, \(df=18,385, F=1.63, NS\)), despite the tendency for trypsin and \(\alpha\)-amylase activities to increase gradually through time in post-prandial MM lobsters.

Differences in SP concentration between the dietary treatments for the combined post-prandial or simulated feeding times, provided further evidence of a significant effect of feeding on digestive enzyme secretion (Table 5.1.). Although no significance was found when comparing the five treatments (one-way ANOVA, \(df=4,126, F=1.22, NS\)), for the four feeding treatments (i.e., MM, MF, FM, and FF) combined, the ingestion of food from the last feeding event increased the overall SP concentration of the FG (i.e., fed lobsters, \(107.1 \pm 5.7 \text{ mg ml}^{-1}\); MC, \(85.0 \pm 6.8 \text{ mg ml}^{-1}\) (\(df=1,129, F=5.46, P<0.05\)). This was achieved despite the tendency for a dilution effect caused by food ingestion (i.e., FG volume of fed MM, MF, FM and FF lobsters, 223.5 ± 9.2 µl versus MC lobsters, 203.7 ± 11.9 µl) (Table 5.1.). When expressed as total SP (i.e., FG, SP concentration \(\times\)
FG fluid volume; DG, SP concentration × DG weight), the proportion in the FG was significantly lower for MC than the four feeding treatments (df=4,126, $F=16.50$, $P<0.001$) (Table 5.1.). In contrast, the SP concentration of the DG and total SP of the two organs (FG and DG) per lobster, were significantly higher (one-way ANOVAs, $df=4,149$, $F=15.62$, $P<0.001$) for MC (i.e., 63.9 ± 3.4 mg g$^{-1}$; 119.6 ± 6.8 mg lobster$^{-1}$) compared to MM (i.e., 46.4 ± 2.4 mg g$^{-1}$; 93.3 ± 4.8 mg lobster$^{-1}$) and MF (i.e., 42.7 ± 2.6 mg g$^{-1}$; 92.3 ± 5.2 mg lobster$^{-1}$) (Table 5.1.).

Table 5.1. Mean ± S.E. lobster weight, digestive gland (DG) weight, DG index, foregut (FG) fluid volume and soluble protein (SP) of *Jasus edwardsii* juveniles in five different dietary treatments$^1$. Significant differences between means within the same row are marked by different letters ($P<0.05$).

<table>
<thead>
<tr>
<th>Mean ± S.E.</th>
<th>MC$^2$</th>
<th>MM$^3$</th>
<th>MF$^4$</th>
<th>FM$^5$</th>
<th>FF$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobster weight (g)</td>
<td>29.8 ± 0.7$^a$</td>
<td>30.1 ± 0.7$^a$</td>
<td>30.1 ± 0.6$^a$</td>
<td>30.6 ± 0.7$^a$</td>
<td>31.2 ± 1.0$^a$</td>
</tr>
<tr>
<td>DG weight (g)</td>
<td>1.6 ± 0.0$^a$</td>
<td>1.6 ± 0.0$^a$</td>
<td>1.6 ± 0.0$^a$</td>
<td>1.7 ± 0.1$^a$</td>
<td>1.7 ± 0.1$^a$</td>
</tr>
<tr>
<td>DG index (%)$^7$</td>
<td>5.4 ± 0.1$^a$</td>
<td>5.3 ± 0.1$^a$</td>
<td>5.4 ± 0.1$^a$</td>
<td>5.7 ± 0.1$^a$</td>
<td>5.5 ± 0.2$^a$</td>
</tr>
<tr>
<td>FG fluid volume (µl)</td>
<td>203.7 ± 11.9$^a$</td>
<td>213.9 ± 16.0$^a$</td>
<td>226.0 ± 15.1$^a$</td>
<td>203.3 ± 22.2$^a$</td>
<td>261.7 ± 22.9$^a$</td>
</tr>
<tr>
<td>SP conc. of FG (mg ml$^{-1}$ fluid)</td>
<td>85.0 ± 6.8$^a$</td>
<td>109.1 ± 9.7$^a$</td>
<td>110.5 ± 9.7$^a$</td>
<td>96.8 ± 14.8$^a$</td>
<td>101.2 ± 10.1$^a$</td>
</tr>
<tr>
<td>SP conc. of DG (mg g$^{-1}$ wet tissue)</td>
<td>63.9 ± 3.4$^a$</td>
<td>46.4 ± 2.4$^b$</td>
<td>42.7 ± 2.6$^b$</td>
<td>61.5 ± 2.9$^a$</td>
<td>64.0 ± 2.4$^a$</td>
</tr>
<tr>
<td>Total SP$^8$ (mg lobster$^{-1}$)</td>
<td>119.6 ± 6.8$^a$</td>
<td>93.3 ± 4.8$^b$</td>
<td>92.30 ± 5.2$^b$</td>
<td>69.7 ± 4.8$^b$</td>
<td>78.5 ± 3.7$^b$</td>
</tr>
<tr>
<td>Proportion of total SP in FG$^9$</td>
<td>14.3 ± 1.2$^a$</td>
<td>21.5 ± 1.4$^b$</td>
<td>25.1 ± 1.4$^{bc}$</td>
<td>24.9 ± 1.7$^{bc}$</td>
<td>31.6 ± 2.1$^c$</td>
</tr>
</tbody>
</table>

$^1$Treatments: MC - lobsters reared on fresh mussel and then fed mussel flesh, except on the last meal where they were disturbed to simulate a feeding event; MM - lobsters reared on fresh mussel and then fed mussel flesh; MF - lobsters reared on fresh mussel and then fed the dry formulated diet; FM - lobsters reared on the dry formulated diet and then fed mussel flesh; FF - lobsters reared on and then fed the dry formulated diet

$^2$n=36; $^3$n=33; $^4$n=39; $^5$n=19, FG: n=12; $^6$n=20, FG: n=12

$^7$DG weight / lobster weight × 100

$^8$SP concentration in FG × FG fluid volume (ml) + SP concentration in DG × DG weight (g)

$^9$SP in FG / total SP × 100
Fig. 5.2. Changes in soluble protein (SP) concentration of the foregut (FG) and digestive gland (DG) of *Jasus edwardsii* juveniles reared on fresh mussel (M lobsters) over time following a feeding event (MM = mussel flesh; MF = dry formulated diet) or disturbance simulating a feeding event (MC = unfed control).
Fig. 5.3. Changes in the total activities for total protease, trypsin, $\alpha$-amylase, and $\alpha$-glucosidase of the foregut (FG) of *Jasus edwardsii* juveniles reared on fresh mussel (M lobsters) over time following a feeding event (MM = mussel flesh; MF = dry formulated diet) or disturbance simulating a feeding event (MC = unfed control).
Fig. 5.4. Changes in the total activities for total protease, trypsin, α-amylase, and α-glucosidase of the digestive gland (DG) of Jasus edwardsii juveniles reared on fresh mussel (M lobsters) over time after a feeding event (MM = mussel flesh; MF = dry formulated diet) or disturbance simulating a feeding event (MC = unfed control).
5.3.2. Soluble protein concentration and enzyme activities of lobsters reared on the dry formulated diet

A period of six months of rearing on the dry formulated diet (i.e., FM and FF) depressed specific and total activities of most digestive enzymes tested in comparison to long-term rearing on fresh mussel (i.e., MC, MM and MF), with only α-glucosidase showing a significant increase in total activity in the DG of FM and FF (Table 5.2.). The F (i.e., FM and FF) and M (i.e., MC, MM and MF) lobsters all shared similar DG index (i.e., 5.5 ± 0.1%) (one-way ANOVA, df=4,149, F=1.47, NS) (Table 5.1.). The SP concentration of the DG was higher in FM and FF (i.e., 61.5 ± 2.9 and 64.0 ± 2.4 mg g⁻¹) compared to MM and MF (i.e., 46.4 ± 2.4 and 42.7 ± 2.6 mg g⁻¹), but did not differ significantly from MC (63.9 ± 3.4 mg g⁻¹) (df=4,149, F=14.88, P<0.001) (Table 5.1.).

α-amylase showed the greatest differences in specific and total activities between feeding treatments (Table 5.2.). Three weeks of experimental feeding on the dry formulated diet (i.e., MF c.f. MM) or mussel flesh (i.e., FM c.f. FF) was sufficient to observe rapid changes in regards to the α-amylase activity in the FG and DG, but not for the other digestive enzymes tested (Table 5.2.). Increasing exposure to the formulated diet reduced α-amylase activity whereas the opposite effect was observed with mussel flesh (Table 5.2.).
Table 5.2. Mean ± S.E. specific (U mg⁻¹) and total activities of the digestive enzymes of the digestive gland (DG; U g⁻¹) and foregut fluid (FG; U ml⁻¹) of *Jasus edwardsii* juveniles in five different dietary treatments¹. Significant differences between means within the same row are marked by different letters (P<0.05).

<table>
<thead>
<tr>
<th>Specific activity (U mg⁻¹)</th>
<th>Organ</th>
<th>MC²</th>
<th>MM³</th>
<th>MF⁴</th>
<th>FM⁵</th>
<th>FF⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protease</td>
<td>FG</td>
<td>40.87 ± 1.84ᵃ</td>
<td>38.48 ± 2.18ᵃ</td>
<td>40.01 ± 1.96ᵃ</td>
<td>18.42 ± 1.99ᵇ</td>
<td>20.04 ± 1.17ᵇ</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>25.76 ± 1.86ᵇ</td>
<td>28.63 ± 2.04ᵇ</td>
<td>34.11 ± 2.34ᵇ</td>
<td>8.84 ± 0.41ᶜ</td>
<td>8.61 ± 0.43ᶜ</td>
</tr>
<tr>
<td>Trypsin</td>
<td>FG</td>
<td>0.35 ± 0.02ᵃ</td>
<td>0.30 ± 0.02ᵃ</td>
<td>0.33 ± 0.02ᵃ</td>
<td>0.21 ± 0.03ᵇ</td>
<td>0.26 ± 0.02ᵇ</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.28 ± 0.02ᵃ</td>
<td>0.36 ± 0.02ᵃ</td>
<td>0.29 ± 0.02ᵃ</td>
<td>0.19 ± 0.02ᵇ</td>
<td>0.16 ± 0.01ᵇ</td>
</tr>
<tr>
<td>α-amylase</td>
<td>FG</td>
<td>1.31 ± 0.12ᵇ</td>
<td>1.79 ± 0.22ᵃ</td>
<td>0.83 ± 0.08ᵇ</td>
<td>0.72 ± 0.10ᶜ</td>
<td>0.45 ± 0.07ᵈ</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>1.77 ± 0.22ᵃ</td>
<td>1.15 ± 0.09ᵃ</td>
<td>0.48 ± 0.05ᵇ</td>
<td>0.48 ± 0.04ᵇ</td>
<td>0.24 ± 0.03ᶜ</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>FG</td>
<td>0.0020 ± 0.0001ᵃ</td>
<td>0.0018 ± 0.0001ᵇ</td>
<td>0.0017 ± 0.0001ᵇ</td>
<td>0.0017 ± 0.0001ᵇ</td>
<td>0.0014 ± 0.0001ᵇ</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.0036 ± 0.0003ᵃ</td>
<td>0.0047 ± 0.0004ᵇ</td>
<td>0.0050 ± 0.0004ᵇ</td>
<td>0.0042 ± 0.0004ᵇ</td>
<td>0.0040 ± 0.0004ᵇ</td>
</tr>
</tbody>
</table>

| Total activity            | FG    | 3332.6 ± 261.3ᵃ | 3860.5 ± 331.9ᵃ | 4010.2 ± 256.7ᵃ | 1592.7 ± 169.7ᵇ | 2019.4 ± 206.5ᵇ |
|                           | DG    | 1454.4 ± 32.6ᵇ | 1199.3 ± 43.6ᵇ | 1256.9 ± 42.8ᵇ | 533.4 ± 26.3ᶜ | 547.1 ± 31.8ᶜ |
| Trypsin                   | FG    | 29.4 ± 3.0ᵇ | 31.3 ± 3.4ᵇ | 34.7 ± 2.8ᵃ | 18.4 ± 2.5ᵇ | 25.8 ± 2.6ᵇ |
|                           | DG    | 16.2 ± 0.5ᵃ | 15.9 ± 1.1ᵃ | 11.2 ± 0.5ᵇ | 11.5 ± 1.0ᵇ | 10.5 ± 1.0ᵇ |
| α-amylase                 | FG    | 112.6 ± 13.7ᵇ | 191.4 ± 32.7ᵇ | 85.9 ± 8.7ᵇ | 61.5 ± 7.2ᶜ | 41.1 ± 5.9ᶜ |
|                           | DG    | 83.7 ± 5.0ᵃ | 51.1 ± 3.5ᵇ | 17.6 ± 1.4ᶜ | 29.1 ± 2.4ᵈ | 14.5 ± 1.7ᶜ |
| α-glucosidase             | FG    | 0.16 ± 0.01ᵃ | 0.18 ± 0.02ᵃ | 0.17 ± 0.01ᵃ | 0.15 ± 0.02ᵃ | 0.14 ± 0.01ᵃ |
|                           | DG    | 0.21 ± 0.01ᵃ | 0.20 ± 0.01ᵃ | 0.19 ± 0.01ᵃ | 0.25 ± 0.01ᵇ | 0.25 ± 0.01ᵇ |

¹ Treatments: MC - lobsters reared on fresh mussel and then fed mussel flesh, except on the last meal where they were disturbed to simulate a feeding event; MM - lobsters reared on fresh mussel and then fed mussel flesh; MF - lobsters reared on fresh mussel and then fed the dry formulated diet; FM - lobsters reared on the dry formulated diet and then fed mussel flesh; FF - lobsters reared on and then fed the dry formulated diet
² n=36; ³ n=33; ⁴ n=39; ⁵ DG: n=19, FG: n=12; ⁶ DG: n=20, FG: n=12
5.3.3. Post-prandial changes in digestive gland structure and digestive fluid pH of lobsters reared on the dry formulated diet and fresh mussel

Table 5.3. Mean ± S.E. lobster weight, digestive fluid pH, digestive gland (DG) F- and B-cell number per tubule, tubule and lumen areas of Jasus edwardsii juveniles reared on either fresh mussel (MC and MM) or dry formulated diet (FF), after a feeding event of either mussel flesh (MM) or dry formulated diet (FF), or disturbance simulating a feeding event (MC). Significant differences between means within the same row are marked by different letters (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>MC 1</th>
<th>MM 2</th>
<th>FF 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobster weight (g)</td>
<td>46.2 ± 1.4ab</td>
<td>47.3 ± 1.3a</td>
<td>42.2 ± 1.6b</td>
</tr>
<tr>
<td>FG pH</td>
<td>6.15 ± 0.03a</td>
<td>5.96 ± 0.03b</td>
<td>6.12 ± 0.04a</td>
</tr>
<tr>
<td>DG pH</td>
<td>6.20 ± 0.03ab</td>
<td>6.34 ± 0.03a</td>
<td>6.20 ± 0.05b</td>
</tr>
<tr>
<td>B-cell tubule⁻¹</td>
<td>5.6 ± 0.8a</td>
<td>5.8 ± 0.6a</td>
<td>7.2 ± 0.9a</td>
</tr>
<tr>
<td>F-cell tubule⁻¹</td>
<td>7.0 ± 0.5a</td>
<td>5.9 ± 0.5a</td>
<td>6.4 ± 0.4a</td>
</tr>
<tr>
<td>Tubule surface area (10³ μm²)</td>
<td>161.4 ± 13.6a</td>
<td>147.2 ± 11.5a</td>
<td>129.2 ± 8.9a</td>
</tr>
<tr>
<td>Tubule lumen to whole tubule surface area ratio (%)</td>
<td>11.0 ± 1.0a</td>
<td>7.5 ± 0.9b</td>
<td>14.1 ± 0.9a</td>
</tr>
</tbody>
</table>

¹ n=12; ² n=24

Feeding on both mussel and formulated diets resulted in a significant gradual increase in pH through time in the FG (i.e., 5.94 at 1 h to 6.23 at 24 h) and a decrease (i.e., 6.41 at 1 h to 6.01 at 24 h) in the pH of the DG (two-way ANOVA, interaction organ × time, df=7,64, F=5.32, P<0.001) (Fig. 5.5.). The FF lobsters had a higher overall pH (6.12 ± 0.04) in the FG and lower pH (6.20 ± 0.05) in the DG than MM lobsters (FG, 5.96 ± 0.03; DG, 6.34 ± 0.03), but FF did not differ significantly from MC lobsters (FG, 6.15 ± 0.03; DG, 6.20 ± 0.03) (two-way ANOVA, interaction dietary treatment × organ, df=2,114, F=9.62, P<0.001) (Table 5.3.). The pH of the digestive fluid from the FG and DG of MC lobsters did not vary through time post-disturbance (df=3,16, F=0.39, NS) (Fig. 5.5.). There was a small but significant difference between the pH of the diets (dry formulated diet pH, 6.58 ± 0.00; mussel mantle, pH = 6.45 ± 0.04; mussel gonad pH, 6.14 ± 0.03) (one-way ANOVA, df=2,6, F=62.15, P<0.001).
Fig. 5.5. Mean digestive fluid pH of the foregut (FG) and digestive gland (DG) of *Jasus edwardsii* juveniles reared on either fresh mussel (MC and MM) or dry formulated diet (FF), after a feeding event of either mussel flesh (MM) or dry formulated diet (FF), or disturbance simulating a feeding event (MC). MC lobster only sampled at 1, 4, 10 and 24 h post-disturbance.
Fig. 5.6. Transverse section (TS) of digestive gland tubules of *Jasus edwardsii* juveniles reared on either fresh mussel (MC and MM) or dry formulated diet (FF), after a feeding event of either mussel flesh (MM) or dry formulated diet (FF), or disturbance simulating a feeding event (MC). (A) 1 h post-disturbance (MC). (B) 8 h post-prandial (MM). (C) 8 h post-prandial (FF). (D) 18 h post-prandial (FF). Lu, tubule lumen; Ct, connective tissue; Hs, haemolymph space; Hc, haemocyte; Lv, lipid vacuole; F, F-cell; R, R-cell; B, B-cell. (D) Whole tubule and tubule lumen surface areas are shown.

From a visual assessment there were no obvious changes in lipid vacuolation in the DG after feeding or simulated feeding. However, FF lobsters appeared to have relatively fewer, but larger lipid vacuoles in their R-cells compared to MM and MC lobsters (Fig. 5.6.). There were no changes in the number of F-cells through time and no difference in the overall number of F-cells among dietary treatments (two-way ANOVA, NS) (Table 5.3.). The number of B-cells per tubule increased significantly in FF lobsters at 18 h post-prandial (i.e., 269% higher than in MM lobsters at the same time), but remained unchanged through time for MM
lobsters (two-way ANOVA, interaction dietary treatment × time, $df=7,32, F=3.85, P<0.05$) and MC lobsters (one-way ANOVA, $df=3,8, F=0.55, NS$) (Fig. 5.6.D and 5.7.). Tubule surface area, and the ratio of the surface area of the tubule lumen and the whole tubule, also did not vary through time post-prandial or with simulated feeding ($NS$). The MM lobsters had an overall significantly smaller tubule lumen to whole tubule surface area ratio ($7.5 ± 0.9\%$) than FF lobsters ($14.1 ± 0.9\%$) and MC lobsters ($11.0 ± 1.0\%$) (one-way ANOVA, $df=2,57, F=14.30, P<0.001$) (Table 5.3.).

Fig. 5.7. Mean B-cell number per tubule of the digestive gland (DG) of *Jasus edwardsii* juveniles reared on either fresh mussel (MC and MM) or dry formulated diet (FF), after a feeding event of either mussel flesh (MM) or dry formulated diet (FF), or disturbance simulating a feeding event (MC). MC lobster only sampled at 1, 4, 10 and 24 h post-disturbance.
5.4. Discussion

The results show that food ingestion in juvenile spiny lobsters induces increased enzyme secretion, a phenomenon that has been found for other crustaceans using histological methods (Barker & Gibson, 1977; Al-mohanna et al., 1985). Sampling of the foregut at various times post-prandial elucidated a common peak of enzyme activity at 4 h after feeding on a meal of dry formulated diet or mussel flesh, and a second peak at 18 h post-prandial for the mussel fed lobsters. Three secretory phases were observed in the digestive gland of the European lobster, *H. gammarus*, at 0-15 min, 1-2 h and 3.5-5 h after a meal (Barker & Gibson, 1977), whereas only two within 6.5 h of a meal for the narrow clawed crayfish, *A. leptodactylus* (see Barker & Gibson, 1977), or one continuous phase of secretion 1-4 h after feeding in the green tiger prawn, *P. semisulcatus* (Al-mohanna et al., 1985). However, in these latter studies there was some ambiguity in the interpretation of results as there were no unfed controls and the activity of the enzyme secretions were not quantitatively determined. Quantitative evidence for a single phase of gastric secretion reaching a peak in enzyme activity at 4 h post-feeding was found for the African catfish, *Clarias gariepinus* (Uys et al., 1987). Results from the current study indicate that the first peak of digestive enzyme in juvenile lobsters (i.e., within 30 min), and possibly previous studies, can be attributed to a disturbance event (i.e., short illumination, water movement) rather than being the direct consequence of food ingestion (Ceccaldi, 1997). Despite the absence of significant post-prandial changes in enzyme activity measured in the digestive gland, it is believed that the peaks in activity of the foregut are related to secretion originating from the F-cells of the digestive gland of Crustacea (Hopkin & Nott, 1980). There were no clear temporal changes in activity among the enzymes measured suggesting that all major digestive enzymes were secreted or activated at the same time in response to food ingestion (Barker & Gibson, 1977). Nevertheless, the other digestive enzymes found in *J. edwardsii* such as lipase, esterase, individual proteases (i.e., chymotrypsin and carboxypeptidase), and other carbohydrases (i.e., β-glucosidase, β-galactosidase, cellulase, cellobiase, laminarinase, and chitinase) were not tested in this current study (Johnston, 2003). The lack of temporal changes in activity of the digestive gland was unexpected given that previous histological studies observed the enzyme secretions in the lumen of the digestive gland tubules (Barker & Gibson, 1977; Al-mohanna et al., 1985). However, enzyme secretion may have been masked by the process for preparing
the digestive gland for enzyme analyses in the current study. Histological methods have the advantage of conserving the structure and content of the digestive gland cells, whereas the preparation of the digestive gland for quantitative enzyme analyses results in the mixing and confounding of secreted and stored enzymes (Al-mohanna et al., 1985; Sánchez-Paz et al., 2003). Such phenomenon could have mislead Ong and Johnston (2006) to suggest that feeding had little influence on digestive enzyme production in the giant tiger prawn, *P. monodon*. The more rigorous approach of examining both foregut and digestive gland enzymes, as adopted in this current study, suggests that enzyme secretion events may be best indicated by measured changes in enzyme activity in the foregut of decapod crustaceans.

Ingestion of the dry formulated diet by lobsters resulted in an enzyme secretion response at 4 h that was comparable with the response at 4 h for lobsters fed mussel flesh, implying that the necessary specific components (e.g., amino acids) or digestion products were present in both diets to induce enzyme secretion (Ceccaldi, 1997). The lower dry matter digestibility of the formulated diet (i.e., 61.2%) compared to fresh mussel (i.e., 89.2%) is therefore not the consequence of a shortcoming in enzymatic secretion (Simon & Jeffs, 2008). Future improvements in dissolution profiles of dietary ingredients upon entering the foregut may assist in improving formulated diet digestibility (see also Simon, 2009d). Previously it was hypothesised that entry of seawater upon ingestion of the dry formulated diet could impair digestion and reduce the physical capacity of the foregut for food intake (Simon & Jeffs, 2008; Simon, 2009a). The pH results of this study appear to contradict this hypothesis since foregut pH following ingestion of the formulated diet was similar to unfed lobsters. However, foregut fluid was relatively more acidic in the first hours following mussel flesh ingestion, despite the small differences in the pH of the two diets. Therefore, the foregut pH of the formulated diet fed lobsters may have been maintained to pre-feeding level by the presence of substantial quantities of acidic gastric fluid. Foregut fluid is found at all times, even during starvation, in the western spiny lobster, *P. longipes* (Dall, 1975), and the American lobster, *H. americanus* (Hoyle, 1973). Under continuous feeding conditions, it is possible that ingestion of seawater in conjunction with the food would have had a greater effect, but in this current study lobsters were fed three times a week and only 50% of the satiation ration of the lobster was allotted after a non-feeding period of four days. The gastric fluid volume of the unfed lobster group was equivalent on average to 24% of the foregut capacity (Simon, 2009a). Such volume represents a potential cutback in foregut capacity.
available for the intake of dry formulated diets, which unlike fresh food would expand in contact with the gastric fluid. This could explain the discrepancies in moisture content of the dry formulated diet prior- (39-52% after 0.5-2 h immersion) and post-ingestion (61-69% moisture for S1-S3 juveniles) (Simon, 2009a).

The absence of a second peak in enzyme activity in the foregut at 18 h post-feeding on the dry formulated diet, in contrast to mussel fed lobsters, suggests that the type of diet influences the temporal digestive response of juvenile *J. edwardsii*. Appetite revival has been linked to the supply of new digestive fluid to the foregut in the freshwater crayfishes *A. astacus* (Vogt et al., 1989) and *Cherax quadricarinatus* (Loya-Javellana et al., 1995). The slow appetite revival on the formulated diet (>18 h) compared to mussel flesh (12 h), and the negative effect of increasing feeding frequency of the formulated diet on growth and consumption, may relate to the digestive system of the lobsters being unable to efficiently process the arrival of formulated food immediately after the evacuation of the former meal (i.e., 10 h) (Simon & Jeffs, 2008). The high number of B-cells per tubule 18 h post-feeding on the formulated diet is a likely indicator of excessive intracellular digestion and/or elimination of waste products (e.g., toxic mineral elements, digestive enzymes, undigested particles) from the digestive gland (Brunet et al., 1994). This may be the result of the poor digestibility of the diet generating an overload of undigested fine particles in the digestive gland (Al-mohanna & Nott, 1987). The larger lumen surface area to tubule ratio and more acidic pH of the digestive gland in formulated diet fed lobsters compared to mussel flesh fed lobsters provides further evidence for the intensified digestive effort on the formulated diet that could have affected the return of appetite. Presumably, the greater fecal production rate on the formulated diet (Simon & Jeffs, 2008) would also result in a greater loss of digestive enzymes associated with undigested food material (Córdova-Murueta et al., 2003). Nevertheless, the typical 24 h B-cell digestive cycle found in other crustaceans such as the green tiger prawn, *P. semisulcatus* (Al-mohanna & Nott, 1986), the European green crab *Carcinus maenas* (Hopkin & Nott, 1980) and the amphipod *Corophium volutator* (Icely & Nott, 1985) occurs in *J. edwardsii* even after feeding on the formulated diet, as B-cell numbers dropped back to low levels by 24 h post-prandial. The level of activity of foregut enzymes in lobsters fed the formulated diet was also similar to mussel fed lobsters at this time. Therefore, the improved growth efficiency and consumption when delivering the dry formulated diet once a day (Simon & Jeffs, 2008) is arguably the result of a better match with the temporal digestive abilities exhibited by *J.*
edwardsii on this diet. This may also be the case for other lobster species such as H. americanus (Bordner & Conklin, 1981), P. argus (Cox & Davis, 2006), P. ornatus (Jones, 2007) and P. cygnus (Johnston et al. 2008), which do not display an increase in food intake or growth rates when fed more often than once daily. Providing food at the time of peak digestive enzyme secretion was found to improve the culture performance of the Kuruma prawn, Marsupenaeus japonicus (Cuzon et al., 1982).

Trypsin and α-glucosidase specific activities of cultured J. edwardsii measured in this study were comparable to wild J. edwardsii (Johnston, 2003), and the California spiny lobster, P. interruptus (Celis-Guerrero et al., 2004), as well as other crustaceans including the red claw crayfish, C. quadricarinatus (Figueiredo et al., 2001), the whiteleg shrimp, Litopenaeus vannamei (Córdova-Murueta et al., 2003), and six species of crabs collected from the wild (Johnston & Freeman, 2005). Total protease and α-amylase activities could not be compared directly between studies owing to the different substrates used, but were in agreement with the high activities of these digestive enzymes observed in other crustaceans (Johnston & Freeman, 2005; Carrillo-Farnés et al., 2007). Lobsters reared on the dry formulated diet for six months showed a reduction in activity of all major digestive enzymes in comparison to lobsters reared on mussels, suggesting that they were in poorer nutritional and digestive condition. This was also suggested by the lower level of lipid droplet accumulation in the R-cells of lobsters reared on the formulated diet (Johnston et al., 2003). Previous research has shown that short-term fasting can result in an accumulation of enzymes in lobsters (Hoyle, 1973). The similar F-cell numbers and soluble protein concentration in the digestive gland of lobsters reared on the dry formulated diet and 96 h unfed lobsters might be reflective of mild food deprivation conditions on the formulated diet regime. The nutritional condition of lobsters on the dry formulated diet is likely to improve if the feed is delivered daily rather than three times a week (Simon & Jeffs, 2008; Simon, 2009a). The relative size of the digestive gland (i.e., DG index) is a well recognised indicator of the nutritional status of crustaceans (Vogt et al., 1985; Cockcroft, 1997; Jones & Obst, 2000). However, it is worthwhile to note that this is not the case for cultured J. edwardsii juveniles, given the lack of difference in digestive gland indexes between treatments found here and in previous nutritional studies (Johnston et al., 2003; Ward et al., 2003).
Further research is required to understand the effect of feeding frequency and food availability on soluble protein concentrations and enzyme activities in *J. edwardsii*. Lobsters in this current study were fed three times a week, and starved for 96 h prior sampling to ensure they reached basal metabolic rate and that there would be no residual effect from prior feeding that would influence the results (Radford et al., 2004). However, it would be interesting to investigate the effect of the diets under more continuous feeding conditions. Temporal variations in digestive enzyme activity have also been shown in shrimps fed continuously, with generally two major peaks in enzyme activity following circadian rhythms (Cuzon et al., 1982; Hernández-Cortez et al., 1999). Previous studies investigating the effect of short-term starvation in Crustacea have shown that this period of restricted feeding may be accompanied by an increase in enzyme specific activity as an adaptation to lower nutrient status and a decrease in total activity to save energy (Comoglio et al., 2004). However, this is not always the case and inconsistencies in the relationship between digestive enzyme activity and feeding rate have been reported (Meyer et al., 2002). The duration of the fasting period and the metabolite reserves of the individual species would affect this relationship (Meyer et al., 2002; Comoglio et al., 2004). For example, Sánchez-Paz et al. (2003) have shown that the trypsin-encoding RNA levels of the whiteleg shrimp, *L. vannamei*, varies after starvation, initially increasing up to 24 h after the initiation of fasting, but then decreasing to a lower level compared to those obtained in fed shrimp (Sánchez-Paz et al., 2003). Under continuous feeding conditions (~ 1% BW day\(^{-1}\) in *J. edwardsii* of this size; Simon & Jeffs, 2008), it is possible that ingestion of the formulated diet would have had a greater, compounded negative effect on enzyme activities, digestive gland structure and digestive fluid pH in comparison to mussel flesh.

Three weeks of feeding on mussel flesh were not sufficient to markedly improve the nutritional condition and enzyme activities of lobsters reared on the dry formulated diet, except for their \(\alpha\)-amylase activities which displayed rapid and substantial adaptive changes to the diets. The decrease in specific activity of \(\alpha\)-amylase after feeding on the formulated diet would indicate that dietary starch inclusion level (i.e., 25% by dry weight) was above optimal level judging from previous results obtained with penaeid shrimps (Van Wormhoudt et al., 1980; Ceccaldi, 1997). However, \(\alpha\)-amylase activity increased markedly in the foregut and digestive gland of lobsters fed mussel flesh, which contained around 20% glycogen by dry weight (Simon & James, 2007), suggesting that mussel glycogen may be of higher nutritional
value than tapioca starch for *J. edwardsii*. Previously α-amylase activity has been found to be positively correlated to weight gain in the Atlantic white shrimp *L. setiferus* (Guzman et al., 2001). A better digestibility and utilisation of the non-protein dietary energy from fresh mussel may be an important factor influencing the better protein retention and higher growth rates exhibited on this diet compared to experimental formulated diets in culture (Crear et al., 2000; Ward et al., 2003; Simon & Jeffs, 2008). In a subsequent study, mussel glycogen was found to be better digested that native tapioca starch, and better utilised than the partially cooked tapioca starch found in the dry formulated diet (see Simon, 2009c).

This study represents the first attempt at investigating the effect of feeding and diet type on the digestive response of spiny lobsters. Despite high natural variation in enzyme activity between individuals the results were sufficient to indicate that juveniles of the spiny lobster *J. edwardsii* display a “natural” enzymatic secretory response to formulated diet, which represents an important prerequisite for the development of successful diets for commercial culture. There is some evidence that ingestion of the formulated diet leads to difficulties in digestion, particularly at the intracellular level in the digestive gland, which may play a part in the poor appetite revival exhibited on this diet in a previous study (Simon & Jeffs, 2008). Six months rearing on this diet also results in a decrease in the digestive capacity (i.e., total enzyme activity in the foregut and digestive gland) of lobsters which would limit their ability to obtain nutrients from the diet. Given the rapid adaptive changes in α-amylase activity observed in experimental lobsters in this study, improvements of the current dry formulated diet is foreseen by reducing starch inclusion level, or selecting other carbohydrate sources, to improve the digestibility and utilisation of the carbohydrate fraction for growth. Improving the dissolution of dietary ingredients in the foregut will be paramount to maximise the overall digestibility and performance of formulated diets for spiny lobsters.

The digestibility and utilisation of various carbohydrate sources included in formulated diets were investigated in subsequent studies and chapters presented in this thesis (Simon, 2009c, d).
Plate 6. *In vitro* and *in vivo* identification of digestible carbohydrate sources in *Jasus edwardsii* juveniles. A) Enzyme homogenates used for *in vitro* carbohydrate digestion assays; B) Carbohydrate substrates; C) Incubation of carbohydrates and enzyme homogenates using a rotary mixer in a temperature-controlled chamber; D) Semi-purified diets containing different carbohydrate sources and inclusion levels used to measure *in vivo* post-prandial haemolymph glucose concentrations; E) A semi-purified moist formulated diet bound with transglutaminase and cut into small cubes for feeding; F) Direct reading of haemolymph glucose concentration from a Lifescan One-touch Basic® Profile glucometer.

Published as:


### 6.1. Introduction

There is still a paucity of information about the nutritional requirements of spiny lobsters, many aspects of which are being assumed from previous research done on nutrition in shrimp aquaculture (Williams, 2007). Starches have been used in diets for spiny lobsters, often aimed at providing inert bulk to compensate for different levels of protein inclusion (Glencross et al., 2001; Smith et al., 2003, 2005a; Ward et al., 2003). This has been done with relatively little knowledge about carbohydrate digestion and nutrition for the particular lobster species being studied. In lobsters, as in penaeids, several digestive carbohydrases have been identified including α-amylase, α-glucosidase, α-maltase, α-saccharase, galactosidase, chitinase, chitobiase and cellulase, which suggest an ability to digest a wide range of carbohydrate sources (Glass & Stark, 1995; Ceccaldi, 1997; Johnston, 2003). The presence of the digestive enzymes α-amylase and α-glucosidase (Simon, 2009b) would be sufficient to hydrolyse most starches to glucose making it readily available for transport and metabolism (Cuzon et al., 2000). However, since starch is not widely present in algae or other natural marine food sources (glycogen from marine animals being a more common carbohydrate source) the benefit of including starch in the diet of marine crustaceans has been subject to some debate (Cousin et al., 1996). The rationale for the inclusion of starches in crustacean formulated diets is to reduce overall production cost and pollution, via sparing some of the dietary protein for somatic growth rather than being catabolised for energy (Davis & Arnold, 1993; Cousin et al., 1996; Rosas et al., 2000). The derived glucose also plays an integral role in the moulting of Crustacea, combining with ammonia produced by the catabolism of amino
acids to form glucosamine, which accumulates in the hypodermis in preparation for ecdysis (Cuzon et al., 2000). Starch also improves pellet binding and water stability through gelatinisation during diet extrusion (Cuzon et al., 1994; Thomas et al., 1998b). Studies on commercially important shrimp species have demonstrated that the inclusion level, extent of processing, and the source of plant starch affect consumption, digestibility, growth and survival (Catacutan, 1990; Davis and Arnold, 1993; Cousin et al., 1996; Shiau, 1997; Guo et al., 2006). Research has also indicated that more complex carbohydrates (e.g., starches, dextrins), and to a lesser extent disaccharides (e.g., sucrose, trehalose), which require some enzymatic hydrolysis before assimilation, appear better utilised than monosaccharides such as glucose (Alava & Pascual, 1987; Shiau & Peng, 1992; Rosas et al., 2000). Dietary glucose is absorbed quickly across the digestive tract leading to prolonged hyperglycaemia and negative physiological effects (Abdel-Rahman et al., 1979).

Gelatinised maize starch appears to be poorly digested and utilised in *Jasus edwardsii* juveniles fed fishmeal-based dry diets (Johnston et al., 2003; Ward et al., 2003). This agrees with the tendency of carnivorous crustaceans to utilise dietary protein to satisfy most of their energy requirement via a well-developed gluconeogenic pathway (Oliveira & Da Silva, 1997; Rosas et al., 2000; Johnston et al., 2003; Oliveira et al., 2004). Nonetheless, respiratory oxygen to nitrogen ratio (O:N) supports the contention for a protein-sparing effect of dietary carbohydrate in the American lobster, *Homarus americanus* (Capuzzo & Lancaster, 1979; Brown, 2006) and the spiny lobster, *J. edwardsii* (Radford et al., 2008). Several authors have suggested that algal carbohydrates may provide a source of energy for juvenile lobsters in the wild (Brown, 2006; Radford et al., 2007). The presence of macroalgae and carbohydrases able to hydrolyse complex algal polysaccharides in the gut of lobsters provide further evidence for this hypothesis (Glass & Stark, 1995; Johnston, 2003). Clearly, the effectiveness of other sources of carbohydrate has to be tested to ensure the best ingredients are used as non-protein energy sources in lobster diets (Johnston et al., 2003). Lobsters are known to display longer rates of gut clearance (Barker & Gibson, 1977; Kurmaly et al., 1990; Simon & Jeffs, 2008) compared to smaller decapods such as penaeid shrimp (Nunes & Parsons, 2000). Small foregut capacity and slow rates of filling and evacuation in *J. edwardsii* juveniles suggest that they may have a limited ability to vary the scale of their feed intake in response to poorly digestible diets (Simon, 2009a; Simon & Jeffs, 2008). As daily throughput of food is reduced, maximising the amount of nutrient assimilated per meal via improving the digestibility of
formulated diets is crucial to achieving optimum performance (Simon & Jeffs, 2008). There is a lack of information on the digestibility of carbohydrates for lobster species with aquaculture potential such as *J. edwardsii*, principally because apparent digestibility experiments are difficult to handle, expensive and prone to errors (Omondi & Stark, 1996; Johnston et al., 2003). Nevertheless, *in vitro* digestibility studies such as those employing enzyme extracts offer a cheaper, convenient and faster means of developing and testing potential diet components (Glass & Stark, 1995; Cousin et al., 1996; Omondi & Stark, 1996). Combining this with *in vivo* measurements of post-prandial haemolymph glucose concentration would give further indication about the rate of digestion, absorption and possibly utilisation of the different carbohydrate substrates (Shiau & Peng, 1992; Radford et al., 2005).

The aim of this study is to estimate the digestibility of different carbohydrate sources in *J. edwardsii* juveniles by measuring: 1) rates of hydrolysis *in vitro* using enzyme homogenates, and, 2) post-prandial haemolymph glucose concentrations following ingestion of semi-purified diets containing different carbohydrate inclusion levels and sources.

6.2. Materials and methods

6.2.1. In vitro rates of hydrolysis

6.2.1.1. Carbohydrate substrates

Samples of 14 different carbohydrates (300 mg) were homogenised in 5 ml of Milli-Q® water (20 °C) to achieve a concentration of 6% (w/v) (Table 6.1.). Native starches (i.e., potato, tapioca, maize, wheat) formed suspensions that had to be well mixed prior to use due to their poor water solubility (Cousin et al., 1996). Gelatinised starches, agar, alginate and carboxymethyl cellulose (CMC) formed weak gels at this concentration but not in the final assay mixture. Freeze-dried whole mussel gonads and the dry formulated diet were ground using a mortar and pestle and also prepared and tested in the same manner as the carbohydrate substrates.
Table 6.1. Type and source of the carbohydrate substrates used in the *in vitro* experiment.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>Sigma-Aldrich, cat. No. S8501</td>
</tr>
<tr>
<td>Trehalose</td>
<td>Sigma-Aldrich, cat. No. T9449</td>
</tr>
<tr>
<td>Agar</td>
<td>Cero® Agar Agar powder type 8925, Hawkins Watts Ltd., Auckland, New Zealand</td>
</tr>
<tr>
<td>Alginate</td>
<td>Protanal® LF 5/60 Alginate, Hawkins Watts Ltd., Auckland, New Zealand</td>
</tr>
<tr>
<td>Carboxymethyl cellulose (CMC)</td>
<td>Image Holdings Ltd., Auckland, New Zealand</td>
</tr>
<tr>
<td>Native potato starch</td>
<td>J.C. Sherratt &amp; Co. Ltd., Wellington, New Zealand</td>
</tr>
<tr>
<td>Native maize starch</td>
<td>J.C. Sherratt &amp; Co. Ltd., Wellington, New Zealand</td>
</tr>
<tr>
<td>Native tapioca starch</td>
<td>J.C. Sherratt &amp; Co. Ltd., Wellington, New Zealand</td>
</tr>
<tr>
<td>Native wheat starch</td>
<td>J.C. Sherratt &amp; Co. Ltd., Wellington, New Zealand</td>
</tr>
<tr>
<td>Gelatinised maize starch (BO11C)</td>
<td>Image Holdings Ltd., Auckland, New Zealand</td>
</tr>
<tr>
<td>Dextrin</td>
<td>Sigma-Aldrich, cat. No. D2256</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>Clintose® CR18 18 D.E. Maltodextrin, Hawkins Watts Ltd., Auckland, New Zealand</td>
</tr>
<tr>
<td>Mussel glycogen</td>
<td>Freeze-dried <em>Perna canaliculus</em> purified glycogen, Healtheries of New Zealand Ltd, Auckland, New Zealand</td>
</tr>
</tbody>
</table>

1 Carbohydrates were also tested after being fully gelatinised at 80 °C for 20 min (100 g l⁻¹ deionised water), dried for 48 h at 50 °C and ground (Holm et al., 1988).

6.2.1.2. Gut enzyme extracts

The digestive gland (DG) and foregut (FG) extracts of *J. edwardsii* juveniles (mean ± S.D., wet BW = 29.9 ± 4.2 g; n=80) that had been raised on fresh mussels for six months and fed mussel flesh for three weeks (MM and MC lobsters) in a previous study (Simon, 2009b)
were used for this experiment. All the unused (kept frozen for 6 months) aliquots (400-600 µl of DG and FG extracts per lobster) were combined to make up two homogenous extract mixtures of DG and FG and stored at –20 ºC before the in vitro assays. Soluble protein concentrations of the pooled DG and FG extracts were determined as per Simon (2009b).

6.2.1.3. In vitro assays

In vitro assays were performed using a modified method described by Cousin et al. (1996). Carbohydrate substrates (250 µl of the 6% w/v solutions or suspensions) and enzyme extract (150 µl of DG or 200 µl of FG extract) were diluted to 1.6 ml in a 100 mM citrate-phosphate buffer (pH 5.0) in three replicate 2 ml Eppendorf® centrifuge tubes. Immediately after inoculation with the enzyme extract the tubes were incubated in a rotary mixer for 60 min at 37 ºC. This incubation temperature was chosen to produce comparable results with previous studies (Wigglesworth & Griffith, 1994; Glass & Stark, 1995; Cousin et al., 1996). Incubation for all carbohydrate substrates was also performed at 20 ºC for 360 min with the DG extract to obtain hydrolysis rate data at a similar temperature to the optimum water temperature for this lobster species (Thomas et al., 2000). At the end of the digestion period, 20 µl aliquots were removed, diluted to 200 µl in a 50 mM sodium-phosphate buffer (pH 7.4) and stored frozen at –20 ºC until glucose determination. Substrates and enzyme extracts were assayed separately to determine amount of glucose present in each fraction. Preliminary assays showed that the rate of hydrolysis of carbohydrates was optimum at pH 5.0 and linear over the first 60 min at 37 ºC (aliquots sampled at 0, 15, 30, 60, 90 min) or 360 min at 20 ºC (aliquots sampled at 0, 10, 20, 30, 60, 90, 180, 360 min). The pH of the assays (pH 5.0) was in agreement with the pH optima (i.e., 4.5-5.5) of carbohydrases of juvenile J. edwardsii (Johnston, 2003). Glucose was determined using an Amplex® Glucose Oxidase Assay Kit from Invitrogen New Zealand Ltd. (Cat. No. A22189). The assay mixture contained a final concentration of 0.1 mM Amplex® Red reagent, 0.2 U ml⁻¹ of horseradish peroxidase and 2 U ml⁻¹ glucose oxidase. Assays (200 µl) were performed in duplicate with glucose concentrations quantified from a D-glucose standard curve by measuring absorbance at 560 nm over 30 min.
The rate of hydrolysis of each substrate for the different experimental treatments (i.e., DG extracts at 20 and 37 °C, and FG extracts at 37 °C) was calculated as follows:

\[
HR \ (\text{nmol glucose min}^{-1} \ \text{mg}^{-1} \ \text{SP}) = (\left[\text{glucose}\right]_F - \left[\text{glucose}\right]_O) / t / SP
\]

Where \([\text{glucose}]_F\) is the final glucose concentration after incubation with enzyme extracts, \([\text{glucose}]_O\) is the original glucose concentration present in the substrate and enzyme extracts, \(t\) is the incubation duration (min) and \(SP\) is the soluble protein content (mg ml\(^{-1}\)) of the enzyme extracts.

To standardise results between experimental treatments, the relative rate of hydrolysis (%) was calculated as follows:

\[
\text{RHR} \ (%) = HR / \text{HR}_{\text{MAX}} \times 100
\]

Where \(HR\) is the hydrolysis rate for a particular substrate and \(\text{HR}_{\text{MAX}}\) is the maximum hydrolysis rate achieved within each experimental treatment. \(\text{HR}_{\text{MAX}}\) corresponded to the hydrolysis rate of the gelatinised tapioca substrate for the three experimental treatments.

6.2.2. In vivo haemolymph glucose concentrations

6.2.2.1. Experimental diets

Fresh mussel gonads (27% glycogen, dry weight), the dry formulated diet (25% tapioca starch, dry weight) (Simon, 2009a) and 14 semi-purified diets formulated to contain various sources of carbohydrates were used for the experiment (Table 6.2.). All semi-purified diets contained a total of 32% carbohydrate by dry weight (i.e., 5% from raw ingredients and 27% supplemented), except for the control diet (i.e., contained only the 5% carbohydrate fraction present in raw ingredients), the 7% glucose and 7% BO11C diets (i.e., 12% carbohydrate) (Table 6.2.). Dietary carbohydrates supplemented at 27% (dry weight) were sucrose, trehalose, agar, CMC, \(P.\ canaliculus\) mussel glycogen, pre-gelatinised maize starch (BO11C), dextrin, and native starch from potato, maize, tapioca and wheat (Table 6.2.). Dry
ingredients were blended and added to a mixture of fresh *P. canaliculus* mussel liquor (i.e., liquid collected while shucking fresh mussels) and fish oil heated at 60 °C. Additional deionised water (60 °C) was added in varying proportion depending on the type of carbohydrate to reach a similar dough consistency. The dough was kept below 60 °C to prevent starch gelatinisation (Cousin et al., 1996). The diets were left at room temperature for 2 h and overnight at 2 °C to allow for transglutaminase binding reaction before being cut into 1 cm³ cubes and kept frozen at −20 °C for a maximum period of three months. Moisture content of diets averaged 49% but ranged from 38.5% (trehalose diet) to 55.9% (agar diet) (Table 6.2.). A high level of available lysine and glutamine residues in the soluble sodium caseinate allowed fast cross-linking (De Jong & Koppelman, 2002) and high water stability of the diets (i.e., >90% dry matter stability after 2 h immersion) as determined by a method described in Simon (2009a).

6.2.2.2. *Experimental design and feeding procedure*

Cultured intermoult *J. edwardsii* juveniles (60.7 ± 6.4 g, n=55) that had been raised on a diet of fresh *P. canaliculus* mussels were acclimated to the experimental conditions for 10 days before the start of haemolymph sampling. Lobsters were held in the same experimental tanks (6 l transparent-plastic aquaria) and conditions as in Simon (2009a). They were fed mussel flesh to satiety every 48 h during the acclimation and recovery period. Following a starvation period of three days, lobsters were fed a similar amount in dry matter of their respective diets (n=3 per diet, 16 diets) that corresponded to 1% of their BW within a 2 h period (Simon, 2009a). Lobsters that did not ingest the complete allotted ration were excluded from further analyses. The starvation period ensured all lobsters reached basal metabolic rate and that there would be no residual effect from prior feeding that would influence the results (Radford et al., 2005). Some lobsters (n=6) were not fed to serve as controls (i.e., preprandial haemolymph glucose concentrations). Lobsters were each used twice, following a recovery period of two weeks between trials and a random reallocation of dietary treatments.
### Table 6.2. Ingredient composition and calculated proximate composition of the semi-purified diets. Values are based on dry weight.

<table>
<thead>
<tr>
<th>Ingredients in semi-purified diets (g kg(^{-1}) of diet)</th>
<th>Control</th>
<th>7% Glucose</th>
<th>7% BO11C</th>
<th>Other diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate supplement(^1)</td>
<td>0</td>
<td>70</td>
<td>70</td>
<td>270</td>
</tr>
<tr>
<td>Diatomaceous earth</td>
<td>270</td>
<td>200</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>Sodium caseinate(^2)</td>
<td>290</td>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
<tr>
<td>Squid meal(^3)</td>
<td>85</td>
<td>85</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Mussel meal(^4)</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Fish oil</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Soy bean lecithin</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Transglutaminase(^5)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin mix(^6)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Other dried ingredients(^7)</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
</tbody>
</table>

#### Calculated proximate composition\(^8\)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>7% Glucose</th>
<th>7% BO11C</th>
<th>Other diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Crude lipid (%)</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>5</td>
<td>12</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>32</td>
<td>25</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>48</td>
<td>49</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Energy (kJ g(^{-1}))</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>P/E ratio (g CP MJ(^{-1}))</td>
<td>32</td>
<td>30</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>

---

\(^1\) Carbohydrates included at 270 g kg\(^{-1}\) in diets were: sucrose, trehalose, agar, CMC, mussel glycogen, BO11C, dextrin, native potato, native maize, native tapioca and native wheat starches (Table 6.1.).

\(^2\) Sodium caseinate A185 ALANAT, Davis Trading Company Ltd., Auckland, New Zealand.

\(^3\) Frozen mantle tubes of the squid *Todarodes pacificus*, Pacific Catch, Wellington, New Zealand. Tubes were low heat dried (45 °C for 48 h) and ground (<700 µm) before use (Córdova-Murueta & García-Carreño, 2002).

\(^4\) Freeze-dried *P. canaliculus* mussel powder, Aroma New Zealand Ltd., Christchurch, New Zealand.

\(^5\) Transglutaminase TG-B (99% caseinate), Image Holdings Ltd., Auckland, New Zealand.

\(^6\) Image Holdings, New Zealand (g kg\(^{-1}\) vitamin mix in active form): thiamin, 1.5; riboflavin, 2.0; niacin, 7.5; pyridoxine, 1.5; cyanocobalamin, 0.005; Biotin, 0.05; retinol, 600000 IU kg\(^{-1}\); cholecalciferol, 120000 IU kg\(^{-1}\); menadione, 1.0; calcium pantothenate, 5.0; folic acid, 0.5; DL α-tocopherol, 20; choline, 50.0; inositol, 10; and ascorbic acid, 10.

\(^7\) Others ingredients (g kg\(^{-1}\) diet): CMC-casein-gelatin coated AA (arginine, 44%; methionine, 20%; threonine, 10%), 47 (Alam et al., 2004); CMC, 20; vitamin C-coated, 10; betaine, 7; Naturose (astaxanthin 1.5%), 5; vitamin E, 2; choline chloride, 2; ethoxyquin, 0.5.

\(^8\) Calculated from proximate composition of ingredients. Energy calculated as 21.3 kJ g\(^{-1}\) for protein, 17.6 kJ g\(^{-1}\) for carbohydrate and 39.5 kJ g\(^{-1}\) for lipid (Cuzon & Guillaume, 1997). Moisture content measured as per Simon (2009a), other diets moisture content: 49 ± 1.4% (mean ± S.E., n=11)
6.2.2.3. Haemolymph glucose measurements

The introduction of food in the tanks corresponded to 0 h. Haemolymph sampling began 2 h after food introduction/feeding, with additional samples taken at 4, 7, 12, 24, and 30 h from the same lobster. Haemolymph was not sampled prior to feeding as this was found to affect feed intake. Haemolymph samples (~ 30-50 µl) were taken from the sinus of the 5th walking leg using a standard 1 cc Terumo insulin syringe (27-gauge needle) (Tlusty et al., 2005). The effect of serial sampling on haemolymph glucose concentration was found be negligible in juvenile *J. edwarsii* of similar weight (Radford et al., 2005). Lobsters appeared unaffected by the procedure and were feeding directly after the last sampling (i.e., fresh mussel overnight). The glucose concentration (mmol l\(^{-1}\)) of the haemolymph samples was read directly (within 45 s of sampling) from a Lifescan One-touch Basic® Profile glucometer (Life-Scan, Inc., Milpitas, USA) (Tlusty et al., 2005). The meter reading was calibrated with results from a glucose HK assay kit (Sigma-Aldrich, USA) obtained from samples (n=22) that were chilled on ice and centrifuged at 16110 × g for 5 min. Results from the two instruments were strongly correlated (Pearson correlation, \(r=0.98, r^2=0.95, P<0.001\)). Overall, \(n=5\) lobsters per dietary treatment were analysed, except for the 7% glucose, agar, native wheat starch and glycogen diets where \(n=4\) (i.e., a total of 528 haemolymph glucose measurements).

6.2.3. Statistical analyses

The effects of experimental treatment (i.e., DG extracts at 20 and 37 °C, and FG extracts at 37 °C), carbohydrate substrate and the interaction (experimental treatment × carbohydrate substrate) on HR (nmol glucose min\(^{-1}\) mg\(^{-1}\) SP) and RHR (%) were tested using two-way ANOVAs.

The effects of dietary treatment, time and the interaction (dietary treatment × time) on *in vivo* haemolymph glucose concentration were tested with a repeated measures ANOVA. A Geisser-Greenhouse correction was used to adjust for violations of the sphericity assumption (Zar, 1999). Differences among peak post-prandial glucose concentrations for the different dietary treatments were tested by one-way ANOVA. Prior to analyses, the ANOVA assumptions of normality of residuals and homogeneity of variances were tested using the Shapiro-Wilk and Levene tests respectively. Data were square-root transformed to satisfy the
assumption when necessary. Tukey-Kramer test was used for post hoc comparisons of means following significant ANOVA ($P<0.05$) (Zar, 1999). When $P>0.05$, non-significant results were stated as $NS$. All analyses were performed using the Statistical & Power Analysis Software NCSS 2004 (Utah, USA).

6.3. Results

6.3.1. In vitro rates of hydrolysis

Digestive enzyme extracts from the FG and DG of the juvenile lobsters resulted in the liberation of glucose from all the carbohydrate sources tested, including the dry formulated diet and freeze-dried whole mussel gonads (Table 6.3.). The HR of the different carbohydrate substrates increased significantly with temperature, and were higher in foregut extracts compared with digestive gland extracts (two-way ANOVA, $df=2,101$, $F=62.85$, $P<0.001$) (Table 6.3.). Overall, DG extracts resulted in $62.9\pm 4.9\%$ (i.e., mean $\pm$ S.E.) of the hydrolysis of FG extracts at 37 °C for an equal amount of soluble protein (Table 6.3.). In some instances (i.e., dry formulated diet and sucrose), the rates of hydrolysis using FG and DG extracts were similar at 37 °C but the overall interaction effect (carbohydrate substrate × experimental treatment) was non-significant (two-way ANOVA, $df=32,101$, $F=1.03$, $NS$). Gelatinised tapioca starch displayed the highest rates of hydrolysis of all the carbohydrate substrates tested for each of the three experimental treatments (i.e., $36.25\pm 5.71$, $80.61\pm 4.51$, and $131.36\pm 21.15$ nmol glucose min$^{-1}$ mg$^{-1}$ SP for DG at 20 and 37 °C, and FG extracts at 37 °C, respectively) (Table 6.3.).

There was no significant difference in the RHR of each carbohydrate substrate between the three experimental treatments (two-way ANOVA, $df=2,101$, $F=0.10$, $P=0.90$; interaction $df=32,101$, $F=0.83$, $P=0.72$). Therefore (i.e., $P>0.25$), RHR data for the three experimental treatments were combined (Table 6.3.) (Winer et al., 1991). The lowest RHR (<20%) was found for the two disaccharides, trehalose and sucrose, as well as alginate and the native starches potato, tapioca and maize. Agar, native wheat starch, the dry formulated diet and whole mussel gonads displayed intermediate RHR (20 to 50%). The CMC, purified mussel glycogen, dextrin and gelatinised starch had the highest RHR (≥70%) (two-way ANOVA, $df=16,101$, $F=33.01$, $P<0.001$) (Table 6.3.). The plant source of starch tended to
have an influence on the in vitro hydrolysis rate whether native (i.e., potato < tapioca, maize < wheat) or gelatinised (i.e., potato < tapioca), with gelatinisation significantly improving subsequent hydrolysis by digestive enzymes.

Table 6.3. In vitro hydrolysis rates (HR) for digestive gland (DG) and foregut (FG) extracts at two temperatures (i.e., 20 and 37 °C) for different carbohydrate substrates, included in the dry formulated diet and mussel gonad. Significant differences between relative hydrolysis rate (RHR) means for the different carbohydrate substrates are marked by different letters within the column (P<0.05).

<table>
<thead>
<tr>
<th>Carbohydrate sources</th>
<th>Mean HR ± S.E. (n=3)</th>
<th>DG / FG ratio</th>
<th>Mean RHR ± S.E. (n=9) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DG extracts 20 °C</td>
<td>DG extracts 37 °C</td>
<td>FG extracts 37 °C</td>
</tr>
<tr>
<td>Trehalose</td>
<td>4.23 ± 2.91</td>
<td>1.76 ± 0.45</td>
<td>2.37 ± 1.80</td>
</tr>
<tr>
<td>Alginate</td>
<td>4.08 ± 2.16</td>
<td>4.08 ± 1.50</td>
<td>10.17 ± 6.46</td>
</tr>
<tr>
<td>Potato</td>
<td>2.01 ± 1.27</td>
<td>8.64 ± 4.59</td>
<td>14.57 ± 2.90</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.30 ± 0.35</td>
<td>8.75 ± 4.32</td>
<td>8.93 ± 1.59</td>
</tr>
<tr>
<td>Tapioca</td>
<td>4.01 ± 0.62</td>
<td>10.65 ± 2.62</td>
<td>33.76 ± 7.67</td>
</tr>
<tr>
<td>Maize</td>
<td>5.41 ± 2.21</td>
<td>11.59 ± 5.75</td>
<td>28.22 ± 11.64</td>
</tr>
<tr>
<td>Agar</td>
<td>2.92 ± 1.00</td>
<td>18.49 ± 5.10</td>
<td>43.20 ± 7.48</td>
</tr>
<tr>
<td>Wheat</td>
<td>12.60 ± 3.37</td>
<td>20.44 ± 0.98</td>
<td>40.62 ± 10.55</td>
</tr>
<tr>
<td>Formulated diet</td>
<td>10.58 ± 1.31</td>
<td>31.44 ± 3.48</td>
<td>30.43 ± 11.38</td>
</tr>
<tr>
<td>Mussel gonad</td>
<td>11.58 ± 1.20</td>
<td>42.73 ± 10.60</td>
<td>72.64 ± 25.90</td>
</tr>
<tr>
<td>Glycogen</td>
<td>27.27 ± 1.52</td>
<td>63.16 ± 13.42</td>
<td>77.10 ± 25.54</td>
</tr>
<tr>
<td>CMC</td>
<td>29.20 ± 0.88</td>
<td>54.54 ± 4.20</td>
<td>94.96 ± 8.42</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>32.32 ± 3.66</td>
<td>57.41 ± 6.36</td>
<td>95.42 ± 27.71</td>
</tr>
<tr>
<td>BO11C</td>
<td>29.12 ± 4.14</td>
<td>60.37 ± 11.98</td>
<td>107.09 ± 47.34</td>
</tr>
<tr>
<td>Dextrin</td>
<td>34.07 ± 6.35</td>
<td>64.98 ± 21.54</td>
<td>92.31 ± 50.35</td>
</tr>
<tr>
<td>Potato gelatinised</td>
<td>32.92 ± 1.75</td>
<td>78.84 ± 0.77</td>
<td>94.40 ± 3.37</td>
</tr>
<tr>
<td>Tapioca gelatinised</td>
<td>36.25 ± 5.71</td>
<td>80.61 ± 4.51</td>
<td>131.36 ± 21.15</td>
</tr>
</tbody>
</table>

1 DG / FG ratio was calculated as: mean glucose produced from DG extracts (37 °C) / mean glucose produced from FG extracts (37 °C) × 100
6.3.2. *In vivo* haemolymph glucose concentrations

Feeding each of the semi-purified diets (i.e., including the control diet), fresh mussel gonads and the dry formulated diet tended to elicit a rise in haemolymph glucose concentration compared to the unfed controls (Fig. 6.1.). Unfed lobsters did not exhibit a significant change in haemolymph glucose concentration over the 30 h period of sampling (repeated measures ANOVA, $df=5.55, F=1.84, NS$) (Fig. 6.1.). Repeated measures ANOVA of all 17 post-prandial glucose fluxes indicated significant effects on haemolymph glucose concentration of diet ($df=16, 71, F=47.53, P<0.001$), time ($df=5.355, F=28.82, P<0.001$) and the interaction (diet × time) ($df=80.355, F=11.22, P<0.001$). The fresh mussel gonad, the dry formulated diet, and the semi-purified diets containing 27% sucrose, native wheat starch, BO11C, dextrin and mussel glycogen resulted in significant increases in haemolymph glucose concentration over the entire 30 h sampling period (repeated measures ANOVA, $P<0.05$). These diets also resulted in significantly higher haemolymph glucose peaks than the unfed lobsters and lobsters fed the control diet (one-way ANOVA, $df=16,71, F=29.56, P<0.001$) (Fig. 6.1. and Table 6.4.). The fresh mussel gonad and sucrose diets were the only diets that resulted in a marked decrease in haemolymph glucose concentration after 12 h post-prandial (Fig. 6.1.). Increasing the inclusion level of BO11C had a significant positive effect on the duration of the glycaemic response and the extent of the glucose peak (i.e., control diet with 0% BO11C: 7 h response, peak = $1.46 \pm 0.32 \text{ mmol l}^{-1}$ at 2 h post-prandial; 7% BO11C: 12 h, $1.87 \pm 0.06 \text{ mmol l}^{-1}$ at 7 h; 27% BO11C: >30 h, $6.97 \pm 0.70 \text{ mmol l}^{-1}$ at 24 h) (Table 6.4.). Distinctive haemolymph glucose fluxes were obtained with the semi-purified diets containing native tapioca (peak = $2.14 \pm 0.56 \text{ mmol l}^{-1}$ at 24 h), maize (3.04 ± 0.81 mmol l$^{-1}$ at 30 h) and wheat starch (5.52 ± 1.19 mmol l$^{-1}$ at 24 h) starch which all had relatively low haemolymph glucose early on in digestion and later post-prandial ($\geq 24$ h) peaks (Fig. 6.1.). The plant source of starch affected haemolymph glucose peaks in agreement with the relative order of hydrolysis rate (RHR) observed in the *in vitro* assays for the different native starch (i.e., potato < tapioca < maize < wheat) (Tables 6.3. and 6.4.). However, native wheat starch appeared to be better digested *in vivo* (Table 6.4. and Fig. 6.1.) than *in vitro* (Table 6.3.) when compared to other carbohydrates such as BO11C, dextrin and mussel glycogen which performed equally well in both experiments. Relatively better *in vivo* results compared to their *in vitro* hydrolysis were also observed for the semi-purified diet containing sucrose, the fresh mussel gonad and dry formulated diets. In contrast, feeding CMC resulted in haemolymph
glucose concentrations that were significantly lower than expected from the high RHR of this carbohydrate source (Table 6.3.).

Table 6.4. Timing and extent of post-prandial peaks for *in vivo* haemolymph glucose in *Jasus edwardsii* juveniles left unfed, or fed (i.e., 1% BW in dry matter) fresh mussel gonads, the dry formulated diet, or one of 14 semi-purified diets containing different carbohydrate sources and inclusion levels (i.e., BO11C at 7% or 27% dry weight). Significant differences between mean peak glucose concentration for the different diets are marked by different letters within the column (*P*<0.05).

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Mean peak [glucose] ± S.E. (mmol l⁻¹)</th>
<th>Time of peak (h)</th>
<th>Duration of glycaemic response (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfed¹</td>
<td>0.75 ± 0.10ᵃ</td>
<td>7</td>
<td>n.a.</td>
</tr>
<tr>
<td>Control²</td>
<td>1.46 ± 0.32ᵃᵇ</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>7% Glucose³</td>
<td>1.66 ± 0.21ᵃᵇ</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>7% BO11C²</td>
<td>1.87 ± 0.06ᵃᵇ</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Trehalose²</td>
<td>1.13 ± 0.08ᵃᵇ</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Agar³</td>
<td>1.28 ± 0.11ᵃᵇ</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>CMC²</td>
<td>1.60 ± 0.19ᵃᵇ</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Sucrose²</td>
<td>2.74 ± 0.43ᵇᶜ</td>
<td>7</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Potato²</td>
<td>1.16 ± 0.11ᵃᵇ</td>
<td>4</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Tapioca²</td>
<td>2.14 ± 0.56ᵃᵇ</td>
<td>24</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Maize²</td>
<td>3.04 ± 0.81ᵇᶜ</td>
<td>30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Wheat³</td>
<td>5.52 ± 1.19ᵃᵈ</td>
<td>24</td>
<td>&gt;30</td>
</tr>
<tr>
<td>BO11C²</td>
<td>6.97 ± 0.70ᵃᵈ</td>
<td>24</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Dextrin²</td>
<td>6.13 ± 0.66ᵃᵈ</td>
<td>24</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Glycogen³</td>
<td>6.61 ± 0.87ᵃᵈ</td>
<td>24</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Mussel gonad²</td>
<td>6.13 ± 0.49ᵃᵈ</td>
<td>12</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Formulated diet²</td>
<td>8.88 ± 0.71ᵃᵈ</td>
<td>12</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

¹ *n*=12; ² *n*=5; ³ *n*=4
Fig. 6.1. *In vivo* haemolymph glucose fluxes in *Jasus edwardsii* juveniles left unfed, or fed (i.e., 1% BW in dry matter) fresh mussel gonads, the dry formulated diet, or one of 14 semi-purified diets containing different carbohydrate sources and inclusion levels (i.e., BO11C at 7 or 27% dry weight). Significant differences between mean glucose concentrations over the entire sampling duration for the different diets are marked by different letters (*P*<0.05). Note: y-axes (haemolymph glucose concentrations) have varying scale.
6.4. Discussion

Results from the in vitro carbohydrate hydrolysis experiment using foregut and digestive gland extracts from cultured juvenile J. edwardsii are comparable for the most part with previous investigations in crustaceans such as the European lobster H. gammarus (Glass & Stark, 1995), the European green crab Carcinus maenas (Kristensen, 1972), and the shrimps Crangon crangon (Kristensen, 1972), Litopenaeus vannamei (Cousin et al., 1996), Fenneropenaeus indicus (Omondi & Stark, 1996), and Penaeus monodon (Wigglesworth & Griffith, 1994). The liberation of D-glucose from all the carbohydrate sources tested in this study indicates a widely diverse carbohydrase profile in cultured juvenile J. edwardsii which concords with enzymatic activities determined in wild-caught juveniles (Johnston, 2003). The stronger hydrolysing ability of foregut extracts compared to digestive gland extracts suggests that the foregut is the major site of carbohydrate digestion in J. edwardsii, as in H. gammarus (Glass & Stark, 1995). Disaccharides such as sucrose and trehalose tended to be equally hydrolysed by the two organ extracts. This is consistent with the fact that the later stages of digestion of shorter oligosaccharides occur in the digestive gland prior to absorption (Glass & Stark, 1995). The poor hydrolysis of these disaccharides in vitro suggests that their use as an energy source might be limited in J. edwardsii, a finding in contradiction with work on the Indian prawn, F. indicus (Omondi & Stark, 1996) and the giant tiger prawn, P. monodon (Pascual et al., 1983; Alava & Pascual, 1987; Wigglesworth & Griffith, 1994). However, lobsters fed the semi-purified diet containing sucrose displayed a significant increase in haemolymph glucose concentration. Whether exogenous sucrase activity due to bacteria present within the gut of J. edwardsii resulted in the breakdown of sucrose from the diet but not from the raw substrate in vitro is questionable. Trehalose was poorly digestible in both experiments.

Soluble storage polysaccharides (i.e., gelatinised starch, dextrin, glycogen) but also the structural polysaccharide CMC were the most digestible sources in vitro. Hydrolysis of CMC was also observed in the European lobster, H. gammarus (Glass & Stark, 1995) and in the mud crab, Scylla serrata (i.e., lower than in this study, 24 nmol min$^{-1}$ mg$^{-1}$ at 55 °C) (Pavasovic et al., 2004) but not in the prawns F. indicus (Omondi & Stark, 1996) and P. monodon (Wigglesworth & Griffith, 1994). The apparent cellulase activity in J. edwardsii and some decapod species may indicate the presence of a β-glucosidase with broad specificity that
facilitates the digestion of carbohydrates like laminarin (i.e., storage polysaccharide of brown algae) (Dall & Moriarty, 1983). The structural algal polysaccharide agar was also hydrolysed to some extent providing further evidence for the potential role of algae as an energy source for *J. edwardsii* (Radford et al., 2005). Carbohydrates such as agar, alginate and CMC are commonly used as binders in experimental diets for Crustacea (Volpe et al., 2008). Previous nutritional studies on *J. edwardsii* have used alginate-based binders in experimental diets (Johnston et al., 2003; Ward et al., 2003) but results from this study indicate a better digestibility of CMC compared to agar and alginate. Therefore, CMC may be a more appropriate binder for future experimental diet development for *J. edwardsii* to provide digestible caloric intake while also improving the overall digestibility of the diets through the breakdown of the binder.

Gelatinisation of starch markedly improved its rate of hydrolysis *in vitro*, which suggests that pre-treatment of the dietary starch source would influence the digestibility of diets for *J. edwardsii*. Cousin et al. (1996) showed that the rate of hydrolysis of starch *in vitro* using enzyme homogenates from the whiteleg shrimp, *L. vannamei*, correlated well with apparent starch digestibility. Heat treatment of starch granules improves their water solubility which maximises their susceptibility to amylolytic degradation and improves their potential nutritional quality (Davis & Arnold, 1993; Glass & Stark, 1995; Cousin et al., 1996). However, the plant source of starch remains important regardless of the pre-treatment (Cousin et al., 1996). Native wheat starch was the best digested starch among the various plant sources of starch tested in this study as has previously been found for fish (Stone, 2003) and some other crustaceans, such as *H. gammarus* (Glass & Stark, 1995) and *L. vannamei* (Davis & Arnold, 1993; Cousin et al., 1996). The better nutritional quality of wheat starch arises from its naturally higher amyllopectin content (>80%) compared to other plant sources (Cruz-Suárez et al., 1994; Cuzon et al., 2000). Early studies used native corn starch as a source of carbohydrate in experimental diets for lobster despite it being poorly digested (62%) (Capuzzo & Lancaster, 1979; Boghen et al., 1982; Bordner et al., 1983; Koshio et al., 1992). In agreement with this study, wheat flour was found recently to have a better apparent digestibility (82.3%) in large *Panulirus ornatus* lobsters (Irvin & Williams, 2007) and has been used successfully in the best-performing experimental diets for *P. ornatus* (Williams, 2007). Glycogen from the mussel *P. canaliculus* in its native or purified form was well digested which is consistent with fresh mussel being an important natural food source for *J.*
edwardsii in New Zealand and an excellent aquaculture diet (Simon & James, 2007; Simon & Jeffs, 2008). Structurally, glycogen is very similar to amylopectin with alpha acetal linkages, but with more branching and more glucose units present than in amylopectin (Garrett & Grisham, 1999). Dextrins, despite being “pre-digested” oligosaccharides, did not display faster hydrolysis rates compared to the more complex gelatinised starches or glycogen. The same phenomenon was observed in vivo with these carbohydrate sources producing similar post-prandial fluxes of haemolymph glucose. This would suggest that the later stage of carbohydrate digestion (i.e., oligosaccharide to D-glucose) is rate limiting, which is consistent with the much higher activity of α-amylase compared to α,β-glucosidase in J. edwardsii juveniles (Johnston, 2003; Simon, 2009b).

In vivo haemolymph glucose fluxes obtained after feeding the diets in this study were more pronounced (i.e., longer glycaemic response and higher glucose concentrations for digestible carbohydrate sources) than in a previous study in juvenile J. edwardsii by Radford et al. (2005). They found that juvenile spiny lobsters fed diets containing disaccharides, glycogen, alginate or agar, had haemolymph glucose concentrations that peaked at 0.9-1.3 mmol l⁻¹ and returned to baseline level 12-24 h postfeeding (Radford et al., 2005). Common starches were not tested and their effect on the post-prandial glycaemic response was unclear until the present study. It is unlikely that the disparities in lobster sizes (i.e., 15-40 g c.f. 60 g in this study) and water temperatures (i.e., 13 °C c.f. 18 °C in this study) would have resulted in the differences observed. Unfed lobsters from the two studies had similar haemolymph glucose concentrations (i.e., 0.6 mmol l⁻¹). The discrepancies in the results are more likely due to the type of diets used. Radford et al. (2005) developed jelly diets using either gelatine or algal carbohydrates (i.e., agar, alginate and carrageenan) as the gelling agents. As these algal polysaccharides have different gel strengths, they were dissolved in differing quantities to achieve consistent diet firmness. The high moisture content of these diets (i.e., 91-96%) clearly resulted in lower and varying levels of carbohydrate consumption (i.e., 0.11-0.14% BW) before haemolymph sampling compared to this study which provided consistent substrate levels (i.e., 0.27% BW). The diets developed in the present study were also attractive and readily ingested within 2h, which facilitated the delivery of a constant amount of dry matter (1% BW). The present results indicate the importance of digestible carbohydrate inclusion level on post-prandial haemolymph glucose fluxes, a factor largely overlooked by Radford et al. (2005). Differences in protein quality and inclusion level, as found between the
two studies, are also known to affect the time course of haemolymph glucose appearance resulting from gluconeogenesis of the amino acids released by protein digestion (Cruz-Ricque et al., 1989; Shiau & Peng, 1992).

Consumption of the dry formulated diet, glycogen, dextrin, gelatinised maize starch and native wheat starch diets resulted in higher glucose concentrations (>5 mmol l\(^{-1}\)) and longer hyperglycaemic responses (>24 h) in juvenile \(J. \text{ edwardsii}\) than in previous studies with the Kuruma prawn, \(M. \text{ japonicus}\) (<0.61 mmol l\(^{-1}\); 12-24 h) (Abdel-Rahman et al., 1979), the giant tiger prawn, \(P. \text{ monodon}\) (<4.2 mmol l\(^{-1}\); 10 h) (Shiau & Peng, 1992), and the whiteleg shrimp, \(L. \text{ vannamei}\) (<4.1 mmol l\(^{-1}\); 6-7 h) (Rosas et al., 2000). These findings indicate a poor regulation of glucose homeostasis when fed diets rich in digestible carbohydrate (Cuzon et al., 2000) but are also consistent with the long digestive period and gut throughput time (>34 h) found in \(J. \text{ edwardsii}\) at 18 °C (Simon & Jeffs, 2008). There may be a possible association between haemolymph glucose fluxes and appetite revival (Even & Nicolaidis, 1986). Appetite revival in 42 g juvenile lobsters fed the dry formulated diet and mussel flesh was 24 h and 12 h, respectively (Simon & Jeffs, 2008), which is similar to the duration of hyperglycaemia on these diets in this present study. It is yet to be determined whether appetite revival could be enhanced by reducing either the digestible carbohydrate level or the hyperglycaemic period. A decrease in feed intake with increasing level of dietary starch has previously been observed in the shrimps \(L. \text{ stylirostris}\) (Rosas et al., 2000) and \(L. \text{ vannamei}\) (Guo et al., 2006).

Glycogen from \(P. \text{ canaliculus}\) when purified and included in a semi-purified diet, as opposed to ingested directly from mussel gonads, resulted in a longer hyperglycaemic response. The two diets had similar glycogen content (i.e., 27% dry weight) so it is unlikely that differences in ingested glycogen quantities could explain such trend. In fresh food, the glycogen is trapped in cellular structures which may slow down its breakdown and lengthen the period over which it is digested. This could result in a more constant flow of glucose into the lobster haemolymph which would be better absorbed and utilised by the digestive gland (Cuzon et al., 2000). However, the haemolymph glucose concentration rises somewhat faster within the first 12 h after feeding on fresh mussel gonads than on the semi-purified mussel glycogen diet. Therefore, it is more likely that the trend reflects a better utilisation or regulation of haemolymph glucose when fed fresh mussel gonads rather than a difference in
glycogen breakdown between diets. Protein synthesis is known to be an energy-consuming process representing as much as 80% of the total post-prandial energy expenditure (Houlihan et al., 1995; Thor, 2000). However, if the amino acid profile of the dietary protein is suboptimal, which is the case when casein is fed to crustaceans, protein synthesis will be reduced and amino acids broken down for energy (Mente et al., 2002). Differences in the energy requirements during post-prandial protein metabolism may be at the origin of the differences in haemolymph glucose concentrations obtained when experimental lobsters were fed on fresh mussel (good protein source) and the casein-based glycogen diet (poor protein source). This hypothesis could be tested by measuring oxygen consumption after feeding on the two diets (SDA) and using a protein synthesis inhibitor such as cycloheximide to directly determine the aerobic cost of protein synthesis (Houlihan et al., 1995; Thor, 2000). The differences in haemolymph glucose concentrations could also originate from differences in regulation mediated by the crustacean hyperglycaemic hormone (CHH) produced by the sinus gland/X organ located in the eyestalks (Santos & Keller, 1993). Whether differing expression rates of the CHH between the fresh mussel and formulated diets could relate to the differences in hyperglycaemic responses needs to be investigated. Ingestion of fresh mussel may inhibit CHH expression and increase uptake of glucose in the muscles and glycogen synthesis (Santos & Keller, 1993). In lobsters, the CHH has also a physiological relevant role in inhibiting ecdysteroid synthesis and moulting, acting as a functional moult inhibiting hormone (MIH) (Chung & Webster, 2003). Further investigation into the endocrinology of cultured spiny lobsters, possibly in relation to carbohydrate and glucose metabolism, may provide useful insights into the reasons why formulated diets perform poorly in comparison to fresh food such as mussels (Crear et al., 2000; Glencross et al., 2001; Ward et al., 2003; Simon & James, 2007).

The timing of glucose appearance in the haemolymph has been suggested to affect glucose utilisation for growth (Abdel-Rahman et al., 1979; Cuzon et al., 2000). Even though glucose availability was generally improved by gelatinising starch in the current study, previous studies in the blue shrimp, *L. stylirostris*, have shown that pre-cooked starch did not perform as well as native wheat starch in sparing protein for growth possibly because glucose appeared earlier in the haemolymph (Cuzon et al., 2000). Metabolic problems associated with a rapid rise of glucose in haemolymph, such as a reduction of amino acid absorption (Alvarado & Robinson, 1979) or a saturation of hexokinase by substrate have been suggested.
(Cuzon et al., 2000). In this regard, the results of the current study suggest that using native starch in diets for *J. edwardsii*, particularly wheat starch which appears to be the most digestible among the several plant sources tested, may have a beneficial effect on the utilisation of glucose for growth. In contrast, the dry formulated diet led to a rapid rise of haemolymph glucose to high concentrations, which may have had detrimental effect on growth and consumption (Simon & James, 2007; Simon & Jeffs, 2008).

The current study indicates that replacing glycogen by cheaper carbohydrate alternatives such as dextrin and gelatinised maize starch does not affect digestibility *in vitro* and the rate and extent of haemolymph glucose appearance in *J. edwardsii*. Soluble starch, dextrin and glycogen when included at 19.5% in semi-purified diets resulted in equivalent hepatopancreatic glycogen levels and growth in the Kuruma prawn, *M. japonicus* (Abdel-Rahman et al., 1979). A similar finding might be expected for *J. edwardsii* juveniles, but the lengthy post-prandial hyperglycaemic responses (>30 h) possibly suggest that these carbohydrate sources are poorly utilised when incorporated in the semi-purified diets of the current study. Using lower inclusion levels of these carbohydrates or incorporating digestible carbohydrate sources delivering slower appearance in haemolymph glucose (i.e., native wheat starch, CMC) into commercial diets may have the potential to improve glucose utilisation for growth in *J. edwardsii*. In a growth trial, a greater number of moulting events was achieved by feeding lobsters the semi-purified diets containing 7% BO11C and 27% native wheat compared to 27% BO11C (unpublished data). Results from the current study suggest that the increase in the degree of gelatinisation of starch during commercial diet manufacturing (Thomas et al., 1998b) is expected to have a positive effect on digestibility. However, the associated effect on haemolymph glucose concentration may preclude the use of a large amount of starch (i.e., 27%) in favour of CMC which would not be affected by the heat treatment and is well digested in *J. edwardsii*. Further research is required to better understand the effect of haemolymph glucose fluxes on consumption, appetite revival, and the utilisation of glucose for metabolic energy in *J. edwardsii* juveniles. A priority is the need to verify the digestibility estimates obtained in the present study. This was performed in the subsequent chapter by measuring the apparent digestibility of selected carbohydrate sources in practical formulated diets. These latter diets were based on fishmeal, instead of sodium caseinate, to ensure the apparent digestibility estimates would be obtained in association with a better, more practical protein source, for direct application of the results to future commercial diets (see Simon, 2009d).
Plate 7. Apparent digestibility of formulated diets in large *Jasus edwardsii* juveniles. A) Experimental system used to house large juvenile lobster individually; B) A semi-moist formulated diet bound with gelatine and vacuum packed; C) Fixing of the plastic ring (left) around the anal pore of a large (115 g) lobster juvenile; in tank with a collection device attached (right); D) Faecal collection device; E) Chromic oxide determination using diphenyl carbazide; F) Elemental analyser (left) used to measure nitrogen and carbon content in small quantities (<1.5 mg DM) of diets and faeces (right).
7. Chapter seven – Apparent digestibility of different carbohydrates, binders and fishmeal particle sizes in formulated diets

Published as:


7.1. Introduction

In a previous study, the digestibility of several carbohydrate sources for juveniles of the spiny lobster *Jasus edwardsii* have been estimated by measuring their rates of hydrolysis *in vitro* using enzyme homogenates and post-prandial haemolymph glucose concentrations following ingestion of semi-purified diets (Simon, 2009c). These techniques offered an inexpensive and efficient means of testing a wide range of carbohydrate sources, but determining the *in vivo* apparent digestibility of selected carbohydrate sources remains necessary to ascertain their usefulness for incorporation into commercial formulated diets (Cousin et al., 1996; Omondi & Stark, 1996). Carbohydrate digestibility is affected by a range of intrinsic (i.e., level of inclusion and extent of processing) and extrinsic factors (i.e., associative effect with other feedstuffs, changes in physical and biochemical conditions in the gastrointestinal track and in the throughput rate of the digesta), which often cannot be replicated accurately by *in vitro* assays (Weurding et al., 2001).

Measuring the apparent digestibility of several carbohydrate sources and inclusion levels in spiny lobsters is needed to better understand their bioavailability when incorporated in formulated diets and their potential influence on the digestibility of other major macro-ingredients such as protein. In particular, algal carbohydrates have been used as binders (up to
6% by dry weight) in the manufacture of experimental diets for spiny lobsters (Glencross et al., 2001; Johnston et al., 2003; Smith et al., 2003; Ward et al., 2003; Radford et al., 2007), but their effect on diet digestibility has been largely overlooked. The effect of particle size on diet digestibility is another aspect that needs further research. Finer grinding of dietary ingredients has been shown to improve digestibility in shrimps (Obaldo et al., 1998). For *J. edwardsii* juveniles, it has been suggested that the size of dietary particles may create difficulties for processing and digesting the dry formulated diet (Simon & Jeffs, 2008).

Apparent digestibility experiments in crustaceans can be prone to errors (Johnston et al., 2003) due to the methods employed to collect faeces (i.e., settlement, filtration) which result in leaching losses (Irvin & Tabrett, 2005). The accurate determination of apparent digestibility relies on the collection of faecal samples with composition reflective of the post-absorption digesta (Irvin & Tabrett, 2005). For this reason a faecal collection device modified from Irvin and Tabrett (2005) was used in the present study to collect uncontaminated faecal samples in large (115 g) juvenile *J. edwardsii* spiny lobster.

The aim of this study is to assess the effects of; 1) carbohydrate source, 2) carbohydrate inclusion level, 3) binder type, and 4) fishmeal particle size on the apparent digestibility (i.e., dry matter, total starch, nitrogen and carbon) of formulated diets for *J. edwardsii* juveniles

### 7.2. Materials and methods

#### 7.2.1. Experimental dietary treatments

A total of 12 semi-moist diets (Table 7.1.) were formulated to produce the various dietary treatments required. In addition to these experimental diets, the digestibility of the baseline dry formulated diet (Tables 2.1. and 3.1.) and the casein-based semi-purified diet containing 27% BO11C (Table 6.2) was also tested for comparison.
7.2.1.1. Carbohydrate source

Six carbohydrate sources were incorporated at 35% by dry weight (i.e., dextrin, carboxymethyl cellulose (CMC), pre-gelatinised maize starch (BO11C), and native starch from wheat, potato and maize). One control diet was produced by substituting the supplemented carbohydrates by an inert filler of diatomaceous earth (ash).

7.2.1.2. Carbohydrate inclusion level

In addition to the 35% BO11C diet, two diets were produced with 15% and 55% BO11C by dry weight at the expense of fishmeal (62% and 22% respectively).

7.2.1.3. Binder type

All diets contained 8% gelatine by dry weight as binder except for two additional diets composed of alginate (7% by dry weight + 1% sodium hexametaphosphate) and agar (8% by dry weight). These diets had an intended composition similar to the 15% BO11C diet for direct comparison of the effect of binder on digestibility.

7.2.1.4. Ingredient particle size

The 15% BOC11 diet containing 62% fishmeal was produced in duplicate with two fishmeal particle size distributions (<500 and <106 μm). The fishmeal used in this study was firstly sieved through a 500 μm screen for all diets (Smith & Tabrett, 2004), and further through a 106 μm screen for the duplicate 15% BOC11 diet. The original particle size distribution of the fishmeal was 30% <106 μm, 62% at 106-500 μm and 8% >500 μm. Proximate analyses of the two 15% BO11C diets (<500 and <106 μm) showed no difference in protein content (54.6% and 54.2% respectively) and only a small decrease in ash content with finer sieving (19.7% and 17.2% respectively).
7.2.2. Diet preparation

Chromic oxide ($\text{Cr}_2\text{O}_3$) was included in all diets at 0.3% of dry weight to serve as an inert marker for apparent digestibility calculation (Table 7.1.), except for the baseline dry formulated diet for which the digestibility was calculated using the ash content (Section 7.2.6.). For the 12 experimental semi-moist formulated diets, all dry ingredients except the binders were blended vigorously in closed plastic bags for 20 min until a homogenous green taint was observed throughout to ensure the complete mixing of the chromic oxide marker (Smith & Tabrett, 2004). The dry powder was then blended to the fish oil and a 1:1 liquid mixture of fresh mussel liquor (i.e., liquid collected from shucked fresh *Perna canaliculus* mussels) and fish hydrolysate to serve as diet attractants. The binders were dissolved in a minimum amount of boiling deionised water and added to the rest of the mixture, with further hot water (60 °C) used in varying proportion depending on the type of carbohydrate and binder to reach a similar dough consistency. The dough was kept below 60 °C to prevent starch gelatinisation (Cousin et al., 1996). Gelatine diets, as well as the agar diet, were allowed to set overnight in flat trays at 4 °C. The alginate diet was first allowed to set in a water bath of 10% calcium chloride for 10 min before being kept refrigerated overnight. Semi-moist diets were then stored frozen at –20 °C under vacuum (Foodsaver® Vac 550, Sunbeam), being cut into 1 cm$^3$ cubes and thawed just prior feeding.
Table 7.1. Ingredient composition and proximate composition of the 12 semi-moist experimental diets. Values are based on dry weight. Refer to Tables 2.1. and 3.1. for the composition of the baseline dry formulated diet and Table 6.2. for the composition of the semi-purified diet used in the present study.

<table>
<thead>
<tr>
<th>Ingredients (g kg(^{-1}) of diet)</th>
<th>Control</th>
<th>Carbohydrates</th>
<th>55% BO11C</th>
<th>15% BO11C</th>
<th>Alginate diet</th>
<th>Agar diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate supplement(^1)</td>
<td>0</td>
<td>350</td>
<td>550</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Diatomaceous earth</td>
<td>350</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fishmeal(^2)</td>
<td>420</td>
<td>420</td>
<td>220</td>
<td>620</td>
<td>620</td>
<td>620</td>
</tr>
<tr>
<td>Fish hydrolysate(^3)</td>
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<td>30</td>
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<td>30</td>
</tr>
<tr>
<td>Gelatine(^4)</td>
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<td>80</td>
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</tr>
<tr>
<td>Alginate(^5)</td>
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<td>0</td>
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<td>0</td>
<td>80</td>
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</tr>
<tr>
<td>Agar-agar(^6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Fish oil</td>
<td>51</td>
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<td>51</td>
<td>51</td>
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</tr>
<tr>
<td>Soy bean lecithin</td>
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<td>15</td>
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</tr>
<tr>
<td>Cholesterol</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mix(^7)</td>
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<td>20</td>
<td>20</td>
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<td>20</td>
</tr>
<tr>
<td>Chromic oxide(^8)</td>
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<td>3</td>
<td>3</td>
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<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Other dried ingredients(^9)</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Proximate composition (%)(^{10})</th>
<th>&lt;500 μm</th>
<th>&lt;106</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>37.1</td>
<td>38.9 ± 0.7</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.2</td>
<td>32.2 ± 1.1</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ash</td>
<td>46.9</td>
<td>15.4 ± 0.8</td>
</tr>
<tr>
<td>Moisture</td>
<td>38.2</td>
<td>32.9 ± 2.1</td>
</tr>
</tbody>
</table>

\(^1\) Carbohydrates included at 350 g kg\(^{-1}\) in diets were: dextrin, carboxymethyl cellulose (CMC), gelatinised maize starch (BO11C), native wheat, native potato and native maize starches (see Table 6.1. for source). BO11C was also included at 550 g kg\(^{-1}\), and 150 g kg\(^{-1}\) in four other diets (i.e., gelatine with two fishmeal particle size distributions, alginate and agar).

\(^2\) Hoki white fishmeal (69.2% CP, 6.6% lipid, 20.1% ash, 5.7% moisture), Talleys Fisheries Ltd, New Zealand

\(^3\) Liquid fish hydrolysate (80% moisture) prepared from Hoki fishmeal by bacterial fermentation, Independent Fisheries Ltd., Christchurch, New Zealand

\(^4\) Protanal® LF 5/60 Alginate (see Table 6.1. for source). Final amount included 10 g kg\(^{-1}\) sodium hexametaphosphate.

\(^5\) Cero® Agar Agar powder type 8925 (see Table 6.1. for source)

\(^6\) Refer to Simon (2009c) for vitamin mix composition

\(^7\) Chromic oxide Cr\(_2\)O\(_3\), Sigma-Aldrich, Cat. No. 393703, U.S.A.

\(^8\) Others ingredients (g kg\(^{-1}\) diet): vitamin C-coated, 10; betaine, 7; Naturose (astaxanthin 1.5%), 5; vitamin E, 2; choline chloride, 2; ethoxyquin, 0.5.

\(^9\) Proximate compositions measured as per section 7.2.5. Crude lipid calculated from proximate composition of ingredients. Other diets: mean ± S.E. (n=6).
7.2.3. Experimental design and feeding procedure

Large intermoult *J. edwardsii* juveniles (mean ± S.D. 115.4 ± 11.4 g, *n*=40) cultured on a diet of fresh *P. canaliculus* mussels (fed three times weekly) were used for the experiment. Lobsters were held individually in 27 l black polyethylene tanks identical to those used by Simon and James (2007) which received partially recirculated filtered seawater (70 µm) at a constant flow-rate of 2.3 l min⁻¹ and at a temperature of 18 ºC. Photoperiod was maintained at L8:D16 with a light intensity at the water surface of 2-4 µmol photons s⁻¹ m⁻². High water quality was maintained throughout the experiment (i.e., dissolved oxygen, >95% saturation; pH, 7.80-8.10; ammonia, <0.05 mg l⁻¹; nitrite, <0.01 mg l⁻¹; nitrate, <0.2 mg l⁻¹). No structures were provided in the tanks to allow easy handling and feeding of lobsters. Lobsters were acclimated to the experimental conditions for three weeks before the start of the faecal collection. Lobsters received one satiation ration per day (i.e., 1% BW day⁻¹) of their respective diets (*n*=3 lobsters per diet) at 14:00 h. After allowing the lobsters to feed for 1 h, all uneaten food was siphoned carefully. Lobsters that ingested at least half of their allotted ration were selected for faecal collection. Samples of diets that had been immersed for 1 h were kept for chemical analyses and digestibility determination.

7.2.4. Faecal collection procedure

A modified faecal collection device was used to collect uncontaminated faecal samples in the present study (Irvin & Williams, 2007). Caps of 2 ml Eppendorf® tubes were drilled through the middle leaving a ring of plastic with a raised flange that was then fixed to the flexible integument around the anal pore of the lobster with cyanoacrylate glue (Loctite® 406) (Irvin & Tabrett, 2005). The entire process was quick to perform and non-invasive as the attached ring would be discarded with the exoskeleton at the next moult. Modified 1.5 ml screw-cap storage tubes (Axygen® Scientific, Cat. No. SCT-150-SS-A) were then attached to the plastic caps as faecal collection devices (25 mm length × 10 mm outer diameter, screw on and off). Faeces produced overnight accumulated in the device without leaching and were collected at 10:00 h. The 20 h post-prandial collection period ensured most of the indigestible material was collected (>75%) after each daily feeding event (Simon & Jeffs, 2008). Collecting the entire faecal production was not necessary because feed digestibility was
measured using the chromic oxide marker method (Lee & Lawrence, 1997). The faecal material was then carefully removed from the tubes and stored at –20 °C. Successive faecal samples collected from the same lobster were combined over several days and lobsters were then re-allocated randomly to a new diet and new faecal samples collected after a recovery period (i.e., one week on fresh mussel, two weeks on the new diet). No lobster received the same diet more than once. This collection protocol ensured each faecal sample (n=3 per diet) was the combined faecal output of two lobsters to minimise individual variation (Lee & Lawrence, 1997) and that sufficient material was collected for the analyses. Overall, the experiment lasted three months (i.e., >100 g faecal wet weight collected in total). To maintain the integrity of the faecal sample, any collection contaminated with water was discarded. If a lobster moulted or the Eppendorf® cap was dislodged, a new cap would be fitted during intermoult and the collection continued as before.

### 7.2.5. Composition analyses of faeces and diets

Samples of the original diets (n=1 per diet), diets that had been immersed for 1 h (n=1 per diet), and the corresponding faeces collected (n=3 per diet) were freeze-dried and finely ground using a mortar and pestle. Per cent dry matter was analysed by weight change following drying at 105 °C to a constant weight (AOAC 930.15), ash by weight change following furnace incineration in crucibles at 550 °C for 4 h (AOAC 942.05), %N and %C with a CN elemental analyser coupled to a mass spectrophotometer (DeltaPlus® Thermoquest, Finnigan) (Fernández et al., 1998). Total crude protein was calculated as %N × 6.25. Total starch (AOAC 996.11) was analysed via enzymatic digestion of samples using heat-stable amylase (EC 3.2.1.1.) and amyloglucosidase (EC 3.2.1.3) (McCleary et al., 1994), followed by D-glucose determination using an Amplex® Glucose Oxidase Assay Kit from Invitrogen New Zealand Ltd. (Cat. No. A22189) as per Simon (2009c). Chromic oxide was determined as chromium via diphenylcarbazide colorimetry (i.e., VersaMax™ microplate reader set at 540 nm) after potassium permanganate oxidation of the ashed samples (Saltzman, 1952). However, the method was modified by conducting the oxidation step on 2-4 mg of the ashed samples and increasing to 4 h boiling with 2 M potassium permanganate followed by decolourisation using 1.5 ml 5% sodium azide, in order to ensure high levels of recovery of
the chromium (>90%). Chromic oxide determination was performed in triplicate for each sample.

7.2.6. Calculation of apparent digestibility and statistical analyses

The dry matter (DM), total starch (S), nitrogen (N) and carbon (C) apparent digestibility (AD) of the diets containing chromic oxide were calculated using the equation (Cousin et al., 1996):

$$AD(\%) = 100 \times [1 - (c_D / c_F) \times (n_F / n_D)]$$

Where $c_D$ and $c_F$ are the concentrations (on a DM basis) of chromic oxide in the ingested diet and faeces respectively, and $n_D$ and $n_F$ are the concentrations (on a DM basis) of the nutrient in the ingested diet and faeces respectively.

Calculations of digestibility were performed using the mean value from both diet conditions (i.e., original and leached for 1 h) for chromic oxide and nutrient concentrations so that the ingested food would represent the average condition of the diet over the period of consumption (Table 7.2.). For the baseline dry formulated diet, which contained no chromic oxide, the ash-ratio technique was used to determine digestibility (Leavitt, 1985; Lee & Lawrence, 1997). This consisted of utilising the ash content of the food and faeces as the inert indigestible marker, replacing $c_D$ and $c_F$ in the formula above by $a_D$ and $a_F$ which are the concentrations (on a DM basis) of ash in the ingested diet and faeces respectively. This technique is based on the assumption that only the organic component of the food is significantly affected by the digestion process (Leavitt, 1985). This assumption was valid in view of the similarity in dry mater digestibility estimates obtained with this technique and previously with total gravimetric collection (Simon & Jeffs, 2008).

Significant differences ($P<0.05$) in mean $AD_{DM}$, $AD_{S}$, $AD_{N}$ and $AD_{C}$ between diets were tested by one-way ANOVAs after the raw per cent apparent digestibility data were arcsine square root transformed. When $P>0.05$, non-significant results were stated as NS. The ANOVA assumptions of normality of residuals and homogeneity of variances were satisfied.
(Shapiro-Wilk and Levene tests respectively, NS). Tukey-Kramer test was used for post hoc comparisons of means following significant ANOVA (Zar, 1999). Differences in mean concentrations of moisture, ash, starch, carbon and protein between the original diets and diets immersed for 1 h were identified using t-tests. Data were arcsine square root transformed prior to analyses. The possible relationship between BO11C inclusion level and total starch digestibility was tested by a Pearson correlation test. All analyses were performed using the Statistical & Power Analysis Software NCSS 2007 (Utah, USA).

7.3. Results

7.3.1. Leaching losses of diets after immersion

There was no significant difference in chromic oxide and nutrient concentrations (on a DM basis) between the original diets and the diets immersed for 1 h during feeding (t-tests, NS). Only the mean moisture content of all diets increased significantly post-immersion from 37% to 52% moisture in average (Table 7.2.).

Table 7.2. Changes in mean composition of all diets used in this experiment after a 1 h immersion duration corresponding to the feeding period. Significant differences between means within the same row are marked by different letters ($P<0.05$). Values are based on dry weight.

<table>
<thead>
<tr>
<th>Mean composition (%) ± S.E ($n=14$)</th>
<th>Diets not immersed</th>
<th>Diets immersed for 1 h</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromic oxide$^1$</td>
<td>0.33 ± 0.01$^a$</td>
<td>0.31 ± 0.01$^a$</td>
<td>$t=1.28$, $P=0.21$</td>
</tr>
<tr>
<td>Moisture</td>
<td>36.8 ± 3.5$^a$</td>
<td>52.4 ± 1.6$^b$</td>
<td>$t=-3.61$, $P&lt;0.01$</td>
</tr>
<tr>
<td>Ash</td>
<td>17.5 ± 2.6$^a$</td>
<td>18.6 ± 2.6$^a$</td>
<td>$t=-0.36$, $P=0.72$</td>
</tr>
<tr>
<td>Starch</td>
<td>25.5 ± 3.8$^a$</td>
<td>25.8 ± 3.9$^a$</td>
<td>$t=-0.03$, $P=0.97$</td>
</tr>
<tr>
<td>Carbon</td>
<td>42.3 ± 1.3$^a$</td>
<td>42.1 ± 1.2$^a$</td>
<td>$t=0.11$, $P=0.91$</td>
</tr>
<tr>
<td>Protein</td>
<td>41.4 ± 2.1$^a$</td>
<td>40.9 ± 2.0$^a$</td>
<td>$t=0.19$, $P=0.85$</td>
</tr>
</tbody>
</table>

$^1n=13$ as the baseline dry formulated diet did not contain the chromic oxide marker
7.3.2. Carbohydrate source

The source of carbohydrate was found to have a significant effect on the AD_{DM} (ranging 62-79\% for the six carbohydrate sources) (ANOVA, \(df=6,14, F=17.68, P<0.001\)), AD_{S} (ranging 59-99\%) (\(df=4,10, F=49.415, P<0.001\)) and AD_{C} (ranging 71-88\%) (\(df=6,14, F=10.217, P<0.001\)) of formulated diets for *J. edwardsii* juveniles (Table 7.3.). Replacing of the indigestible inert filler (control diet) by digestible carbohydrates resulted in a significant improvement of AD_{DM}, except for the native maize starch (Table 7.3.), and no difference in AD_{N} (ranging 82-88\% for all diets) (\(F=1.05, NS\)). Native starch from maize (59\%) and potato (60\%) were poorly digested compared to gelatinised starch (84\%), native wheat starch (91\%) and dextrin (99\%) (Table 7.3.). The digestibility of CMC *per se* could not be determined because the starch determination method was not applicable to this non-starch carbohydrate. However, the intermediate levels of AD_{DM} and AD_{C} of the CMC diet between those of the native wheat and dextrin diets indicated almost complete digestion (i.e., ~94\%) of this carbohydrate source.

Table 7.3. Dry matter, starch, nitrogen and carbon apparent digestibility of formulated diets containing different carbohydrate sources (i.e., 35\% dry weight) fed to *Jasus edwardsii* juveniles. Significant differences between means within the same column are marked by different letters (\(P<0.05\)).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dry matter (%)</th>
<th>Starch (%)</th>
<th>Nitrogen (%)</th>
<th>Carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>79.0 ± 2.1\textsuperscript{a}</td>
<td>99.4 ± 0.2\textsuperscript{a}</td>
<td>88.0 ± 1.2\textsuperscript{a}</td>
<td>87.9 ± 1.1\textsuperscript{a}</td>
</tr>
<tr>
<td>CMC\textsuperscript{2}</td>
<td>76.4 ± 2.2\textsuperscript{ab}</td>
<td>n/a</td>
<td>88.0 ± 1.7\textsuperscript{a}</td>
<td>85.0 ± 1.6\textsuperscript{a}</td>
</tr>
<tr>
<td>Wheat</td>
<td>73.2 ± 1.1\textsuperscript{ab}</td>
<td>91.2 ± 2.5\textsuperscript{b}</td>
<td>85.4 ± 2.1\textsuperscript{a}</td>
<td>82.8 ± 1.3\textsuperscript{ab}</td>
</tr>
<tr>
<td>BOC11</td>
<td>71.1 ± 1.2\textsuperscript{abc}</td>
<td>83.7 ± 3.1\textsuperscript{b}</td>
<td>86.8 ± 1.2\textsuperscript{a}</td>
<td>81.1 ± 1.7\textsuperscript{ab}</td>
</tr>
<tr>
<td>Potato</td>
<td>68.0 ± 3.0\textsuperscript{bc}</td>
<td>59.7 ± 3.0\textsuperscript{c}</td>
<td>88.2 ± 1.9\textsuperscript{a}</td>
<td>76.5 ± 2.5\textsuperscript{bc}</td>
</tr>
<tr>
<td>Maize</td>
<td>62.1 ± 2.6\textsuperscript{cd}</td>
<td>59.1 ± 4.2\textsuperscript{c}</td>
<td>82.0 ± 3.8\textsuperscript{a}</td>
<td>70.8 ± 2.7\textsuperscript{c}</td>
</tr>
<tr>
<td>Control\textsuperscript{2}</td>
<td>52.5 ± 2.0\textsuperscript{d}</td>
<td>n/a</td>
<td>85.3 ± 1.2\textsuperscript{a}</td>
<td>81.0 ± 0.5\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

\textsuperscript{1} All diets were produced to contain 42\% fishmeal
\textsuperscript{2} CMC and control (diatomaceous earth) diets contained no starch
7.3.3. Carbohydrate inclusion level

Increasing inclusion level of BO11C from 15% to 55% at the expense of fishmeal (decreasing from 62% to 22%) reduced significantly the AD\textsubscript{DM} (ranging 68-73%) (ANOVA, \(df=2.6, F=6.66, P<0.001\)), AD\textsubscript{S} (ranging 79-92%) (\(F=12.95, P<0.01\)) and AD\textsubscript{C} (ranging 76-84%) (\(F=10.32, P<0.05\)) of formulated diets for \textit{J. edwardsii} juveniles (Table 7.4.). The AD\textsubscript{N} (85-89%) of the diets tended to decrease with increasing level of BO11C (or reducing level of fishmeal) but this was not significant (\(F=4.49, NS\)) (Table 7.4.). There was a significant negative correlation between BO11C inclusion level and AD\textsubscript{S} (\(n=21\), Pearson’s \(r=-0.89, r^2=0.79, P<0.001\)), regardless of the other feedstuffs present in the diets (i.e., casein or fishmeal as the main protein source; gelatine, agar or alginate as binders) (Fig. 7.1.).

Table 7.4. Dry matter, starch, nitrogen and carbon apparent digestibility of formulated diets containing different inclusion levels of gelatinised starch (BO11C) fed to \textit{Jasus edwardsii} juveniles. Significant differences between means within the same column are marked by different letters (\(P<0.05\)).

<table>
<thead>
<tr>
<th>Diet(^1)</th>
<th>Dry matter (%)</th>
<th>Starch (%)</th>
<th>Nitrogen (%)</th>
<th>Carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>73.4 ± 1.1(^a)</td>
<td>92.3 ± 0.9(^a)</td>
<td>88.9 ± 0.3(^a)</td>
<td>84.3 ± 0.6(^a)</td>
</tr>
<tr>
<td>35%</td>
<td>71.1 ± 1.2(^{ab})</td>
<td>83.7 ± 3.1(^b)</td>
<td>86.8 ± 1.2(^a)</td>
<td>81.1 ± 1.7(^{ab})</td>
</tr>
<tr>
<td>55%</td>
<td>68.3 ± 0.4(^b)</td>
<td>79.2 ± 1.2(^b)</td>
<td>85.0 ± 1.1(^a)</td>
<td>75.5 ± 1.6(^b)</td>
</tr>
</tbody>
</table>

\(^{1}\)Starch inclusion was serially increased at the expense of fishmeal
Chapter 7

Fig. 7.1. Relationship between gelatinised starch (BO11C) inclusion level (%) and mean apparent starch digestibility (%) ± S.E. in formulated diets for *Jasus edwardsii* juveniles. AD<sub>S</sub>, apparent starch digestibility (%), and S, inclusion level of BO11C (%). Note: y-axis scale ranges 75-100%. Diets contained different levels of BO11C but varied in protein source, fishmeal protein particle sizes and binder types.

7.3.4. Binder type

Using gelatine instead of agar or alginate as dietary binder (7-8% inclusion level) was found to have a significant positive effect on the AD<sub>DM</sub> (ranging 61-73%) (ANOVA, df=2,6, F=14.01, P<0.01) and AD<sub>C</sub> (ranging 77-84%) (F=14.44, P<0.01) of formulated diets for *J. edwardsii* juveniles (Table 7.5.). Diets made with gelatine also tended to have higher AD<sub>N</sub> (89%) compared to the two algal carbohydrate binders (85-87%) but this was not significant (F=3.51, NS). The AD<sub>S</sub> of BO11C was not affected by the type of binder used (F=0.51, NS) (Table 7.5.).
Table 7.5. Dry matter, starch, nitrogen and carbon apparent digestibility of formulated diets with different binder types fed to *Jasus edwardsii* juveniles. Significant differences between means within the same column are marked by different letters (*P*<0.05).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dry matter (%)</th>
<th>Starch (%)</th>
<th>Nitrogen (%)</th>
<th>Carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatine</td>
<td>73.4 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.3 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alginate</td>
<td>68.1 ± 2.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>92.2 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.5 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.5 ± 1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agar</td>
<td>60.8 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.2 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Diets were produced to contain 62% fishmeal and 15% BO11C. Binders were included at either 8% for gelatine and agar or 7% for alginate (+1% sodium hexametaphosphate)

### 7.3.5. Particle size and diet type

Differences in fishmeal particle sizes (<500 µm or <106 µm) had no significant effect on the nutrient or overall digestibility of the final diets (ANOVA, *NS*) (Table 7.6.). The formulated diets developed in this study showed better AD<sub>DM</sub> than the baseline dry formulated diet used in previous research, except for those diets containing algal carbohydrates, the diatomaceous earth filler, native potato and maize starches. Low AD<sub>C</sub> (67%) and AD<sub>N</sub> (72%) of the baseline dry formulated diet accounted for the poor overall digestibility, which was unrelated to starch content given the high AD<sub>S</sub> (94%) of this diet (Table 7.6.). The semi-purified diet containing 27% BO11C showed the best AD<sub>DM</sub> (86%) and AD<sub>N</sub> (97%) of all diets (*df*=16,35, *F*=20.52; 9.42, *P*<0.001) (Table 7.6.).
Table 7.6. Dry matter, starch, nitrogen and carbon apparent digestibility of formulated diets with two different fishmeal particle size distributions (<500 µm and <106 µm) fed to *Jasus edwardsii* juveniles. The digestibility of the baseline dry formulated diet and the semi-purified diet (27% BO11C) are included for comparison. Significant differences between means within the same column are marked by different letters (*P*<0.05).

<table>
<thead>
<tr>
<th>Diet¹</th>
<th>Dry matter (%)</th>
<th>Starch (%)</th>
<th>Nitrogen (%)</th>
<th>Carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-purified diet</td>
<td>86.4 ± 1.2ᵃ</td>
<td>86.3 ± 1.7ᵃ</td>
<td>96.6 ± 0.9ᵃ</td>
<td>88.7 ± 1.4ᵃ</td>
</tr>
<tr>
<td>Dry formulated diet</td>
<td>60.1 ± 2.4ᵇ</td>
<td>94.4 ± 1.7ᵇ</td>
<td>72.4 ± 2.9ᵇ</td>
<td>66.8 ± 3.1ᵇ</td>
</tr>
<tr>
<td>Fishmeal &lt;500um</td>
<td>73.4 ± 1.1ᶜ</td>
<td>92.3 ± 0.9ᵃ</td>
<td>88.9 ± 0.3ᶜ</td>
<td>84.3 ± 0.6ᵃ</td>
</tr>
<tr>
<td>Fishmeal &lt;106um</td>
<td>73.7 ± 1.0ᶜ</td>
<td>93.4 ± 0.2ᵃ</td>
<td>86.3 ± 0.6ᶜ</td>
<td>83.1 ± 0.2ᵃ</td>
</tr>
</tbody>
</table>

¹Diets varying in fishmeal particle size distributions were prepared to contain 62% fishmeal and 15% BO11C

### 7.4. Discussion

The source and inclusion level of carbohydrates have a marked effect on the digestibility of diets for *J. edwardsii* juveniles. Results suggested a poor digestibility of native uncooked maize and potato starches (59-60%), but not native wheat starch (91%), and a positive effect of cooking and hydrolysis (i.e., gelatinised and dextrin) on starch digestibility. Similar results have been observed in the whiteleg shrimp, *Litopenaeus vannamei* (Cousin et al., 1996). Native maize starch was also found to have a similar apparent digestibility (62%) in a hybrid clawed lobster *Homarus* sp. (Bordner et al., 1983). In the present study, CMC appeared to have high apparent digestibility (~94%) in *J. edwardsii* juveniles, as previously predicted by a fast rate of hydrolysis *in vitro* (Simon, 2009c). Among the various carbohydrate sources tested, dextrin was the only source to be completely digested at the 35% inclusion level. The higher the inclusion level, the less digestible maize gelatinised starch became when incorporated in formulated diets for *J. edwardsii*. This trend is also observed in various fish species and is thought to be due to a saturation of the digestive carbohydrase enzymes by substrate (Stone, 2003).

The apparent digestibility results were for the most part in agreement with digestibility estimates derived from the rates of hydrolysis *in vitro* and post-prandial haemolymph glucose
concentrations (Simon, 2009c). Therefore, these latter methods appear suitable to provide fast and relatively accurate estimations of carbohydrate digestibility in spiny lobster. Nevertheless, in vitro tests should be considered with care as they do not encompass all the factors that may influence digestibility at the level of the organism (Cousin et al., 1996). In the present study, gelatinised starch was less digestible (84%) than dextrin (99%), which was at odds with the comparable in vitro hydrolysis rates and effects on haemolymph glucose concentration of the two carbohydrate sources (Simon, 2009c). However, gelatinised starch acts as a binder when included in diets whereas dextrin, containing low molecular weight soluble oligosaccharides, does not (Thomas et al., 1998b). This marked difference in binding properties is not a factor for in vitro assays which examine the digestibility of the substrates in isolation (Weurding et al., 2001). The higher inclusion level of gelatinised starch used in the present study (35%) than previously (27%) for haemolymph glucose measurements (Simon, 2009c) may also have further reduced the digestibility of this carbohydrate source in comparison to dextrin, possibly via greater substrate inhibition of α-amylase (Spannhof & Plantikow, 1983). The apparent digestibility of native starches was generally better than expected from their rates of breakdown in vitro (Simon, 2009c), which is in agreement with findings in the whiteleg shrimp, *L. vannamei* (Cousin et al., 1996). Native starches tend to coagulate easily in solution which may impair in vitro digestion (Weurding et al., 2001). The improved digestion of native starches in *J. edwardsii* juveniles may also originate from a longer digestion process in vivo (>34 h at 18 °C) (Simon & Jeffs, 2008) compared to the one carried out in vitro (6 h at 20 °C) (Simon, 2009c). Other digestive processes occurring in vivo such as the intracellular uptake of the products (e.g., glucose) have the potential to further inhibit the activity of α-amylase (Hill et al., 1997). Additional physical processing occurring during diet manufacturing (Cousin et al., 1996), food ingestion and trituration in the foregut are also essential in improving the susceptibility of starches to enzymatic hydrolysis (Weurding et al., 2001).

Native wheat starch and CMC appear as the best potential carbohydrate sources for improving digestibility and utilisation of formulated diets for *J. edwardsii* juveniles, given their high apparent digestibility (>91% at 35% inclusion level) but also their reduced hyperglycaemic effects compared to dextrin and gelatinised starch (Simon, 2009c). Wheat flour (i.e., including gluten) shows the best apparent digestibility (82%) among various marine and terrestrial feed ingredients for the spiny lobster *Panulirus ornatus* (Irvin &
Williams, 2007). Native wheat starch is also generally thought to be the best digested botanical source of starch for fish (Stone, 2003) and other crustaceans (Davis & Arnold, 1993; Glass & Stark, 1995; Cousin et al., 1996). Altogether this warrants the suitability of wheat feedstuffs (i.e., wheat flour, starch, gluten) for inclusion in spiny lobster diets. However, more peculiar to J. edwardsii juveniles is the excellent apparent digestibility of soluble cellulose (CMC). The clawed lobsters, H. gammarus (Glass & Stark, 1995) and H. americanus (Koshio et al., 1992), the mud crab, Scylla serrata (Pavasovic et al., 2004), the crayfish, Cherax quadricarinatus (Xue et al., 1999) and the shrimp Macrobrachium rosenbergii (González-Peña et al., 2002a) all share a capacity to digest fibre, but ostensibly to a lesser extent than J. edwardsii juveniles. It was expected that at 35% inclusion level, the strong binding action of CMC (Ruscoe et al., 2005), and the increased viscosity of the diet (Thomas et al., 1998b), would reduce apparent digestibility. High fibre diets have been shown to decrease protein digestibility and utilisation in humans and laboratory animals (Shiau et al., 1988).

Nevertheless, in J. edwardsii, apparent nitrogen digestibility of the fishmeal diet was unaffected by CMC inclusion. Increasing α-cellulose (i.e. non-soluble cellulose) level also had no effect on protein digestibility, but reduced dry matter apparent digestibility in non-ablated lobster, H. americanus (Koshio et al., 1992), and the shrimps M. rosenbergii (Fair et al., 1980), Penaeus monodon (Catacutan, 1990) and L. vannamei (Shiau, 1997).

For M. rosenbergii, up to 10% α-cellulose in the feed increases growth rates by increasing digesta residence time in the gastrointestinal tract, thereby increasing absorption (González-Peña et al., 2002b). Whether CMC increases digestive tract retention time in J. edwardsii needs to be rigorously tested, as it could have the potential benefit of improving the apparent digestibility of formulated diets. Longer gut transit time of CMC may also result in an overestimation of its true apparent digestibility, particularly if the transit speed of the chromic oxide marker is faster than the digesta (Leavitt, 1985). In the present study, occasional differences in colour patterns of faeces throughout a single collection event were observed for all formulated diets, as previously found in other crustaceans such as the American lobster, H. americanus (Leavitt, 1985), and the crayfishes Procambarus clarkii (Brown et al., 1986) and Cherax destructor (Jones & De Silva, 1997). Although there was the possibility of a differential transit speed between the marker and faeces, the collection of the majority of the faeces after each meal and the pooling of faeces over several days as performed in this study would have reduced the potential for this source of error (Jones & De
Silva, 1997; Smith & Tabrett, 2004). Overall, CMC appears to show good promise as an inexpensive but highly digestible energy source and efficient binder for future diet development for *J. edwardsii*, but further research on evacuation rate may be necessary to further confirm this conclusion.

Algal carbohydrates (i.e., agar and alginate) when used as binders were found to decrease the digestibility of the diets compared to gelatine in *J. edwardsii* juveniles. These substrates were also poorly digested *in vitro* (Simon, 2009c). A similar trend was found for the rainbow trout, *Salmo gairdneri*, where alginate inclusion reduced the apparent protein and lipid digestibility of the feeds (Storebakken, 1985). A 1% alginate-bound crab diet also showed lower dry matter digestibility (41%) than a 2% gelatine-bound crab diet (79%) in *Octopus maya* juveniles, with the resulting reduction in nutrient absorption being sufficient to cause weight loss and severe mortalities of the octopuses (Rosas et al., 2008). Alginate has been used at 6% inclusion level in previous experimental diets for spiny lobsters including *J. edwardsii* (Johnston et al., 2003; Ward et al., 2003), *P. ornatus* (Smith et al., 2003) and *P. cygnus* (Glencross et al., 2001). Agar was also used as a binder in experimental diets for *J. edwardsii* on the basis of inducing significant post-prandial glycaemic responses (Radford et al., 2005; Radford et al., 2007). Alginate is likely to have reduced the apparent dry matter digestibility (ranging 53-64%) of previous *J. edwardsii* diets, given no difference was found over the entire range of protein / BO11C inclusion levels examined (Ward et al., 2003). Using gelatine and CMC as binders in future diets for *J. edwardsii* is likely to improve performance by optimising protein and overall diet digestibility.

Finer grinding of dietary ingredients has been shown to improve growth rates and food conversion ratios of shrimp by increasing diet water stability and digestibility (Palaniswamy & Ali, 1991; Obaldo et al., 1998). A reduction in particle size from 450-1000 µm to <450 µm also improved digestibility of diets for the abalone *Haliotis midae* (Sales & Britz, 2002). The present results suggest that reducing the fishmeal (62% by dry weight of the diet) particle size distribution from <500 µm to <106 µm does not improve the digestibility of formulated diets for *J. edwardsii*. It appears unlikely from these results that insufficient grinding of dietary ingredients could have reduced markedly the digestibility of the baseline dry formulated diet used in recent research (Simon & Jeffs, 2008). The same conclusion can be drawn about the starch content which showed excellent digestibility here and in a previous study (Simon,
The low apparent dry matter digestibility may relate to the poor nitrogen (72%) and carbon (67%) digestibility of some ingredients used in this previous experimental diet including the fishmeal, seaweed meal, yeast, and the carrageenan binder (Simon, 2009a). As there was no reduction in enzyme secretion after ingestion of the baseline dry formulated diet, it is also possible that poor ingredient solubility and foregut processing are major factors impairing the digestibility of this diet in *J. edwardsii* juveniles (Simon, 2009b). The semi-purified diet containing 27% BO11C displayed excellent nitrogen digestibility (97%) because the major protein source (sodium caseinate) is completely water soluble (Simon, 2009c). In this regard, the incorporation of more soluble and pre-hydrolysed protein sources in diets for spiny lobsters may greatly improve digestibility which is critical in the species of lobster where overall food intake is limited (Simon & Jeffs, 2008; Simon, 2009a).

In summary, the results of this present study indicate that the digestibility of formulated diets for *J. edwardsii* will be maximised by; 1) selecting particular carbohydrates such as dextrin, CMC and native wheat starch for digestible energy sources, 2) reducing inclusion level of gelatinised maize starch, 3) using gelatine and CMC instead of algal-carbohydrates as binders, and, 4) possibly incorporating more soluble protein sources. Among the wide range of digestible carbohydrate sources tested, both CMC and wheat starch have the most prospect for use in formulated diets for this species of lobster (Simon, 2009c), but the potential importance of digestible carbohydrate sources to spiny lobster nutrition needs to be confirmed through comparative growth experiments.
Plate 8. Advancing the nutrition of *Jasus edwardsii* in aquaculture. A) *J. edwardsii* juveniles close to the Japanese market size cultured in the prototype sea-cage on fresh mussels; B) Biofouling is likely to be an important supplementary food source in sea-cage, note the absence of biofouling inside the sea-cage; C) A moist formulated diet format developed in this study using transglutaminase; D) Measuring feed intake rate on an individual lobster; E) Accurate food consumption measurement in spiny lobster requires the collection of the fine uneaten manipulated particles (bottom tray); F) A large juvenile *J. edwardsii* feeding on the baseline dry formulated diet of this study.
8. Chapter eight – General discussion

8.1. The nutritional benefits of biofouling

The present research represents the first attempt at culturing *Jasus edwardsii* juveniles in experimental sea-cages on formulated diets (Simon & James, 2007). There is strong evidence from the research that supplementary nutrition from biofouling was essential in improving lobster growth in the prototype sea-cages. Lobsters left feeding only on the biofouling of the prototype sea-cages grew slowly (i.e., SGR=0.38 BW day\(^{-1}\)) and achieved moderate survival (66%) over eight months. Lobsters fed three times a week in the prototype sea-cages grew significantly faster, and displayed a darkened exoskeleton pigmentation (Plate 2), compared with lobsters cultured in the experimental tanks. This was particularly the case for those fed on the dry formulated diet regime (SGR=0.78% BW day\(^{-1}\) > 0.30% BW day\(^{-1}\) in tanks) (Simon & Jeffs, 2008). The considerable quantities of predated barnacle and mussel shells retrieved at each census, and the lower coverage of biofouling in the sea-cage areas accessible to lobsters, suggest that some of the sea-cage biofouling, perhaps also including small shell-less organisms and slow-moving crustaceans, was selectively consumed by the cultured lobsters. This additional source of nutrients, natural pigments and active constituents (i.e., bacteria, active enzymes and hormones) may have helped to increase the overall mass of food ingested between experimental feeding allotments and possibly ameliorated the nutritional inadequacies of the formulated diet. Further research that accurately defines the nutritional contribution of biofouling using stable isotopes and gut analyses is needed to help identify the main species ingested and the specific components improving the lobsters’ nutrition. Expensive key components of formulated diets, such as the astaxanthin pigment, could then be spared to some extent by biofouling, which would reduce overall feed cost (Barclay et al., 2006). Crear et al. (2002) and Johnston et al. (2007) have overcome some of the nutritional limitations of formulated diets by providing fresh mussel as a supplementary food source once or twice weekly, thereby reducing production costs without reducing lobster performance. In the case of sea-cage culture, fresh mussel supplement may not be needed because the presence of natural biofouling may be sufficient to achieve commercially viable growth rates on formulated diets (Simon & James, 2007). Best performing practical diet formulations should be further tested in sea-cages to mimic as far as possible the future on-
farm conditions (Tacon, 1996). Feeds will have to be delivered once daily rather than three times a week to maximise consumption and the scope for growth (Simon & Jeffs, 2008). It would then be important to assess the nutritional benefits of biofouling under more frequent feeding regimes and over a range of juvenile sizes up to market size. However, current results suggest that biofouling is likely to remain beneficial even at higher feeding frequencies of formulated diet, given that lobsters grew faster in the prototype sea-cage than in the experimental tanks, even when the food was delivered daily, or more frequently, in the tanks.

8.2. Towards the commercialisation of sea-cage technology for spiny lobster ongrowing

Ongrowing juvenile *J. edwardsii* in sea-cages to a size suitable for the Japanese niche market (i.e., 100-300 g) is likely to have the best prospects for commercial aquaculture development in New Zealand. This size of lobster is well under the minimum harvestable legal size (i.e., around 0.6-1 kg) and therefore would not compete with the existing commercial lobster fisheries. Targeting such a niche market would allow aquacultured lobsters to attract premium prices for the shortest growing time, ensuring a fast turnaround of cultured stocks while reducing business risks and production uncertainties, and improving potential profitability. Ongrowing early juveniles to the smallest market size (60 mm CL; 100 g) would take around 22 months in the prototype sea-cage, when feeding fresh mussels three times a week at the relatively low water temperatures (9-18 °C) recorded in the Wellington Harbour (Simon & James, 2007). This is a conservative estimate as the growth extrapolation is based on the growth rates achieved in the last two months of the sea-cage experiment, where daily water temperatures were on average 12.8 °C. In a scenario where the farmer could access sufficient waste mussels, the ongrowing operation may become viable if a practical and cost-effective system is developed to remove the mussel shells (either before or after feeding), which would otherwise accumulate rapidly within the sea-cages. In the more likely case where a cheap and reliable supply of fresh mussels cannot be assured, a cost-effective nutritionally-adequate formulated diet delivered into sea-cages daily by automatic feeders is required for *J. edwardsii* culture to become economically viable. The dry formulated diet tested in the present research was clearly outperformed by fresh mussel, even with biofouling as a supplementary source of nutrition (Simon & James, 2007) or when the formulated diet
was fed up to five times a day in tanks (Simon & Jeffs, 2008). The present research identifies several digestive issues relating to formulated diets that need to be resolved in order to improve their nutritional qualities (Section 8.3.). However, it is interesting to note that the dry formulated diet, by containing only relatively low cost ingredients (i.e., total ingredient cost of NZD 2.31 kg⁻¹), may already be cost-effective for commercial culture compared to purchased fresh mussel (i.e., NZD 2 kg⁻¹ wet weight including shell, which equates to a dry tissue weight of around NZD 20 kg⁻¹) or more expensive commercial kuruma shrimp diets (i.e., 1500 yen kg⁻¹ ≈ NZD 24 kg⁻¹) (Lim et al., 1997; Crear et al., 2002). An extrapolation of growth based on the last two months of culture in sea-cages suggests the lobsters would reach the smallest market size (100 g) on the current baseline formulated diet in 29 months, with a similar rate of survival to lobster cultured on fresh mussel alone. This extra seven months of culture is reasonable given that sea-cages will have lower running costs than land-based facilities, which may ultimately allow improved production cost on this cheaper formulated diet, particularly during periods of low water temperatures (~ 12.8 °C) when the scope for growth differences between formulated diets and a diet of fresh mussel is reduced (Simon & James, 2007). Further growth experiments that ongrow early juvenile lobsters up to market size, combined with detailed economic analyses, are required to determine the most cost-effective scenarios for sea-cage culture (Jeffs & Hooker, 2000).

Modifying the design of the experimental prototype sea-cage into a more practical design for commercial operation is another important issue that should be addressed through future applied research. This needs to be achieved without losing some of the design characteristics (i.e., complex internal shelter matrix and the feeding station) which are believed to have improved culture performance in comparison to the previously used commercial sea-cages (Simon & James, 2007). Submerged sea-cages (>5-10 m depths), possibly integrated with mussel longline culture systems to benefit from the proximity of the waste mussel and settling spat, appear to have a better prospect for commercialisation than coastal raft systems under the current New Zealand aquaculture legislation. Integration of lobster sea-cages into existing mussel farms would certainly avoid conflict with competing maritime users, which is one of the major reasons for the difficulties in aquaculture expansion in New Zealand (Jeffs, 2003b). Submerged sea-cages would also benefit from receiving high quality seawater without large fluctuations in temperature and salinity. This may be particularly important for potential farming areas in northern New Zealand, where the ambient
water temperature offers the best prospects for obtaining maximum growth rates from the spiny lobsters, but may reach lethal levels especially towards the surface (>24 °C) during the summer months (Jeffs, 2003a). Practical modifications to the prototype sea-cage could include the following:

- Further expansion of the internal matrix via an increase in the number and size of the stack perforated trays. A high internal surface to volume ratio is essential to achieve commercially viable stocking densities.

- Multiplying feeding stations and feed delivery tubes to reduce food wastage and competition (Thomas et al., 2003). The importance of these elements was revealed by personal observations using infrared cameras in large tanks containing a similar internal matrix. Directly delivering the food to dedicated feeding stations situated at the bottom of the cage ensures lobsters reliably locate the available food, thereby avoiding food wastage. Maximising consumption efficiency will be very important for both economic and ecological reasons at the commercial scales of future lobster aquaculture. The relatively high FCR values (1.3-2.6) achieved with *J. edwardsii* feeding on formulated diets (Crear et al., 2002; Simon & Jeffs, 2008) suggest that with correct feed management (automated feeders located at the surface), most of the delivered food can be converted to lobster growth to minimise environmental pollution. The food delivery tubes could also be useful for facilitating the stocking of the sea-cage with early juveniles at the beginning of the ongrowing period.

- Ensuring the perforations of the internal matrix allow vertical movements of lobsters throughout the ongrowing period. Personal observations indicate that non-feeding lobsters approaching ecdysis move up and away from the feeding cohort, which tends to remain near the feeding station. Allowing this natural partitioning behaviour is essential to avoid cannibalism of post-moult lobsters.

- Using a single outer net with the mesh tailored to the lobster size to avoid escapees and predation by fish. The outer net would surround the internal matrix at some distance (e.g., attached to spacers to avoid predation of protruding limbs) and remain easily accessible by the lobsters that would benefit from the supplementary nutrition from biofouling, whilst providing a convenient means of cleaning the cage (reducing labour cost). A practical system would need to be developed to rapidly open and change the outer net during
cleaning and harvesting by the servicing boat, as this was found to be a major time-consuming process with the experimental sea-cage design used in the present research.

8.3. Feeding and its relation to digestive physiology in cultured Jasus edwardsii juveniles

The understanding of spiny lobster nutrition is fragmentary and incomplete, with most recent efforts having been devoted to the development of formulated diets without a specific focus on the reasons for ongoing presence of poor growth rates on such diets. Currently, the main explanation for poor growth relates to inadequate food consumption due to problems arising prior, or during ingestion, such as poor chemoattraction, feeding stimulation and palatability of the diets (Glencross et al., 2001; Williams et al., 2005; Nelson et al., 2006; Johnston et al., 2007). However, there has been contradictory evidence for this hypothesis, including the observation that feeding lobsters several meals a day does not improve food consumption or growth (Bordner & Conklin, 1981; Thomas et al., 2003; Cox & Davis, 2006; Jones, 2007; Simon & Jeffs, 2008; Johnston et al., 2008). Typically more frequent delivery of feed should increase attraction and palatability, thereby resulting in higher food consumption and growth (Sedwick, 1979).

The present study indicates that issues in digestive processing may provide a better explanation for the low formulated feed consumption in juvenile spiny lobsters. This research is the first investigation into the capacity, filling and evacuation dynamics of the digestive tract of spiny lobsters (Simon & Jeffs, 2008; Simon, 2009a), as well as the biochemical processes occurring in their foregut and digestive gland (Simon, 2009b), in response to feeding on natural and formulated diets. The research has identified two fundamental issues impairing the food consumption of the dry formulated diet in cultured J. edwardsii juveniles over a large size range (20-80 g);

1) The lack of a nutrient density advantage of feeding dry pellets (ranging 61-69% moisture post-ingestion), in terms of the extent of dry matter intake in a single meal (0.8-1.2% BW). This is exacerbated by the reduced physical capacity (2.5-3% BW),
and slow rates of filling (1-2 h) and evacuation (10 h) of the foregut (Simon & Jeffs, 2008; Simon, 2009a).

2) Slow rates of appetite revival (ranging 18-48 h), which are consistent with the lack of improvement in food consumption (1-1.2 %BW day⁻¹) at higher feeding frequencies, and relate to the digestive system of the lobsters being unable to efficiently process the arrival of formulated food immediately after the evacuation of a former meal.

Lobsters feeding on the dry formulated diet displayed an intensified digestive effort in their digestive gland in comparison to those fed mussel flesh (i.e., 88% larger tubule lumen size, 269% more B-cells per tubule at 18 h post-feeding, and lower digestive fluid pH). This may be caused by, and in turn exacerbate, digestive processing issues in the foregut and the poor digestibility of some of the ingredients used (e.g., fishmeal, algal binders). Instead of the foregut digestive enzymes migrating with the digested particles (<1 µm) into the tubule of the digestive gland for further intra-luminal digestion (Dall & Moriarty, 1983), these may be lost by being bound to small indigestible food particles that are rapidly purged from the foregut into the hindgut for defecation (Córdova-Murueta et al., 2003; Simon & Jeffs, 2008). The increased cost of digestion in the digestive gland would in turn compromise the synthesis of fresh digestive enzymes to be delivered to the foregut in anticipation of the next feeding event, as suggested by the lack of a second peak at 18 h in enzyme activity in the foregut of lobsters fed the dry formulated diet (Simon, 2009b). Difficulties in producing fresh digestive enzymes for the next meal would lengthen appetite revival (Loya-Javellana et al., 1995).

It is important to note one major limitation of this research; the use of only one baseline dry formulated diet for comparison with the best natural diet to date (fresh mussel). The use of this diet for the research was due to its advanced performance over previous iterations of research diets tested in New Zealand. However, the formulated diet contained uncommon ingredients such as seaweed meal, lupin meal and yeast, which may have played an uncertain influence on aspects of feeding and digestion identified in the present research. Further research is needed to test for the potential effect of the individual components, as this was beyond the scope and timeframe of the present research. Still, many attributes of the baseline formulated diet, such as the dry extruded format with fishmeal as the main protein source, and its effect on growth, survival, food consumption and digestibility, shared strong
similarities with previous experimental and commercial diets fed to *J. edwardsii* (Ward et al., 2003). It is thus reasonable to assume that similar issues in digestive processing would have been present with previous formulated diets. For example, the clear similarities in food consumption (~ 1% BW day\(^{-1}\)) found across previous studies regardless of the feeding frequency (Crear et al., 2002; Thomas et al., 2003; Tolomei et al., 2003; Simon & Jeffs, 2008) show that slow rates of appetite revival might be a relatively common phenomenon with formulated diets. This explains why *J. edwardsii* juveniles have a limited ability to vary the scale of their maximum feed intake beyond the capacity of one foregut load per day in response to poorly digestible diets (Ward et al., 2003). This is in contrast to penaeid shrimps which can compensate for reduced diet digestibility and deficiencies in particular ingredients by a higher ingestion rate (Kureshy & Davis, 2002). At present, the increased formulated feed retention time in the digestive tract of spiny lobsters (Simon & Jeffs, 2008) is also not correlated with significantly better apparent digestibility (Ward et al., 2003; Simon, 2009d) in comparison to penaeid shrimps (Lee & Lawrence, 1997). Results of the present research indicate that the poor overall diet digestibility is strongly affected by the source and inclusion level of carbohydrates, including the use of algal binder, as well as the solubility of the dietary protein sources (Simon, 2009b, d). Furthermore, the results suggest dry matter digestibility is not related to a limitation in digestive enzyme secretion (Simon, 2009b) or a potentially suboptimal ingredient particle size (Simon, 2009d). Further testing is required to investigate if digestive enzyme secretion is impaired under more continuous feeding conditions than three times a week (Simon, 2009b). The effect of dietary particle size on digestibility also requires further investigation, as in the present study experimental comparisons of different particle sizes were only made for the fishmeal component of the diet.

### 8.4. Carbohydrate digestion in *Jasus edwardsii* juveniles

For the culture of juvenile *J. edwardsii*, where daily food intake is limited by foregut capacity and slow appetite revival, maximising the amount of nutrient assimilated per meal by improving the digestibility and utilisation of formulated diets is crucial to improving their performance (Simon and Jeffs, 2008; Simon, 2009a, b). One of the least digested and utilised components of experimental lobster diets has been the carbohydrate fraction (Bordner et al., 1983; Johnston et al., 2003; Ward et al., 2003), yet little research has been devoted to finding
an appropriate carbohydrate source for spiny lobsters that could act as a lower cost alternative to protein and lipid as an energy source (Johnston et al., 2003; Radford et al., 2005). The present research represents the most exhaustive investigation of carbohydrate digestibility in spiny lobsters. It demonstrates that several carbohydrate sources may be valuable ingredients as a low cost source of energy to spare the protein fraction for growth without compromising overall diet digestibility.

Three different methods were used to determine the digestibility of various sources of carbohydrate in *J. edwardsii* juveniles;

1) *In vitro* hydrolysis rates of substrate using enzyme homogenates from the foregut and digestive gland (Simon, 2009c).

2) *In vivo* post-prandial haemolymph glucose concentrations (Simon, 2009c).

3) *In vivo* apparent digestibility (Simon, 2009d).

The post-prandial haemolymph glucose fluxes were also useful in providing some information about utilisation, knowing the rate of breakdown and digestibility of the carbohydrates.

The research shows that disaccharides (i.e., sucrose and trehalose) and algal carbohydrates (i.e, alginate and agar) are poorly digested in juvenile *J. edwardsii* (relative rate of hydrolysis <20%) (Simon, 2009c), which suggests that their use as an energy source is limited in *J. edwardsii*. Algal carbohydrates also do not appear suitable as dietary binders because they reduce overall diet apparent digestibility (agar, 60.8%; alginate, 68.1%) in comparison to gelatine (73.4%) (Simon, 2009d); an important finding given that algal carbohydrates have been used extensively in the manufacture of experimental diets for spiny lobsters (Glencross et al., 2001; Ward et al., 2003; Johnston et al., 2003; Smith et al., 2003; Radford et al., 2007).

Native starches vary in digestibility for *J. edwardsii*, with potato, tapioca, maize and wheat being increasingly digestible. Among these plant sources of starch, only wheat starch appears useful for future incorporation in formulated diets due to the high apparent digestibility of its native form (91.2% at a 35% inclusion level) (Simon, 2009d), which may relate to a higher amylopectin content (Cuzon et al., 2000). Wheat starch also appears as the
best utilised starch source, with lower post-prandial haemolymph glucose concentrations early in digestion (i.e., reaching a peak of 5.52 mmol l\(^{-1}\) only after 24 h post-prandial) than would be expected considering its high digestibility (Simon, 2009c). Cooking (gelatinisation) improves the digestibility of native starches by *J. edwardsii*, but the plant source of starch was found to remain important regardless of the pre-treatment because tapioca starch was apparently more digestible than potato starch whether native (relative hydrolysis rate = 16.7 % versus 9.1%, respectively) or cooked (100.0% versus 86.8%, respectively) (Simon, 2009c), and the apparent digestibility pre-gelatinised maize starch (83.7%) was lower than native wheat starch (91.2%) (Simon, 2009d). Starch gelatinisation also results in the production of high-glycaemic compounds, their rapid digestion leading to elevated post-prandial haemolymph glucose concentrations (Fig. 6.1.), which may affect appetite revival and the utilisation of the derived glucose (Simon, 2009c). A similar phenomenon occurs when the starch is provided in a hydrolysed form such as dextrin (Fig. 6.1.).

Carboxymethyl cellulose, the solubilised structural carbohydrate of plant and algal cell walls, showed excellent digestibility both *in vitro* (73.5%) (Simon, 2009c) and *in vivo* (~94%) (Simon, 2009d) in juvenile *J. edwardsii*. Although dietary fibre, such as cellulose, is traditionally believed to provide no nutritive value for crustaceans (Ceccaldi, 1997), soluble cellulose appears to be one of the best digested carbohydrate sources in *J. edwardsii*, second most digested carbohydrate (~94%) after dextrin (100% digested). At a 35% inclusion level of carboxymethyl cellulose, apparent dry matter (76.4%) and protein (88.0%) digestibility were significantly better than for previous fishmeal-based experimental diets fed to *J. edwardsii* (Ward et al., 2003). There has been increasing evidence for the digestibility of fibre and its positive effect on digestion and growth in aquatic animals, such as the red sea bream, *Pagrus major*, and the shrimp, *Macrobrachium rosenbergii* (Morita et al., 1982; Ceccaldi, 1997; González-Peña et al., 2002a). In *J. edwardsii* juveniles, carboxymethyl cellulose also shows the best prospect for utilisation among the digestible carbohydrate sources identified in the present research because of its comparatively reduced effect on the post-prandial glycaemic response of lobsters (i.e., 12 h response with a peak reaching 1.60 mmol l\(^{-1}\) at 7 h), which would suggest a good utilisation of the derived glucose for energy or storage as glycogen in the digestive gland (Cuzon et al., 2000). Unlike native wheat starch, carboxymethyl cellulose would not be affected by heating during diet manufacturing, and therefore may be better utilised in extruded commercial formulated diets for *J. edwardsii*. Carboxymethyl cellulose
has also unmatched diet binding characteristics (Forster, 1972; Ruscoe et al., 2005), which are essential to the production of highly water stable and palatable diets for spiny lobsters.

Glycogen isolated from *Perna canaliculus* was another carbohydrate source found to be highly digestible (relative rate of hydrolysis = 70.8%) (Simon, 2009c) in the present research. This is consistent with fresh mussel being well digested (89.2%) (Simon & Jeffs, 2008) and an excellent food source for *J. edwardsii* and other spiny lobsters. The glucose produced via the breakdown of the glycogen present in fresh mussel gonads was also apparently better utilised (within 24 h the glucose concentrations returned to pre-feeding levels after reaching a peak of 6.13 mmol l$^{-1}$ at 12 h) than the glucose originating from feeding on the semi-purified diets containing digestible carbohydrate sources (Simon, 2009c). However, mussel glycogen is very expensive to obtain in a refined form, and its similar effect on haemolymph glucose fluxes when purified and incorporated in a diet to other compounds like dextrin and gelatinised starch indicates that it may have no additional nutritional benefits as a source of energy (Simon, 2009c).

The difference in the utilisation of mussel glycogen from the natural and formulated diets suggests that the utilisation of carbohydrate for energy in spiny lobsters is also influenced by extrinsic factors to the particular carbohydrate source. Several hypotheses were proposed to explain this phenomenon, including an interaction effect with the utilisation of the protein source and/or a better regulation of glucose homeostasis mediated by an inhibition of the crustacean hyperglycaemic hormone (CHH) when feeding on fresh mussel (Simon, 2009c). Both possibilities deserve further investigation as they may provide some explanation for the poor growth of spiny lobsters under formulated diet regimes (Simon, 2009c). In lobsters, CHH expression inhibits moulting and growth (Chung & Webster, 2003). Hence, quantifying haemolymph CHH concentrations via ELISA methods would be recommended in future growth studies with spiny lobsters fed a range of natural and formulated diets. These ELISA methods could be facilitated by the use of existing antibodies developed previously for a homologous CHH from the American lobster, *Homarus americanus* (Chang et al., 1998). It may also be interesting to test whether biofouling as a source of supplementary nutrition has an effect on CHH expression in cultured lobsters, which could explain the higher growth rates in sea-cages. If the haemolymph CHH concentrations are found to be lower in lobsters feeding on natural food sources, it might be possible to inhibit the expression of this hormone on formulated diet regimes via the
development of a routine surgical method for eye-stalk ablation, which has previously been found to improve food consumption and growth rates on fresh food in the American lobster, *H. americanus* (Koshio et al., 1989), and in the spiny lobsters *Panulirus homarus* (Radhakrishnan & Vijayakumaran, 1984) and *P. ornatus* (Juinio-Meñez & Ruinata, 1996). However, given the fundamental bottlenecks in digestive processing of formulated diets, such treatment may not improve the growth of lobsters until a suitable formulated diet is developed that can meet the needs of a heighten metabolism, requiring greater nutrition and causing higher stress levels (Verghese et al., 2008).

Further research should focus on accurately determining the metabolic utilisation of the several digestible carbohydrate sources identified in the present study. Research into carbohydrate and glucose utilisation should proceed by measuring respiratory oxygen to nitrogen ratios (O:N) to determine the type of substrate used for energy (Capuzzo & Lancaster, 1979; Radford et al., 2008), measuring glycogen concentration in the digestive gland (Rosas et al., 2000), assessing histological changes in digestive gland structure (Pascual et al., 1983; Guo et al., 2006), and performing growth experiments to accurately determine the scope for energy utilisation and protein sparing of each carbohydrate source (Alava & Pascual, 1987; Cruz-Suárez et al., 1994). The study of enzymes regulating the digestion of carbohydrates (e.g., α-amylase, α, β-glucosidase), glycolysis (e.g., hexokinase, pyruvate kinase) and gluconeogenesis (e.g., phosphoenolpyruvate carboxykinase) would also be useful because they can help understand the variations in carbohydrate digestion and utilisation in response to the source and inclusion level of dietary carbohydrates and proteins (Cuzon et al., 2000). For instance, in the present study, the decrease in α-amylase specific activity with increasing feeding time on the baseline dry formulated diet (e.g., feeding only on fresh mussel without formulated diet supplementation, 1.79 and 1.15 U mg⁻¹ in foregut and digestive gland respectively; after three weeks feeding on formulated diet, 0.83 and 0.48 U mg⁻¹; after six months feeding on the formulated diet, 0.45 and 0.24 U mg⁻¹) was a major finding suggesting the starch source and inclusion level were unsuitable for *J. edwardsii* juveniles (Wormhoudt et al., 1980; Ceccaldi, 1997).
8.5. Towards the successful development of formulated diets for spiny lobsters

The complexities of developing formulated diets to address the digestive physiology of spiny lobsters offer a large scope for further research, as there has been very little research done prior to the present study. Three broad strategies for improving the nutrition of cultured *J. edwardsii*, and possibly other spiny lobster species, can be drawn from the present research;

1) Ameliorating food consumption, via improving the extent of food intake in a single meal and the rate of appetite revival between meals.

2) Improving the digestibility of dietary ingredients in formulated feeds to maximize the amount of nutrient assimilated per meal.

3) Incorporating digestible carbohydrates in formulated feeds that are utilised as a cheap source of energy to spare protein for growth.

8.5.1. Improving the extent of food intake on formulated diets

The only solution to improve the extent of food intake within a 1-5 h feeding period is to reduce the moisture content of the digesta in the foregut. Currently, both fresh mussel flesh and the dry formulated diet result in digesta moisture levels of around 60-70% (Simon, 2009a). The scope for improvement in food intake is somewhat limited because the gastric fluid volume prior to ingestion takes on average 24% of the capacity of the foregut (Simon, 2009b). However, while the gastric fluid volume combines with the overall digesta volume when feeding on the dry formulated diet (40-50% moisture post-immersion but 61-69% post-ingestion), it is displaced when feeding on fresh mussel flesh (~70% moisture post-immersion and ingestion) (Simon, 2009a). It is believed that semi-moist pellets could be ingested in larger pieces by a “sucking and cutting action”, as observed when lobsters are feeding on mussel flesh, rather than the typical “grinding action” of the mandibles on dry formulated pellets (pers. obs.). This different feeding process observed on mussels may reduce the uptake of seawater during ingestion and the mixing with gastric fluid during early foregut digestion, reducing digesta expansion to allow further intake without regurgitation (Simon, 2009a).
Ingesting larger pieces of food would also reduce manipulative wastage during feeding as found for the crayfish *Cherax quadricarinatus* (Ruscoe et al., 2005). Improvement in dietary format is foreseen by manufacturing semi-moist diets with a malleable texture, and an overall moisture content that should remain around or bellow 50% to improve dry matter intake in comparison to mussel flesh. This may be assisted with the incorporation of carboxymethyl cellulose and small quantities of other digestible binders such as transglutaminase and gelatine. In addition to improving dietary format, omitting particular ingredients such as gluten and starch, which absorb considerable seawater upon immersion, may also increase the extent of food intake in a single meal.

8.5.2. Improving appetite revival on formulated diets

8.5.2.1. Reducing the intracellular digestive load of the digestive gland

There is strong evidence in this study that the slow appetite revival on the dry formulated diet is due to intensified intracellular digestion in the digestive gland preventing lobsters from efficiently processing the arrival of new formulated food immediately after a former meal (Simon, 2009b). Difficulties in producing fresh digestive enzymes for the next meal lengthen appetite revival (Loya-Javellana et al., 1995). Lobsters may then associate the chemical stimuli with the nutritional quality of the food, and respond accordingly by minimising the energetic costs of handling, ingestion and digestion of poorly digestible and nutritive diets (Kurmaly et al., 1990; Lee & Meyers, 1996). This could explain the discrepancies found between previous studies investigating the chemoattraction of spiny lobsters to formulated diets. When only the extracts are tested (Tolomei et al., 2003), formulated diets are found to be as attractive as fresh mussel to *J. edwardsii* because no post-ingestive feedback mechanism develops. However, the attraction to formulated diets may reduce as soon as lobsters are allowed to ingest the test feeds (Williams et al., 2005). In the future appetite revival and consumption may be enhanced alongside improvements in foregut processing, digestibility and the overall nutritional quality of formulated diets (Section 8.5.3).
8.5.2.2. Reducing the post-prandial hyperglycaemic response

The complete return of appetite in 42 g juvenile lobsters fed the baseline dry formulated diet and mussel flesh occurred at 30 h and 12 h respectively (Simon & Jeffs, 2008), which matched the onset of the decline of the hyperglycaemic response of these diets (Simon, 2009c). Appetite revival may be enhanced by reducing the hyperglycaemic period, for example, by minimising cooked starch inclusion level. Several species of shrimp have been found to reduce feed intake with increasing levels of dietary starch, which also correlate with more intense and prolonged hyperglycaemia and higher digestive gland glycogen levels (Rosas et al., 2000; Guo et al., 2006). In vertebrates, such as cultured fish, the glucostatic theory proposes that short term hunger and satiety regulation is mediated by the utilisation of glucose at the cellular level (Le Bail & Boeuf, 1997; Even & Nicolaidis, 1986; Bornet et al., 2007). However, in crustaceans the control of haemolymph glucose levels is limited (Cuzon et al., 2000), thus the potential for glucose per se to be the crucial substance involved in appetite regulation in spiny lobsters appears unlikely. Appetite may instead be regulated by a more general mechanism controlling total metabolisable energy intake (Johansen et al., 2002; Geurden et al., 2006).

8.5.3. Improving the digestibility of formulated diets

The digestibility of formulated diets can be improved by selecting particular sources of carbohydrates (i.e., dextrin, carboxymethyl cellulose, wheat starch, glycogen), heat-treating native starches during diet manufacturing, reducing the inclusion level of pre-gelatinised starch, and avoiding the use of algal carbohydrates for binding (e.g., agar, alginate) or as an energy source (e.g., seaweed meal). Digestive processing issues in the foregut may also be related to the buffering of the acidic gastric fluid by seawater intake while feeding on dry pellets. These issues would be addressed by developing new dietary formats that reduce seawater uptake (Section 8.5.1.), and adjusting dietary pH to more acidic levels of around 5.0, to improve the enzymatic digestion of carbohydrates (Simon, 2009b).

Improving the solubility of other dietary macro-ingredients, such as the protein sources, is also likely to improve feed processing and digestibility (Simon, 2009c, d).
The protein digestibility (97%) of the semi-purified diets developed by Simon (2009c) was greater than other fishmeal based formulated diets fed to lobsters (73-83% in Ward et al., 2003; 82-88% in Simon, 2009d) because of the presence of refined water soluble proteins such as sodium caseinate, freeze-dried mussel meal, low-heat dried (hydrolysed) squid and wheat gluten with low ash content. Protein solubility has been identified as an essential factor affecting the digestibility of diets for fish larvae (Carvalho et al., 2004; Tonheim et al., 2007). Fish and squid meal have a much lower water solubility than krill and mussel meal (Nankervis & Southgate, 2006), and remarkably spiny lobsters appear to be able to better digest the proteins from mussel (89-98%) and krill (89%) meals than fish (63-84%) and squid (7.3-59%) meals (Williams, 2007). Future in vitro testing of protein digestibility using the pH-stat method would assist in the identification of cheaper alternative protein sources that are digestible by spiny lobsters (Ezquerra et al., 1997; Lemos & Nunes, 2008). Pre-hydrolysis treatment of dietary proteins prior to inclusion in formulated diets (Córdova-Murueta & García-Carreño, 2002), or upon immersion via the supplementation of active exogenous proteases (Dabrowski & Glogowski, 1977), may also assist in improving their digestibility.

Even though finer sieving of fishmeal below 500 µm did not appear to have a beneficial effect on the digestibility of formulated diets in this present study, the effect of dietary particle size on digestibility needs to be further studied in spiny lobsters, especially in relation to foregut evacuation and growth rates. In the shrimp *Litopenaeus vannamei*, reducing particle size to 124 µm improves growth, but further grinding down to 69 µm reduces growth significantly (Obaldo et al., 1998). Large ingredient particle size (>500 µm) may have lower digestibility due to the time required for breakdown by the gastric mill and digestive enzymes, but very small particle sizes may be purged over-rapidly from the foregut into the hindgut of lobsters. In fish, dry formulated diets are not digested as efficiently as fresh food because they are composed of small particles of high energy which are emptied too quickly from the stomach and subsequently overload the digestive and absorptive capacities of the intestine (Jobling, 1986). In the present study, the dry formulated diet was also found to be purged quickly from the foregut after an initial delay of around 4 h, and defecated faster than for fresh mussel for the three size-classes of *J. edwardsii* juveniles (Simon & Jeffs, 2008). The large pieces of ingested mussel flesh may be more easily retained in the foregut to allow sufficient time to complete digestion (Simon & Jeffs, 2008). Differences in the speed of foregut evacuation would have an important effect on digestibility in Crustacea as the purged
material completely by-passes the absorptive area of the digestive gland (Dall & Moriarty, 1983). The development of semi-moist diets with an adequate amount of viscous carboxymethyl cellulose (Section 8.5.1.) may also assist in improving feed retention in the foregut for more efficient digestion (Simon, 2009d). Including 10% of non-soluble cellulose in formulated diets has been shown to improve digestive tract retention, absorption efficiency and growth rate in the shrimp *M. rosenbergii* (González-Peña et al., 2002b).

### 8.5.4. Improving growth via the protein sparing-effect of carbohydrates

Carnivorous crustaceans such as lobsters tend to utilise dietary protein to satisfy most of their energy requirement via a well-developed gluconeogenic pathway (Rosas et al., 2000; Oliveira et al., 2004), but there is some evidence of a protein-sparing effect of dietary carbohydrate in the American lobster, *Homarus americanus* (Capuzzo & Lancaster, 1979; Brown, 2006) and the spiny lobster, *J. edwardsii* (Radford et al., 2008). The linear growth response to protein inclusion level observed in the spiny lobsters *P. ornatus* (Smith et al., 2005) and *P. cygnus* (Glencross et al., 2001), and the good growth rate achieved in *J. edwardsii* using commercial shrimp feeds high in crude protein (Crear et al., 2002), suggest that a limited amount of dietary protein is diverted into somatic growth in spiny lobsters. For most cultured animals, there is a certain protein inclusion level beyond which growth is not further supported, and may even decrease due to the cost of deamination and excretion of the absorbed amino acids (Jauncey, 1982; Siddiqui & Khan, 2009). However, in spiny lobsters this phenomenon is suppressed (but see Ward et al., 2003) for two potential reasons:

1) Not enough food is consumed and the diets are poorly digestible, hence overall protein intake is reduced.

2) Non-protein energy sources, particularly carbohydrates, are not utilised efficiently to spare protein for growth, and therefore most of the energy requirement is met by the catabolism of the dietary protein.

The present research provides help to resolve these two issues in order to develop cost-effective (lower protein content) nutritionally-adequate diets for commercial culture. Several carbohydrate sources that are digestible, and potentially utilised, have been identified
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(Section 8.4.) to ensure the best ingredients are used as non-protein energy sources in future spiny lobster diets. Early lobster studies have used native corn (maize) starch as a source of carbohydrate in experimental diets for clawed lobsters (Capuzzo & Lancaster, 1979; Boghen et al., 1982; Bordner et al., 1983; Koshio et al., 1992), but this source is poorly digested (Bordner et al., 1983; Simon, 2009d), and therefore little energy is provided to spare protein for growth. More recent studies on spiny lobsters have all used pre-gelatinised maize starch as a carbohydrate source (Glencross et al., 2001; Smith et al., 2003; Ward et al., 2003; Johnston et al., 2003; Smith et al., 2005; Johnston et al., 2007, 2008), but despite being more digestible than native maize starch (Simon, 2009 c,d), the present research suggests it may not be well utilised because it leads to a fast rise in haemolymph glucose and prolonged hyperglycaemia (Simon, 2009c). In the latest study on formulated diet development for *P. ornatus*, Barclay et al. (2006) have switched to wheat flour as a carbohydrate source, and the diet developed is considered the best to date (Williams, 2007). This is in agreement with the present research on *J. edwardsii*, which indicates that wheat starch is the best digested and utilised source of starch (Simon, 2009c, d). Further improvement using wheat starch may be achievable by reducing the processing of the feed during extrusion to prevent extensive gelatinisation. This study also indicates that carboxymethyl cellulose is another carbohydrate source showing great potential for digestion and utilisation in *J. edwardsii* juveniles. Further growth studies testing diets containing different levels of wheat starch and carboxymethyl cellulose at a constant protein level, thereby varying total energy and the protein to energy ratio, are highly recommended to determine the best inclusion level of these energy sources.

Unfortunately, previous growth experiments investigating the effect of protein inclusion level have confounded results by varying the amount of protein at the expense of gelatinised starch (Glencross et al., 2001; Smith et al., 2003; Ward et al., 2003; Smith et al., 2005). It remains to be tested whether there is a negative effect of increasing starch level over and above the decrease in protein, with the support of data regarding the activity of key digestive carbohydrases and metabolic enzymes involved in glycolysis and gluconeogenesis (Van Wormhoudt et al., 1980; Ceccaldi, 1997).
8.6. Conclusion

The broad, multidisciplinary, applied research approach of the present study was successful in addressing the two major issues (i.e., rearing system and nutrition) currently constraining the commercial ongrowing of *J. edwardsii* juveniles. An effective sea-cage rearing system was developed that provided significant growth advantages over tank culture systems, especially for formulated diets, by addressing some of the dietary inadequacies via the supplementary nutrition derived from biofouling. Significant advances in the understanding of the feeding, digestive physiology and carbohydrate digestion of spiny lobster juveniles have been made through this current research, and these will have important implications for their successful aquaculture. The research has also found commercially relevant results and identified a number of new research directions towards advancing the nutrition of *J. edwardsii* juveniles, and possibly other spiny lobsters. Similar investigations in other spiny lobster species of commercial interest (e.g., *P. argus*, *P. ornatus*, *P. cygnus*) are highly recommended to accurately characterise their specific scope for food ingestion and digestion.
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