LIBRARY
Te Tumu Herenga
THE UNIVERSITY OF AUCKLAND

RESEARCHSPACE@AUCKLAND

http://researchspace.auckland.ac.nz

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.
http://researchspace.auckland.ac.nz/feedback

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.

Note : Masters Theses

The digital copy of a masters thesis is as submitted for examination and contains no corrections. The print copy, usually available in the University Library, may contain corrections made by hand, which have been requested by the supervisor.

The sea cucumber *Australostichopus mollis*: Juvenile feeding ecology and habitat

Matthew James Slater

Thesis submitted in partial fulfilment of the requirements for the degree
of Doctor of Philosophy in Marine Science,
University of Auckland, 2009

Abstract

The aspidochirote sea cucumber *Australostichopus mollis* is a commercially valuable species of research interest as a candidate for aquaculture or polyculture. Understanding juvenile habitat and feeding biology is essential to furthering aquaculture efforts and expanding knowledge of the ecology of the species. Surveying within the Mahurangi Harbour, New Zealand, revealed highly patchy distribution of juvenile *A. mollis* at one site within an area of high adult density. High juvenile density correlated with sediment qualities favouring epibenthic detritivorous deposit-feeding. Juvenile *A. mollis* showed no distinct spatial separation from adults and no association with dense macroalgae or preference for shallower depths than adults, as may be expected from studies on other sea cucumber species.

Wild caught juveniles displaced to non-juvenile habitats with novel surface sediments showed rapid growth. The results indicate that the highly patchy distribution of juveniles in the harbour is not due to differences in the food quality of sediments but rather other localised effects. Juvenile *A. mollis* show an ability to exploit a variety of benthic sediment food sources, with growth rates comparable to other cultured species.

Juvenile *A. mollis* exhibited positive selectivity for organic particles both in situ and in the laboratory. Juveniles exhibited an ability to distinguish between different sediment TOM levels, an ability which became limited once TOM exceeded 3%. The results reiterate the specific nutrient recycling role of deposit-feeding sea cucumbers and may help explain the patchy distribution of juvenile sea cucumbers in heterogeneous habitats. In addition the research indicates that diet homogeneity and stability will be important in development of *A. mollis* artificial diets while a broad range of artificial diet grain sizes will be consumed. Juvenile *A. mollis* fed a high rate of mussel waste diet over three months exhibited significantly higher specific growth rate (SGR) than those fed natural sediment or algal diets. The results indicate the suitability of mussel waste as a juvenile diet and show that rapid growth can be expected among juvenile sea cucumbers cultured beneath mussel farms provided sea cucumber stocking rates are managed appropriately. Bioavailability of potential artificial diet ingredients was also systematically tested for juvenile *A. mollis*. Apparent digestibility was moderate for common carbohydrate sources and excellent for a range of artificial protein sources including low-cost protein sources. However, growth on artificial diets was poor and diet performance may need to be improved using fermentation steps or similar before they become viable. Rapid growth and flexible feeding preferences show *A. mollis* to be well suited to future aquaculture and polyculture.

Frontispiece

A kaka song infused summer breeze
washes our words -
the conversations we have
carried in our hearts over
distances measured in latitude
and time zones.

It carries them down
the pohutakawa studded hill side,
away from our seat that
is slowly reverting to moss
and the elements of grass and cicada melody,
over a ruffled aqua Pacific Ocean.

Memories, laughter, regrets
settle like fine dust on the water's surface.
Beneath,
schools of blue maomao dart
and whirl like blue summer streamers
drawing eastward with them these
spoken pieces of us,
past pastel black horizon-bound islands.

Untitled, 2008 by S. Fairweather

Acknowledgements

I owe a great deal to the many people who have helped and guided me over the past three years. First and foremost, heartfelt thanks must go to my primary supervisor Andrew Jeffs for his support and guidance throughout. I greatly appreciate the enormous amount of time and effort and help you have provided!

Thanks also to my secondary supervisor Mary Sewell for her help, time, excellent suggestions and directional guidance over the past three years and to Guy Carton for the first year of supervision and support at the beginning of my PhD research. Many thanks also to Richard Taylor for listening to my occasional musings and for very valuable input to analysis of several chapters and to Rich Ford my advisor.

Enormous thanks to Natalie Usmar for her help at the start of this research and allowing me to tag along on sampling trips. The surveying work would have been impossible without your help.

Thanks to the many people at the Leigh Marine Laboratory who have helped with advice and practical elements of my work: Dr. Rich Ford, Murray Birch, Peter Browne, Brady Doak, Brian Dobson, Jo Evans, John Atkins, Arthur Cozens, Malwenn Lassudrie, Charlie Bedford, Craig Radford, Richie Griffiths, Atsuko Fukunaga, Shahriman Ghazali, Leo Zamora, Jenny Stanley, Kat Subedar, Jenna Martin, Kathy Dunlop, Darren Parsons and Adam Cowles – the most over-qualified park ranger there will ever be, you are an inspiration...

Thanks to the Lord, for his gifts and care, and for constantly reminding me the value of humility.

To my office mates Denham and Alicia and to my dear friend Amy, thanks for putting up with me in the cramped pseudo-armpit that came to be our office. Thanks also to Amy's Mum for the ceaseless over-supply of lollies and slim jims. The day the doctors inform me of my sugar induced diabetes I shall think of you and smile.

Craig, Amy, Charlie, Shah, Adam, Richie, Atsuko, Rich, Emma, Nat, Agnes and Murray thank you all for your wonderful friendship.

Outside the lab, an enormous thanks to my family especially Mum, Dad, Kerry and Ethan for always being there and always supporting my endeavours, no matter how far-fetched they may seem. None of this would have happened without you. Lastly to Corinna - this thesis is for you.

This research was funded through a University of Auckland doctoral fellowship.

Table of Contents

List of Figures

List of Tables

Chapter 1: General Introduction

1.1 Sea cucumber exploitation

Sea cucumber is a high value seafood product that is exploited in wild fisheries and commercially aquacultured. Many of the commercially exploited species of sea cucumber are aspidochirote deposit-feeding species. Traditionally they are consumed as a food and as a dietary supplement in Asian traditional medicines or as extracts and tonics (e.g., gamat oil). Sea cucumber tissue and derivatives are now also used more widely around the world in dietary supplements. The global sea cucumber fishery catch peaked at 23,400 t in 2000 and total exports are valued at between $US56 and $US130 million annually (Ferdouse, 2004). High value species can fetch hundreds of NZ dollars per kg as end products if they are of the desired size and are appropriately processed. There is a global fishery for sea cucumber with the largest areas of production and fishery being the Northwest and Southwest Pacific Ocean. Aquaculture production is dominated by the rapidly developing pond culture of *Apostichopus japonicus* in mainland China (Chang and Yu, 2004; Chen, 2004). Around the world sea cucumber fisheries are more diverse. These range from artisanal fisheries, where tropical species are taken by hand in shallow sub-tidal areas or by free-divers, through to industrial scale fisheries using combined SCUBA/hookah diving and dredge fisheries for temperate species, such as *Parastichopus californicus* in Canada and the United States (Kinch, 2002). The aspidochirote sea cucumber *Australostichopus mollis* is a valuable seafood species found around most of New Zealand and parts of Australia. It is the focus of a burgeoning fishery and considerable research efforts towards commercial culture (Archer, 1996; Morgan, 2004; Slater and Carton, 2007; Maxwell et al., 2009; Stenton-Dozey and Heath, 2009).

Exploited sea cucumber populations have proven very susceptible to overfishing at both local and regional scales (Dalzell et al., 1996; Skewes et al., 2000; Toral-Granda and Martínez, 2000; Uthicke, 2000; Kinch, 2005; Uthicke and Conand, 2005). Extremely high consumer demand and good economic returns for fishers, combined with low capital input requirements for harvesting, mean that local populations of sea cucumbers can become rapidly depleted (Uthicke and Conand, 2005). Once depleted, sea cucumber populations take long periods of time and careful management to recover, if they recover at all (Purcell, 2004; Uthicke et al., 2004). Furthermore, many local populations in enclosed coastal waters may have limited recruitment, possibly due to self-seeding, and depleted broodstock numbers. These limitations, combined with strong “Allee” effects with breeding success falling as the density of populations decreases, may greatly reduce the rate of stock recovery (Uthicke, 2000; Uthicke and Benzie, 2001). Sea cucumber stock depletion also raises concerns regarding the ecological impact resulting from the loss of their detrital benthic feeding activities. Deposit-feeding sea cucumbers fulfil an important ecological role in driving nutrient fluxes at the sediment water interface and in physical movement and resorting of benthic material (Crozier, 1918; Bakus, 1973; Uthicke, 1999; 2001; Slater and Carton, 2009).

1.2 Sea cucumber culture and polyculture

Spawning and larval rearing methods for the temperate species *A. japonicus* were developed in the 1940's and 1950's and were initiated in reaction to overexploitation of stocks of these sea cucumbers in Japanese waters (Imai and Inaba, 1950). Mass production of viable juveniles has since been applied to coastal restocking and ranching projects in Japan since the 1980's (Imai and Inaba, 1950; Ito and Kitamura, 1997; Yanagisawa, 1998). This species is extensively cultured in single species pond culture in the People's Republic of China where production now reportedly exceeds 80,000 t annually (Chang and Yu, 2004; Chen, 2004).

Successful culture of *A. japonicus* required the development of new aquaculture methods in four key areas; spawning induction; larval rearing; settlement and metamorphosis; and juvenile rearing. The methods developed for each of these areas are apparently effective with only small adjustments for culture efforts and research on other species. Currently wild collected adults are spawned using thermal induction or water spray induction. Larvae are then tank-reared with appropriate provision of a microalgal diet and control of harpacticoid pests (Ito and Kitamura, 1997; Chang and Yu, 2004). Settlement is carried out on polyethylene plates or similar coated with a diatom film which also acts as a post-metamorphic diet for early juveniles (Ito and Kitamura, 1997). Finally, juveniles move from the settlement plates to soft sediment, and then rearing is carried out in nursery tanks, using a variety of supplement feeds, prior to release in restocking programmes or culture ponds (Imai and Inaba, 1950; Ito and Kitamura, 1997; Battaglene, 1999; Battaglene et al., 2002; Chang and Yu, 2004; Stenton-Dozey and Heath, 2009).

Several sea cucumber species other than *A. japonicus* are the focus of scientific and economic efforts towards development of successful restocking programmes and aquaculture

(James et al., 1994; Battaglene and Bell, 1999; Pitt and Duy, 2004; Purcell, 2004; Asha and Muthiah, 2006; Slater and Carton, 2007; Stenton-Dozey and Heath, 2009). Increasing consumer demand and decreasing wild supply has driven global pricing rapidly upwards. This has provided strong economic stimulus for the development of aquaculture of new species of sea cucumber. In addition, new stimulus to conserve wild populations has been provided by evidence of stock collapses and an increasing awareness of the important ecological role of sea cucumbers in their natural habitats (Crozier, 1918; Uthicke, 1999; 2001; Slater and Carton, 2009). Previous advances made in *A. japonicus* culture have formed the basis of research into closing the life cycle of several other sea cucumber species, in particular the high-value tropical species *Holothuria scabra* (James et al., 1988, 1994; Battaglene et al., 1999). However, advances in spawning and larval rearing of valuable tropical species in particular have yet to be translated into commercial culture on a scale approaching that of *A. japonicus* despite small scale farming as well as numerous releases of juveniles in restocking and stock enhancement projects (Battaglene, 1999; Battaglene et al., 1999; Tuwo, 2000; Dance et al., 2003; Purcell, 2004; Purcell and Simutoga, 2008).

1.3 Juvenile habitat current knowledge

Survival and growth of released or farm-stocked juveniles are key to success of restocking and culture programmes (Purcell, 2004; Purcell and Kirby, 2006). Knowledge of juvenile ecology and biology are invaluable in improving survival and growth rates of cultured animals, yet in situ research on juvenile sea cucumbers is limited (Shiel, 2004; James, 2005). The few studies on juveniles in situ have revealed that sea cucumbers have a general association with planar surfaces in the first 1 to 3 months after settlement followed by migration to soft substrate or complex substrate. During this substrate transition their diet changes from benthic biofilm to surface sediment, i.e., deposit-feeding (Cameron and Fankboner, 1989; Wiedemeyer, 1994; Ito and Kitamura, 1997; Battaglene et al., 1999; Mercier et al., 2000a; Yaqing et al., 2000; FAO, 2003). Juvenile sea cucumbers are highly cryptic, a trait which is maintained in some species to maturity. Juveniles are generally associated with complex substrate in shallower water than adult conspecifics (Choe, 1963; Mosher, 1980; Yingst, 1982; Hamel and Mercier, 1996; Mercier et al., 1999; Mercier et al., 2000a; Dance et al., 2003; Yamana et al., 2006). Juvenile and non-juvenile habitats often appear to be distinct and spatially separated (Cameron and Fankboner, 1989; Conand, 1993; Mercier et al., 2000b; FAO, 2003). However, there is only a small amount of habitat data available for only a few commercially valuable species. Overall, the ecology of juveniles, of the majority of sea cucumber species remains poorly understood, especially their habitat and diet preferences. Therefore, advancing our knowledge in this area is a priority for improving the management of wild populations and the performance of restocking and aquaculture initiatives (Shiel, 2004; James, 2005).

1.4 Juveniles in culture

While there are a limited number of studies of sea cucumber growth and survival in wild populations there is an increasing amount of research into cultured juvenile sea cucumbers (Battaglene et al., 1999; Tanaka, 2000; Dance et al., 2003; Purcell, 2004; Purcell et al., 2006b). Mortality rates of wild released juveniles have been shown to vary with the habitat type where juvenile sea cucumbers are released, although it remains unclear whether this is due to differences in rate of predation, lack of food, or other abiotic factors such as lack of shelter or excessive wave energy (Dance et al., 2003). Detachment, handling and transportation methods for juveniles can also affect survival (Tanaka, 2000; Purcell et al., 2006b). Survival of juvenile *A. japonicus* and *H. scabra* released into the wild and into culture ponds improves with increasing size of juveniles at release, lending weight to the argument for improved nursery on-growing (Tanaka, 2000; Pitt et al., 2004; Purcell and Simutoga, 2008).

Currently juveniles are on-grown in nursery ponds or tanks to a size which is considered suitable for release to the wild for restocking. Low-densities of juveniles can be on-grown in ponds with little or no additional feeding, although significantly improved growth can be achieved if supplementary diets such as macroalgae, macroalgal extract, or powdered microalgae are provided (Chen, 2004; Giraspy and Ivy, 2008). A broad range of potential diets have been tested for juvenile sea cucumber survival and growth, including dredged muds, as well as artificial feeds containing fish meal, waste plant crops, corn, beans and aquaculture waste (Huiling et al., 2004; Pitt and Duy, 2004; Yuan et al., 2006; Zhou et al., 2006; Liu et al., 2009; Maxwell et al., 2009; Qin et al., 2009). However, the approach to diet development for juvenile sea cucumbers has, in many senses, been ad hoc. There are no systematic studies available regarding the digestibility of specific diet ingredients that could be included in artificial feeds supplied to nursery-held juveniles. This constitutes a considerable hurdle to development of artificial diets optimised to the digestive capabilities and meeting the nutritional needs of juveniles.

1.5 Challenges for future polyculture

In addition to novel monoculture efforts, deposit-feeding sea cucumbers are excellent candidates for polyculture with commercial shellfish and finfish farms (Inui et al., 1991; Wu, 1995; Ahlgren, 1998; Kang et al., 2003; Pitt et al., 2004; Purcell et al., 2006a; Zhou et al., 2006; Slater and Carton, 2007; Stenton-Dozey, 2007; Qin et al., 2009). Ecologically beneficial polyculture of sea cucumbers with abalone, scallops, oysters, mussels, and shrimp has been successfully piloted and generally results in reductions in benthic organic enrichment and excellent sea cucumber growth (Kang et al., 2003; Zhou et al., 2006; Slater and Carton, 2007; Paltzat et al., 2008; Slater and Carton, 2009; Zheng et al., 2009). *Apostichopus japonicus* is reportedly grown in polyculture on a commercial scale with shrimp in land-based ponds in the People's Republic of China, although there is no information available regarding the ecological benefits of this practice, and co-culture with shrimp has proved to be problematic for other sea cucumber species (Yaqing et al., 2000; Bell et al., 2007). Polyculture of the New Zealand sea cucumber *A. mollis* has been piloted successfully and is the focus of significant on-going research effort (Slater and Carton, 2007; Stenton-Dozey and Heath, 2009). Sea cucumber ranching beneath the extensive areas of Greenshell mussel farms in New Zealand (total area now >11,000 hectares) has been suggested as the method best suited to current New Zealand aquaculture infrastructure and farming techniques (Slater, 2006). This method of culture will involve the hatchery production or wild collection of large numbers of juvenile sea cucumbers and their release or "seeding" to mussel farms where mussel biodeposits would be the main sea cucumber diet. The success of this aquaculture method requires determining the suitability of mussel biodeposits as a food source for the juveniles on release, and determining survival, potential growth rates and suitable stocking densities.

1.6 *Australostichopus mollis* fishery

Australostichopus mollis is the only common aspidochirote holothurian present in shallow New Zealand waters and is also a high value food species, returning up to NZ$30 kg^{-1} green weight if appropriately handled and processed (Pawson, 1970). There is a small existing fishery for *A. mollis* in New Zealand. In the North Island sea cucumbers are hand-collected by divers and supplied to the local market as live seafood (S. Kereopa, pers. comm.), and processed for supply to Asian export markets (A. Jeffs, A. McCully pers. comm.). In the South Island, fisheries existed in the Marlborough Sounds and Fiordland between 1991 and 2003, and a small fishery existed sporadically in Kaikoura, but exports rarely exceed a few tonnes of dried sea cucumber a year (Morgan and Archer, 1999). The *A. mollis* fishery is managed within New Zealand's quota management system (QMS). While the current annual catch still does not exhaust the available total allowable commercial catch limit around the country, the exploitation of quota in the North Island is expanding. There have been attempts to circumvent catch limitations, resulting in the Ministry of Fisheries implementing a rapid increase in species deemed value for sea cucumbers taken without quota over the past year (A. McCully pers. comm.). Developing functional and economically viable polyculture of *A. mollis* prior to a boom and apparently inevitable bust in the wild fishery would be a significant and positive development in management of a sea cucumber fishery (Conand, 2000; 2006). Global sea cucumber fisheries (and indeed many other fisheries) appear to exist within an overexploitation paradigm by which aquaculture is often considered impossible, unviable or simply not worthwhile unless a wild stock is already decimated. While it is often difficult for an aquaculture system to compete economically with a viable wild fishery, aquaculture areas in New Zealand present a competitive existing framework into which sea cucumber culture may be integrated.

1.7 *Australostichopus mollis* previous research

Originally classified as *Stichopus mollis* the species has recently been assigned to the new genus *Australostichopus* on the basis of its unique glycoside component (Moraes et al., 2004). This deposit-feeding sea cucumber is distributed around the entire New Zealand coastline and associated islands, and parts of southern Australia. It inhabits a wide range of depths and a broad variety of habitats ranging from rocky reefs to mudflats (Pawson, 1970). In northern New Zealand, *A. mollis* is observed in waters between 2-25 m and is predominantly observed in association with reef structures, although this is likely due to the low frequency of dive searches in mudflat areas (Sewell, 1990). It is usually seen in crevices or depressions within the reef where deposited or collected sediments accumulate (Sewell, 1990). Mladenov and Campbell (1998) reported densities of *A. mollis* of up to 2.5 ind m^{-2} in Fiordland, New Zealand, although reported densities are lower in more recent research in the same fiord area (Wing et al., 2008). Slater and Carton (2007) found densities of this species in the North Island to be highly variable and to correlate strongly with the rate of sedimentation, with natural reef densities occasionally exceeding 1 ind m^{-2}. *Australostichopus mollis* feeds on nutrient-rich sediments, presumably assimilating food value from detrital matter, bacteria and possibly decomposed macroalgae from within the sediment (Wing et al., 2008). It is the largest deposit feeder commonly found in New Zealand coastal waters. It is unknown whether the species exhibits any form of selective feeding or selective movement based on organic content, grain size or other sediment characteristics, however, adult *A. mollis* densities are higher where rates of sedimentation are high and adults exhibit high growth rates when exposed to organically enriched sediments (Slater and Carton, 2007).

Populations of *A. mollis* exhibit a 1:1 sex-ratio and a uni-modal size-frequency distribution from 13-25cm (Sewell, 1990) and mean size at first sexual maturity is 91g wet weight (Raj, 1998). No common predators of *A. mollis* are known, although predation by the asteroids *Luidia varia* and *Coscinasterias muricata* has been observed (Sewell, 1990). *Australostichopus mollis* colouring is permanent and exhibits unique patterns allowing photo-identification (Raj, 1997). The elongate body is covered with raised papillae on the dorsal surface. The ventral surface is dominated by locomotory podia. The mouth is anterior and ventral, with shield-like buccal tentacles surrounding the mouth opening, the anus is terminal (Sewell, 1987; Moraes et al., 2004). The dermis is spiculated with regular tables, while irregular plates and rods occur in the tentacles, podia, pedicels and in mesenteries of internal organs (Sewell, 1987). The general anatomy of *A. mollis* is typical of the Stichopodidae with some variation in the structure of the calcareous ring and a singular polian vesicle (Sewell, 1987). Buccal tentacles (typically 20) surround the mouth, the calcareous ring forms an attachment surface for muscles controlling the tentacles and for the large longitudinal muscles used in body movement and contraction. The mouth extends through to the stomach which is indistinct from the beginnings of the small intestine, the small intestine continues into the large intestine, terminating at the cloaca. The intestines are associated with haemal vessel networks linked to the respiratory trees via the respiratory-haemal plexus. The right and left respiratory trees extend from the cloaca along the body cavity. The gonads of both sexes are similar in appearance; the gonad is anchored in the mesentery and extends as tubules from the anterior end of the animal. The gonad becomes enlarged and increasingly branched as maturity is reached prior to the annual spawning period, the gonad tubules are completely reabsorbed following spawning (Sewell, 1992).

Sewell (1987) reported an annual reproductive cycle with a peak during the summer period, with synchronous gonad development in both sexes. Archer (1996) was unable to induce gonad maturation or spawning with a range of methods and collected wild-spawned eggs and sperm for laboratory analysis. Egg size is similar to comparable aspidochirote species at approximately 180 µm, while time to settlement is slightly longer than comparable species at 25 days. Archer (1996) described an indirectly developing planktonic larva, with an embryo developing into a feeding auricularia, followed by a non-feeding doliolaria which settles to the benthos as a pentactula and ultimately a benthic juvenile.

Thermal shock treatment has been successfully used to induce spawning in *A. mollis* with fully developed gonads, as determined by observation of oocyte germinal vesicle breakdown, gonad colour and movement of motile spermatozoa (Morgan, 2009). The periodicity of spawning was shown to be loosely correlated with lunar phase, but could only be reliably predicted with monitoring of reproductive maturity in the form of gonad biopsy followed by gonad index estimates. Morgan (2004) showed that hatchery raised larvae are susceptible to overfeeding due to an inability to control gut passage rates, and suggested maternal origin factors may be important in predicting successful larval cycle completion. Survival of larvae beyond settlement has recently been reported by the National Institute for Water and Atmospheric Research using methods developed for *A. japonicus* larval rearing, although current on-growing methods produced extremely variable results and are reportedly incapable of producing commercially viable juvenile growth (Stenton-Dozey and Heath, 2009, P. Heath pers. comm.).

Slater and Carton (2007) showed *A. mollis* survives and grows when caged below mussel farms at densities exceeding those observed in natural reef conditions. Adult *A. mollis* were also shown to readily consume sediment collected from directly beneath mussel farms, with growth of adults averaging a modest 0.07g day^{-1} over six months in cage experiments at mussel farms. This growth rate increased to 0.19g day^{-1} in tank experiments when adults were fed an excess of mussel farm sediment (Slater, 2006). Feeding rates for adults were estimated at 6.7g wet weight sediment per day, although feeding rates were observed to be highly variable and the measured rates were considered to be an underestimate (Slater, 2006). Slater (2007) also showed that *A. mollis* feeding reduces the organic carbon content and phaeopigment content of mussel farm impacted sediments. Maxwell et al. (2009) calculated energy budgets for *A. mollis* fed artificial abalone diet and abalone waste from artificial and natural diets and found organically rich abalone waste may be suitable for on-growing small sea cucumbers. A three week study of the growth of caged *A. mollis* under kingfish (*Seriola lalandi*) farms in the Marlborough Sounds, New Zealand, provided inconclusive growth results but reported very high densities of naturally occurring *A. mollis* in association with salmon farms (Fern, 2006).

1.8 Juvenile *Australostichopus mollis*

The literature contains no data regarding the juvenile biology or feeding behaviour of *A. mollis*. Sewell (1987) collected three juveniles between 1.8 and 4 gm in weight but the juveniles did not gain weight in laboratory growth experiments. Observations of juveniles are extremely limited, although some juvenile *A. mollis* have been encountered under boulders and in crevices in the same depth range and sites as adult specimens. Juveniles have also been reported to be present under boulders and rocks in shallower areas adjacent to subtidal areas where adults are present (Sewell, 1987; 1990; Mladenov and Gerring, 1991). It remains unclear whether juveniles are simply not present in areas where large numbers of adult *A. mollis* are found or whether juveniles are simply not observed due to their small size or cryptic behaviour. Addressing the lack of knowledge regarding juvenile *A. mollis* ecology and feeding biology will not only help further understanding of the ecological role of sea cucumbers in the marine environment it will also directly support the development of the species for future aquaculture.

1.9 Current research aims

The current research specifically aims to address a number of limitations in biological knowledge which need to be overcome in order to develop successful *A. mollis* culture and polyculture.

(1) The natural habitat of juvenile *A. mollis* remains poorly understood. The first chapter of this thesis provides a thorough description of juvenile *A. mollis* distribution, densities and associated habitat in the Mahurangi Harbour, a small 25 km^2 shallow harbour in northeastern New Zealand. Beam trawls, followed by SCUBA surveying and sampling of sediments describes the density of juvenile *A. mollis* within the diversity of benthic habitats found throughout the harbour. Sediment sampling and assaying provide a profile of sediment

and other abiotic habitat characteristics which are associated with juvenile *A. mollis* natural habitat and which may favour *A. mollis* settlement, growth and survival.

(2) Once the characteristics of juvenile sea cucumber habitats are determined, this research attempts to identify the factors determining the spatial distribution of *A. mollis*. The spatially variable presence of post settlement juveniles may be due to differences in survival due to the availability of suitable sediment diets, the supply of settling juveniles or behavioural aspects. The second chapter of this thesis aims to gain an insight into the effect of available sediment diets on juvenile distribution by exposing juvenile *A. mollis* to novel sediments. Transplantation experiments enable comparisons of juvenile survival and growth at sites where juveniles are present and where they are naturally absent. This helps to determine if spatial differences in food availability or quality are influencing the spatial distribution of juvenile sea cucumbers. By monitoring the transplantation experiments over a year the study also provides the first long-term growth rate estimates for juvenile *A. mollis* in situ.

(3) Juvenile *A. mollis* biology is also scantly described, in particular with regard to feeding behaviour in response to grain size and organic content of sediments. In addition to improving the understanding of juvenile biology, information about feeding behaviour can aid the development of juvenile diets and on-growing methods for *A. mollis* aquaculture. Juvenile *A. mollis* may change their position within their habitat in response to availability of organic material for deposit-feeding, and/or they may feed selectively within the habitat in which they reside. The aim of the third chapter of this thesis is to determine the level of feeding selectivity of juvenile *A. mollis* in situ and in controlled laboratory feeding experiments. Feeding selectivity for grain size and organic matter content of sediment by juvenile sea cucumbers is determined using methods which build on earlier work on aspidochirote selectivity, whilst also attempting to overcome some of the uncertainties associated with these methods. This chapter determines whether juvenile *A. mollis* can selectively feed on specific grain sizes from their sediment diets and/or whether they can selectively feed on the organic content from the sediment. In addition, the ability of juvenile *A. mollis* to adjust position within their environment based on variation in organic content of sediment is determined in a series of simple laboratory experiments.

(4) Proposed polyculture will depend on the palatability and growth performance of diets that juvenile *A. mollis* will encounter when co-cultured with other aquaculture species, such as green-lipped mussels. It is necessary to test the dietary value of fresh mussel faeces

for juvenile *A. mollis*. This is determined in the fourth chapter of this thesis by following the growth and survivorship of juvenile sea cucumber in the laboratory over three months while they are fed a range of natural, artificial and polyculture diets including varying amounts of green-lipped mussel waste. The resulting data can reveal the suitability of waste from green-lipped mussels as a juvenile sea cucumber diet, and allow the calculation of expected growth rates and optimum stocking densities below mussel farms to ensure high sea cucumber yields.

(5) The digestibility and growth performance of potential artificial diet ingredients is unknown for juvenile *A. mollis*. Determining effective artificial diet ingredients for rapid on-growing of hatchery-produced juveniles will be a key to improving the future culture of the species. The fifth chapter of this thesis develops methods to determine the apparent digestibility of carbohydrate and proteins commonly used in artificial aquaculture diets. An initial selection of such carbohydrate and protein sources is experimentally examined for both digestibility and growth performance in juvenile sea cucumbers. Palatable diets are developed that will help with the process for identifying effective artificial diet constituents for future diet formulation. The performance of artificial diets in growth experiments also allows their evaluation as potential on-growing diets for juveniles prior to release and as supplement feeds for possible pond culture.

Overall, the research presented in this thesis aims to provide clear insights into the ecology, distribution and feeding biology of juvenile *A. mollis*. From an aquaculture perspective the research presented within this thesis will describe feeding behaviour, dietary requirements, assay methods for potential ingredients for artificial diets, feeding rates, and provide basic juvenile holding parameters that are sufficient to enable the successful on-growing of hatchery-produced juvenile *A. mollis* in nursery ponds or tanks. The research describing juvenile sea cucumber habitat can also be used to ensure that successful nursery rearing can be followed by release of animals to suitable habitats or suitable culture environs including polyculture units. Thus, if combined with successful hatchery production, the data in this thesis will significantly contribute to fulfilling the requirements for *A. mollis* culture or polyculture programmes. Beyond these aquaculture applications, the data in this thesis will also contribute to our understanding of *A. mollis* biology and ecology and provide a description of juvenile habitat which allows recruitment areas to be defined and suitably protected within a burgeoning fishery for an ecologically important benthic species.

Chapter 2: Highly Localised Distribution Patterns of Juvenile *Australostichopus mollis*

Revised version submitted for publication as: Slater, M.J., Carton A.G. , Jeffs A.G. Highly localised distribution patterns of juvenile sea cucumber *Australostichopus mollis*, New Zealand Journal of Marine and Freshwater Research.

2.1 Introduction

Many deposit-feeding sea cucumber species are heavily fished and exhibit rapid depletion in fished areas followed by slow population recovery (Skewes et al., 2000; Kinch, 2002; Uthicke et al., 2004; Hearn et al., 2005; Conand, 2006). Effective stock management is often limited, among other things, by a dearth of knowledge of juvenile habitat preferences and ecology. Such knowledge is essential to ensure important juvenile habitats can be effectively protected and juvenile stocks conserved. The majority of deposit-feeding sea cucumber species are seldom observed as juveniles in their natural habitat and descriptions of juvenile habitats are often limited to anecdotal information or chance observations revealing little more than depth, physical complexity of habitat and proximity to areas of high adult densities (Shiel, 2004; James, 2005). Nonetheless, chance events or intensive surveying have facilitated studies of a few deposit-feeding sea cucumber species *in situ* (Cameron and Fankboner, 1989; Wiedemeyer, 1994; Mercier et al., 2000b; Yamana et al., 2006). In addition, nursery research and experiments involving the release of nursery-produced juveniles provide some information on juvenile sea cucumber survival in varying habitats (Battaglene, 1999; Tanaka, 2000; Dance et al., 2003; Purcell, 2004; Dong and Dong, 2006).

Both in the hatchery and in the natural environment, pentactula juvenile sea cucumbers show a preference to settle to planar surfaces or macroalgae with an associated bio-film, as opposed to soft sediment or complex substrates (Cameron and Fankboner, 1989; Wiedemeyer, 1994; Ito and Kitamura, 1997; Battaglene et al., 1999; Mercier et al., 2000a; Toral-Granda et al., 2008). This solid substrate is abandoned, generally within 3 months, at which time juveniles migrate to adjacent complex or soft sediment substrate to begin feeding on particulates rather than biofilms or diatom films (Mercier et al., 2000a; Yaqing et al., 2000; Purcell, 2004). The size at which juveniles move away from the settlement substrate varies widely between species and early forced migration from settlement substrate to alternative substrates negatively affects survival and growth (Wiedemeyer, 1994; Battaglene et al., 1999; Mercier et al., 2000a; Purcell, 2004; Purcell and Simutoga, 2008). Migration from solid substrate appears to be primarily due to ontogenetic diet change as it does not correspond with any recorded increase in ability to avoid predation by size or biochemical composition.

Juveniles remain highly cryptic in their behaviour after migration presumably as a predator avoidance mechanism (Hamel and Mercier, 1996; Mercier et al., 1999; Mercier et al., 2000a; Hamel et al., 2001; Dance et al., 2003). They are generally described in association with complex structure, frequently macroalgae, but also mangrove branches, the underside of rocks and coral fragments - as opposed to adults selecting a more exposed habitat (Choe, 1963; Mosher, 1980; Yingst, 1982; Yamana et al., 2006). Yamana et al. (2006) describe high densities (6.7 m^{-2}) of juvenile *Apostichopus japonicus* in the intertidal in association with large amounts of the macroalgae *Sargassum* spp. and *Ulva* spp., moderate eutrophication and protection from 'environmental fluctuation' (desiccation and high wave energy) during low tide. In addition to an association with complex structure, there is frequently, but not exclusively, a distinct spatial separation between juvenile and non-juvenile habitats with most deposit-feeding sea cucumber species exhibiting movement from shallow to deeper water as they increase in size from juvenile to adult (Cameron and Fankboner, 1989; Conand, 1993; Mercier et al., 2000b; Hamel et al., 2001).

The juvenile distribution, habitat preferences and ecological requirements of the Australasian sea cucumber *Australostichopus mollis* are poorly understood and observations of juveniles are uncommon. Sewell (1987) reported that there is no depth-correlation in the size distribution of *A. mollis*. Isolated individuals have been encountered under boulders and in crevices in the same depth range and sites as adult specimens (pers. obs.) and three juveniles have previously been collected from under boulders adjacent to adult habitats (Sewell, 1990; Mladenov and Gerring, 1991). Increasing fishing pressure and the obvious potential of *A. mollis* for aquaculture in New Zealand and Australia have recently stimulated increased research interest. In particular, research is focussed on larval biology and aquaculture methods with the aim of fulfilling the requirements for successful breeding and nursery rearing for developing commercial culture (Slater and Carton, 2007; Slater et al., 2009; Stenton-Dozey and Heath, 2009). Nonetheless, the almost complete lack of knowledge of juvenile habitat remains a significant shortfall in understanding of this species. As with many other commercially important deposit-feeding sea cucumber species, the efficacy of *A. mollis* stock management, aquaculture and stock enhancement efforts will be greatly increased by improving knowledge of juvenile habitat requirements.

The current research investigates anecdotal reports and uses multiple scales of survey data from a harbour in northern New Zealand to refine the search for *A. mollis* juvenile habitat. The juvenile density data collected, combined with descriptions of sediment facies and sediment characteristics determined in physicochemical assays will allow us to test several null hypotheses formed on the basis of data presented for other deposit-feeding sea cucumber species: $H_0$1) that juvenile *A. mollis* habitat does not differ from adult conspecifics as observed in other sea cucumber species, $H_0$2) that juvenile *A. mollis* does not exhibit association with complex substrates and/or macroalgae also as observed in other species, and $H_0$3) that depth does not correlate with the size (juvenile to adult) of *A. mollis.*

2.2 Methods

2.2.1 Juvenile distribution surveying

Beam trawls

The current study was conducted in the Mahurangi Harbour, a 25 km^2 shallow harbour on the east coast of the North Island of New Zealand (36° 27' 0 S, 174° 43' 0 E). The harbour was selected because of the reported high densities of *A. mollis* and availability of data from an extensive beam trawl survey covering all habitat types in the Mahurangi Harbour for juvenile fishes. Trawls sampling 600 m^2 per trawl had been carried out using a beam trawl net (4 m wide beam with 3 m net opening, 6 m net length, 9 mm net mesh, 200 m tow length) towed by a shallow draught research vessel. Surveys were conducted on a quarterly basis between December 2005 and March 2007 with 36 or 48 trawls carried out per survey (total 252 trawls). Recorded data provided trawl position, trawl depth (1 to 18.5 m) and total number of adult sea cucumbers caught per trawl. Where sea cucumber numbers exceeded 50 per trawl (i.e., catch density of >0.08 m^{-2}), sites were plotted on a digital map to create an overview of high densities of sea cucumbers (Figure 2.1). The March 2007 sampling event was the only set of trawls set that were available for sampling sea cucumbers and therefore the contents of catches from this beam trawl survey (36 trawls) were analysed for presence of sub-adult or juvenile sea cucumbers. The location of sites where any sea cucumbers under 7 cm (juvenile) in length were captured was recorded and used to identify suitable locations for subsequent high detail SCUBA transects sampling for juvenile densities and a range of habitat parameters. The maximum length for juveniles (<7 cm) corresponds to an average weight of less than 40 g and the size at first sexual maturity for this species which is in excess of 90 g (Raj, 1998). A total of nine sites were selected by three criteria. Firstly, sites were selected on the basis of beam trawl results for the presence of juveniles (any site where juveniles were captured was included). Secondly, sites were selected where adult sea cucumber densities were in excess of 0.1 m^{-2}. Thirdly, additional sites were included in areas where adult density was low and no juveniles were encountered in beam trawls. This third group of sites ensures the inclusion of sampling in all benthic habitat types within the harbour; sand, subtidal mud, sand, shell hash and horse mussel dominated facies (Morrison, 2000).

SCUBA transects

A series of three 50 m^2 SCUBA transects was then conducted at each of the nine sites within the Mahurangi Harbour in December 2007 and January 2008 (Sites A-I, Figure 1). Transects were conducted by a SCUBA diver searching for animals 1 m either side of a 25 m transect tape laid in a straight line along a randomly selected compass bearing. The start of each transect was selected at a random point within a 5 m radius of the anchor of the diver support vessel. The body length and position along the transect of any *A. mollis* found by a diver was measured and all animals 7 cm or less in length were recorded as juveniles. In addition, the depth, transect bearing and a sediment facies description was recorded for each transect. The presence or absence of adult sea cucumbers was recorded, as was the presence of any macroalgal growth. In May 2008 an additional set of three transects was conducted at the highest density juvenile site (D) to determine adult density and population structure at the site. The lengths of all *A. mollis* encountered 1 m either side of a 25 m tape were recorded.

2.2.2 Surface sediments

Surface sediment samples (approx 10 g WW) were taken at 5, 15 and 25 m along each transect adjacent to the tape. Samples were taken by inserting a 30 ml plastic vial 2-3 mm into the sediment surface and scraping the vial sideways to fill it with surface sediment. Vials were sealed and taken to the surface at the end of the transect dive. Samples were stored in a sealed chiller bin on the boat and returned to the laboratory within 2 h. Samples were divided into three approximately equal sub-samples in the laboratory by twice drawing off approximately one third of each sample into clean marked vials from the original sample whilst constantly shaking. All sub-samples were then wrapped in aluminium foil to protect them from light and stored at -80°C until their use in physicochemical assays.

Elemental carbon and nitrogen

Surface sediment sub-samples (approx 0.5 g) were dried in a 60°C oven to constant weight (48 hrs). Dried samples were sieved over a 355 µm sieve to remove shell fragments, ball ground and stored in 1.5 ml Eppendorf tubes before being analysed for total carbon and nitrogen using a Leco TruSpec elemental determinator.

Phytopigment content

Chlorophyll-*a* and phaeopigment were determined using a variation of the spectrophotometric method initially described by Lorenzen (1967). Sub-samples (1 g WW of sediment) were extracted with 100% acetone (1.8 ml) and stored overnight at 4°C. Distilled water (0.2 ml) was then added and the sample centrifuged at 4°C for 9 min at 3000 rpm. Extinction of 1 ml of the supernatant was measured at 665 and 750 nm against a 90% acetone reference. HCl (40 µl 10%) was then added to the supernatant for 4 min. Extinction of the acidified supernatant was then measured at 665 and 750 nm against a 90% acetone reference (mean of three repeat measurements). Samples were then dried at 80°C for 48 h and weighed. Chlorophyll-*a* and phaeopigment concentrations were calculated as follows:

Chlorophyll-*a* µg/g = [(AK(665o-665a)v)/Vl]
Phaeopigment µg/g = [(AK((R*665a)-665o)v)/Vl]

Where:
A = 11.0µg, the inverse extinction coefficient for chlorophyll-*a* in 90% acetone
R = 1.7, maximum absorbance ratio for 665o/665a without phaeopigment
K = 2.43
665o and 665a are the absorbances before and after acidification respectively
v = volume of acetone used in the extraction in ml
V = dry sediment weight in grams
l = cuvette pathlength

Grain size distribution

Surface sediment sub-samples (approx. 1.5 g WW of sediment) were treated for 4 h in a sodium metaphosphate solution (5 g l^{-1}) to de-clump clay particles. Grain size distribution was then examined using a Malvern Mastersizer 2000 Particle Size Analyser using laser and blue light diffraction detection to determine sample grain size distribution (mean of three repeat measurements). Particles were grouped into six size ranges: 0 - 63 µm, 64 - 125 µm, 126 - 250 µm, 251 - 500 µm, 501 - 1000 µm and 1001 – 2000 µm.

2.2.3 Statistical analyses

All sediment chemistry data were averaged by transect to simplify analyses, in particular for the correlation with number of juveniles present. Data for each measure were log transformed where necessary to fulfil ANOVA assumptions and analysed for significant differences between sites using a one-way ANOVA. A Tukey HSD test post hoc analysis ($\alpha = 0.05$) was carried out on significant ANOVA results to identify pair-wise significant differences between site means. For each physicochemical characteristic of the sediment (including site depth) a rectangular matrix correlation analysis was used to test for significant correlation with the juvenile density. In addition, following the normalisation of variables, resemblance matrices were created for all sediment physicochemical characteristics (including site depth) measured for each transect on the basis of Euclidean distance. Multidimensional scaling analysis was used to plot transect similarity by multiple factors. Eigenvector values were calculated using Spearman rank correlation coefficients to reveal the direction and scale of effect of individual variables on distribution of transects in multivariate space.

2.3 Results

2.3.1 Juvenile distribution

Analysis of all existing beam trawl data indicated the highest densities of adult sea cucumbers were centred on a region in the mid-harbour although high numbers also extended to near the harbour mouth (Figure 2.1). Analysis of March 2007 beam trawl catches, which were directly observed, showed that five juvenile sea cucumbers were captured in a single trawl. The location of the trawl site was accordingly included in sites selected for SCUBA transects (site D). SCUBA transect results confirmed the pilot beam trawl results including the presence of juvenile *A. mollis* at site D (Figure 2.1). This area of relatively high density (0.1 - 0.18 m^{-2}) of juvenile sea cucumbers is in mid-harbour in 11-12 m of water on a silt and mud dominated facies with occasional large horse mussel shell fragments (*Atrina zelandica*, shell >10 cm length). The site also had a high density of adult *A. mollis* (0.72 – 1.14 m^{-2} Figure 2.2). Juveniles were also encountered at much lower densities (<0.05 m^{-2}) in three further transects at site F and site H.

None of the sites surveyed by SCUBA exhibited macroalgal growth although very occasional macroalgal debris was observed at sites D to I (mid-harbour to harbour mouth). Juveniles were situated on the sediment surface and frequently in small holes and gaps formed by large shell fragments. The size of juveniles ranged from 3 to 7 cm length. There was no obvious single year cohort structure to the population at site D. Sea cucumber length at the site ranged from 3 to 17 cm with a mean size of approximately 11 cm length.

2.3.2 Surface sediment characteristics

One-way ANOVA analyses revealed significant differences amongst sites for all of the sediment chemistry measures. Post-hoc analyses revealed complex groupings with the high density juvenile site D located in homogenous groups (Tukey HSD $\alpha > 0.05$) with downstream sites G, F, H or I for all measures apart from phaeopigment content (Figure 2.3). Elemental carbon content of surface sediments ranged from 0.59% C (± 0.03 SE) at site E to 1.82% C (± 0.08 SE) at site I, and mean nitrogen content ranged from 0.11% N (± 0.002 SE) at site E through to 0.18% N (± 0.002 SE) at site D.

Phytopigment values from the sampled sediments were highly variable between sites (Figure 2.3). Chlorophyll-*a* was lowest at site D (3.7 µg chl*a* g^{-1} sediment ± 0.6 SE) while site E had both the highest sediment chlorophyll-*a* content (10.2 µg chl*a* g^{-1} sediment ± 0.5 SE) and the lowest phaeopigment concentration (2.53 µg phaeopigment g^{-1} ± 0.2 SE). Site H exhibited the highest mean phaeopigment concentration (6.37 µg phaeopigment g^{-1} sediment ± 0.43 SE). The phaeopigment to chlorophyll-*a* ratio was also highly variable. Site D exhibited the highest value and was significantly different from all sites except site B (Tukey HSD $\alpha < 0.05$).

Sites H and D were shown to be silt and clay dominated (0-63 µm) with a further large proportion of very fine sand (64-125 µm) (Figure 2.4). Sites F, G, B and C exhibited a more equal proportioning between silt and very fine sand with increased fine sand content at site C. Sites I, A and E had a high proportion of silt, but with a higher content of medium sand and coarse sand.

2.3.3 Correlation of site characteristics with juvenile density

Total nitrogen, phaeopigment/chlorophyll-*a* ratio, depth and grain size analysis (GSA) silt fraction all exhibited significant positive correlations with increasing number of juveniles (Table 2.1). Chlorophyll-*a* content was the only factor that exhibited a significant negative correlation with increasing juvenile numbers (Table 2.1). A multidimensional scaling plot of all sediment characteristics showed strong grouping (Euclidean distance 3.8) among transects at sites D and H where juveniles were encountered (Figure 2.5). The eigenvector plot shows a strong positive MDS y contribution for silt content and nitrogen content as opposed to fine sand content which is strongly negative (Figure 2.6). The MDS x positive consists approximately equally of phaeopigment content, phaeopigment:chlorophyll-*a* ratio, depth and carbon content with a strong negative contribution by chlorophyll-*a* content.

2.4 Discussion

The recruitment processes of many commercially important deposit-feeding sea cucumber species remain poorly understood due to a lack of knowledge of juvenile habitat choices (Shiel, 2004; James, 2005). The current research describes the highly localised distribution of juveniles of the sea cucumber *A. mollis* associated with specific habitat and sediment characteristics. Nine sites which included all predominant benthic habitat types within the Mahurangi Harbour were selected to be surveyed. The three sites where juvenile sea cucumbers were encountered shared similar grain size distributions, high phaeopigment concentrations and high nitrogen content compared to all other sites surveyed. Multidimensional scaling plots of sediment characteristics also closely grouped all of the sites where juveniles were encountered - sites D, H and F (Figure 2.5). Site D is of particular interest due to the presence of much higher density of juveniles than any other site surveyed (0.1 – 0.2 m^{-2}). It differs most strongly from all other sites in its high nitrogen content, low chlorophyll-*a* and high phaeopigment:chlorophyll-*a* ratio. Multidimensional scaling plots tightly grouped transects in which juveniles were present with the exception of one transect at site F (Figure 2.5). Silt and clay content, nitrogen content, increasing depth, phaeopigment content and phaeopigment:chlorophyll-*a* ratio show positive eigenvector contributions towards increasing juvenile densities (Figure 2.6). This supports the correlation results for the individual physiochemical variables in relation to juvenile density, with the exception of phaeopigment.

Overall, these results appear to indicate a superiority of food value for detritivores at the sites where juveniles are present, in particular site D. The smaller grain size profile of the sediment at site D is likely to offer high food quality not only through associated bacterial activity, but also through the surface-rich nature of smaller grains with an associated organic monolayer offering a rich food source (Mayer et al., 1985; Yamamoto and Lopez, 1985). Conversely, the high content of nitrogen, carbon and phaeopigment in sediment at site D in comparison to most other sites surveyed indicates considerable allochthonous inputs including algal detrital and terrigenous detrital input. The low chlorophyll-*a* content of sediment at site D shows comparatively low benthic primary productivity, which is logical given the depth (11-12 m), high turbidity and low sediment incident light levels encountered at this site (Gibbs et al., 2005). Yet high chlorophyll *a* content has been shown to be preferable in food sediments for certain tropical species (Uthicke and Karez, 1999). It is however probable that *A. mollis*, which inhabits waters to depths exceeding 200 m exhibits more distinctly detritivorous feeding preferences than shallow-water tropical species (Pawson, 1970). The high phaeopigment:chlorophyll-*a* ratio at site D is indicative of a more advanced degradative state of particulate organic matter reaching the sediment, but may also indicate high rates of grazing at the sediment surface (Welschmeyer and Lorenzen, 1985). High nitrogen content at sites D and H in comparison to other sediments within the harbour may also indicate higher bacterial activity in sediments and increased availability of amorphous (fine) sedimentary organic matter content as a result of physical, bacterial and fungal decomposition (Lopez, 1987). The increased decomposition and bacterial content of both terrigenous and macroalgal detritus greatly improves the digestibility of organic matter to holothurians (Yingst, 1976). Bacterial content is selectively consumed by several tropical aspidochirote species (Moriarty, 1982). Bacterial carbon itself, however, is unlikely to be the primary carbon source in the sediment for detritivores, but rather the amorphous aggregated sedimentary organic matter resulting from bacterial digestion and exoenzyme activity (Lopez, 1987; Nealson, 1997).

The subtidal macrofauna in Mahurangi Harbour is dominated by detritivorous species *Theora lubrica* along with oligochaetes and the polychaete *Pironospio* sp. (Ellis et al., 2006). However, species diversity is significantly lower in the subtidal Mahurangi Harbour than in the intertidal and in comparable harbours in north-eastern New Zealand (Ellis et al., 2006). The density of detritivorous macrofauna is only 25% that of intertidal sites in the Mahurangi Harbour and in comparable harbours despite high organic content and suspended sediments (Gibbs et al., 2005; Ellis et al., 2006). Juvenile sea cucumbers at site D face markedly lower competition with other detritivores for surface sediment organic. Alternatively, large numbers of juvenile and adult sea cucumbers feeding on the available sediment detritus may simply be excluding other detritivores from the site.

These results show a strong association of juvenile density with specific sediment and facies characteristics. Distribution and density of juveniles correlates well with increasing organic matter availability in diet sediments as reported previously for adult sea cucumbers in sandy or muddy environments (Massin, 1982; Sibuet, 1984). However, they do not exclude the role of other potential factors determining juvenile distribution such as the hydrodynamics influencing larval distribution, which have been shown to concentrate larvae of other benthic species in mid-harbour within the Mahurangi Harbour, settlement surface preferences, or the influence of early juvenile mortality as a result of predation or other unknown environmental factors (Harrison, 1974; Martin and Foster, 1986; Osman et al., 1992; Gosselin and Qian, 1997). Equally the stochastic settlement and transport of larvae cannot be fully discounted as a cause for the disjunct distribution, despite the observation and collection of large numbers of juveniles in the same location over three consecutive years of settlement events.

Previous studies have reported a distinct separation between juvenile deposit-feeding sea cucumbers and adult populations (Cameron and Fankboner, 1989; Mercier et al., 2000a). In comparison, we found large numbers of adult sea cucumbers in the area where juveniles were present (Figure 2.2). Despite there being no distinct separation between the two size groups, there is a size-related element to distribution of *A. mollis* within the harbour, with adults present in considerable numbers in several areas where juveniles are not present. The separation of juveniles and adults of deposit-feeding sea cucumber species has frequently been described as following a depth gradient with juveniles in shallow waters or even the intertidal (Bulteel et al., 1992; Conand, 1993; Hamel and Mercier, 1996; Conand, 2006; Yamana et al., 2006). The current research shows an opposite trend, with increasing juvenile density positively correlated to increasing depth, with large numbers of *A. mollis* within one of the deepest areas of mid-harbour and absent in considerably shallower adjacent areas.

Holothuria scabra associates with seagrass at settlement and early post-settlement, while *P. californicus* is associated with filamentous algal mats and *A. japonicus* is found considerably later in juvenile development associated with the seagrass *Zostera japonica,* and the macroalgae *Ulva* spp. and *Sargassum* spp. (Choe, 1963; Cameron and Fankboner, 1989; Mercier et al., 2000b; Yamana et al., 2006). The association of juvenile *A. japonicus* with seagrass and macroalgae was thought to be due to the availability of algal detritus as a food source (Yamana et al. 2006), while cryptic behaviour of juvenile *P. californicus* in algal mats was interpreted as predator avoidance (Cameron and Fankboner 1989). *Holothuria scabra* settles directly to seagrass blades before migrating to sediment at about 10 mm in length (Mercier et al., 2000a). The area of high juvenile *A. mollis* abundance found in this study has no macroalgae and there is minimal macroalgae present in the adjacent intertidal areas. The lack of macroalgae negates the likelihood of *A. mollis* settling to macroalgae at site D. Clumps of macroalgal detritus were periodically observed being swept through the area by tidal water movement during transect dives, but it is unclear whether these make a significant contribution as a detrital food supply and/or settlement habitat.

If juveniles are settling directly at site D, the most likely settlement surface appears to be shell fragments of the large horse mussel (*Atrina zelandica*) which is prevalent at the site, as they represent the only solid planar surface available. Juveniles were often found in contact with larger shell fragments, either fully enclosed within fragments, or at least with part of the body attached to, or touching shell fragments. This preference for contact with at least one solid planar surface, even when extending the anterior end over soft sediment to feed, has been described previously in adult *A. mollis* (Slater, 2006). It is possible that this behaviour, in particular full concealment of juveniles within large shell fragments, could be a form of predator avoidance, although predators of this species remain unknown (Sewell, 1987). It is possible that larger shell fragments provide a hard settlement surface for larvae and a refuge for small juveniles (<10 mm length) prior to migration to soft sediments (Wiedemeyer, 1994). However, primary benthic productivity, as indicated by chlorophyll *a* content, is low at site D meaning there would be few benthic diatoms available as a food source to post-settlement juveniles prior to early juveniles beginning to feed on soft sediments. In this case, juveniles may be settling and feeding directly on decaying detritus or bacterial biofilms associated with the shell fragments as opposed to the preference of other species for high chlorophyll *a* content in sediments (Uthicke and Karez, 1999). If *A. mollis* alternatively conforms to the general settlement pattern frequently described for sea cucumbers, with settlement to planar surfaces in shallow water followed by juvenile migration to deeper sites and associated deposit-feeding, then site D is more likely to represent an early migration site (Conand, 1993; Hamel and Mercier, 1996; Mercier et al., 2000a). In this case any migration to the site will nonetheless take place from areas with negligible macroalgal growth but offering considerable bivalve shell hash and planar surfaces in the form of subtidal rock wall as potential settlement surfaces.

2.5 Conclusions

The research found a highly localised pattern of recruitment of a commercially important deposit-feeding sea cucumber within a large harbour. Patterns of distribution of juvenile sea cucumbers are explained well by physicochemical properties of the surface sediments at the sites surveyed, in particular several properties commonly linked to increased degradative state and increasing food availability for deposit feeding detritivores. The distribution of the juvenile *A. mollis* differs from that commonly described for other deposit-feeding sea cucumbers in its lack of association with macroalgae, the absence of a distinct separation between adults and juveniles, as well as an unusual association of juveniles with deeper water. The highly localised pattern of recruitment may help to explain the previous lack of observations of juveniles of this species in the wild. The observed highly localised distribution also emphasises the importance of identifying and protecting juvenile habitats in order to effectively preserve fished sea cucumber populations.

Table 2.1 Relationship between juvenile *A. mollis* abundance and a range of physiochemical sediment characteristics for nine sampled sites in the Mahurangi Harbour. Chl *a* and phaeo are abbreviations of chlorophyll a and phaeopigment respectively, GF indicates grain size fraction.

Sediment characteristic	**Correlation (r =)**	**Level of sig. (p=)**
% Nitrogen	.6570	>0.000
% Carbon	.1493	0.457
Chl *a* µg/g sed	-.5594	0.002
Phaeo µg/g sed	.2132	0.286
Phaeo/chl *a* sed	.5896	0.001
0-63 µm GF	.4969	0.008
64-125 µm GF	-.1415	0.481
126-250 µm GF	-.3597	0.065
251-500 µm GF	-.2601	0.190
501-1000 µm GF	-.1217	0.545
1001-2000 µm GF	-.1205	0.549
Depth m	.5015	0.008

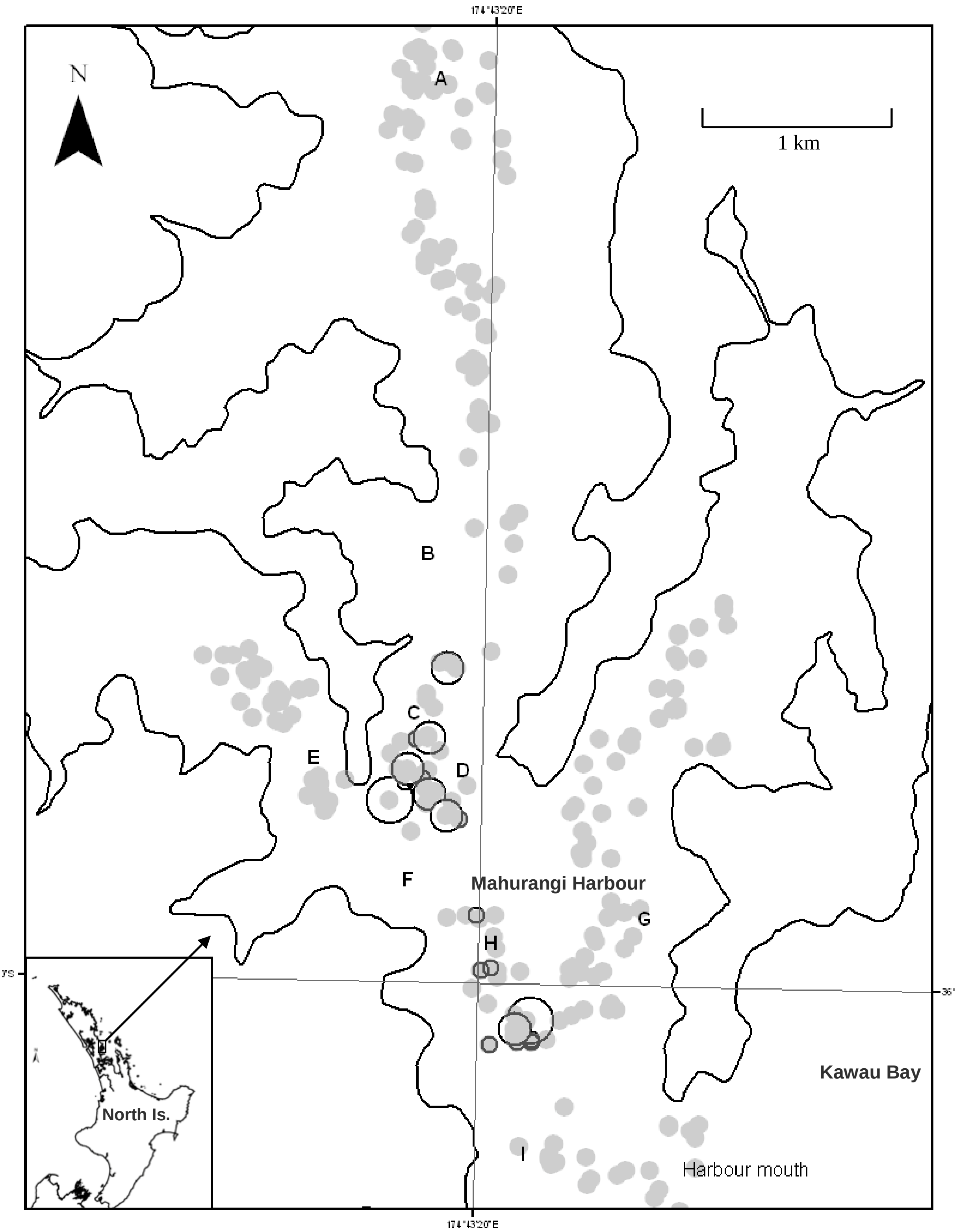

Figure 2.1 The Mahurangi Harbour showing areas of high density adult *A. mollis* as determined by 600 m2 beam trawls; ○ = 0.09-0.16 m-2; ○ = 0.17 - 0.33 m-2; ○ = 0.34-0.54 m-2. Letters indicate sites selected for 50m2 SCUBA transects. Grey circles indicate all beam trawls conducted between December 2005 and March 2007.

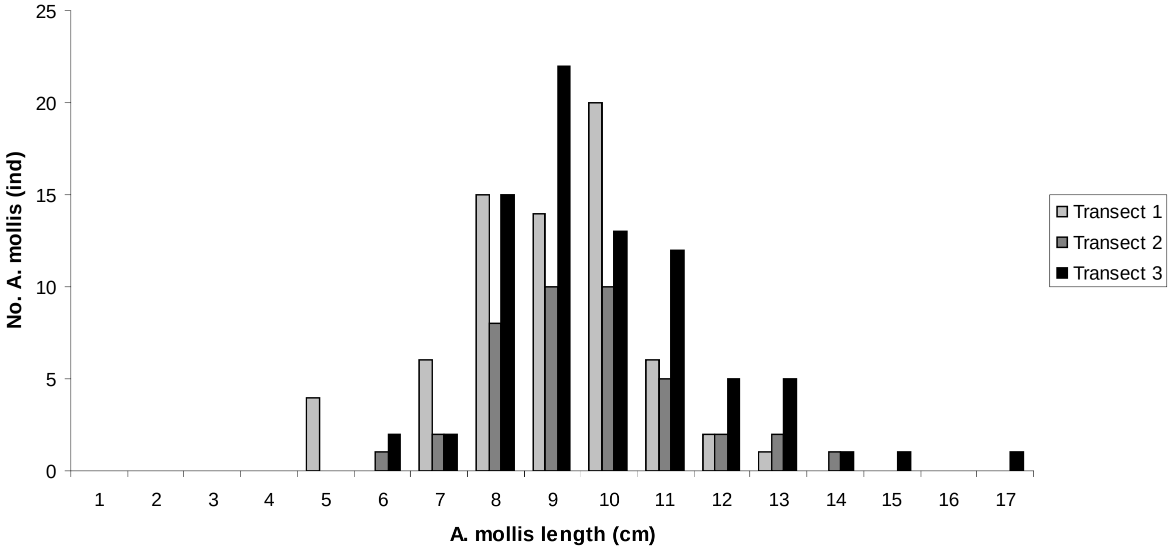

Figure 2.2 Number of *A. mollis* encountered by length class in three 50 m-2 transects at site D in May 2008.

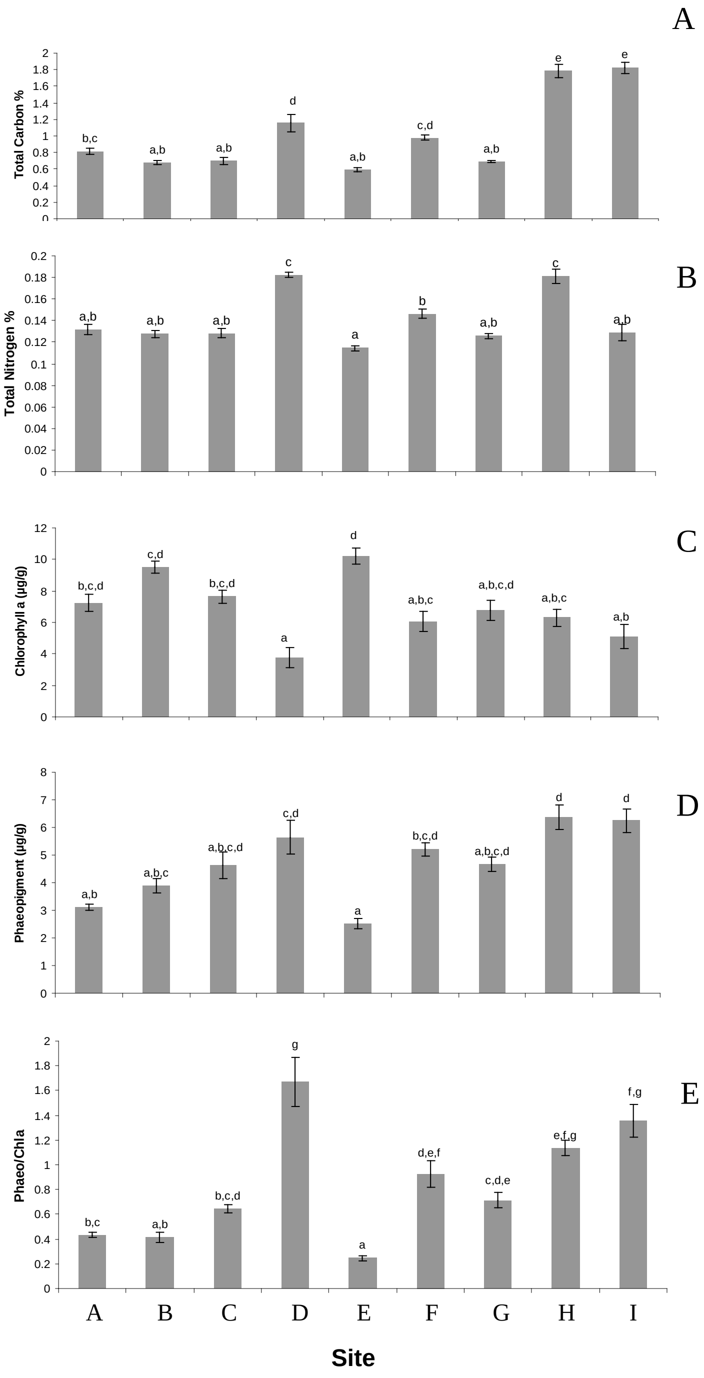

Figure 2.3 Physicochemical characteristics of surface sediments for each survey site; A. Total carbon; B. Total nitrogen; C. Chlorophyll a; D. Phaeopigment; E. Phaeopigment/chlorophyll a ratio; Error bars indicate standard error of the mean. Lower case letters indicate groups after pairwise comparison.

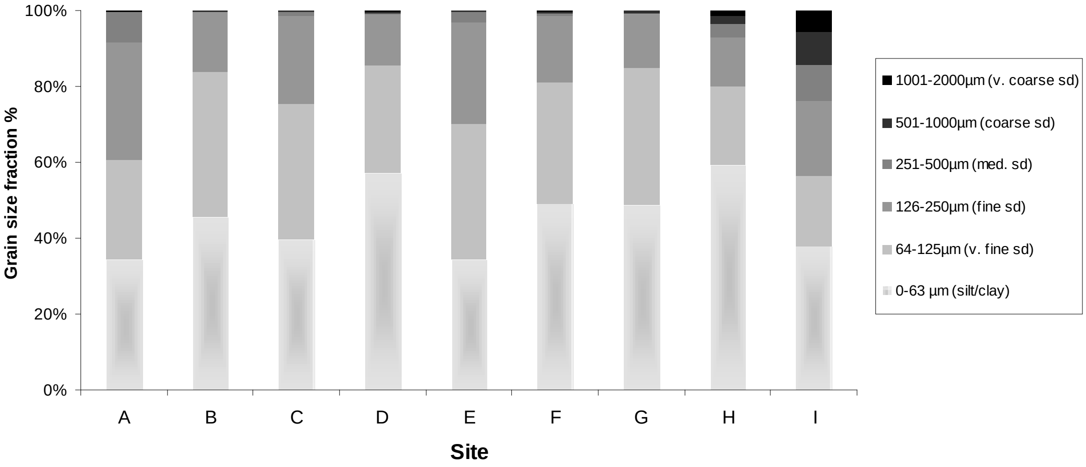

Figure 2.4 Grain size distribution of surface sediments for each survey site.

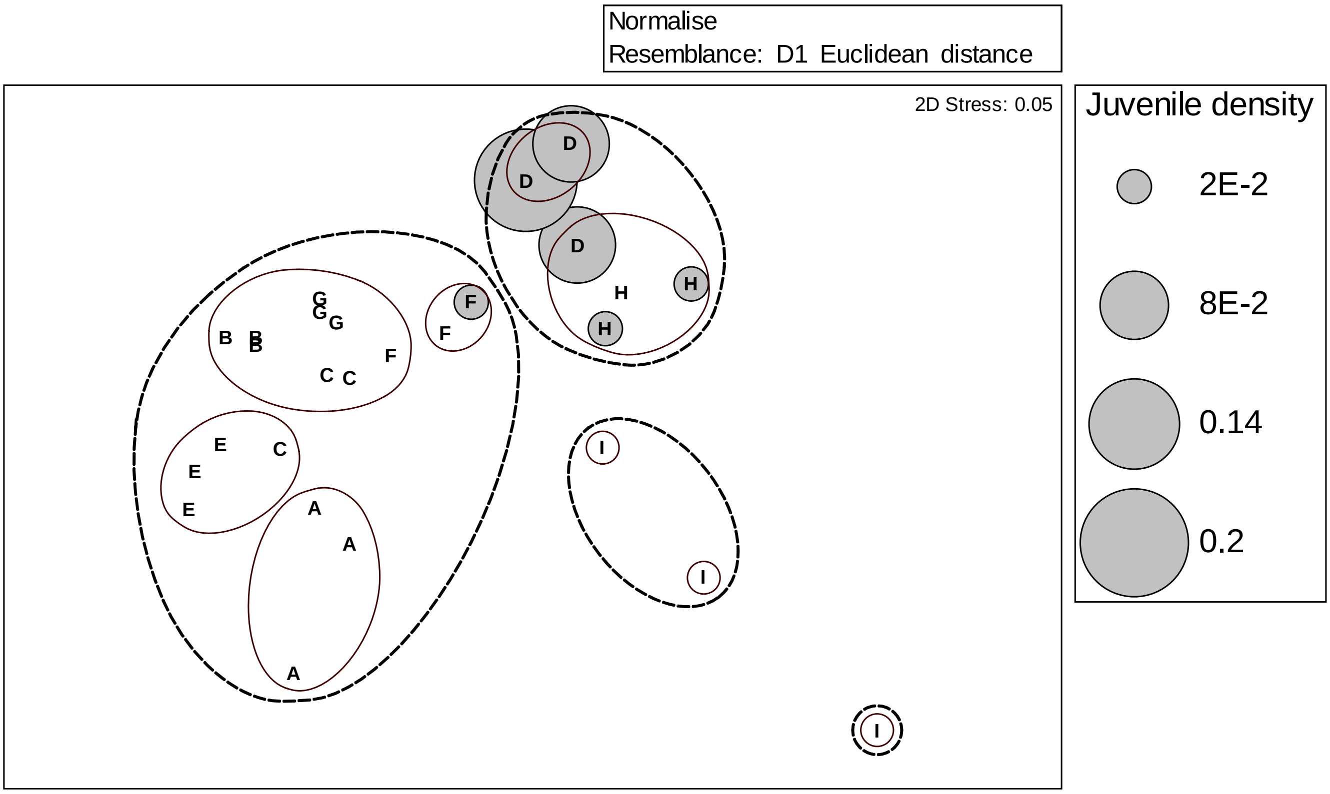

Figure 2.5 MDS plot of 27 transects conducted at nine survey sites within the Mahurangi Harbour. Transects are grouped by Euclidian distance similarity based on cluster analysis of all physicochemical sediment characteristics from surface sediments collected for each transect and depth. Individual transects are marked with site identification letters (A-I). Juvenile density at each transect is indicated by bubble plot.

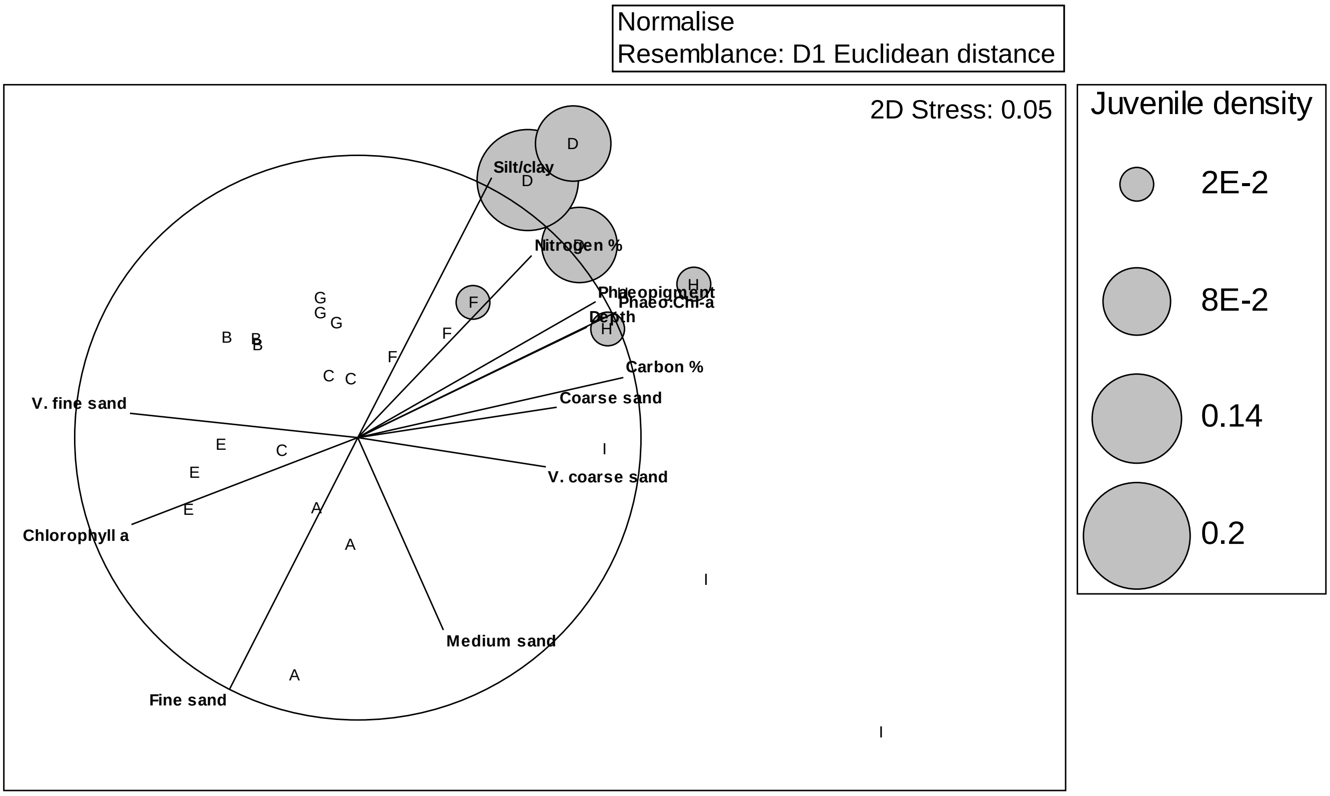

Figure 2.6 MDS plot of 27 transects at nine survey sites within the Mahurangi Harbour. Individual transects are plotted by all physicochemical sediment characteristics from surface sediments collected for each transect and depth. Eigenvectors displayed are Spearman rank correlation coefficients for all variables. Individual transects are marked with site identification letters (A-I). Juvenile density at each transect is indicated by bubble plot.

Chapter 3: The Role of Benthic Sediment Characteristics in Determining the Distribution of Juvenile *Australostichopus mollis*

In press as: Slater, M.J., Jeffs A.G. Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*? Journal of Sea Research.

3.1 Introduction

The juvenile ecology of sea cucumbers has been described for few species despite their commercial importance as both fishery and aquaculture resources. Little is known about the processes involved in determining the natural distribution of juveniles and there remains a lack of data on *in situ* growth rates and life history. Juvenile sea cucumbers have been shown to exhibit habitat preferences which differ distinctly from the adults (Mercier et al., 2000a; Purcell, 2004; Shiel, 2004; James, 2005; Yamana et al., 2006). The highly cryptic nature of juvenile sea cucumbers and their apparent absence from areas where commercially valuable adults are studied and fished means that field observations of juveniles for many species are rare (Shiel, 2004; James, 2005). However, an understanding of the factors that influence the distribution of juvenile sea cucumbers, allowing protection of nursery sites which are key in maintaining exploited adult populations, is critical to improving the management of sea cucumber populations.

Although juvenile sea cucumbers are found in habitats adjacent to areas of high adult densities, their habitats frequently differ from those of adults in terms of substratum type, food type, food availability, and depth (Mercier et al., 2000a; Purcell, 2004; Shiel, 2004; James, 2005; Yamana et al., 2006). Juveniles generally inhabit shallower water than adults, often in association with sediments with higher organic matter content and smaller grain size (Choe, 1963; Bulteel et al., 1992; Mercier et al., 2000b). An association of juvenile sea cucumbers with macroalgae is also common, with the algae being used both as an initial settlement surface for larval sea cucumbers, and as a source of detritus and refuge from predation (Mercier et al., 2000b; Yamana et al., 2006). Attachment to complex substrate other than macroalgae is also common in early juvenile sea cucumbers, as is highly cryptic behaviour (Yingst, 1982; Cameron, 1986; Hamel and Mercier, 1996; Shiel, 2004).

The habitat choices and distribution of sea cucumbers have previously been attributed to a variety of factors including hydrographic processes affecting larval supply, settlement preferences, substratum type, sediment food value and availability, and predatory pressure or avoidance (Sloan and von Bodungen, 1980; Young and Chia, 1982; Cameron, 1986; Massin and Doumen, 1986; Bulteel et al., 1992; Mercier et al., 2000a; Uthicke et al., 2004; Eckert, 2007; Entrambasaguas et al., 2008). Juvenile distribution in particular has been attributed to predation effects, patchy settlement or the presence of settlement surfaces within "nursery" habitats followed by subsequent migration to adjacent suitable adult habitats offering rich detrital food sources (Cameron, 1986; Mercier et al., 2000b). However, these purported processes responsible for determining the natural distribution of juvenile sea cucumbers remain untested.

Australostichopus mollis is a common coastal deposit-feeding sea cucumber in Australasia that is exploited in small-scale fisheries. The juvenile ecology of this species has not been investigated and little is known of juvenile biology, potential growth rates, or the life history of the species. Recent research has revealed a distinctly patchy distribution of juvenile *A. mollis* within the Mahurangi Harbour, New Zealand (Chapter 2), which appears to be typical of the broad but patchy distribution of this species throughout much of its range (Morgan, 2004; Shears and Babcock, 2007; Slater and Carton, 2007). High densities of juveniles are only encountered within a distinct area of the Mahurangi Harbour covering less than 1% of the harbour area despite intensive large and fine scale surveying (Chapter 2). This area of high juvenile density is one of the deeper sites within this harbour. The substratum at the site has large shell fragments (> 10 cm) of the horse mussel (*Atrina zelandica*) and is further characterised by small grain size and high sediment food value compared to other sites within the harbour (Chapter 2). The observed patchy distribution of juveniles may be attributable to highly localised favourable sediment characteristics as a food source facilitating good juvenile survival and growth. Alternatively distribution may be due to larval supply resulting from abiotic factors, suitability of settlement substrate, substrate complexity or predation, however, none of these factors has been investigated to date.

The aim of the current research was to determine the potential for localised differences in the quality of the sediment as a food source for deposit-feeders to determine the observed highly patchy distribution of juvenile *A. mollis*.

3.2 Methods

3.2.1 Site designation

This research was conducted within the Mahurangi Harbour, a 25 km^2 shallow harbour on the east coast of the North Island of New Zealand (36° 27' 0 S, 174° 43' 0 E). Three sites within the harbour where abundant sea cucumber juveniles were encountered during SCUBA dive surveys were designated "juvenile sites" (Figure 3.1). A further three sites were designated "non-juvenile sites" where no juvenile sea cucumbers were encountered during previous SCUBA dive surveys. Non-juvenile sites were selected to differ from juvenile sites in their sediment characteristics, i.e., sediment facies, depths and apparent grain size distributions (Table 3.1). The selected sites also varied in mid-water current regime, although the current at the seafloor did not vary significantly between sites (Harrison, 1974; Green et al., 1998). Non-juvenile sites were a minimum of 1.2 km and a maximum of 2.1 km apart while juvenile sites were a maximum of 0.9 km apart (Figure 3.1). The average depth of non-juvenile sites was 6.0 m while the average depth at juvenile sites was 9.7 m.

3.2.2 Cage placement and preparation

Cages (0.90 × 0.90 × 0.23 m, l × w × h), were constructed of metal frames and covered with plastic mesh (6 mm mesh size) and openings were cut into the mesh roof of the cages that were held shut with cable ties during the experimental period. The cages were placed on the seabed at each of the six designated sites in groups of four. Each set of four cages was arranged in a line with a distance of 1.5 m between cages and each line of cages was oriented perpendicular to the channel. The cages were joined by a weighted line so they could be located by SCUBA divers during periods of poor underwater visibility and both ends of the line were moored with 35 kg steel mooring blocks. The lines were marked with a surface buoy at the end closest to shore. Cages were pushed as far as possible into the sediment when being positioned. As cage bases were not able to be forced by divers uniformly though the upper sediment layer, the upper 2 cm of surface sediment from the surrounding area was scraped into the cages to ensure the cage floor was fully covered with sediment to approximately 3 cm depth. The cages were left for more than two weeks to enable the sediment to settle before juvenile sea cucumbers were transplanted into them. The sediment surface in cages became contiguous with the sediment at the cage edge within 2 weeks and no obvious entrainment of sediment or scouring under cages was observed during maintenance or sampling SCUBA dives over the next year.

3.2.3 Collection of juveniles

Juvenile *A. mollis* weighing between 12 and 39 g (mean 24 g) were collected by SCUBA divers from a site with naturally high density of juveniles in the Mahurangi Harbour. The juveniles were photo-identified (Raj, 1997) and blotted dry before being weighed to the nearest gram. Photo-identified animals were then randomly allocated into groups of two, and a group was then allocated to each of the 24 cages which were located at the six sites (four cages per site) within the Mahurangi Harbour (Figure 3.1). The mean wet weight of sea cucumbers per cage varied from 20 - 29 g. SCUBA divers transported and released the juveniles into cages using labelled 1 l containers of fresh ambient sea water and then the opening in each cage roof was sealed.

3.2.4 Growth data and survival

Caged sea cucumbers were monitored every 3 mo over a 9 mo period from 19/06/2008 to 18/03/2009. At each sampling event the sea cucumbers were removed from cages by SCUBA divers and brought to the surface in 1 l containers of sea water where they were re-identified and weighed to the nearest 1 g as described above. Sea water container size was increased to 2 l at the 9 mo sampling to accommodate increases in animal size. The animals were returned to their allocated cages the roofs of which were then resealed. The period of time that animals were outside cages did not exceed 45 min. The daily specific growth rate (SGR d^{-1}) was calculated for each animal as (Ricker, 1979):

$$\text{SGR } d^{-1} = 100 * (\text{LN(sample date weight)}-\text{LN(initial weight)})/\text{number of treatment days}$$

Growth rate was also measured as grams of wet body mass gained per day based on final weights and days from the outset of the experiment. Survival was recorded as presence or absence of individual sea cucumbers in each cage at each sampling time. Any cages where an animal was not present were excluded from further analyses as the density of animals in the cage was no longer comparable with the remaining experimental cages.

At the completion of 9 mo of sampling it was decided to use one juvenile site (Mahurangi West) and one non-juvenile site (Browne's Bay) to examine if increased density of juveniles could be responsible for the decline in growth rate observed in the previous 3 mo period. The number of cages at these two sites was doubled with cages installed as outlined previously and the existing juveniles at each site were randomly re-assigned to cages at a density of one per cage. The growth of individuals was monitored for a further 3 mo using the methods described previously.

3.2.5 Surface sediment analyses

Three surface sediment (upper 2 mm) samples of approximately 5 g of wet weight were taken randomly at each caging site prior to cage placement. Sediments were protected from light with aluminium foil and stored at -80° C for later use in physico-chemical assays. Total organic matter (TOM) was determined using a variation on the combustion method as recommended by Byers et al. (1978). Sub-samples of surface sediment (approx 1 g) were dried in a 60°C oven to constant weight (48 h). Pre-weighed dry samples were ashed in a muffle furnace at 500°C for 6 h. and then re-weighed. Total organic matter was calculated as percentage weight loss following combustion. Sub-samples (approx 0.5 g) of surface sediment were dried in a 60°C oven to constant weight (48 h).

Dried samples were sieved through a 355 µm sieve to remove shell fragments, ball ground and stored in 1.5 ml Eppendorf tubes before being analysed for total carbon and nitrogen using a Leco TruSpec elemental determinator.

Chlorophyll-*a* and phaeopigment were determined using a variation of the spectrophotometric method initially described by Lorenzen (1967) and as described by Slater and Carton (2009).

Surface sediment sub-samples (approx. 1.5 g w.w. of sediment) were treated for 4 h in a sodium metaphosphate solution (5 g l^{-1}) to de-clump clay particles. Grain size distribution was then examined using a Malvern Mastersizer 2000 particle size analyser using laser and blue light diffraction detection to determine sample grain size distribution. Particles were grouped into six size ranges: 0 - 63 µm, 64 - 125 µm, 126 - 250 µm, 251 - 500 µm, 501 - 1000 µm and 1001 – 2000 µm.

3.2.6 Statistical analyses

Normality and homogeneity of variances were confirmed for body weight data using a Shapiro-Wilk’s test and a Levene’s test respectively. A nested ANOVA design with individual sites nested within site type (non-juvenile or juvenile) was applied to test for significant differences in growth at juvenile and non-juvenile sites and between individual sites. This test was applied to mean SGR d^{-1} values (by cage average) at 0 mo (wet weights compared to ensure no bias in the random allocation of experimental animals) after 3 mo, 6 mo and 9 mo. Significant differences between individual site means were identified with pair-wise comparisons using a Tukey HSD post-hoc test. A t-test was used to compare the mean SGR between the two sites sampled at 12 mo and t-tests on dependent samples were used to test for significant differences between 9 mo and 12 mo SGR values for each site. Kaplan Meier survival rates were calculated for juvenile and non-juvenile sites and the survival of juvenile sea cucumbers over 3, 6 and 9 mo was tested for significant differences using a log-rank comparison.

A non-parametric Mann–Whitney U test was used to identify significant differences between each of the measured chemical sediment characteristics (i.e., TOM, carbon, nitrogen, phaeopigment, chlorophyll-*a*) grouped as juvenile versus non-juvenile sites. Where normality and homogeneity of variance assumptions were fulfilled, a one-way ANOVA was used to test for significant differences amongst individual sites for each sediment characteristic, followed by Tukey post-hoc comparison of means ($\alpha = 0.05$). Where ANOVA assumptions were not fulfilled, a Kruskal-Wallis test on ranks was used to test for significant differences amongst sites and where overall results were significant a Tukey post-hoc comparison of rank sums was used to identify differences between sites ($\alpha = 0.05$).

3.3 Results

3.3.1 Juvenile growth

Mean SGR over 9 mo was 0.30% d^{-1} (±0.02 SE) at juvenile sites and 0.45% d^{-1} (±0.02 SE) at non-juvenile sites. The mean SGR was significantly higher at non-juvenile sites after 6 mo ($F=19.02$, $p<0.001$) and 9 mo ($F=24.9$, $p<0.001$). Pair-wise comparisons showed that mean SGR at Browne’s Bay was significantly higher than all other juvenile sites after 6 mo. However, at 9 mo the mean SGR at the Lagoon Bay non-juvenile site was higher than all other juvenile sites (Figure 3.2).

Growth of juvenile *A. mollis* was highly variable across all sites and within individual cages. After 3 mo the SGR of individual animals varied from -0.17% d^{-1} (the only negative SGR recorded during the period) to 1.05% d^{-1}. Over the 3-6 mo period the SGR of individuals varied between 0.06% d^{-1} and 0.87% d^{-1}, and in the 6-9 mo period it varied from -0.38% d^{-1} to 0.43% d^{-1}. Over all sites combined the juveniles gained an average of 45.2 g (± 3.0 SE) over the 9 mo period (Figure 3.3). Total wet body weight increment over 9 mo varied from 90 g (initial weight 20 g, final weight 110 g, daily growth 0.33 g d^{-1}) to a minimum of 16 g (initial weight 32 g, final weight 48 g, daily growth 0.06 g d^{-1}).

When juvenile density was halved at 9 mo mean body weight and SGR recovered significantly at both sites over the 12 mo period when compared with the previous 9 mo period at double the density (t-test Browne’s Bay, $t= 5.9$ $p<0.01$, Mahurangi West, $t=4.0$ $p<0.05$) (Figure 3.4). Mean SGR was –0.11% d^{-1} (± 0.02 SE) and -0.11% d^{-1} (± 0.03 SE) respectively at the Mahurangi West and Browne's Bay sites between 6 and 9 mo, while between 9 and 12 mo it was 0.36% d^{-1} (± 0.08 SE) and 0.44% d^{-1} (± 0.06 SE) at the same sites respectively. The non-juvenile Browne's Bay site continued to exhibit significantly higher growth than the Mahurangi West juvenile site at the final 12 mo sampling ($t= 4.8$; $p<0.001$).

3.3.2 Juvenile survival

The Scott's Landing North juvenile site could not be continued after the first 3 mo sampling event due to a SCUBA dive gear failure during sampling resulting in the loss of animals. In addition, one cage was destroyed by a boat anchor at the Scott's Landing South site after 9 mo. These losses were not included in the survival data. Taken over all sites the caged juvenile sea cucumber survival was high at 94% over the 9 mo experimental period. The Kaplan Meier survival rate for animals at juvenile sites was 0.931 and 0.951 at non-juvenile sites after 3 mo and remained stable until the end of the 9 mo period. A log-rank test comparing survival (at 3 mo) indicated that survival rates did not differ significantly between juvenile and non-juvenile sites ($p > 0.5$).

3.3.3 Total organic matter of surface sediment

Mean TOM was significantly higher in surface sediments collected at juvenile sites (Mann–Whitney U = 13, $n_1 = n_2 = 9$, $p < 0.05$) with a maximum TOM of 7.6 (±0.16, SE) at Scott’s Landing South (Figure 3.5). There were also significant differences between individual sites (ANOVA F=99.8, $p <0.01$). Mean TOM content of sediments did not differ significantly among non-juvenile sites, however, there were significant differences in the TOM content amongst all juvenile sites, with the Mahurangi West site exhibiting the lowest TOM among all of the juvenile sites of 5.5 (±0.14, SE).

3.3.4 Elemental carbon and nitrogen content of surface sediment

Mean carbon content of sediment was highly variable among sites but did not differ significantly between juvenile and non-juvenile sites (Mann–Whitney U = 25, $n_1 = n_2 = 9$, $p > 0.05$). However, there were significant differences between individual sites (Kruskal-Wallis, $p<0.01$, Figure 3.5). Mean nitrogen content was significantly higher in sediments from juvenile sites than for non-juvenile sites (Mann–Whitney U=5, $n_1=7 n_2=9$, $p<0.01$), however, overall there were no significant differences among individual sites (ANOVA F= 3.3, $p >0.05$, Figure 3.5).

3.3.5 Phytopigment content of surface sediment

Mean chlorophyll-*a* content of surface sediment was significantly lower at juvenile sites (Mann–Whitney U=12, $n_1=n_2=9$, $p<0.05$) and differed significantly among all caging sites (ANOVA F= 10.3, $p <0.001$, Figure 3.5). Phaeopigment content did not differ significantly between juvenile and non-juvenile sites (Mann–Whitney U=24, $n_1=n_2=9$, p>

0.05) nor amongst individual sites (ANOVA F= 0.8, p >0.05, Figure 5). The phaeopigment:chlorophyll-*a* ratio was significantly higher at juvenile sites compared to non-juvenile sites (Mann–Whitney U=14, n_1=n_2=9, p<0.05), but there was no significant difference amongst individual sites (ANOVA F= 2.3, p >0.05, Figure 5).

3.3.6 Grain size distribution of surface sediment

The mean of the 0 to 63 µm grain size fraction was significantly higher at juvenile sites compared with non-juvenile sites (Mann–Whitney U=13, n_1=n_2=9, p<0.05), while all other grain size fractions did not differ significantly between grouped juvenile and non-juvenile sites (Figure 3.6). The mean 64 – 125 µm grain size fraction exhibited significant differences amongst all sites (ANOVA F= 26.4, p<0.001) as did the 251-500 µm fraction (Kruskal-Wallis p<0.05).

3.4 Discussion

The factors underlying the patchy spatial distribution of juveniles of commercially important sea cucumbers such as *A. mollis* are poorly described, yet need to be understood to improve the management of fished stocks and to support aquaculture efforts. The current research provides an insight into the importance of the sediments available for deposit-feeding in determining the distribution of juvenile *A. mollis*. The data collected also provide valuable information on growth rates for this species allowing comparison to other high value commercial species.

These data are significant as they follow individual growth of *A. mollis* for the longest period reported to date. Previous growth of tank-held juveniles has ranged from nil (Sewell, 1987) through to maximum 0.21 g d^{-1} for tank-held juveniles fed mussel waste for one month at a final density of 735 g sea cucumber biomass m^{-2} (Slater et al., 2009). In the current experiment average daily weight gain over all sites over the entire 9 mo was 0.17 g d^{-1} (± 0.05 SE) and final density averaged 170 g m^{-2} in natural conditions without diet addition. The mean juvenile *A. mollis* weight at the Browne’s Bay site increased from 25 g (± 3.0 SE) to 125.5 g (± 9.5 SE) over 12 mo, corresponding to a mean growth rate of 0.28 g d^{-1} (± 0.02 SE).

These long term growth rates, similar to other commercially valuable sea cucumber aquaculture species, and the high level of food source adaptability exhibited have positive implications for future aquaculture of *A. mollis* if sea cucumber density and food supply are appropriately managed. For example, commercially cultured *Apostichopus japonicus* is reported to grow at a rate of 0.29 g d^{-1} between 15 and 130 g in their second year when cultured in ponds to comparatively high final densities between 400 and 700 g m^{-2} (Chen, 2003; 2004). Lower growth rates of between 0.15 and 0.26 g d^{-1} for this species were reported when co-cultured at varying densities (ca. 25-50 g m^{-2}) in scallop lantern nets while tank-reared *A. mollis* gained 0.21 g d^{-1} at end densities of 735 g m^{-2} when fed an excess of fresh mussel waste (Zhou et al., 2006; Slater et al., 2009). Growth of 0.11 g d^{-1} has been recorded for *Parastichopus californicus* in co-culture with the pacific oyster *Crassostrea gigas*. Tank-reared juveniles of the considerably larger sea cucumber species *Holothuria scabra* fed fresh and powdered algae were recorded as growing 0.2 g d^{-1} on average in tank rearing, although growth slowed once densities exceeded 225 g m^2 (Battaglene et al., 1999). Similar growth rates (max 0.31 g d^{-1}) have also been found for *H. scabra* when fed shrimp starter food, although growth limitation occurred at densities of 300 g m^2 or greater (Pitt and Duy, 2004).

In the current study, growth appears to have become density-limited as sea cucumber biomass in cages approached 160-170 g m^2 - after 6 mo. This effect was quickly reversed among animals on-grown at lower densities at the Browne's Bay and Mahurangi West sites (Figure 3.4). Growth at high densities has been recorded for this species under heavy sedimentation regimes providing a surfeit of high organic matter in a sediment diet (Slater and Carton, 2007; Slater et al., 2009). Therefore, the observed density limitation is unlikely to be maintained in higher sedimentation regimes as in polyculture or with a supplemented diet as in aquaculture. Growth of juvenile *A. mollis* is also highly variable at the individual level with animals ranging from 32 to 100 g at one experimental site after 6 mo from an initial mean size of 23 g (± 1.8 SE). Variability did not relate to initial weight of animals or size differences between animals within individual cages. Such variable growth requires a more complex grading system at harvest or multiple harvests in aquaculture systems which can be both costly and inefficient. Variability may decrease in the controlled conditions of a pond and with the steadiness of a supplement diet supply, although pond-reared *A. japonicus* continue to exhibit variable growth rates (Chen, 2003).

There is a dearth of information regarding sea cucumber growth and survival in relation to sediment characteristics. This study aimed to measure differences in survival and growth in response to differing sediment characteristics in order to explain the patchy distribution of *A. mollis* within the Mahurangi Harbour. Correspondingly, the sediment characteristics at juvenile sites differed significantly from the non-juvenile sites. TOM values in surface sediments at juvenile sites were significantly higher as was nitrogen content. The chlorophyll-*a* content in juvenile site sediments was significantly lower than at non-juvenile sites and the chlorophyll-*a*:phaeopigment ratio was correspondingly higher. The high TOM and nitrogen values, in particular at the juvenile sites, indicate that they offer favourable sediment food sources with higher energy content that may be required by detritivorous sea cucumbers with higher detritus input and greater bacterial activity (Moriarty, 1982; Kautsky and Evans, 1987; Kang et al., 2003; Zhou et al., 2006). Despite this, survival did not differ between sites and growth was higher at non-juvenile sites with low TOM, carbon and nitrogen values. In particular, animals caged at the non-juvenile Lagoon Bay and Browne's Bay sites showed significantly higher growth than sites with significantly higher TOM where juveniles were naturally abundant (Tukey's HSD $\alpha<0.05$, Figure 2). High organic content values (TOM =37%) have been suggested as important for promoting growth in juvenile *A. mollis* (Maxwell et al., 2009). However the long term growth of *A. mollis* in the current experiment and digestive efficiency data for *A. japonicus* indicate that diet supply rate and digestibility of TOM available in a diet play a more significant role than undifferentiated high organic content alone (Yuan et al., 2006).

Grain size analysis showed an overall trend towards coarser grain size distribution at non-juvenile sites, in particular at the Oaua West site and the 0-63 µm grain size fraction was significantly greater at juvenile sites. There is currently no evidence that *A. mollis* is grain size selective, and selectivity is considered less likely for a deposit-feeding sea cucumber which does not encounter significant competition from co-occurring deposit-feeding species (Sloan and von Bodungen, 1980). Likewise, for other deposit feeding sea cucumbers the role of sediment grain size in facilitating feeding is unclear. For example, sediments with different grain sizes made no difference to the survival or growth of *H. scabra* when cultured in ponds (Bell et al., 2007). Nonetheless, smaller grain sizes favour detritivorous sediment feeding because of the associated bacterial activity and the surface-rich nature of smaller grains with an associated organic monolayer offering a rich food source (Mayer et al., 1985; Yamamoto and Lopez, 1985). However, the significantly coarser grain size distribution, particularly, at the Oaua West site did not result in significantly lower survival rate or SGR in the juvenile sea cucumbers examined in this current study.

The current research is unable to provide causes for the higher growth recorded at non-juvenile sites, however, several deposit feeding sea cucumber species have been shown to select sediments or sediment patches with high benthic microalgal content (Uthicke and Karez, 1999; Hudson et al., 2005). Lagoon Bay and Browne's Bay are at shallower depths than all other sites with correspondingly higher microphytobenthic activity which may help explain the higher SGR amongst juveniles caged at these sites. This effect would be expected to be more pronounced in spring and summer when microphytobenthic activity increases markedly. This possibility was supported by accelerated weight increases observed at the two shallow sites between 3 and 6 mo corresponding to the southern hemisphere spring (Figure 3.4).

Potential determinant factors for the patchy distribution of *A. mollis* juveniles other than sediment remain to be excluded. These include predation on juveniles, although predation on sea cucumbers has previously been disregarded or considered negligible (Bulteel et al., 1992; Yamana et al., 2006). There is effectively no evidence for fish predation on *A. mollis* despite the very large amount of data available on fish diets. Only two cases of attempted predation on *A. mollis* have been recorded, both by the seastar *Luidia varia* (Graham, 1939; Godfriaux, 1969; Colman, 1972; Morton and Miller, 1973; Webb, 1973; Sewell, 1990). However, early juvenile predation has been shown to be a potentially strong determinant in invertebrate distribution, micropredators (nereids, harpacticoid copepods, decapods and small gastropods) prey on early juvenile sea cucumbers in hatchery culture and *in situ*, and there is considerable evidence of predation on late juvenile and adult sea cucumbers by specialised predators, primarily seastars (Osman et al., 1992; Francour, 1997; MedeirosBergen and Miles, 1997; Chen, 2003; FAO, 2003). Cameron and Fankboner (1989) also noted recruitment of sea cucumbers to sites where predatory seastars were absent and the patchy distribution of dendrochite sea cucumbers has been attributed to heavy seastar predation (Eckert, 2007). There are large numbers of two predatory seastars, *Coscinasterias muricata* and *Astropecten polyacanthus*, within the Mahurangi Harbour in a patchy distribution (N. Usmar, unpublished data). Unlike the data presented by Cameron and Fankboner (1989), seastars and juvenile sea cucumbers frequently co-occur in the Mahurangi Harbour, indicating that predation is unlikely to be an important determinant of the observed patchy distribution. However, further study is required to exclude this factor.

Juvenile *A. mollis* are likely to select areas offering complexity and refuge as do other species of sea cucumber (Yingst, 1982; Cameron, 1986; Hamel and Mercier, 1996; Shiel, 2004). The juvenile sites investigated in this caging experiment offer facies complexity in the form of live horse mussels (*Atrina zelandica)* at Mahurangi West and large horse mussel shell fragments at the Scott's Landing North and South sites. The non-juvenile sites offer minimal facies complexity and little refuge. The facies at Lagoon Bay and Browne's Bay are planar, offering effectively no complexity for refuge and these two sites have significantly higher incident light values compared with deeper juvenile sites (Lagoon Bay = approx. 25% incident light at sediment surface versus approximately 3% at Scott's Landing sites) (Gibbs et al., 2005). *Australostichopus mollis* activity and feeding is distinctly, but not strictly, nocturnal, with the animals overwhelmingly avoid exposure to high light conditions and preferring contact with multiple surfaces (Slater, 2006). It is probable that the sediment grain size distribution and more importantly microphytobenthic activity is favourable to *A. mollis* feeding and growth at several non-juvenile sites but lack of refuge and shallow depths resulting in high incident light may be responsible for excluding juveniles from the habitat.

There is insufficient macroalgal growth adjacent to the juvenile sites for macroalgae to be considered a potential settlement surface for *A. mollis* larvae or juvenile refuge as has been observed in other related species of sea cucumber (Mercier et al., 2000b; Yamana et al., 2006). Conversely, the hard shell fragments and rock surfaces adjacent to the area have considerable benthic diatom coverage throughout the period (December – March) when larvae can be expected to settle. Potential settlement surfaces are not exclusively present near the juvenile site although large shell fragments are abundant at the site and in the adjacent shallows and may represent a preferable settlement surface to the mud flat substrate and sand adjacent to non-juvenile sites. A strong concentration of entrained larvae can be expected within the mid-harbour based on both flow data and direct larval sampling (Harrison, 1974; Martin and Foster, 1986). This concentration is likely to have a considerable effect on the distribution of settling sea cucumber larvae focussing it on an area centred on the juvenile sites. Juvenile sites may then simply present sites within this area which are acceptable for larvae to settle to and support subsequent survival to juvenile.

3.5 Conclusions

Growth is rapid and maintained amongst juvenile *A. mollis* in both natal and novel environments but appears to be quickly limited when the quality and/or rate of food supply is exceeded by the density of sea cucumber biomass. Mean growth results indicate that cultured *A. mollis* can be expected to match the growth rate recorded in other valuable cultured sea cucumbers such as *A. japonicus,* but individual growth is highly variable and may complicate harvesting and grading of animals in commercial aquaculture. The results of the current research show that the observed patchy distribution of juvenile *A. mollis* within the Mahurangi Harbour is not caused by selective differences in survival due to the differences in the physico-chemical characteristics of the ambient sediment available for deposit-feeding. Despite significant differences in TOM, nitrogen, chlorophyll-*a* content and grain size distribution of the sediments at non-juvenile sites when compared to juvenile sites, sediment food sources at all sites were sufficient for survival and growth. At several transplant sites the growth of caged juveniles was significantly higher than the growth of those caged at juvenile sites. Further understanding of the factors determining distribution patterns of juvenile sea cucumbers requires data regarding natural settlement substrates and predators for this species, as well as hydrodynamic effects on larval distribution.

Table 3.1 The designations, predominant facies description and depths of juvenile *A. mollis* caging sites.

Site label	**Facies**	**Depth (m)**	**Juvenile habitat**
Scott’s Landing North	Silt/mud, large shell hash	10	Juvenile
Scott’s Landing South	Silt/mud, large shell hash	11	Juvenile
Mahurangi West	Silt/mud, horse mussel (>1 m^2)	8	Juvenile
Browne’s Bay	Mud, horse mussel (<0.2 m^2)	4	Non-juvenile
Oaua West	Small shell hash	10	Non-juvenile
Lagoon Bay	Mud, horse mussel (<0.2 m^2)	4	Non-juvenile

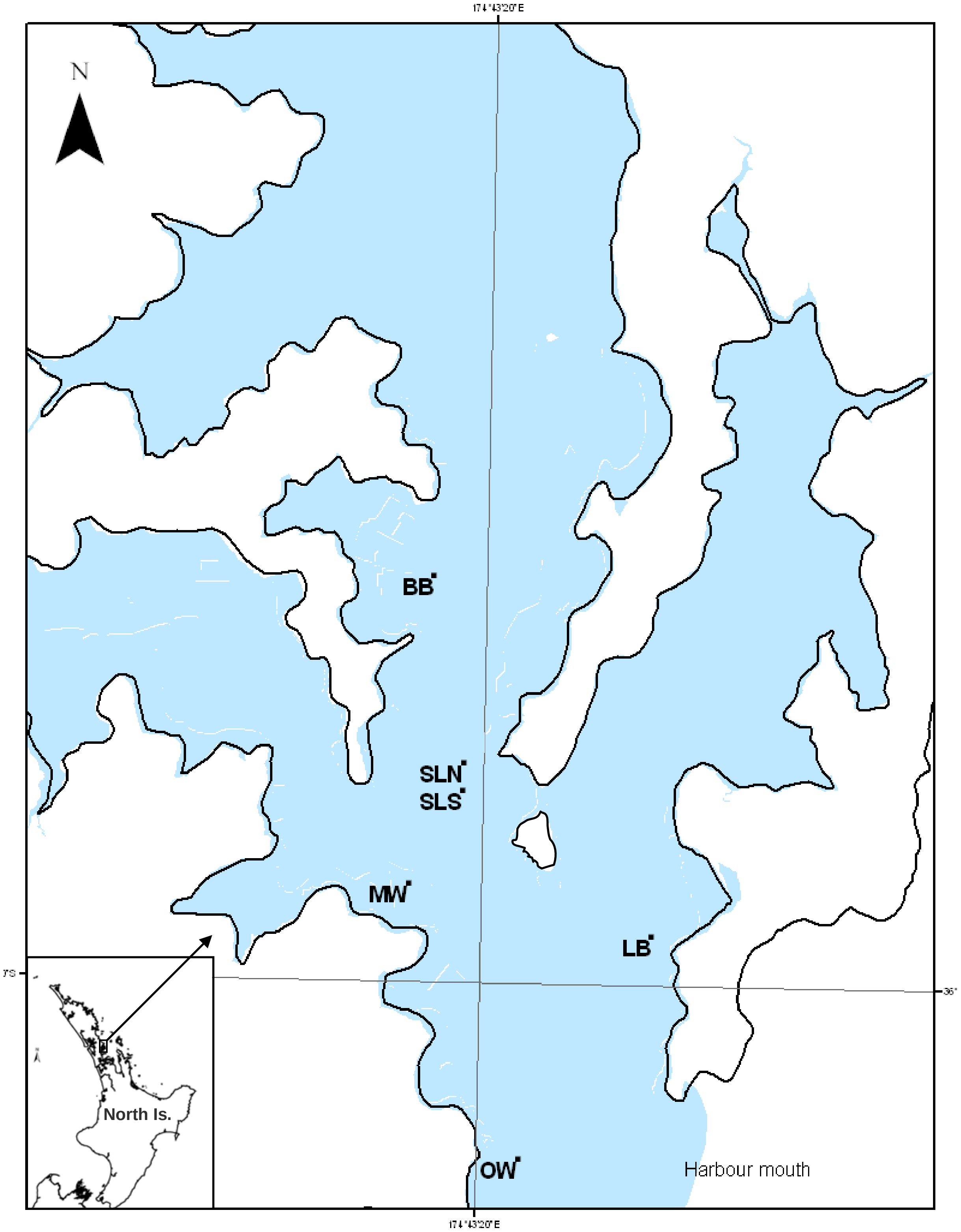

Figure 3.1 Juvenile *A. mollis* caging sites within the Mahurangi Harbour. Displacement sites (non-juvenile); LB, Lagoon Bay; OW, Oaua West; BB, Browne's Bay. Juvenile sites (juvenile); SLN, Scott's Landing North; SLS, Scott's Landing South; MW, Mahurangi West.

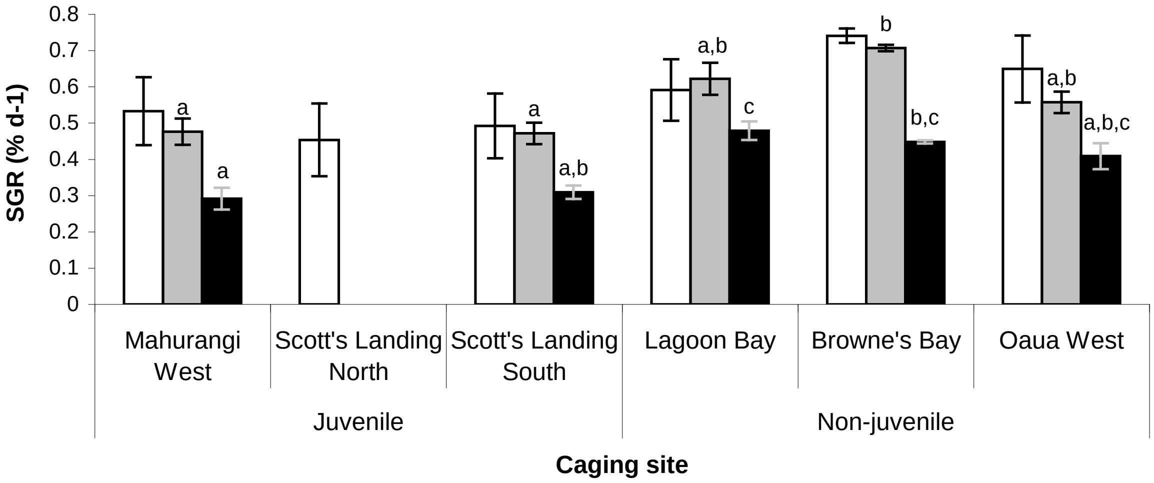

Figure 3.2 Mean daily specific growth rate (SGR d-1) of juvenile *A. mollis* caged at various sites within Mahurangi Harbour, sites are grouped by observed natural juvenile presence (juvenile versus non-juvenile). White bars indicate SGR d^{-1} after 3 mo (n= 44), grey bars indicate SGR d^{-1} after 6 mo (n = 34), and black bars indicate SGR d^{-1} after 9 mo (n = 34). Error bars indicate standard error of the mean. Means with the same lower case letter indicate homogenous groups as determined by Tukey HSD post hoc analysis (α= 0.05).

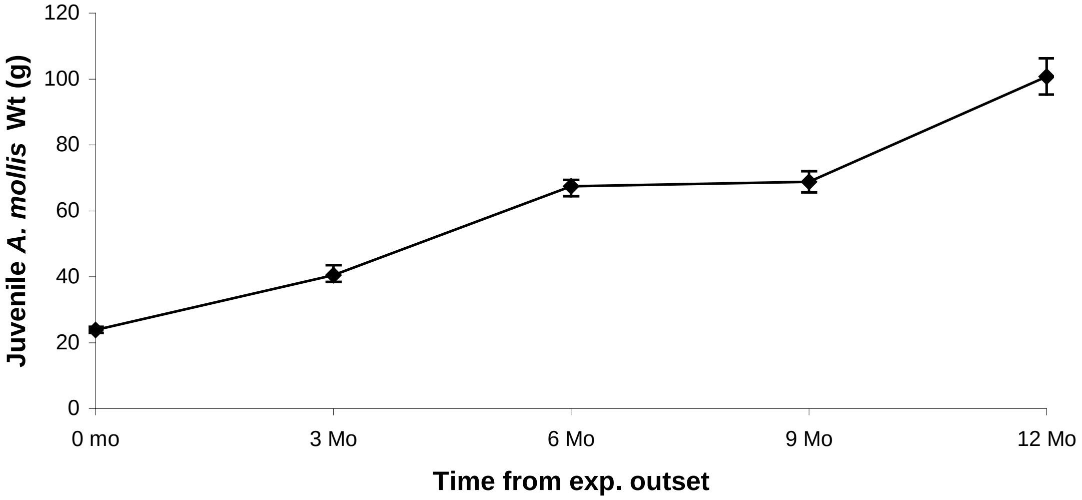

Figure 3.3 Cumulative growth (mean wet weight g) of *A. mollis*, all sites combined, within Mahurangi Harbour as sampled at 3 monthly intervals for one year, error bars indicate standard error of the mean. (n: 3 mo = 44, 6 mo = 34, 9 mo = 34, 12 mo = 12.)

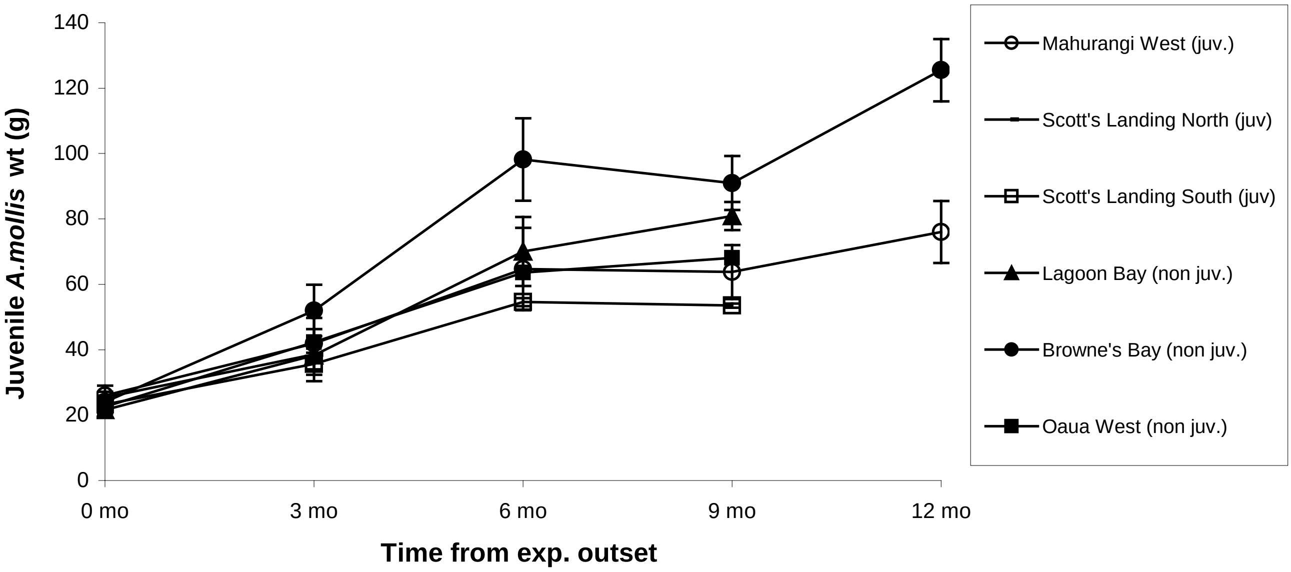

Figure 3.4 Mean wet weight (g) of *A. mollis* caged at various sites within Mahurangi Harbour as sampled at 3 monthly intervals for one year, error bars indicate standard error of the mean. (N: 3 mo = 44, 6 mo = 34, 9 mo = 34, 12 mo = 12.)

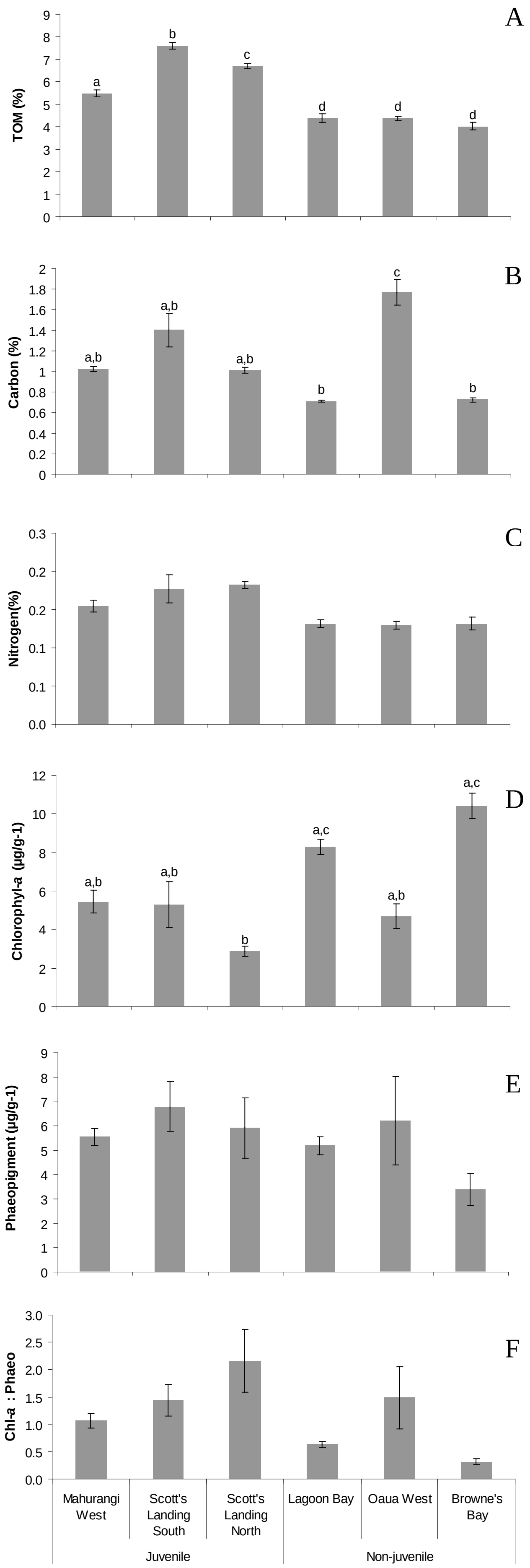

Figure 3.5 Physico-chemical characteristics of surface sediments for caging sites within Mahurangi Harbour; A. Total organic matter; B. Total carbon; C. Total nitrogen; D. Chlorophyll *a*; E. Phaeopigment; F. Phaeopigment/chlorophyll-*a*; Lower case letters indicate homogenous groups after pair-wise comparison (Tukey HSD α = 0.05) Error bars indicate standard error of the mean.

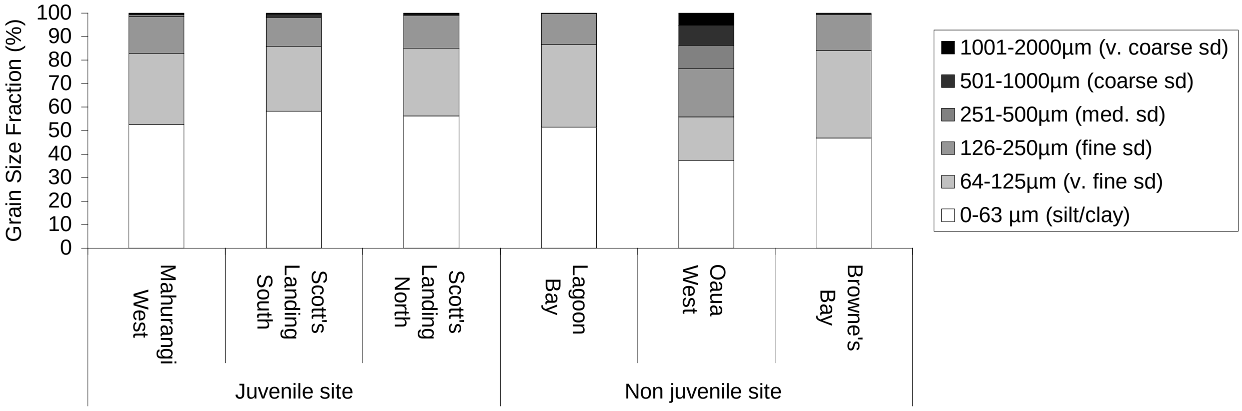

Figure 3.6 Grain size distribution of surface sediments for caging sites within Mahurangi Harbour.

Chapter 4: Organically selective deposit-feeding behaviour of juvenile *Australostichopus mollis*

4.1 Introduction

Deposit feeding sea cucumbers feed on surface sediments, processing large amounts of benthic material, assimilating bacterial, fungal and detrital organic matter while at the same time facilitating significant horizontal redistribution and bioturbation of sediments and recycling of nutrients (Crozier, 1918; Haukson, 1979; Lawrence, 1982; Moriarty, 1982; Uthicke, 1999; 2001; Slater and Carton, 2009). A better understanding of the feeding biology of deposit-feeding sea cucumbers will provide insights into the mechanisms underlying their important role in benthic nutrient cycling and will also aid the development of diets for aquaculture of candidate sea cucumber species.

Surface benthic sediments are generally a low nutrient food source and therefore bulk consumption and efficient assimilation of scarce organic matter is required to fulfil nutritional requirements of sea cucumbers (Lopez, 1987). Any mechanisms that facilitate selection and consumption of organically rich or more easily assimilated fractions within a sediment diet will help optimise nutrient uptake (Lopez, 1987). Several deposit-feeding aspidochirote holothurians have been shown to selectively consume nutrient-rich sediment grains along with bacteria and organic matter from natural sediments or specific grain sizes (Webb et al., 1977; Haukson, 1979; Moriarty, 1982; Roberts and Bryce, 1982; Paltzat et al., 2008). Selective feeding on sediment patches with high chlorophyll or nutrient content and smaller particle sizes is exhibited in some tropical species and recently in abyssal species (Uthicke, 1994; Uthicke and Karez, 1999; Hudson et al., 2005). Yet, reported selectivity varies between aspidochirote sea cucumber species. Broad differences in the abilities of tropical sea cucumber species to select food particles led Levin (1979) to suggest behavioural grouping of species by level of grain size selectivity, and many species have been shown to be non-selective feeders (Yingst, 1974; Sloan and von Bodungen, 1980; Hammond, 1982; Massin, 1982; Roberts and Bryce, 1982; Yingst, 1982; Massin and Doumen, 1986; Uthicke and Karez, 1999).

A variety of opinions are expressed in the literature regarding the existence and degree of aspidochirote sea cucumber feeding selectivity. This is not only due to species differences but also due to the different methods used to determine selectivity. It is argued that apparent feeding selectivity may simply be the result of the ability of sea cucumbers to restrict consumption to the uppermost layers of the available sediment (Yingst, 1982). Hence, difficulty in obtaining accurate diet samples for comparison with gut content (i.e., diet sediments have been sampled deeper than the natural feeding depth of sea cucumbers) may have blurred the results of investigations into selection of grain size selection and organic particles within specific size ranges. In addition, it has been suggested that an inability to exclude digestive fluids from gut organic content analysis may have previously resulted in incorrect inference of organic selectivity (Yingst, 1974; Haukson, 1979; Roberts and Bryce, 1982; Yingst, 1982).

Mechanisms of food particle selection in aspidochirote sea cucumbers are also unresolved. Cameron and Fankboner (1984) provide a description of the tentacular feeding in the aspidochirote, *Parastichopus californicus,* in which the terminal end of peltate tentacles expands or splays over the surface sediment, the tentacle end is pushed into the surface sediment and particles adhere to the tentacle surface. The tentacle is then 'cupped' and retracted toward the mouth. Particles are removed from the tentacle by wiping the terminal or functional end of the tentacle against the pharynx wall. The pharynx wall bulges to facilitate effective wiping to remove the majority of particles from the tentacle (Cameron and Fankboner, 1984). It is unresolved whether tentacle expansion entraps particles or whether adhesive secretions at the tentacle terminal are responsible for adhesion, although it would appear that both mechanisms are used to a greater or lesser degree (Roberts and Bryce, 1982; Cameron and Fankboner, 1984; Roberts et al., 2000). Adhesion is unlikely to allow grain size selectivity at the tentacle terminal whereas tentacle expansion and contraction may allow size selection. It is also unknown whether tentacles have a chemosensory capacity facilitating organic selectivity or whether selection (if any) occurs at the mouth.

It has been shown that where several sea cucumber species are present within a heterogeneous sub-tidal tropical environment each species feeds selectively either within a distinct grain size or nutrient content scale (Roberts, 1979; Roberts and Bryce, 1982; Massin and Doumen, 1986). It is suggested that strong competition between deposit-feeding sea cucumbers drives increased particle size selectivity and allows for increased niche specificity, while organic selectivity simply allows better exploitation of energy in diets irrespective of competitive pressures (Roberts and Bryce, 1982). Consequently low diversity amongst macrobenthic deposit feeders, as is commonly found in New Zealand coastal benthic environments, is expected lead to reduced grain size selectivity by resident sea cucumber species (Sloan and von Bodungen, 1980). These suggestions, however, do not recognise the presence of many other smaller benthic detritivores in both tropical and temperate habitats.

Selective behaviour at larger scales, such as selective movement based on organic content of food sources or patch selection, can also improve deposit-feeding efficiency and may help explain the spatial distribution of sea cucumbers within a habitat or among habitats. For tropical sea cucumber species, variability in distribution have previously been attributed to heterogeneous distribution of sediments (indicating organic availability) and resource partitioning where a number of species share the same habitat (Sloan and von Bodungen, 1980; Roberts and Bryce, 1982; Massin and Doumen, 1986). The random movement of feeding aspidochirote sea cucumbers has been observed within a relatively homogenous horizontal substrate, the behaviour probably reflecting the homogenous over-abundant food source present (Hammond, 1982; Da Silva et al., 1986; Cieciel, 2004). However, larger-scale movement of sea cucumbers appears to be directed, in particular when habitat boundaries such as unsuitable sediment types, strong hydrodynamic regimes or excessive algal density are encountered (Sloan and von Bodungen, 1980; Hammond, 1982; Massin and Doumen, 1986). Furthermore, sea cucumber movement is greatly reduced in the presence of excess deposition of sediment material (Da Silva et al., 1986). Periods of rapid sea cucumber movement when organic content is low may be a search strategy which is quickly abandoned once animals find an area with sufficient food availability for deposit-feeding.

Australostichopus mollis is widely distributed in New Zealand and southern Australia and is generally the sole aspidochirote sea cucumber in the diverse range of habitats where it is found, including shallow rocky reefs out to continental shelf plains at depths of ca. 200 m (Pawson, 1970). It is found living in a broad range of benthic habitats and sediment types including coarse shell substrates (Pawson, 1970; Fenwick and Horning, 1980; Slater and Carton, 2007). Previous research has shown high growth in *A. mollis* when fed rich organic diets. The species has also been shown to reduce organic content in sediments through deposit-feeding and indirectly through bioturbation (Slater and Carton, 2007; 2009). More recently, the patchy distribution of juveniles within harbour habitats has been shown to correlate with specific sediment characteristics, in particular fine grain size and high organic content (Chapter 2). The feeding biology of *A. mollis* remains poorly understood especially in regard to selective mechanisms at any scale. An understanding of feeding biology and preferences, especially in relation to grain size and organic selectivity, are also imperative for future diet development and aquaculture efforts.

This study investigates feeding selectivity for grain size and organic content in juvenile *A. mollis*. The research compares foregut content with accurately sampled diet sediments, combining *in situ* and controlled laboratory-based feeding experiments for the first time. Spatial selectivity is estimated in controlled tank experiments comparing sea cucumber occupation of adjacent patches of sediment set at varying levels of organic content and distance moved over varying levels of organic content is also calculated.

4.2 Methods

4.2.1 Juvenile collection

Juvenile *A. mollis* weighing between 12 and 40 g were collected by SCUBA divers from a site with naturally high density (Chapter 2) of juveniles in the Mahurangi Harbour, a 25 km^2 shallow harbour on the east coast of the North Island of New Zealand (36° 27' 0 S, 174° 43' 0 E – Figure 4.1). The sea cucumbers were transferred to the nearby Leigh Marine Laboratory in tanks of sea water with macroalgae as attachment substrate and then held in tanks with flowing sea water (100 µm filtered, 2-3 $l\ min^{-1}$, polypropylene disc filter). Prior to all experiments the juvenile sea cucumbers were suspended in flowing sea water in 4 mm mesh bags for 24 h to ensure gut contents were evacuated (Sewell, 1990).

4.2.2 Laboratory sediment and foregut sampling

Natural sediment from juvenile sea cucumber habitat was collected with a sediment grab sampler (collection of top 4-6 cm to ensure inclusion of larger grain sizes, Figure 4.1) and transferred to the laboratory. The natural sediment was mixed at equal ratios with mussel waste (faeces and pseudofaeces) collected from beneath tank-cultured green-lipped mussels (*Perna canaliculus*) to increase carbon and nitrogen content in the sediment and improve palatability (Slater et al., 2009). To avoid any size class stratification of grains in sediment to be fed to juveniles, the sediment was mixed thoroughly to a thick paste which was then transferred onto three 0.5 m x 0.3 m plastic trays, spread to ca. 4 mm thick and frozen rapidly at -78°C. Rapid freezing was used to avoid any settling or stratification of sediment within the trays. The three resulting frozen sediment sheets were gently transferred to the base of three replicate polyethylene experimental tanks with a floor area of 0.20 m^2 (0.55 m × 0.35 m × 0.21 m L×W×H) and supplied with filtered sea water (50 µm filtered, 0.2 l min^{-1}) to a depth of 180 mm to fill the tank while avoiding disturbance to the sediment layer. A natural light cycle was maintained during the experiment although shaded from direct sunlight (L= 0.2 - 0.3 µE m^{-2} s^{-1}).

Full experimental tanks were left with sea water inflow for 4 h to ensure the seawater temperature had reached ambient levels. Six juvenile sea cucumbers with evacuated guts and weighing between 20 and 30 g were placed in each tank at 13:00 hrs. Sea cucumbers were allowed to actively feed for approximately 6 h then between 0:00 and 01:00 hrs sea cucumbers actively feeding on the sediment diet were immediately dissected. The gut was divided into two parts: Foregut = sediment in the oesophagus, stomach and beginning of small intestine to half body length (approx. 25 to 30 mm as measured from the pharyngeal bulb). Hindgut = sediment in the large intestine. Any faecal casts in the tanks were also collected. Foregut and hindgut contents of five animals per replicate tank, plus any faecal matter was collected, pooled for each replicate tank and stored at -78°C until analysis.

4.2.3 In situ sediment and foregut sampling

In situ sampling was conducted by SCUBA divers at a site with naturally high density of juveniles in the Mahurangi Harbour (Figure 4.1) (Chapter 2). An area of the benthos was selected where five juvenile sea cucumbers were actively feeding within approximately 2 m^2. Surface sediment samples of the upper 2-3 mm of surface sediment were taken directly in front of the oral end (< 10mm distance) of each juvenile. Juveniles were then collected and taken to the surface and immediately transferred to an adjacent jetty where they were dissected as described previously to obtain a pooled foregut and pooled hindgut sample. This dive collection was repeated twice more to obtain a total of three pooled foregut and three pooled hindgut samples and three pooled diet sediment samples. Samples were pooled in order to ensure that sufficient sample would be available for all required analyses. This pooling was necessary because of the small sample size able to be obtained from animal foregut in particular. All samples were returned to the laboratory within 1.5 h and stored at -78°C until analysis. Juvenile weight varied from 20 to 35 g (non gut-evacuated weight).

4.2.4 Surface sediment grain size distribution

Surface sediment and gut content sub-samples (approx. 0.8 g w.w. of sediment) were treated for 4 h in a sodium metaphosphate solution (5 g l^{-1}) to declump clay particles. Grain size distribution was then examined using a Malvern Mastersizer 2000 Particle Size Analyser using laser and blue light diffraction detection to determine sample grain size distribution (Singer et al., 1988; Sperazza et al., 2004). Particles were grouped into six size ranges: 0 - 63 µm, 64 - 125 µm, 126 - 250 µm, 251 - 500 µm, 501 - 1000 µm and 1001 – 2000 µm for electivity index calculation (Vanderploeg and Scavia’s Relativised Electivity index E_i*). Particle sizes were also grouped (100 groups) along a logarithmic size band from 0.020 to 2000 µm for comparative visual presentation of particle size distributions.

4.2.5 Carbon and nitrogen content

Surface sediment and gut content sub-samples (approx 0.5 g w.w.) were rinsed thoroughly with dH_2O and centrifuged for 5 min at 4000 rpm, the supernatant was discarded and the rinse step repeated to ensure removal of digestive fluids. The resulting pellets were re-suspended in dH_2O and wet sieved over a Prüfsieb DIN 4188 analytical sieve series to separate samples into 0 - 63 µm, 64 - 125 µm, 126 - 250 µm, 251 - 500 µm, 501 + µm size fractions. Fractions were then dried in a 60°C oven to constant weight (48 hrs). Dried samples were ball ground and stored in 1.5 ml Eppendorf tubes before being analysed for total carbon and nitrogen using a Leco TruSpec elemental determinator.

4.2.6 Selective movement

Dry dune sand was collected by hand from Pakiri Beach and transferred to the Leigh Marine Laboratory. This low organic content sand was mixed with varying amounts of mussel waste (section 4.2.2) to produce three sets of “high” and “low” organic content sediments.

Set A: TOM high = 1.76% (± 0.01 SE) versus low = 0.32% (± 0.01 SE)
Set B: TOM high = 2.84% (± 0.05 SE) versus low = 1.88% (± 0.06 SE)

Set C: TOM high = 3.80% (± 0.13 SE) versus low = 3.06% (± 0.11 SE)

A divider plate was placed along the centre line of an experimental tank (0.7 × 0.9 x 0.12 m L×W×H, figure 4.6) with a "sediments" base area (0.7 × 0.7 m L×W) and a small water inflow and sea cucumber "entry" area where the tank base was kept bare. The two sediment mixtures were gently transferred as slurries into the tank, one each to half the sediments base of the tank. Filtered sea water was supplied (100 µm filtered, 1.5 l min^{-1}, polypropylene disc filter) to fill the tank while avoiding disturbance to the sediment layer. Any excess sediment was removed from the entry area using a siphon cleaner. The divider plate was then gently removed. The tank was subjected to a 12:12 L:D light regime. Light intensity was 0.3 - 0.5 µE m^{-2} s^{-1} L= 7:00-19:00 hrs. The tank was illuminated during dark hours by a Tafu Electronic Tech Co® automated infra-red light source (wavelength 840 nm) mounted diagonally 1 m above the tank. A Swann CCD KC S-4140 colour-infrared camera with a low-power integrated infra-red light source (wavelength 840 nm) was mounted 1.7 m directly above the tank. A hard-drive connected to the camera recorded a single frame image of the tank every 10 min.

Eight gut evacuated juvenile sea cucumbers between 20 and 30 g were placed in the "entry" area of the tank at 16:00 hrs. Sea cucumbers were free to move and feed within the tank either in the entry area or within the low organic area or high organic sediment area. Images were analysed between 19:00 and 07:00 hrs for two consecutive nights. The position of the high and low organic content sediments was then reversed, the eight sea cucumbers were replaced with new gut evacuated juveniles and the experiment was repeated. The total number of juvenile sea cucumbers in high or low areas of the tank was recorded every 10 min for 12 hrs. This was repeated for each set of high versus low organic matter sediments, providing four nights footage for each set and a total of 12 nights of sampling.

The straight line distance moved by each individual was also tracked between 0:00 hrs and 7:00 hrs, this time period was selected as it includes the period of highest activity observed in *A. mollis* ex situ. Distance moved was calculated by measuring distance moved between "frames" at the animals anterior end as measured against on-screen scale bars along the tank edges. Movement distance was recorded in 30 mm increments, which proved to be the smallest accurately measurable distance. The position of animals over each sediment patch was also recorded allowing rates of movement in each patch to be calculated. Where animals moved between sediment patches from frame to frame or into the ‘entry area’, no value was recorded. Where animals did not move at all during the period from 0:00 hrs to 7:00 hrs and no interaction was observed with the sediment (i.e. no feeding, no mouth movement, no sediment touching) no values were recorded. The final sediment set (C orientation 2 - High L Low R) was also maintained and recorded over 7 days. Movement distance was quantified for days 4 and 7 of this treatment in order to assess changes in behaviour following the depletion of the food resource.

4.2.6 Statistical analyses

Comparing sediment samples with the aim of determining grain size selectivity was carried out visually using graphic presentation of results. Vanderploeg and Scavia’s Relativised Electivity index (E_i*), were used to calculate electivity based on the selectivity coefficient (W_i) whilst stabilising the index when relative abundance of food types (in this case particle size fractions) varies (Vanderploeg and Scavia, 1979; Lechowicz, 1982). E_i* is calculated as follows:

$$E_i^* = [W_i-(1/n)]/[W_i+(1/n)]$$

$$W_i = (r_i/p_i)/(\Sigma_i r_i/p_i)$$

Where:
r_i = the proportion of a particle size fraction in the foregut
p_i = the proportion of a particle size fraction in the sediment diet
n = the number of size fractions considered

Mean nitrogen and carbon contents of sediment and foregut samples for each size fraction were compared with a t-test. The same was applied to sediment versus hindgut samples for each size fraction. A Kolmogorov-Smirnov test and Browne-Forsythe test were applied to each tested category to ensure normality of distributions and homogeneity of variances. In addition, regression coefficients were calculated for nitrogen, carbon and sediment grain size spectra with sediment content plotted on the x axis (predictive) and foregut content on the y axis (dependent). A t-test was used to test the null hypothesis that samples do not differ, i.e., that the population regression line slope ($ß_o$) = 1 as per Zar (1999).

Mean numbers of juvenile sea cucumbers present in high versus low organic matter areas were calculated for all observations for each orientation (position left or right of tank) of high and low organic content sediments. A Kolmogorov-Smirnov test was applied to ensure normality of distribution of averages and a Browne-Forsythe test was used to ensure homogeneity of variances. A one-way ANOVA was used to test for significant differences between mean values of high versus low TOM.

Daily mean distances moved in high versus low organic matter areas were calculated for each sediment set. A non-parametric Mann–Whitney U test was used to identify significant differences in mean daily distance moved between high and low TOM patches for each sediment set (A, B and C). The same test was applied to all data irrespective of sediment set. All statistical tests were carried out using StatSoft Statistica 6.0 software.

4.3 Results

4.3.1 Particle size selectivity

Visual comparison of particle size spectra of foregut and surface sediment for juveniles collected in situ and laboratory specimens reveals no appreciable selectivity based on particle size (Figure 4.2). Both in situ and laboratory foregut contents exhibit a slight increase in total volume of particles in the range from 10 to 70 µm, however, spectra show very similar shapes and proportions of each grain size fraction by volume. Regression analysis of foregut versus sediment grain size fractions resulted in a regression slope B which did not deviate significantly from 1 (x=y) for in situ ($t_{(16)} = 0.79$ $p>0.25$) or tank samples ($t_{(16)}= 1.17$ $p>0.25$)(Figure 4.3).

Calculated E_i* values for in situ samples showed slightly positive selectivity towards higher size classes (251 – 500 µm). Conversely, laboratory samples exhibited a low level of positive grain size selectivity towards smaller particle size classes (0-63 µm and 64-125 µm) (Table 4.1). These positive selectivity values coincide with very low proportions of particle size fractions in diet sediments in each case (Figure 4.2).

4.3.2 Organic selectivity

In three of six sub-samples of in situ juvenile gut content, the coarser particle size fractions (251-500 and 501+ µm) were of insufficient volume to be accurately assayed. Triplicate assaying was only possible for 0-63 µm, 64-125 µm samples and 126-250 µm samples. Laboratory samples, however, allowed organic content assaying across all size classes.

Foregut carbon and nitrogen content of in situ and laboratory samples were significantly higher than those measured from the surface sediment across the vast majority of particle size fractions (Table 4.2). In situ foregut sample fractions 0-63 µm and 64-125 µm showed a 42% and 53% increase in nitrogen content and a 77% and 30% increase in carbon content respectively (Figure 4.4). Laboratory foregut sample fractions exhibited 82%, 119% and 40% increases in nitrogen content and 145%, 60% and 103 % increases in carbon content across size fractions 0-63 µm, 64-125 µm and 126-250 µm respectively. Highest carbon and nitrogen contents were found in the smallest grain size fraction for laboratory-sourced samples.

Regression analysis of foregut versus sediment carbon resulted in a regression slope b that deviated significantly from 1 (x=y) for in situ ($t_{(10)}$= 1.17 $p>0.25$) and for laboratory samples ($t_{(13)}$ = 6.99 $p< 0.001$) (Figure 4.5). Foregut versus sediment regression analysis for nitrogen content also produced regression coefficients which differed significantly from 1 for both in situ ($t_{(10)}$= 1.84 $p>0.05$) and for laboratory samples ($t_{(13)}$ = 4.22 $p< 0.001$) (Figure 4.6).

Hindgut nitrogen and carbon content was also significantly higher than that of surface sediment across the majority of size fractions assayed with the exception of the 125-250 µm fraction for nitrogen and carbon at both sites (Table 4.2).

4.3.3 Selective movement

Significantly larger numbers of juvenile sea cucumbers were present in high organic content areas for sets A (1.76% versus 0.32% TOM, $F_{(1)}$ = 531.6 $p<0.05$) and B (2.84% versus 1.88% TOM, $F_{(1)}$ = 213.6 $p<0.05$) of sediments tested (Figure 4.7). There was no significant difference between mean number of animals present in high and low patches for sediment set

C (3.80% versus 3.06% TOM, $F_{(1)} = 2.69$ p>0.3). Movement patterns of juveniles were not significantly different between positions of the sediment treatments (left or right) of high and low sediment patches ($F_{(1)}$ p> 0.15 for all cases).

Overall, greater distances were moved by animals over low TOM patches in comparison to their respective paired high TOM patches. Averaged over all observations and all sediment sets juveniles moved an average of 24 mm (± 0.7 SE) every ten minutes when in high TOM patches and 42 mm (± 1.9 SE) when in low TOM patches (Mann–Whitney U = 25, $n_L = 11$ $n_H = 12$, $p < 0.05$) with total distance moved decreasing as TOM increased in low patches from 0.3 to 3.0 % (Figure 4.8). Daily means of distances moved were however highly variable and n values were low, consequently differences were not statistically significant between high and low TOM patches compared at the level of individual sediment sets (A - high vs. low, B - high vs. low and C - high vs. low). Once the entire sediment surface had been grazed in treatment C orientation 2 (recorded for 7 days) the movement increased so markedly, that individual animals could not be identified between consecutive 10 min frames so that the movement of individuals could not be quantified reliably. Feeding ceased almost completely, no coprophagia was observed, and all animals continued to exhibit rapid large scale movements.

4.4 Discussion

The current research applies a novel combination of methods to accurately determine selectivity of feeding in juvenile *A. mollis*. Diet sediment and foregut content analysis reveals organic selectivity for both carbon and nitrogen content and a clear lack of grain size selectivity. The results provide evidence of the ability of aspidochirote sea cucumbers to select organic particles within particle size spectra. In addition, laboratory examination of movement indicates that juvenile *A. mollis* are capable of controlling their movement in response to organic content of sediments, but that movement and the ability to select may have a distinct upper limit of organic content.

The level of enrichment in juvenile *A. mollis* foregut as a result of organically selective feeding is higher than that measured for *Parastichopus parvimensis* (Yingst 1982), similar to *Holothuria atra* and *Stichopus chloronotus* (Moriarty, 1982), and considerably lower than reported for *A. japonicus* (nitrogen 300-500%) (Tanaka, 1958), for *Stichopus tremulus* (organic content >400%) Haukson (1979), and for *Parastichopus californicus* (organic content >300%) (Paltzat et al., 2008). In the current research the percentage organic enrichment in juvenile *A. mollis* foregut did not vary greatly between in situ (30-80%) and laboratory (40-145%) prepared sediment assays, despite the fact that the laboratory diet sediment organic concentration was 300% higher. This indicates that the organic selective feeding ability of this species does not vary greatly with varying sediment organic concentration in the range of diets provided. Thus higher levels of enrichment recorded in other studies are probably due to stronger selective feeding ability in other sea cucumber species.

Methodological differences cannot, however, be ignored as a possible cause of the differences in the enrichment level measured between this and previous studies. Juveniles were used exclusively in the current research, as opposed to adults in all previous studies, and juveniles may exhibit weaker selective feeding abilities (Cameron and Fankboner, 1984). Alternatively, simple differences in sampling (such as dH_2O rinsing of samples to remove digestive fluids, or differences in defining foregut area) and assaying techniques may also be a possible explanation for observed differences in results among studies. Many previous studies of holothurian selective feeding have not rinsed foregut samples to remove digestive fluids despite Yingst (1974) suggesting that observed levels of enrichment in foregut content may be due to mucous added to ingested sediments (Haukson, 1979; Moriarty, 1982). It is difficult to ascertain whether the dH_2O rinsing applied guarantees the removal of all digestive fluids or mucus. Equally, it is not possible to determine whether labile organic fractions are lost at rinsing. However, if enrichment were due to digestive fluids alone, the percentage of enrichment could not be expected to remain the same or even increase (as observed) when animals feed on sediments with significantly higher organic content levels. Combining in situ and laboratory-based experiments in the current study allows it to be shown that digestive fluids are not responsible for the observed foregut enrichment in *A. mollis*. In addition, controlling the stratification of sediments in laboratory-based experiments has allowed surface sediment sampling methods in situ to be validated and in situ results confirmed for both organic and grain size selection.

The organic selectivity exhibited by juvenile sea cucumbers in the current research highlights the important role of *A. mollis* in sub-tidal communities and is useful in explaining the efficient remediation of enriched sediments by deposit-feeding sea cucumbers (Isgoren-Emiroglu and Gunay, 2007; Slater and Carton, 2009; Zheng et al., 2009). Selective feeding by *A. mollis* (which consumes approximately 3 kg sediment per annum as a conservative estimate, Slater, 2006) results in targeted nutrient recycling in several ways. Firstly, it directly reduces the organic content in the selectively consumed sediment as nutrients are assimilated and excreted by the sea cucumber (Uthicke, 2001; Maxwell et al., 2009). Secondly, feeding bioturbates large areas of sediment, exposing new sediment to oxygenated water at the sediment-water interface (Bakus, 1973; Slater and Carton, 2009). Thirdly, organically selective feeding repackages concentrated organic content into faecal casts which lose labile organic content to the water column more quickly and provide greater surface area and enriched substrate for bacterial mineralisation of nutrients (Hargrave, 1976; Hammond, 1982; Uthicke, 1993; Slater, 2006).

The capacity for *Australostichopus mollis* to select its position at small scales based on the organic content of available sediments may help explain natural patterns of distribution of juveniles. While limited in scope and scale, the laboratory observations show that juvenile *A. mollis* spend less or more time in an area in response to variations in the organic content of the sediment at scales of less than a metre. In addition, the results show that juveniles move more quickly on average over areas of low TOM and change behaviour to faster and larger scale search movement when a food source is depleted. Patchy juvenile *A. mollis* distribution has previously been shown to correlate strongly with increasing nitrogen content of ambient sediments and smaller grain sizes (Chapter 2). Spatial selectivity based on organic content may help to explain both the observed distribution and correlation with high nitrogen. While movement of deposit-feeding sea cucumbers has been shown to be random over large scales on lagoon flats, random large-scale movement of *A. mollis* may be a search strategy primarily associated with locating an undepleted area in which to feed (Da Silva et al., 1986). Where organic availability is too low, juvenile *A. mollis* may simply cease feeding and move faster over the sea floor, to find new areas where the level of organic matter in the sediment is sufficiently high to stimulate feeding.

The calculated electivity values differ greatly from the results of visual analysis of graphed grain size spectra and regression slope testing. The variable results appear to be influenced by the low frequency of certain grain sizes. Results indicate that even more robust measures of selectivity such as E_i* are not applicable for analysing grain size selection by deposit feeders, particularly when the grain size spectrum measured is unevenly distributed (Hammond, 1982; Lechowicz, 1982). The lack of grain size feeding selectivity found in juvenile *A. mollis* indicates that this species is unlikely to redistribute specific grain size fractions or alter grain size stratification in benthic sediments. The observed lack of grain size selectivity also seems to support the theory that low diversity of aspidochirote species within a specific area may result in reduced levels of selectivity by individual species (Sloan and von Bodungen, 1980; Roberts and Bryce, 1982).

The lack of particle size selectivity allows for simpler formulation of artificial diets for the aquaculture of *A. mollis* as it appears that diets can be supplied in a wide range of particle sizes. Powdered or micropellet diets up to 1 mm in diameter should be readily consumed if appropriately organically enriched assuming the selective feeding mechanism is sufficiently versatile to detect artificial sources of organic matter. Simple and low-cost powdered or granulated diets that are produced from processing wastes or by-products may even be useful (Kiang, 1998; Bureau et al., 1999). However, rather than supplying powdered feed ingredients separately to sea cucumber cultures, the mixing and binding of formulated feed ingredients may be important to ensure that the diet ingredients are not preferentially selected. The searching behaviour of sea cucumbers observed in this study suggests that the even distribution of formulated feeds in tank or pond culture may be important to ensure the sea cucumbers remain evenly distributed and that stocking levels can be kept suitably high. A lack of grain size selectivity among juvenile *A. mollis* also has potential advantages for polyculture applications where sediment grain size can be significantly altered as a result of biodeposition (Mattsson and Linden, 1983).

4.5 Conclusions

The investigation of feeding selectivity in a deposit-feeding sea cucumber, *A. mollis*, both in situ and in the laboratory has been achieved by carefully controlling sediment grain size and organic stratification in the laboratory and accurate diet sampling. Juvenile *A. mollis* feeding is organically selective, but non-selective for specific grain sizes. These sea cucumbers also show an ability to move in response to varying levels of organic content of benthic sediment. Differences in foregut organic enrichment between sea cucumber species are probably due to lower selective capacity but may also be due to differences in the research methods used for examining the feeding in different species. Results of this study reiterate the importance of *A. mollis* in nutrient recycling and regulation in coastal sediments. The organically selective feeding shown here is one of several mechanisms underlying the effective remediation of organically enriched sediments by *A. mollis* and other aspidochirotes. In addition, the results show the feeding biology of *A. mollis* may suit simple formulation of artificial diets as no optimal diet particle size needs to be provided. However, organic content of formulated diets must be suitably high and supplied in a spatially uniform manner within the culture environment. Further research will be required to understand the underlying mechanisms used for selection and more accurate methods in determining selective movement may reveal more precise selective feeding ability based on organic content.

Table 4.1 Vanderploeg and Scavia's Relativised Electivity index (E_i*) scores for *A. mollis* foregut versus sediment diet (by grain size fractions) in laboratory feeding experiment and *in situ* sampling.

Particle size fraction (µm)	Ei* Lab.	Ei* In situ
0.01-63	0.178	-0.036
63-125	0.244	-0.067
125-250	0.087	-0.095
250-500	0.032	0.260
500-1000	-0.201	0.109
1000-2000	NA	-0.410

Table 4.2 Carbon and nitrogen proportion of foregut and sediment samples from in situ and laboratory sampling of juvenile A. mollis. p 1 = p value foregut versus sediment. p 2 = p value hindgut versus sediment. T-test df = 4. n = 3 (In situ: foregut 251-500 µm n= 2; foregut 501+ µm n = 1). Sed = sediment, SEM = standard error of the mean, Fore = foregut, Hind = hindgut

Sample source	Particle size fraction	Element	Sed. %	SEM	Fore. %	SEM	Hind. %	SEM	p 1	p 2
Laboratory	0-63 µm	Nitrogen	0.56	0.01	1.02	0.08	0.75	0.03	0.005	0.006
Laboratory	64-125 µm	Nitrogen	0.11	0.01	0.24	0.02	0.15	0.01	0.006	0.054
Laboratory	126-250 µm	Nitrogen	0.07	0.00	0.10	0.01	0.07	0.00	0.029	0.334
Laboratory	251-500 µm	Nitrogen	0.07	0.01	0.12	0.01	0.10	0.01	0.005	0.043
Laboratory	501+ µm	Nitrogen	0.08	0.03	0.36	0.07	0.21	0.04	0.018	0.074
In situ	0-63 µm	Nitrogen	0.20	0.00	0.29	0.01	0.25	0.01	0.001	0.004
In situ	64-125 µm	Nitrogen	0.07	0.00	0.11	0.01	0.11	0.00	0.017	0.002
In situ	126-250 µm	Nitrogen	0.12	0.01	0.13	0.01	0.11	0.01	0.424	0.420
In situ	251-500 µm	Nitrogen	0.18	0.01	0.20	0.01			N.V	N.V
In situ	501+ µm	Nitrogen	0.19	0.02	0.25	0.00			N V	N V
Laboratory	0-63 µm	Carbon	4.77	0.07	7.70	0.51	6.45	0.13	0.005	0.006
Laboratory	64-125 µm	Carbon	0.51	0.09	1.25	0.19	0.89	0.05	0.023	0.029
Laboratory	126-250 µm	Carbon	0.26	0.02	0.42	0.04	0.29	0.00	0.016	0.137
Laboratory	251-500 µm	Carbon	0.28	0.04	0.57	0.03	0.45	0.03	0.003	0.025
Laboratory	501+ µm	Carbon	0.44	0.07	1.58	0.29	0.93	0.15	0.018	0.071
In situ	0-63 µm	Carbon	1.55	0.08	2.32	0.05	2.20	0.10	0.001	0.010
In situ	64-125 µm	Carbon	0.50	0.06	0.88	0.04	0.69	0.02	0.007	0.049
In situ	126-250 µm	Carbon	1.08	0.09	1.41	0.05	1.17	0.07	0.036	0.564
In situ	251-500 µm	Carbon	1.97	0.16	3.28	0.44			N.V	N.V
In situ	501+ µm	Carbon	3.25	0.78	4.24	0.00			N V	N V

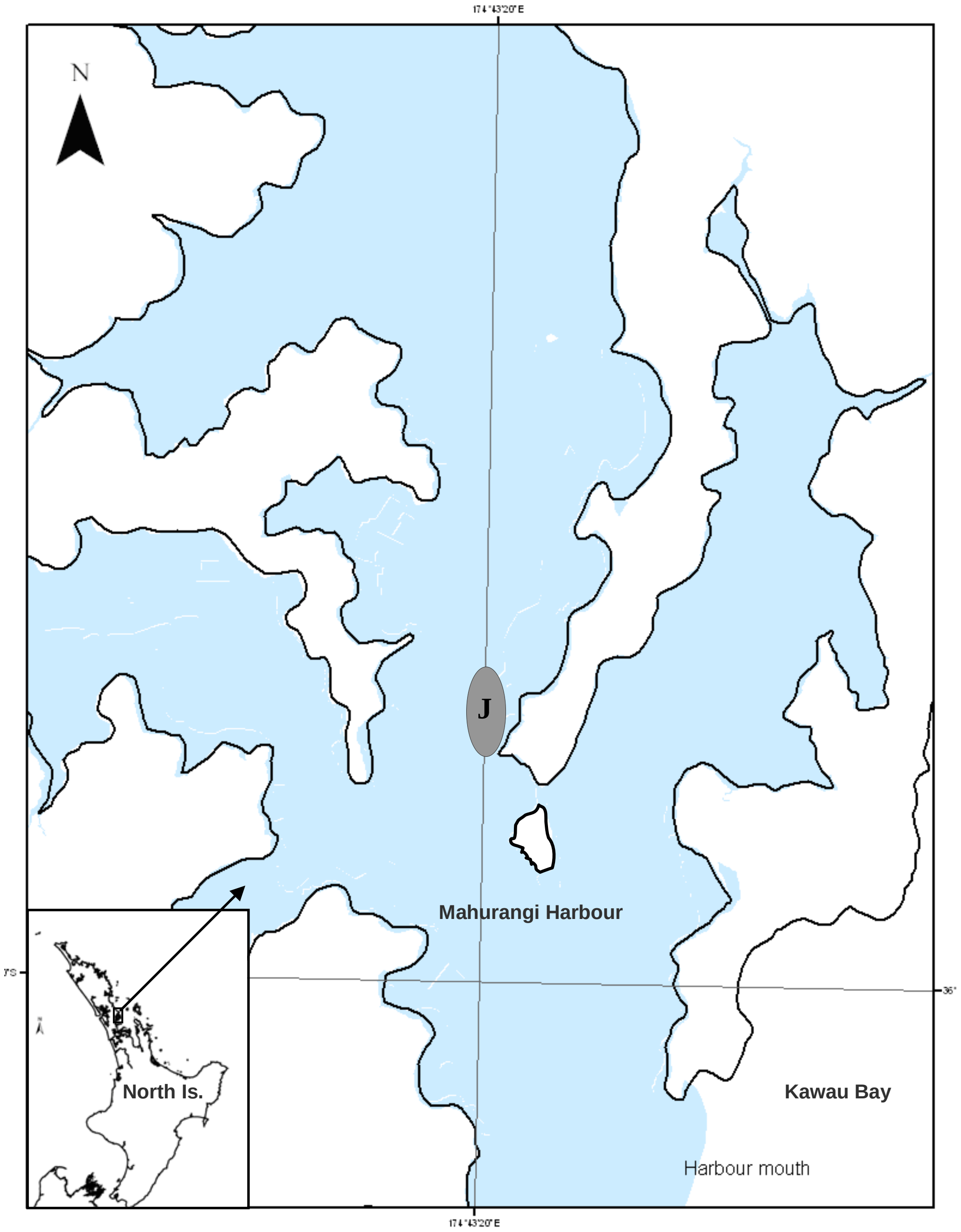

Figure 4.1 The Mahurangi Harbour showing area of high density of juvenile *A. mollis* and where in situ collections were made for sea cucumber gut contents and surface sediment analyses (J).

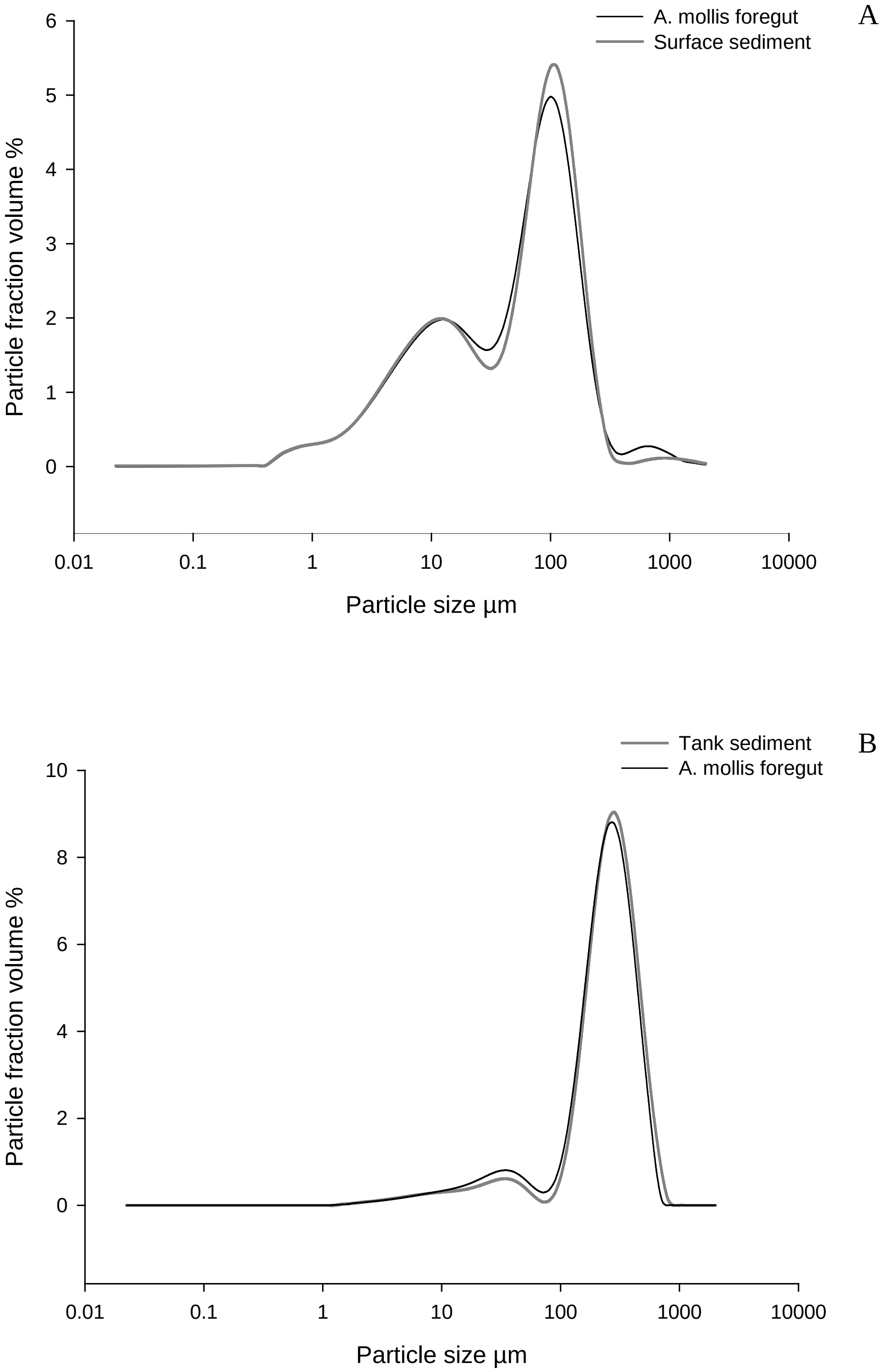

Figure 4.2 Grain size distribution of grazed surface sediments (grey line n = 3) and juvenile *A. mollis* foregut content (black line n = 3) collected in situ in Mahurangi Harbour (A) and in laboratory feeding experiments (B).

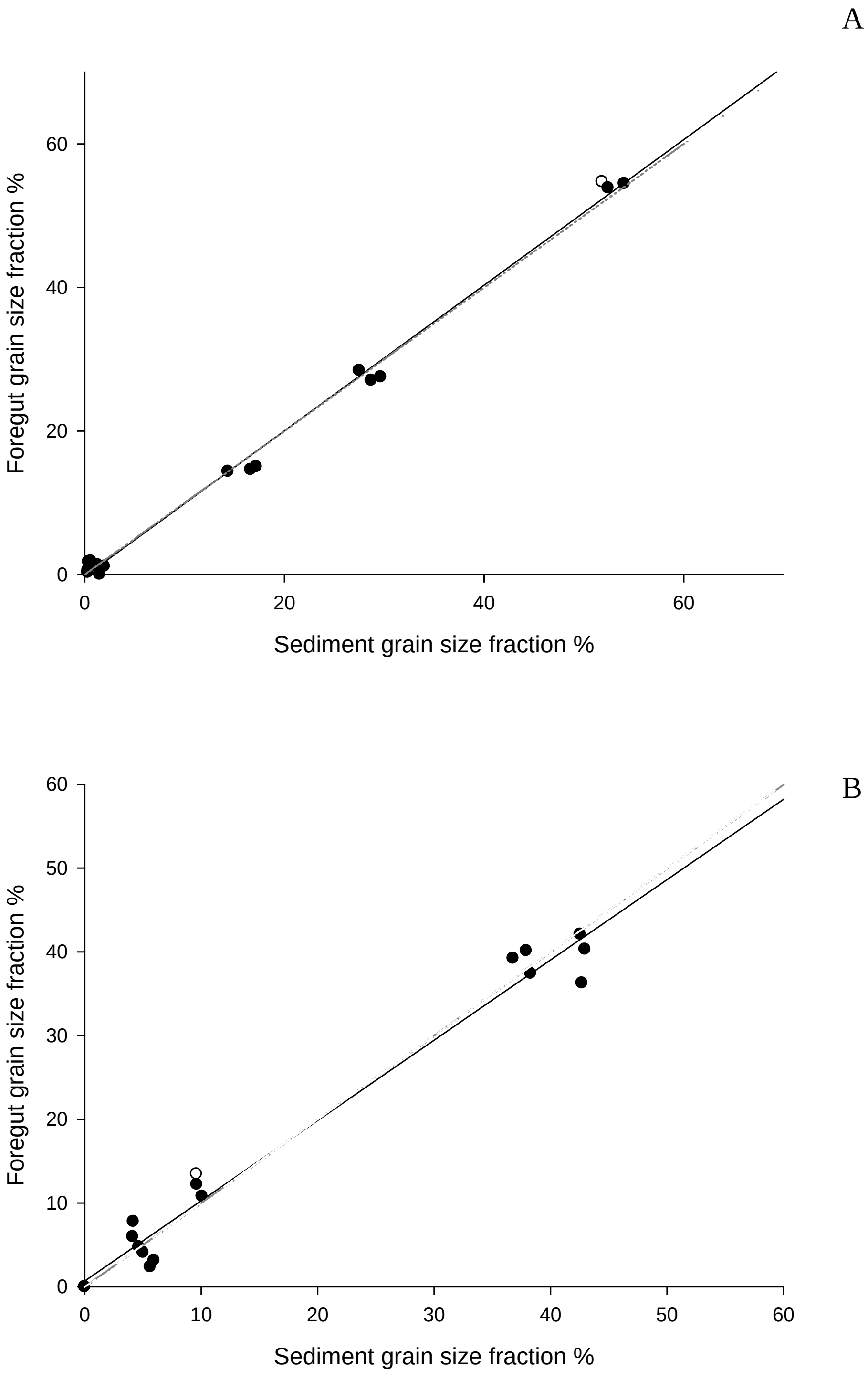

Figure 4.3 Scatterplot and calculated regression line for grain size distribution (% of total sample) in grazed surface sediments (x) versus juvenile *A. mollis* foregut content (y) collected in situ in Mahurangi Harbour (A) (n = 18) and in laboratory feeding experiments (B) (n= 18). Grey dashed lines indicate x = y slope (ßo = 1).

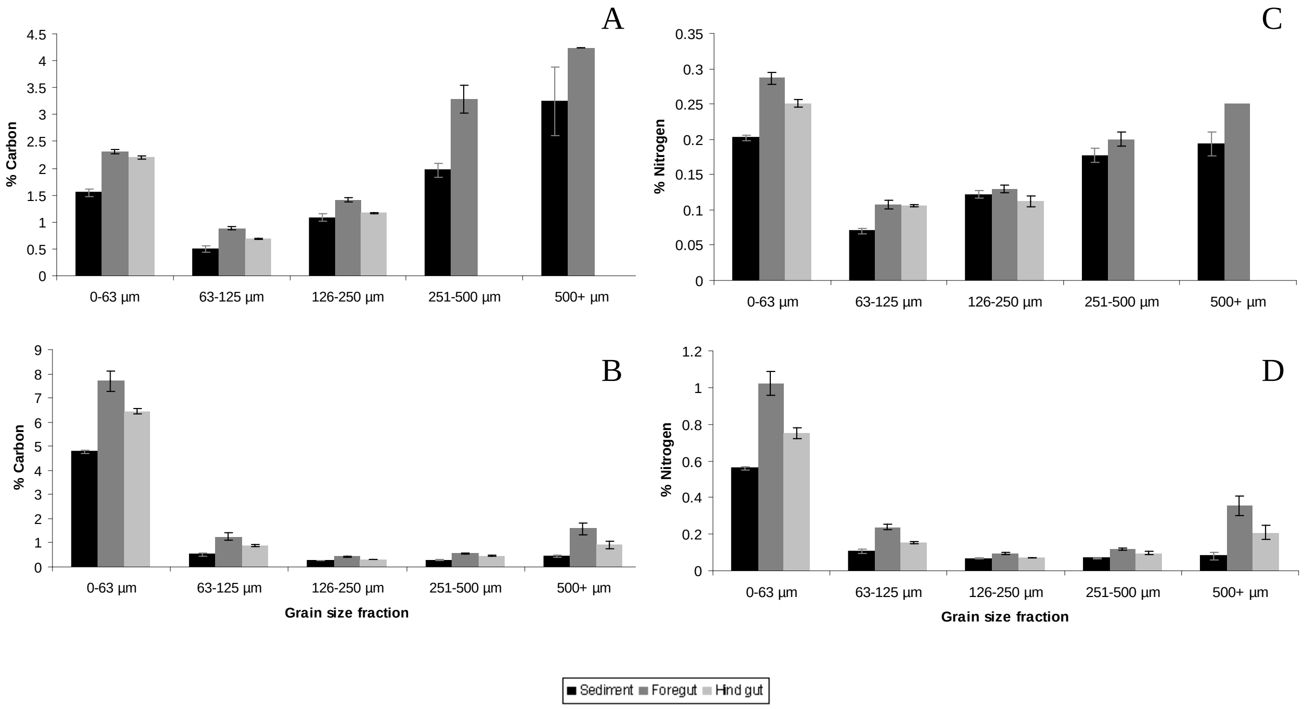

Figure 4.4 Percentage carbon and nitrogen in grain size fractions of surface sediments; *A. mollis* foregut content; and hindgut content collected in situ in Mahurangi Harbour (A, C) and in laboratory feeding experiments (B, D). Error bars indicate standard error of the mean, n = 3 for all bars (n = 2 foregut A, C 250-500µm and n = 1 A, C foregut 500+µm).

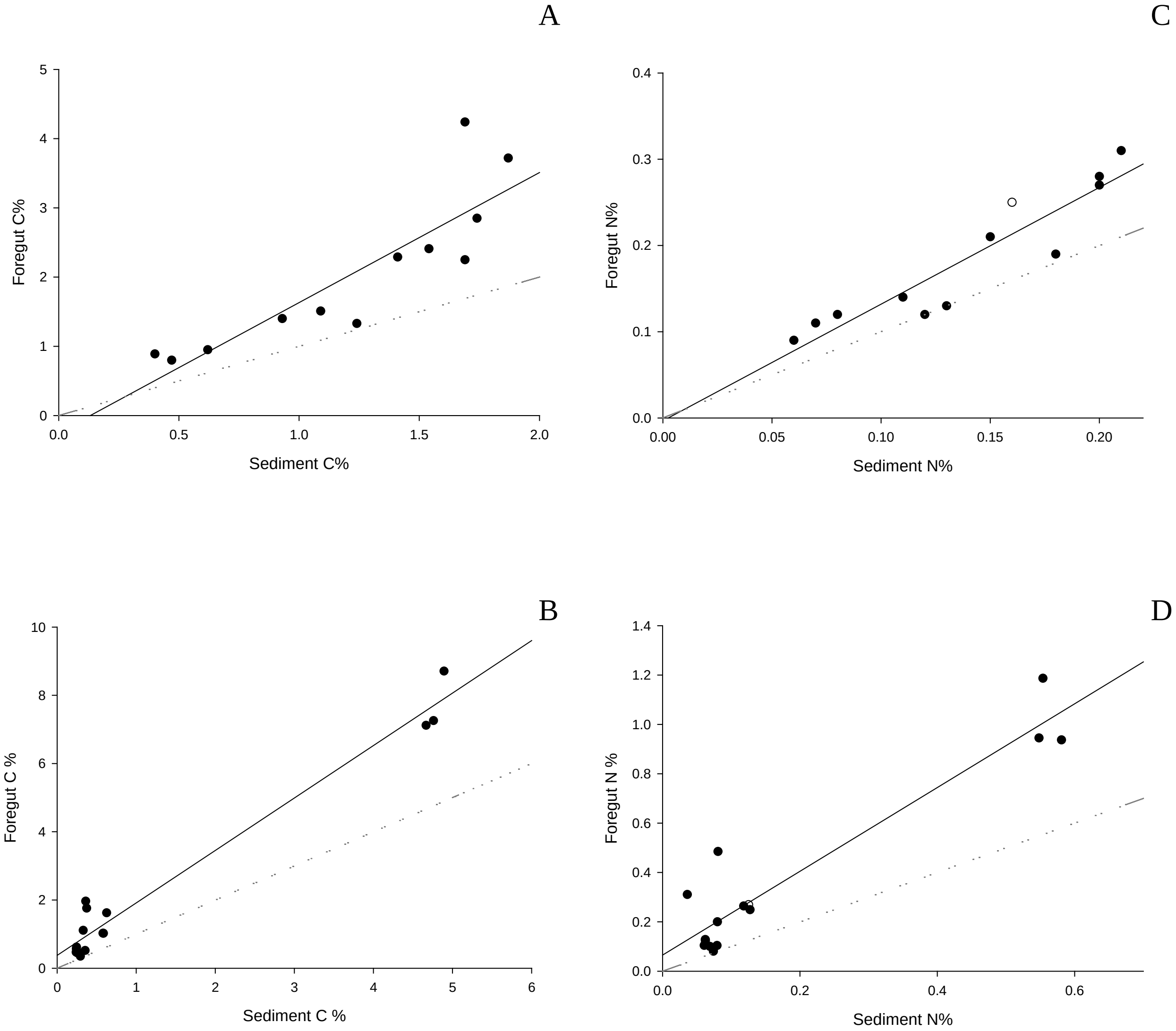

Figure 4.5 Scatterplot and calculated regression line for carbon (A, B) and nitrogen (C, D) content in grazed surface sediments (x) versus juvenile *A. mollis* foregut content (y) collected in situ in Mahurangi Harbour (A, C) (n =12) and in laboratory feeding experiments (B, D) (n =15). Grey dashed lines indicate x = y slope (ßo = 1).

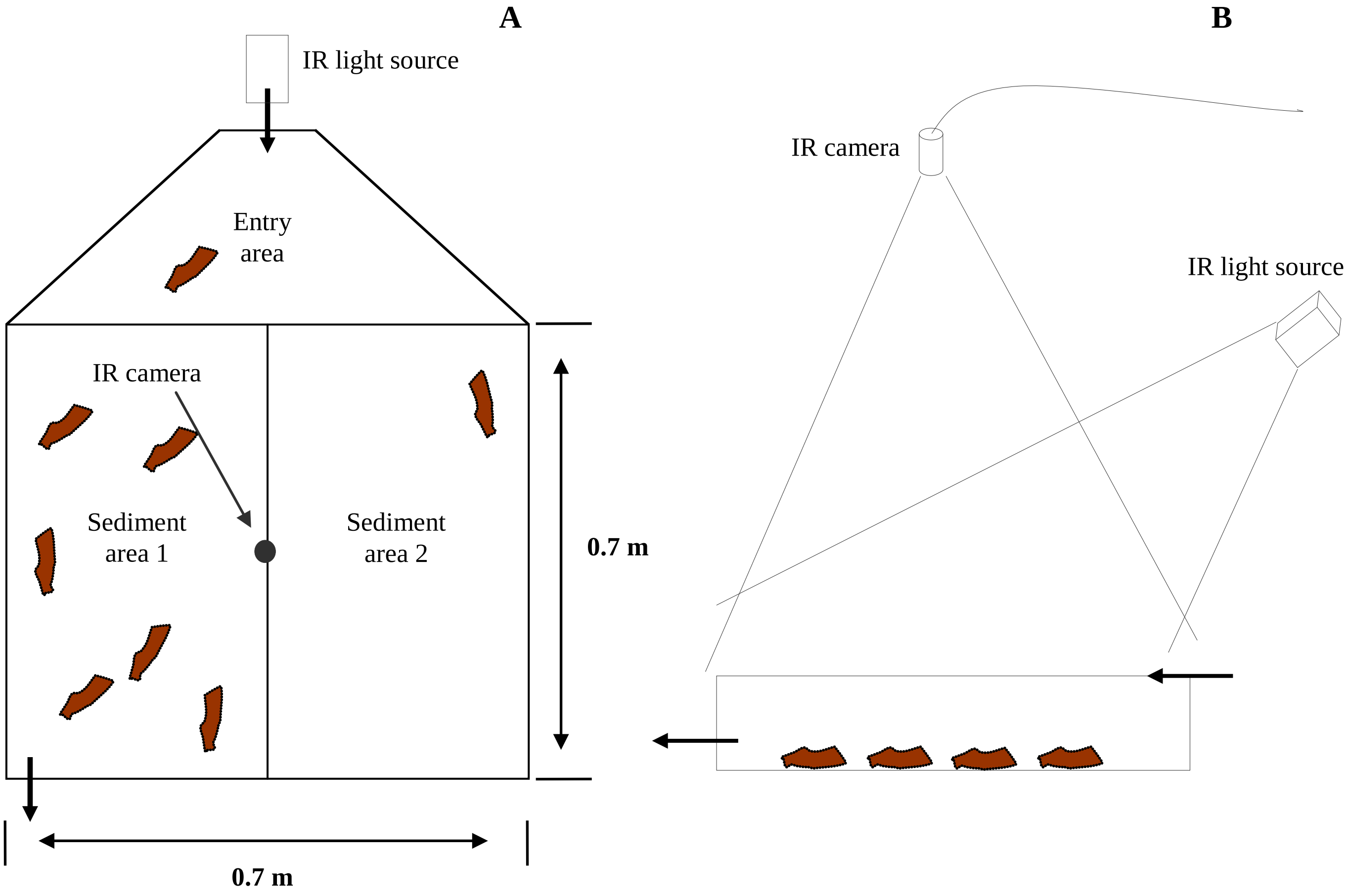

Figure 4.6 Schematic of selective movement tank in relation to experimental sediment enrichment with organic matter. Overhead view showing entry area and behavioural choice arena (A) and side view showing camera and light source mounting (B). Bold arrows indicate filtered seawater inflow and outflow.

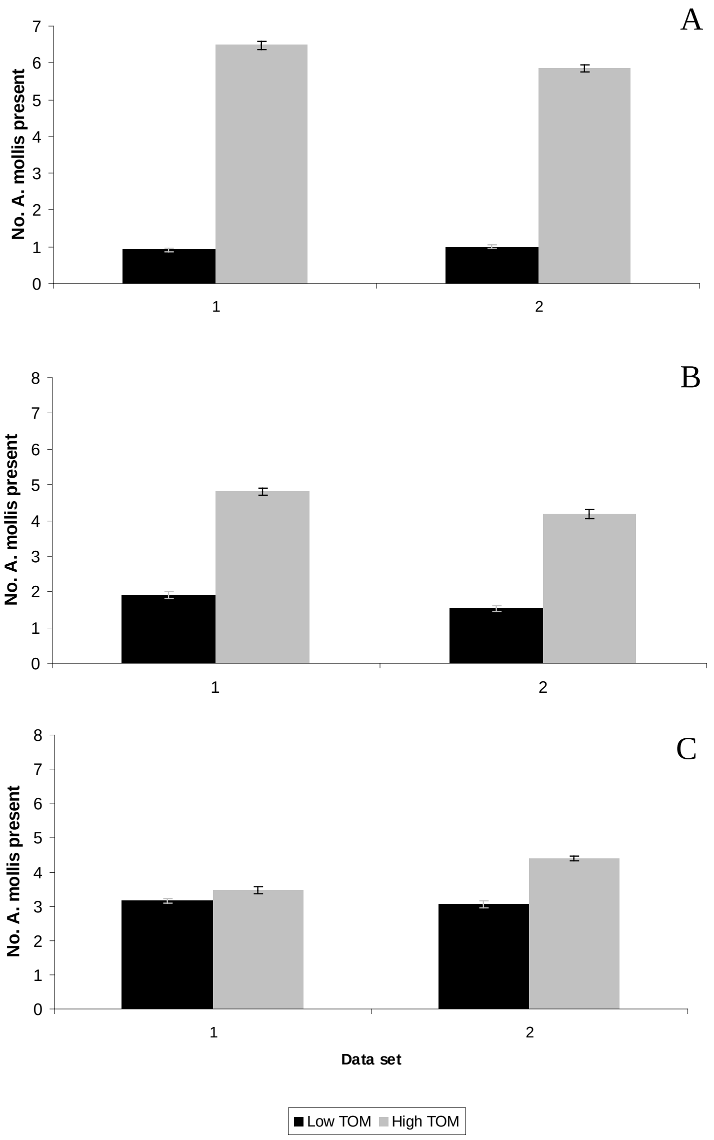

Figure 4.7 Mean number of juvenile *A. mollis* present on low and high organic matter patches (A) TOM 1.85% H vs. 0.32% L; (B) TOM 2.97% H vs. 1.98% L, (C) TOM 3.80% versus 3.06%. Mean calculated from 146 observations (2 nights) per data set.

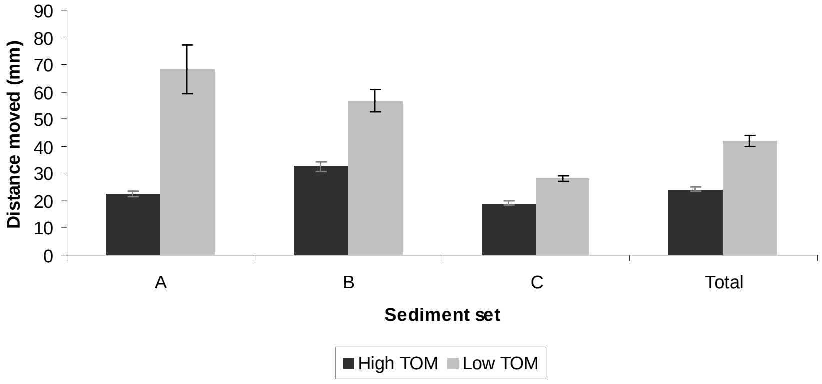

Figure 4.8 Mean distance moved by juvenile *A. mollis* in low and high organic matter patches (A) TOM 1.85% H vs. 0.32% L; (B) TOM 2.97% H vs. 1.98% L; (C) TOM 3.80% versus 3.06% and overall mean distance moved.

Chapter 5: The Use of the Waste from Green-lipped Mussels as a Food Source for Juvenile *Australostichopus mollis*

Published as: Slater, M.J., Carton, A.G., Jeffs, A.G., 2009. The use of the waste from green-lipped mussels as a food source for juvenile sea cucumber, *Australostichopus mollis*. Aquaculture 292, 219-224.

5.1 Introduction

Efforts to develop effective methods to culture the high-value sea cucumber *Australostichopus mollis* are expanding commensurate with its increasing commercial exploitation in New Zealand and Australia (Slater and Carton, 2007; Stenton-Dozey, 2007). Recent studies have shown this species has excellent potential for polyculture by releasing hatchery-raised juveniles beneath mussel farms, while polyculture with commercial abalone farms is not viable (Maxwell, 2006; Slater and Carton, 2007). Successful juvenile sea cucumber rearing requires knowledge of feeding behaviour and dietary requirements. However, there remains a paucity of information regarding the feeding behaviour, growth rate, habitat preferences and general biology of juveniles and sub-adults of sea cucumber species, including *A. mollis* (Sewell, 1990; James, 2005). Furthermore, there is limited information on artificial diets capable of inducing rapid growth in commercially valuable sea cucumbers (Yuan et al., 2006; Zhou et al., 2006; Giraspy and Ivy, 2008). There are no data regarding natural juvenile diets or the value of artificial diets in promoting juvenile growth of *A. mollis* and no data are currently available regarding natural or artificial growth rates. Improved knowledge of juvenile feeding behaviour is essential to assist in nursery culture efforts for laboratory reared juveniles of this species and in improving the success of pilot aquaculture schemes with this species.

A large amount of research has been carried out to develop effective hatchery methods to produce juvenile sea cucumbers for aquaculture supply and for enhancement of wild populations of commercial sea cucumber species. This research has focussed primarily on *Holothuria scabra* and *Apostichopus japonicus* (Imai and Inaba, 1950; Ito and Kitamura, 1997; Battaglene et al., 1999; Tanaka, 2000; Agudo, 2006). Currently post-settlement juvenile sea cucumbers are fed a variety of dried and powdered algae such as *Sargassum* spp and *Ulva lactuca* after they are weaned from larval settlement plates covered in benthic diatoms (James et al., 1994; Yanagisawa, 1998). Success in post-larval rearing using seaweed extracts and fermented seaweeds has been reported (Ito and Kitamura, 1997; Yanagisawa, 1998; Asha and Muthiah, 2007; Giraspy and Ivy, 2008). Juvenile *H. scabra* fed commercially available dried microalgae feeds also showed significant growth (Battaglene et al., 1999). Excellent growth has been achieved for sub-adult *A. japonicus* fed mixed algal diets containing dried bivalve faeces, but a diet of pure dried bivalve faeces (without algae) resulted in negative growth (Yuan et al., 2006).

Further improvement of nursery culture methods for hatchery-reared juveniles for polyculture is vital because sea cucumbers of a larger size at release have better survival (Tanaka, 2000; Purcell and Simutoga, 2008). The higher survival of larger juveniles is probably due to both size-related predation and improved feeding capabilities.

Therefore, the aim of this study was to begin to determine an initial effective nursery diet for juvenile *A. mollis*. Mussel waste and dried *Sargassum polycystum* were used because previous studies have reported that these diets are applied in commercial hatcheries as nursery feed for juvenile sea cucumber species (Yuan et al., 2006; An et al., 2007). Furthermore, the inclusion of several rates of feeding of mussel waste will assist in determining future stocking densities for polyculture of *A. mollis* beneath mussel farms with known waste deposition rates.

5.2 Methods

5.2.1 Experimental animals

Juvenile *A. mollis* weighing between 5 and 40 g were collected by SCUBA divers from a site with naturally high density of juveniles in the Mahurangi Harbour in Northern New Zealand. The sea cucumbers were transferred to the nearby Leigh Marine Laboratory and held in tanks with flowing 100 µm filtered sea water. The juvenile sea cucumbers were suspended in mesh bags for 24 h to ensure gut contents were evacuated.

Gut evacuated juveniles were photo-identified and weighed to the nearest gram after excess external water was removed from the sea cucumbers by placing them on unbleached tissue to blot dry (Raj, 1998). The sea cucumbers were allocated to 18 groups, each of five individuals. Average weight (within each group) ranged from 20.4 g (±4.2 g SD) to 23.2 (±3.9 g SD), overall average weight (all group averages) was 21.7 (±0.8 g SD). The groups of five individuals were then randomly allocated to 18 polyethylene experimental tanks with a floor area of 0.20 m^2 (0.55 m × 0.35 m × 0.21 m L×W×H) and supplied with filtered sea water (100 µm filtered, 0.5 l min^{-1}) to a depth of 180 mm. Natural light cycle was maintained during the experiment although shaded from direct sunlight (L= 0.2 - 0.3 µE m^{-2} s^{-1}). Two empty tanks were also maintained as water filtration controls for assessing POM sedimentation in tanks by gathering, drying and weighing the settled POM at the end of each month of the experiment.

5.2.2 Experimental diets

Three types of experimental diets were used; Natural Sediment from the surface (top 2 cm) of the seafloor collected by a sediment grab sampler from the habitat where the experimental juveniles were collected, Mussel waste material collected from beneath tank-cultured green-lipped mussels (*Perna canaliculus*), and a Dried Seaweed diet made of dried and ground (to pass 100 sieve = 150µm) *Sargassum polycystum* supplied by SAFA Seaweed Products Ltd.

Six dietary treatments were used in this feeding experiment, with natural sediment and mussel waste diets being delivered in two and three different experimental rations respectively (Table 5.1).

The Natural Sediment Low diet feeding rate (diet dry weight supply = 103 g m^2 d^{-1}) was selected to approximate the rate of sedimentation at the site where juveniles were collected (119 - 163 g m^2 d^{-1} dry weight (Forrest, 1991)). Mussel Waste Low was selected as a direct comparison to the wet weight supply of Natural Sediment. Mussel Waste Medium (diet dry weight supply = 91 g m^2 d^{-1}) was chosen to reflect the mean sedimentation rate beneath a typical green-lipped mussel farm in New Zealand (Hartstein, 2003; Hartstein and Stevens, 2005).

5.2.3 Juvenile growth

All experimental animals were weighed each month for 3 mo. The gut contents of sea cucumbers were evacuated followed by photographic identification of individuals and weighing as previously described. Weight data were then used to calculate a specific growth rate (SGR) as follows:

$$\text{SGR} = 100*(\text{LN}(\text{Current Weight})-\text{LN}(\text{Initial Weight}))$$

5.2.4 Survival

Any juveniles which developed obvious signs of illness, (i.e. visible discolouration or deformation of the epidermis), were immediately removed from the tanks. Removed animals were held in separate quarantine tanks and observed. Any deaths were recorded and animals removed to quarantine were excluded from results.

5.2.5 Morphometric parameters

At completion of the experiment (3 mo) the sea cucumbers were placed on ice and opened with a scalpel along the left ventral interambulacrum. The viscera and any remaining coelomic fluid were removed completely and the complete integument (including tentacles and calcareous ring) was immediately stored in individual labelled plastic bags at -80°C for subsequent measurement of morphometric parameters.

The frozen juveniles were later allowed to thaw at 4° C then dabbed dry to remove all fluid and weighed to the nearest 0.1 g to obtain gutted weight. Body wall thickness was measured once at the midpoint between the oral and anal ends to the nearest 0.1 mm using callipers. The widest point of the left dorsal longitudinal muscle band was measured to the nearest 0.1 mm using callipers. Gutted weight to wet weight ratio was calculated for all individuals to confirm the accuracy of wet weight measures applied during the duration of the experiment.

5.2.6 Diet and sediment assays

Three samples of the mussel waste diet were taken during diet preparation and were stored at -13° C before being freeze-dried for 24 h. Freeze dried samples were then analysed for lipid to AOAC standard methods (960.39, 991.36 after (Bligh and Dyer, 1959)), protein to AOAC standard methods (928.08 (modified) and 981.10 Block Digestion Method after Kjeldahl), ash (920.153 - direct ashing, muffle furnace at 550°C for 4 h) and carbohydrate content analysis (calculated by difference).

Total organic matter (TOM) for all diets was determined by a variation of the combustion method recommended by Byers et al. (1978). Samples were oven-dried for 36 hrs at 60° C weighed, and then placed in a furnace at 500° C for 6 hours to ensure complete combustion. Percentage total organic matter was calculated by sample weight loss after combustion.

5.2.7 Statistical analyses

All SGR and morphometric measures data were tested for normality using Shapiro-Wilk and homogeneity of variance using a Levene’s test. Mean wet weights per tank for all diets were compared by a one-way ANOVA at the commencement of the experiment to ensure that there were no significant differences in weight existed among allocated diets. Mean monthly and total SGR was compared among diets using one-way nested ANOVA. Significant differences between diet means were identified with pair-wise comparisons using a Tukey HSD test. Mean values for morphometric measures for various diets were compared by one-way ANOVA, significant differences between diet means were identified with pair-wise comparisons using a Tukey HSD test.

5.3 Results

5.3.1 Palatability and feeding

All experimental animals were directly observed feeding on the diets within 24 h of the first addition of each diet. Throughout the remainder of the experiment evidence of continued feeding was apparent from the production of faeces and fresh disturbance of the sediment surface due to tentacular feeding.

The natural sediment and mussel waste diets were fully consumed at the sediment – water interface at all feeding rates. The *Sargassum* diet was not fully consumed by the juvenile sea cucumbers and tended to decay periodically in small patches at the bottom of tanks. Decay did not cover large areas of the tanks and no effort was made to remove unconsumed diet. The *Sargassum polycystum* feeding rate was maintained as a direct comparison with feeding rates of other diets.

The amount of settled POM was consistent between control tanks and did not exceed 12 g of dry sediment per month in any tank. Therefore, it is unlikely that the small amount of POM arriving in the seawater influenced diet effects or the feeding rates of the sea cucumbers in the experiment.

5.3.2 Juvenile weight monitoring

Overall, the mean SGR differed significantly among diets after the 3 mo experimental period (F= 63, P<0.001) (Figure 5.1). Post-hoc pair-wise comparison revealed significant differences (P<0.05) between all diets with the exception of Natural Sediment Base vs. Low diets and Mussel Medium vs. Dried Seaweed. Juveniles fed the Mussel Waste High diet exhibited the largest increase in weight with a mean SGR for the 3 mo period of 30% (± 7.7 SD). Mean SGR was 27% for these juveniles (±4.2 SD) for the first month, and 37% (±5.4 SD) for the second month. Sea cucumbers fed this diet, nonetheless, exhibited a reduction in average SGR over the third month to 30% (±7.7 SD). This reduction in growth rate in the third month was consistent across all six diets.

The Natural Sediment Low diet exhibited the lowest mean SGR of -12% (±3.9 SD) -26% (±3.4 SD) and -41% (±3.5 SD) over 1, 2 and 3 months respectively.

5.3.3 Survival

Overall, the survival of juvenile sea cucumbers in the experiment was high. The Natural Sediment Base diet and the Mussel Waste Medium and High diets all exhibited 100% survival. Mussel Waste Low and Natural Sediment Low both exhibited survival of 87% with two animals each dying in the last month of the experiment. The sea cucumbers maintained on the Dried Seaweed diet exhibited the lowest survival of 80%, with three sea cucumbers dying in the final month of the experiment.

5.3.4 Morphometric parameters

There were significant differences among diets for mean gutted weight (F= 12,$p<0.001$), maximum longitudinal muscle band width (F= 16, $p<0.001$) and body wall thickness (F= 10, $p<0.001$) (Figure 5.2). Mean gutted weight was highest for juveniles fed the Mussel Waste High diet, the lowest mean gutted weight was recorded for juveniles fed the Natural Sediment Low diet. Body wall thickness and maximum longitudinal muscle band width exhibited the same overall ranking of diets with the highest mean values for both measures recorded for the Mussel Waste High diet and the lowest value for the Natural Sediment Low and Natural Sediment Low or Base diets respectively. Gutted weight to wet weight ratio was calculated for all diets. There were no significant differences among diets for gutted weight to wet weight ratio which supports the accuracy of wet weight measures applied during the duration of the experiment.

5.3.5 Diet and sediment assays

Mussel waste had a lipid content of 1.0% (±0.15 SD), carbohydrate content of 19.6% (±0.25 SD), crude protein 5.1% (±0.06 SD) and ash content of 72.3% (±0.56 SD).
Total organic matter of the diets varied considerably, with mean values for dried *Sargassum polycystum* 23.1% (±0.29 SD), mussel waste 15.1% (±0.42 SD) and natural sediment much lower at 5.6% (±0.15 SD).

5.4 Discussion

Juvenile sea cucumber diets for nursery pond culture or early feeding in polyculture must be palatable and capable of inducing rapid growth. The results of this experiment show that mussel waste is both palatable to juvenile *A. mollis* and is capable of producing and sustaining growth when fed in sufficient quantities. Mussel waste also produced faster growth in juvenile sea cucumbers than natural sediment sourced from the natural habitat of juvenile *A. mollis*. Juvenile sea cucumbers supplied mussel waste at the highest feeding rate outperformed dried algal supplement *Sargassum polycystum* which is a diet commonly used as an artificial feed in other species of sea cucumbers (Yuan et al. 2006). The *S. polycystum* Dried Algae diet was added at a lower rate than the highest rate mussel waste diets, nonetheless the juvenile sea cucumbers could not consume all of this diet and it is unlikely that an increased feeding rate of dried algae would have resulted faster growth. Thus given a sufficient supply, mussel waste is a superior juvenile nursery feed to a commonly used dried algae supplement (Yuan et al. 2006). It remains unclear what the causes of the superior growth are. It seems likely to be due to beneficial bacteria, nutritional content, or predigestion by mussels making nutrient content more available to sea cucumbers, or a combination of these factors.

The results of this research show growth in excess of similar research with *A. japonicus* fed solely bivalve faeces. The fresh mussel faeces diet used in this study clearly outperformed the dried and ground faeces diet supplied by Yuan et al., (2006). The results also support their supposition that the drying process may have greatly reduced the food value of the bivalve faeces diet by removing nutrients, lipids or beneficial bacterial content (Yuan et al., 2006). Yuan et al., (2006) reported a daily SGR of -0.7 % d^{-1} for *A. japonicus* fed dried bivalve faeces and a maximum daily SGR of 2.1% d^{-1} was reported for juveniles maintained on a diet of 75% dried faeces and 25% powdered algae for 35 days (Yuan et al., 2006).

Sea cucumbers fed the Mussel Waste High diet exhibited an average 0.32% d^{-1} (± 0.08 SD) growth over three months. SGR was higher in the first and second months; 0.82% d^{-1} (± 0.12 SD) and 0.59 (± 0.09 SD) respectively. Zhou et al. (2006) reported a specific growth rate maximum of 1.38% d^{-1} after two months for tank-held *A. japonicus* in co-culture with the scallop *Chlamys farreri* as a fresh bivalve faeces source. In the third month of the same experiment a loss of was recorded with specific growth rate decreasing to approximately 0.5% d^{-1} (Zhou et al., 2006). The loss in condition was similar in timing and magnitude to that recorded in the current experiment. The stocking density was a degree of magnitude greater in this experiment than in the study conducted by Zhou et al. (2006), which is likely to explain the slightly slower growth rates in the current study. It is difficult to assess differences in diet provision rate as Zhou et al. (2006) conducted research with unfiltered seawater with scallops feeding directly above sea cucumbers in the same tanks. Nonetheless the overall supply of bivalve faeces and particulate organic matter (POM) were likely to be higher in these previous studies compared to the current research.

The Mussel Waste Low and Medium diets supplied 45g (50% of typical farm biodeposition) and 91g (100% of typical farm daily biodeposition) mussel waste m^2 d^{-1} (dry weight) respectively (James et al., 2001; Hartstein, 2003; Hatton et al., 2005; Slater, 2006). While the diets reflected average biodeposition rates encountered beneath mussel farms, the experimental stocking density of sea cucumbers was high (543 g $m-^2$) compared to the observed natural densities. The Mussel Waste High diet was equivalent to 7.3g mussel waste.sea cucumber.d^{-1} (dry weight) or 33% of initial juvenile weight d^{-1} as opposed to 8% and 17% of initial juvenile weight d^{-1} for the Mussel Waste Low and Medium diets. The greater mussel waste input per animal at the same stocking density, in the Mussel Waste High diet, resulted in significant increases in specific growth rate. If a feeding rate of 33% dry weight per individual is capable of inducing rapid juvenile growth, an average mussel farm has a carrying capacity of juvenile *A. mollis* in excess of 270 g $m-^2$. Juveniles fed the Mussel Waste Medium diet exhibited growth until the feeding rate fell below 15% of juvenile weight d^{-1} (as a result of increased average juvenile weight). If sea cucumbers are capable of maintaining condition at this feeding rate, the carrying capacity of sea cucumbers for a typical green-lipped mussel farm is in excess of 500 g m^{-2},

All artificial diets showed an unexpected loss of weight during the third month of the experiment. For artificial diets, where significant growth was recorded in the first two months, it is possible that the increase in overall stocking density (g m^{-2}) as sea cucumbers grew had a limiting effect on further growth. Individuals fed the Mussel Waste High diet increased the effective stocking density from 557 g m^{-2} at the start of the experiment to 810 g m^{-2} by the end of the second month of the experiment. Such increases would have also reduced the amount of diet available to individual sea cucumbers by wet weight and is likely to have limited growth. Similar studies have shown limitations to growth of juvenile sea cucumbers as stocking density is increased, with growth limitation reported at densities as low as 225 g m^{-2} and 300 g m^{-2} for tank-held juvenile *H. scabra.* (Battaglene et al., 1999; Pitt et al., 2004).

Other potential causes of the loss of condition in the third month of the experiment include the considerable increase in the ambient water temperature in experimental tanks over the duration of the experiment, reaching 23°C in the third month (Figure 5.3). A similar distinct reduction in growth rate was also reported by Zhou et al., (2006) and was thought to be a result of increasing tank water temperatures causing aestivation. Aestivation is, however, not reported for *A. mollis* and the animals in the current study were still observed actively feeding in the last month. The temperature increase may also have exacerbated any effects of oxygen depletion particularly in Dried Algae diet tanks, where the diet was not fully consumed and began to decompose in small amounts.

The current study shows that tank-held *A. mollis* grows well if provided with an appropriate feed in sufficient quantities and at sustainable stocking densities. These results indicate the potential for using artificial feeds for the nursery culture of juvenile *A. mollis* in tanks or pond culture systems. Pond culture of sea cucumbers is practiced broadly in China and may be successful in the New Zealand context for *A. mollis* if appropriate diets are developed (Yaqing et al., 2000; Chen, 2004). Land-based polyculture of *A. mollis* with green-lipped mussels is economically unfeasible due to the enormous flow requirements of feeding mussels, but polyculture with higher value species such as abalone or urchin may be viable if they are fed a natural kelp-based diet (Maxwell, 2006). Further research will, however, be essential to ensure that stocking and feeding rates are appropriate to ensure sea cucumber growth and survival.

5.5 Conclusions

The results of the current study of juvenile *A. mollis* show fresh mussel waste is a palatable and effective artificial diet for juvenile *A. mollis* that outperforms the commonly used diet of *Sargassum polycystum.* Good growth rates recorded in tank culture experiments indicate the potential for using artificial diets in conjunction with tank or pond culture for the culture of juvenile *A. mollis.*

Table 5.1 Diets supplied to individual tanks containing juvenile *A. mollis* including feeding rates and dry weight supply per individual.

Diet	**Rate of feeding (wet weight) g d^1**	**Moisture content %**	**Dry Wt g m^2 d^{-1}**	**Dry Wt g ind d^{-1}**
Natural Sediment Base	1kg at outset only	41%	NA	NA
Natural Sediment Low	35	41%	103	4.1
Mussel Waste Low	35	74%	46	1.8
Mussel Waste Medium	70	74%	91	3.6
Mussel Waste High	140	74%	182	7.3
Dried Seaweed (*Sargassum polycystum)*	35	0%	175	7

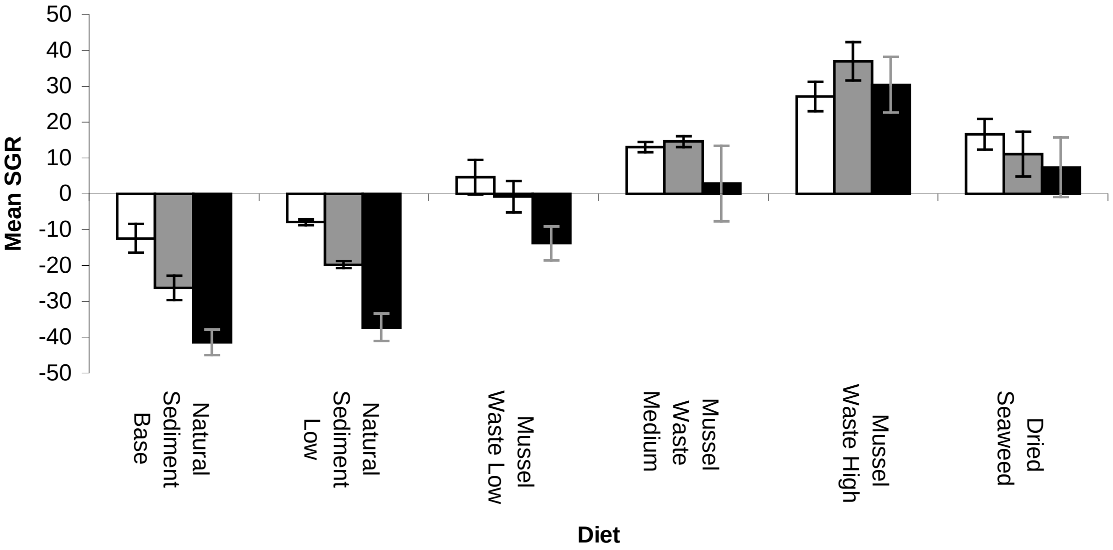

Figure 5.1 Mean cumulative specific growth rate of juvenile *A. mollis* maintained on six diets over 1, 2 and 3 month periods (n=3). White bars indicate month 1, grey month 2 and black month 3 specific growth rates. Error bars indicate the standard deviation of the mean.

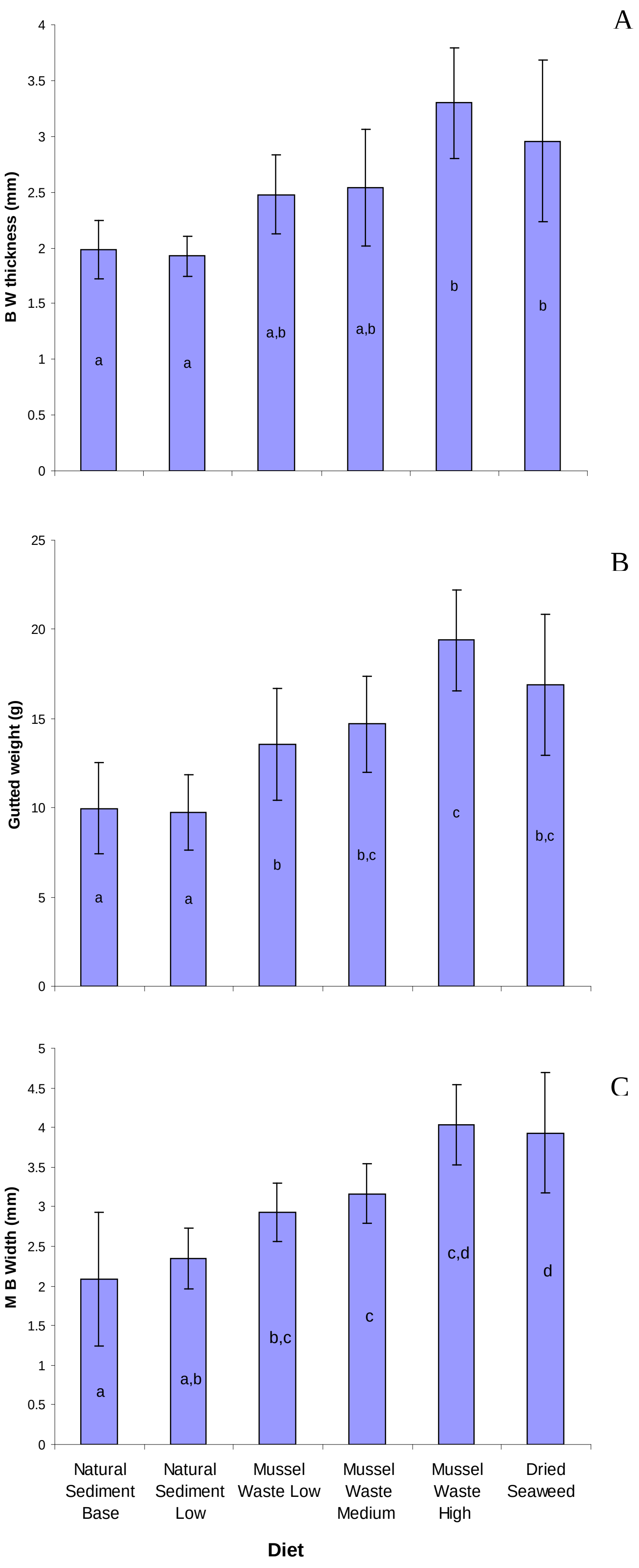

Figure 5.2 Mean morphometric parameters of gutted animals at conclusion of the experiment. A) Body wall thickness. B) Gutted weight. C) Muscle band width. Lower case letters indicate homogenous groups after pairwise comparison (Tukey HSD α = 0.05, Between MS = .25, df = 62.0).

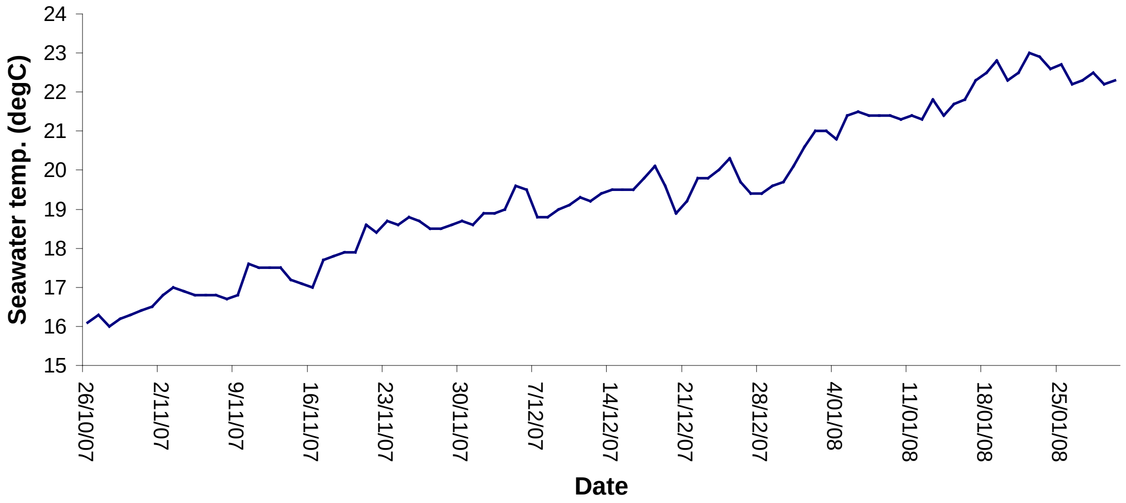

Figure 5.3 Tank seawater temperatures during experimental period.

Chapter 6: Apparent Digestibility of Carbohydrate and Protein Sources for Artificial Diets for Juvenile *Australostichopus mollis*

Currently under review as: Slater, M.J. Lassudrie, M, Jeffs A.G. Apparent Digestibility of Carbohydrate and Protein Sources for Artificial Diets for Juvenile *Australostichopus mollis*. Aquaculture Research.

6.1 Introduction

Several commercially valuable sea cucumber species are cultured for wild release in restocking programmes or for aquaculture, while other species are the focus of research and development efforts towards successful commercial culture (James et al., 1994; Yanagisawa, 1998; Battaglene et al., 2002; Chen, 2004; Purcell, 2004; Asha and Muthiah, 2006; Slater, 2006; Yuan et al., 2006; Paltzat et al., 2008). The nursery culture of juveniles is a costly element of sea cucumber culture, and formulation of lower-cost nursery diets, or improving the performance of existing feeds with supplementary nutrient sources, will make future aquaculture more viable (Pitt and Duy, 2004).

Current sea cucumber farming practise requires the maintenance of vast nursery pond or tank systems coupled with provision of suitable nursery diets to promote juvenile growth prior to pond, cage or restocking release (Pitt and Duy, 2003; Chang and Yu, 2004). Survival of juvenile sea cucumbers at release is significantly improved for larger juveniles, but survival benefit must be weighed against increased nursery costs and length of the production cycle (Tanaka, 2000; Pitt and Duy, 2003; Purcell and Simutoga, 2008). More effective artificial nursery diets can reduce overall production costs by producing larger individuals, with better survival rates, whilst reducing nursery holding times and increasing carrying capacities. A number of commercial diets are currently used, however, these have been developed mostly through trial and error rather than any systematic evaluation of nutrient digestibility.

Commercial nursery diets for *Apostichopus japonicus* and *Holothuria scabra* include fermented algae, powdered algae, expensive prepared algal supplements or shrimp starter diet (Chen, 2003; Pitt and Duy, 2003; Asha and Muthiah, 2007; Giraspy, 2008). Experimental formulated diets have also been tested for growth promotion at the nursery stage and for their ability to increase juvenile stocking densities in nursery ponds. The broad range of experimental diets examined include dredged marine muds, wheat flour, fishmeal, casein, corn, beans, chicken manure, *Sargassum* spp, bivalve biodeposits and shrimp waste (Huiling et al., 2004; Pitt and Duy, 2004; Zhou et al., 2006; Okorie Eme et al., 2008; Liu et al., 2009; Qin et al., 2009; Slater et al., 2009). Additional research has focussed on the energetic requirements of sea cucumbers (including juveniles) when fed artificial diets or the digestibility of diets as determined by the reduction in dry weight of diets after digestion (Yuan et al., 2006; Maxwell et al., 2009). Total nutrient digestibility of whole diet (faeces vs. diet organic content) varies between 30 and 65% for *A. japonicus* for diets containing bivalve faeces and formulated diets respectively (Huiling et al., 2004; Yuan et al., 2006). Yingst (1976) reported up to 40% assimilation efficiency for *Parastichopus parvimensis* consuming decomposed animal and algal detritus.

While the reported results of previous research on nursery diets for juvenile sea cucumbers vary widely and are difficult to compare due to the variety of diets and compositions reported, some useful conclusions can be drawn from the information available. Increasing protein content in artificial diets to between 15 and 40% (= shrimp starter feed) significantly improves growth and survival in juveniles and digestibility of diets (Huiling et al., 2004; Pitt and Duy, 2004; Giraspy and Ivy, 2008). However, not all protein sources appear to induce strong growth (e.g., *Spirulina*) and increasing diet energy levels appears to reduce ingestion rates (Yuan et al., 2006). In addition, natural diets composed of sediment and bivalve faeces, with a low protein (approx. 5%) and high carbohydrate (approx. 20%) content have also consistently been shown to induce rapid growth in juveniles (Yuan et al., 2006; Zhou et al., 2006; Paltzat et al., 2008; Slater et al., 2009). Refractory organic content including complex structural carbohydrates (e.g., from macroalgae), which can form a significant part of deposit-feeding sea cucumber diet in situ, remain undigested unless significantly decomposed and are ineffective in inducing growth amongst juvenile sea cucumbers (Yingst, 1976; Lawrence, 1982; Féral, 1989; Roberts et al., 2000; Gao et al., 2008).

Detritivorous aspidochirote sea cucumbers ingest the upper few millimetres of surface sediment, mainly assimilating carbon from bacteria and fungi as well as detrital carbon made labile as a result of microbial and fungal degradation (Yingst, 1976; Massin, 1982; Lopez, 1987). Sea cucumber digestion is considered primarily enzymatic, although the potential digestive role of symbiotic bacteria found in the gut and gut epithelium is yet to be elucidated, and whilst broad in range, digestive efficiency is considered to be low (Lawrence, 1982; Ward-Rainey et al., 1996; Roberts et al., 2000). A broad suite of digestive enzymes, including carbohydrases, such as amylase, reflects the natural low nutrient detritivorous diet of sea cucumbers (Lawrence, 1982). As detrital feeders it could be expected that sea cucumbers would be capable of digesting most nutrient sources including starches (Choe, 1963; Fu et al., 2005; Gao et al., 2008). Alginase and cellulose activity is, however, weak or undetectable in most species examined and stable isotope analysis strongly indicates that *Australostichopus mollis* derives organic matter from refractory algal and terrestrial carbon sources only after bacterial recycling (Lawrence, 1982; Gao et al., 2008; Wing et al., 2008).

No published studies exist on digestibility and bioavailability of specific ingredients with the potential to be used in artificial diets for juvenile sea cucumbers. Digestibility and utilisation efficiencies for proximate diet components (carbohydrate, protein, lipid) are also unreported for sea cucumbers. Such studies can provide further insight into digestive capacity and nutritional requirements of holothurians and are an important first step in developing formulated artificial nursery diets. Comparatively low-cost dietary sources of carbohydrate may provide a cheap energy source for metabolic maintenance in growing animals (although echinoderm respiratory energy cost is comparatively low) allowing more protein from diets to be utilised in growth (Lawrence, 1982). Likewise, low-cost dietary protein sources remain untested for digestibility and their potential to contribute directly to the somatic growth of juvenile sea cucumbers.

The sea cucumber *A. mollis* is a commercially valuable sea cucumber which is subject to increasing fishing pressure and is currently the focus of active research towards culture and polyculture (Slater and Carton, 2007; Maxwell et al., 2009; Stenton-Dozey and Heath, 2009). The current research investigated the digestibility of selected carbohydrate and protein sources for *A. mollis* and their efficacy in inducing rapid juvenile sea cucumber growth to determine the value of specific ingredients in formulated diets and to gain insight into the digestive abilities of juveniles of this species.

6.2 Methods

6.2.1 Juvenile sea cucumber collection

Juvenile *A. mollis* weighing between 9 and 40 g were collected by SCUBA divers from a site with naturally high density of juveniles in the Mahurangi Harbour in northern New Zealand. The sea cucumbers were transferred to the nearby Leigh Marine Laboratory and held in tanks with flowing 100 µm filtered sea water. The juvenile sea cucumbers were suspended in mesh bags for 24 h to ensure gut contents were evacuated before use in feeding and digestibility experiments (Sewell, 1990).

6.2.2 Apparent digestibility of artificial carbohydrate sources

Four experimental diets using different artificial carbohydrate sources were tested; wheat starch, tapioca starch, carageenans, pre-gelatinized maize starch and a fifth control diet, containing only acid washed sand and gelatine (Table 6.1).

Diets were prepared as follows:

An aliquot of 10 g of bovine gelatine was added to 100 ml dH_20 and heated in a 250 ml beaker in a 600 W microwave at low setting to approx. 100°C to ensure gelatine was fully dissolved. The solution was allowed to cool to 40°C before 49.5 g carbohydrate source, 198 g acid washed sand and 2.5 g Cr_2O_3 was added rapidly under constant stirring. The mixture was refrigerated for 12 h to ensure complete gelling before being blended for 2 min in a standing electric blender to ensure homogeneity. The homogenate was rapidly frozen to -80°C and ground to < 1500 µm and stored at 13°C prior to use in feeding. All diets contained 19% carbohydrate by dry weight and 3.8% gelatine to help ensure the carbohydrate source remained evenly distributed throughout the sedimentary substrate upon which the experimental juvenile sea cucumbers fed (Table 6.2).

Juvenile tank holding digestibility

Gut evacuated juveniles were weighed to the nearest gram after excess external water was removed from the sea cucumbers by placing them on unbleached tissue to blot dry. The juvenile sea cucumbers were sorted into two weight classes; 20-30 g and 31- 40 g and then allocated into 18 groups of four individuals consisting of two individuals from each size class.

The groups of four were then randomly allocated to 18 polyethylene experimental tanks with a floor area of 0.20 m^2 (0.55 m × 0.35 m × 0.21 m L×W×H) and supplied with filtered sea water (temperature maintained at 17°C, 50 µm filtered, 0.5 l min^{-1}) to a depth of 180 mm. Natural light cycle was maintained during the experiment although tanks were shaded from direct sunlight (L= 0.2 - 0.3 µE m^{-2} s^{-1}). A further two tanks were maintained with water flow only to measure any settling debris delivered via the water supply.

Feeding and sampling

A diet treatment was supplied (40 g d^{-1}) at 17:00 hrs to each of three tanks randomly selected from the 18 holding tanks. The water supply was interrupted and thawed ground diet was distributed over the entire bottom of the tank and allowed to settle for 45 min before the water supply was restored. All faecal matter was collected from each tank each morning (within 7 h of defecation) for three consecutive days. Faecal matter was stored at -13°C for further analysis. In addition a 10 g sample of each diet was placed in a replicate tank for 24 h before being sampled and stored for analysis of carbohydrate loss from diets by dissolution.

Survival

Any juveniles that developed obvious signs of illness, (i.e., visible discolouration or deformation of the epidermis), were immediately removed from the tanks. Removed animals were held in separate quarantine tanks and observed. Any deaths were recorded and animals removed.

Chemical analysis

Faecal matter samples, diet samples and diet carbohydrate loss samples were analysed for total carbohydrate concentration using the colorimetric method of Dubois et al. (1956) as modified by (Taylor, 1995). Samples were dried to constant weight before 73.5 mg sample was boiled for approx 30 sec in 180 ml dH_2O then made up to 200 ml with dH_2O. A 0.5 ml aliquot of this solution was acidified with 2 ml H_2SO_4 and mixed thoroughly before being cooled rapidly to ambient temperature in a water bath. Then 25 ml of phenol was added and mixed thoroughly. After 30 min the absorbance of samples was determined at 480 nm (corrected by abs. 600 nm) against a dH_2O blank. Dilution standards were created for each carbohydrate source and standard curves plotted to determine equations to calculate concentration of assayed samples.

Apparent digestibility coefficient

Chromic oxide was added as an inert dietary marker for use in calculating apparent digestibility of different carbohydrate sources, however, initial assaying of Cr_2O_3 concentrations using the method developed by Petry and Rapp (1971) showed high variability of results. Less variable results were achieved using the quantity of acid washed sand as a dietary marker (Bureau et al., 1999). Sand concentration in diet and faecal matter samples was determined by ashing samples in a muffle furnace for 7 h at 500°C to remove organics and calculating sand percentage concentration as 100 × end weight / initial weight. Ash content was assumed not to vary markedly between diet and faecal matter.

Apparent digestibility coefficient (ADC) was calculated after Conover (1966) as:

$$ADC = 100-100\times(SD/SF) \times (CF/CD)$$

Where SD = sand marker percentage in diet; SF = sand marker percentage in faeces; CF = carbohydrate percentage in faecal matter and CD = carbohydrate percentage in diet. The carbohydrate percentage in diet was adjusted by the 24 h solubility loss factor determined for each diet prior to calculation of apparent digestibility (Table 6.3).

6.2.3 Apparent digestibility of artificial protein sources

Steps under 6.2.2 (excluding chemical analysis) were repeated to determine apparent digestibility of five artificial protein sources; wheat gluten, mussel meal, caseinate, meat meal, fish meal, and a sixth control diet, containing only acid washed sand and agar (Table 6.2). Diets were prepared as previously described for carbohydrate but with agar replacing gelatine as the binder to help ensure the protein source remained evenly distributed throughout the sedimentary substrate upon which the experimental juvenile sea cucumbers fed. Protein content was determined using the Bradford method as modified by Zor and Selinger, (1996) as follows:

A dried sample (95 mg) was added to 4 ml NaOh and heated for 4 hr at 56°C. Samples were then centrifuged at 4000 rpm for 3 min before the supernatant was transferred to new tubes. The pellet was retained and washed twice in 1 ml NaOh with heating and centrifuge steps repeated and supernatant added to the original 4 ml supernatant (final volume 6 ml). 500

µl supernatant was transferred to new tubes and 4.5 ml dH_20 to dilute NaOH to 0.1 M for spectrophotometric determination. A 0.2 mL sample solution was added to 0.8 mL Bradford reagent and triplicate measures of absorbance at 595 over 450 nm (abs ratio 595 nm / 450 nm) were taken for each sample with dH_20 as a blank. Acid washed sand was used as a dietary marker and apparent digestibility of proteins was calculated as previously described for carbohydrate sources.

6.2.4 Growth on artificial carbohydrate sources

Four experimental carbohydrate diets were tested for induction of growth in juvenile sea cucumbers. Wheat starch, tapioca starch, carageenans and pre-gelatinized maize starch (Table 6.1). Diets consisted of 19% carbohydrate by dry weight supplemented with 4% dry weight casein as a protein source (in addition to 3% gelatine as a binder) and 1% dry weight of high lipid fish meal to provide approx 0.2% lipid content by dry weight (Table 6.4). The inclusion of carbohydrate at around 20% of dry weight was based on previous measures of the carbohydrate content of a mussel waste diet which produced good growth in juveniles of this species of sea cucumber (Slater et al., 2009).

Juvenile holding

Gut evacuated juvenile sea cucumbers were photo-identified and weighed to the nearest gram after excess external water was removed from the sea cucumbers by placing them on unbleached tissue to blot dry (Raj, 1998). The sea cucumbers were allocated into 15 groups, each of three individuals. Average weight (within each group) ranged from 17 g (±4.2 g SD) to 25 (±8.1 g SD), overall average weight (all group average) was 23 (±6.2 g SD). The groups of three individuals were then randomly allocated to 15 polyethylene experimental tanks with a floor area of 0.20 m^2 (0.55 m × 0.35 m × 0.21 m L×W×H) and supplied with filtered sea water (temperature maintained at 17°C, 50 µm filtered, 0.5 l min^{-1}) to a depth of 180 mm. Natural light cycle was maintained during the experiment although shaded from direct sunlight (L= 0.2 - 0.3 µE m^{-2} s^{-1}).

Juvenile feeding and growth

Dietary treatments were randomly allocated to three of the 15 tanks and 25 g of diet was added to each tank per day. Diet was never fully consumed and uneaten diet and faeces were siphoned out of experimental tanks every second day prior to addition of fresh diet.

Survival was monitored as per 2.2.3. After 31 d the gut contents of sea cucumbers were evacuated followed by photographic identification of individuals and weighing as previously described. Weight data were then used to calculate specific growth rate (SGR) as follows:

$$SGR = 100 \times (LN(\text{Current Weight}) - LN(\text{Initial Weight}))$$

6.2.5 Statistical analyses

All data were initially tested for normality and homogeneity of variances to ensure ANOVA assumptions were fulfilled. A one-way ANOVA was used to test for significant differences between ADC values for different carbohydrate sources. A Kruskal Wallis ANOVA was used to test for significant differences between protein sources as data did not fulfil homogeneity of variances assumptions despite transformation. Significant results were further tested with a Tukey HSD post hoc test on ranks ($\alpha = 0.05$) to identify homogenous groups.

Juvenile growth data was compared between diet treatments using a nested ANOVA design with means for tanks nested within treatments. Where results were significant, a Tukey HSD post hoc test ($\alpha = 0.05$) was applied to identify homogenous groups. The same nested ANOVA design was applied to initial weight data of randomly allocated animals to ensure no initial weight bias at the outset of the experiment.

6.3 Results

6.3.1 Apparent digestibility of artificial carbohydrate sources

Dietary carbohydrate solubility losses were high for maize (26.5%) and tapioca (17.8%, Table 6.3). Overall, the mean apparent digestibility after correction for dissolution was relatively low for carbohydrate sources and did not vary significantly (ANOVA F=1.22, $p > 0.3$) between carbohydrate sources - ranging from 46.2% (± 2.9 SE) and 37.6% (± 10.3 SE) for carageenans and wheat respectively to the lowest ADC values of 30.8% (± 2.1SE) for tapioca and 31.6% (± 6.9 SE) for maize (Figure 6.1). Survival was 100% for all treatments.

6.3.2 Apparent digestibility of artificial protein sources

The mean ADC values differed significantly between protein sources (Kruskal Wallis, H= 11.1, $p< 0.05$) with casein (98.1 % ± 0.2 SE) and mussel meal (91.9 % ± 2.4 SE) recording the highest mean ADC of all diet constituents. Fish meal and meat meal recorded the lowest mean ADC amongst protein sources, 75.9 % (± 1.7 SE) and 77.2 % (± 2.0 SE) respectively. Post hoc analysis revealed significant differences between mean ADC values for casein and fish meal (Figure 6.2). Survival was 100% for all treatments.

6.3.3 Juvenile feeding and growth carbohydrate sources

Initial mean juvenile weights did not differ significantly between treatments or between tanks nested within treatments (ANOVA $F = 0.26$ and 0.61, $p> 0.5$). Mean SGR differed significantly between dietary treatments (ANOVA $F = 8.54$, $p<0.01$). While results were highly variable within dietary treatments, the overall growth performance was consistently poor for the juveniles maintained on the carbohydrate diets for 31 d. Growth was negative for juveniles fed carageenans, tapioca and wheat diets, while animals fed the maize diet recorded the only positive mean SGR 0.5% (±4.7 SE). Mean SGR was lowest for the wheat diet (-27%, ± 4.5 SE). Post hoc analysis revealed significant differences between mean SGR of wheat versus carageenans, wheat versus maize and tapioca versus maize (Figure 6.3). Survival was 100% for all treatments.

6.4 Discussion

There is little published information regarding the ability of sea cucumbers to digest and assimilate the nutrients provided through diet ingredients commonly used in formulated aquaculture feeds. Developing successful artificial nursery feeds requires data on the digestibility of a broad range of economically viable nutrient sources and their ability to induce growth. This study presents methods to quantify digestibility and growth values for some common carbohydrate and protein sources fed to juvenile *A. mollis*. While this study has only assessed a relatively small number of potential nutrient sources, the results show that juvenile sea cucumbers could be relied upon to consume all of the dietary ingredients that were presented in this format. The demonstration of the methodology will allow relatively rapid future assessment of other potential dietary ingredients and facilitate experimental determination of levels of inclusion of ingredients, such as through protein:energy determination.

In this study experimental diets consisting of a relatively high proportion of a single carbohydrate-source had moderate to low carbohydrate digestibility when fed to juvenile *A. mollis*. The highest digestibility was recorded for carageenans, which are complex structural carbohydrates derived from macroalgae (ADC = 46.2%). In other species of deposit-feeding sea cucumbers, where these algal-derived carbohydrates can form a significant part of the available diet, this nutrient source remains basically undigested unless significantly decomposed prior to consumption (Yingst, 1976; Lawrence, 1982; Gao et al., 2008). Previous research indicates that *Australostichopus mollis* is also only capable of assimilating many refractory terrestrial carbon sources after recycling by bacteria (Wing et al., 2008). Our results suggest *A. mollis* has the ability to directly hydrolyse complex algal polysaccharides such as carageenans. Although the digestibility of low-cost carbohydrates sources such as wheat starch and maize were comparatively low (ADC = 37.6% and 31.6% respectively) these carbohydrates may still prove useful as low-cost energy sources in artificial diet formulation. Furthermore, adjustment of inclusion of these carbohydrate sources to lower levels may also lead to improved digestibility due to limited availability or rate limited carbohydrases. The higher digestibility of wheat starch in this sea cucumber compared to other botanical starches (maize, tapioca) is paralleled in many cultured fish and crustaceans where it is considered to be one of the best digested botanical sources of starch (Davis and Arnold, 1993; Glass and Stark, 1995; Cousin et al., 1996; Stone, 2003).

The relatively low carbohydrate digestibility was also reflected in poor overall growth performance of juvenile *A. mollis* in this growth experiment, which compared poorly with previous growth data for juveniles of this species and other sea cucumber species fed commercial diets or bivalve biodeposits (Huiling et al., 2004; Yuan et al., 2006; Giraspy and Ivy, 2008; Liu et al., 2009; Slater et al., 2009). The overall poor growth in this experiment regardless of the inclusion of various carbohydrate sources strongly suggests that the rudimentary diet formulation was insufficient for generating growth in juvenile sea cucumbers. Juvenile *A. mollis* may have a protein requirement similar to closely related *A. japonicus* which is reported to require around 21% protein in artificial diets (Huiling et al., 2004; Okorie Eme et al., 2008). However, the proximate composition of our rudimentary diet was based on the composition of bivalve waste with approximately 20% carbohydrate, 5% protein and 1% lipid which had previously been found to produce excellent growth in juvenile *A. mollis* (Slater et al., 2009). Given the overall poor growth performance on the experimental diets the differences between dietary carbohydrate treatments should be treated cautiously. Inconsistencies between digestibility and growth results may be explained either by differences in palatability and ingestion rates of the experimental diets, or interference by the carbohydrate sources in the digestion and assimilation of other dietary nutrients. Maxwell et al. (2009) found ingestion rates were lower for *A. mollis* fed abalone waste derived from formulated feeds when compared to natural kelp-derived abalone waste. Quantifying ingestion rates for the different experimental diets may have provided an insight into the differences in diet growth performance, although faeces production was similar in volume across treatments in growth and digestibility experiments (pers. obs.).

The apparent digestibility data from the current study show *A. mollis* can digest a variety of potential protein sources efficiently. This corresponds well with previous research which has shown improved growth performance and improved digestibility with increasing protein content in artificial diets for juvenile sea cucumbers (Huiling et al., 2004; Giraspy and Ivy, 2008). In this study casein and mussel meal were extremely well digested (ADC=98.1 and 91.9% respectively) but lower-cost protein sources, such as meat meal (ADC=77.2%), also proved to be highly digestible. Protein digestibility results indicate that dietary protein is important in formulated sea cucumber feeds and that there is potential for more expensive marine protein sources to be substituted with lower-cost terrestrial protein sources for formulated sea cucumber diets.

Higher rates of protein digestion and assimilation could be expected to be highly advantageous for deposit-feeding sea cucumbers given the very low availability of protein in natural sediments where they live in the wild (Mayer et al., 1986). Sea cucumbers generally feed by a plug-flow mechanism, they have minimal gut retention times, rely on enzymatic digestion and have poor comparative absorption rates (Lawrence, 1982; Roberts et al., 2000). High ingestion rates increase overall gut throughput when sea cucumbers feed on low organic content diets (Yuan et al., 2006). Considering the low organic content of natural sediment diet of *A. mollis* and other deposit-feeding sea cucumbers, this appears to be a favourable digestive strategy by which a broad suite of relatively low activity enzymes is capable of digesting the small amounts of organic material available within large amounts of ingested material. This strategy allows for rapid, comparatively efficient, processing of low energy diets but not for slower efficient processing of energy rich diets. There is some evidence that sea cucumbers may reduce feeding rates and digestion when supplied with an excessively high energy diet (Roberts et al., 2000; Yuan et al., 2006). If ingestion rates drop accordingly with higher energy diets, high protein content is required as only easily digested protein is capable of inducing growth.

The importance of bacterial vectors within sediments in making nutrients available for assimilation by detritivores should not be neglected and may be of importance in future development of formulated feeds for *A. mollis* and other juvenile sea cucumbers. Bacterial biomass is strongly correlated with protein concentration in surface sediments despite contributing only a small percentage of total protein (Mayer et al., 1986; Fabiano and Danovaro, 1994). It has also been shown previously that deactivating bacteria by drying diet sources reduces growth induction and digestibility in otherwise effective diets (Yuan et al., 2006; Zhou et al., 2006; Slater et al., 2009). It is possible that appropriately fermented carbohydrate sources would provide a more digestible diet source for juvenile feeding, however, the practical and economic implications of this additional process in diet preparation are yet to be investigated. Furthermore, the potential of carbohydrates as supplements to improve performance of natural diets - or to be directly added to ponds to stimulate the growth of bacteria as a sea cucumber food source - is worthy of further investigation (Hari et al., 2004). This approach would require reduction of dissolution losses and attention to ensure oxygen demand at surface sediments did not affect juvenile survival.

6.5 Conclusions

Artificial carbohydrate sources for diet formulation were only moderately digested by juvenile *A. mollis* and failed to induce growth when included as a significant nutritional component of a rudimentary formulated nursery diet. In comparison, a range of artificial protein sources were well digested and consequently there is considerable scope for diversification of protein sources for inclusion in formulated diets for juvenile *A. mollis*. High energy diets appear poorly suited to juvenile sea cucumber feeding mechanisms which are adapted to bulk consumption of comparatively low energy surface sediments. Fermentation of carbohydrate energy sources prior to feeding may improve digestibility and effectiveness in juvenile diets. The provision of larger amounts of potentially more palatable and digestible lower energy feeds with suitable bacterial vectors may prove more effective in inducing high ingestion rates, improving digestibility and producing growth in juveniles of this sea cucumber species.

Table 6.1 Diet constituents and sources.

Diet constituent	Source
Wheat starch (native)	J.C. Sherratt & Co. Ltd, NZ
Tapioca starch (native)	J.C. Sherratt & Co. Ltd, NZ
Carageenans (kappa)	Marine Colloids Inc., Philippines
Maize starch (pre-gelatinized)	Image Holdings Ltd, NZ
Acid washed sand	Ajax Finechem Pty Ltd, NZ
Wheat Gluten	Healtheries of New Zealand Ltd, NZ
Sodium caseinate	Davis Trading Co. Ltd, NZ
Mussel meal	Aroma New Zealand Ltd, Christchurch, N.Z.
Fish meal	PVL Proteins Ltd, NZ
Fish meal (high lipid)	NRM Stockfeeds, NZ
Meat meal	PVL Proteins Ltd, NZ
Gelatine	Ajax Finechem Pty Ltd, NZ
Cr_2O_3	Aldrich Chemical Co, USA
Agar (coarse)	Ajax Finechem Pty Ltd, NZ

Table 6.2 Diet composition carbohydrate for apparent digestibility coefficient experiment.

Diet constituent	% d.w.	% w.w.
CHO/Protein	19.0	13.8
Binder Gelatine/Agar	3.8	2.8
Sand	76.2	55.0
Cr_2O_3	1.0	0.7
Total dry weight	100.0	72.2
H_2O mL		27.8
Total wet weight		100.0

Table 6.3 Carbohydrate loss factor (24 h tank holding with seawater flow no feeding).

Carbohydrate source	Carbohydrate loss %
Carageenans	4.72
Maize	26.51
Wheat	11.97
Tapioca	17.84

Table 6.4 Diet composition for carbohydrate growth experiment.

Diet constituent	% d.w.	% w.w.
Sand	72.0	54.5
CHO	20.0	15.2
Protein source	4.0	3.0
Fish meal (HL)	1.0	0.8
Gelatine	3.0	2.3
Total dry weight	100.0	75.8
H_2O mL		24.2
Total wet weight		100.0

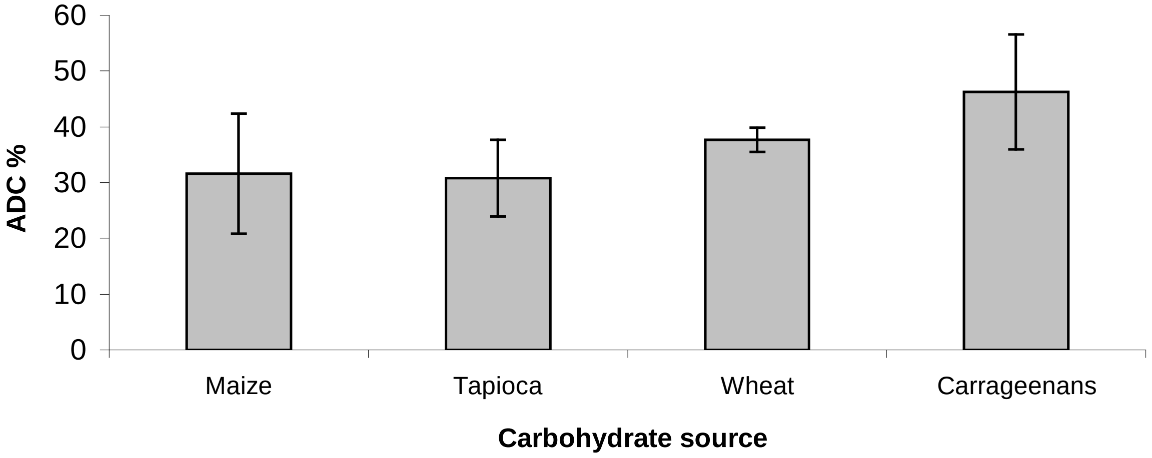

Figure 6.1 Mean apparent digestibility of artificial carbohydrate sources fed to juvenile *A. mollis* (n= 9).

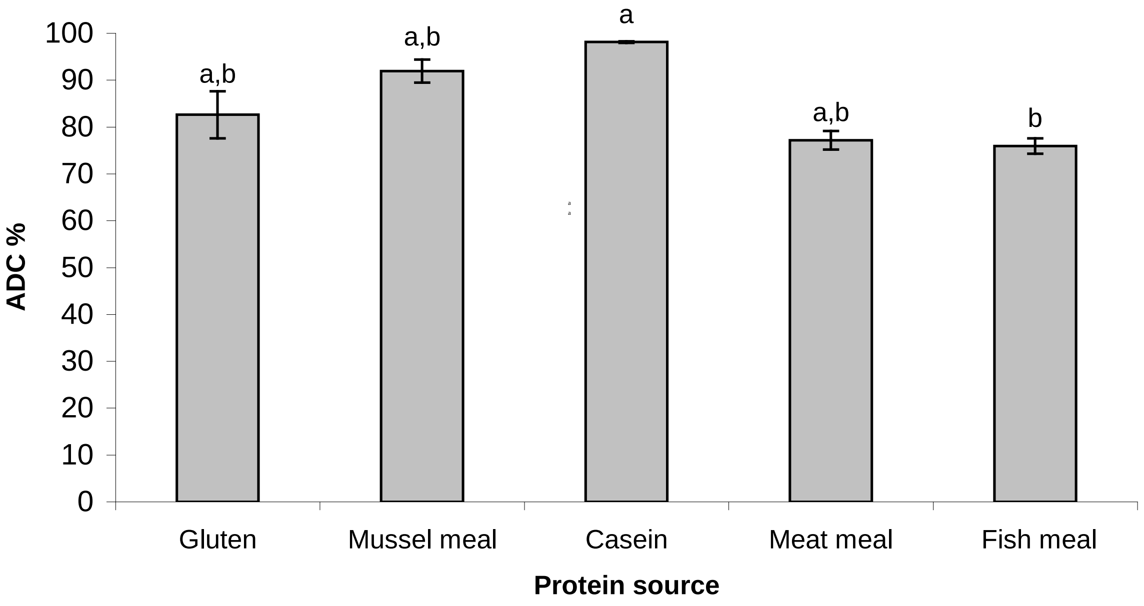

Figure 6.2 Mean apparent digestibility of artificial protein sources fed to juvenile *A. mollis* (n= 9).

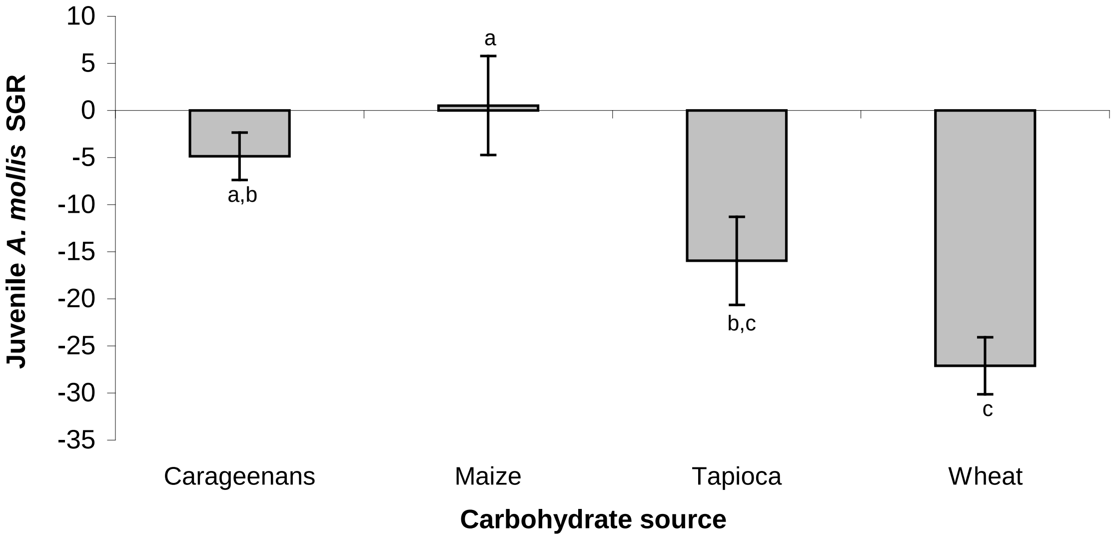

Figure 6.3 Mean juvenile *A. mollis* SGR after 31 d feeding with artificial carbohydrate source diets (n= 9).

Chapter 7: General Discussion

7.1 Summary

Overall, this thesis presents data on the ecology, distribution, feeding biology and diet preferences of juvenile *Australostichopus mollis* which will further the understanding of the general biology of this species and will contribute to future efforts to aquaculture this species. The research presented in this thesis first provides a description of the natural habitat of juveniles with regard to sediment characteristics and sediment facies and then examines the potential factors that may underlie the spatial distribution of juvenile sea cucumbers. These data expand our knowledge of the general ecology of the species and also provide information to allow areas of high natural recruitment to be identified and suitably managed. The selective feeding mechanisms used by juvenile *A. mollis* when consuming a natural sediment diet are investigated and described which assists in the formulation of artificial diets, and provides some insight into the ability of juveniles to perceive and react to varying organic content of diets at small scales. Finally, this research applies feeding assay methods in the laboratory to better understand the dietary requirements of juveniles, and to aid in the development of successful nursery rearing and to test the efficacy of various artificial diets. This final aspect of the thesis aims to increase the understanding of dietary requirements, and growth potential of juvenile *A. mollis* specifically by improving the nutrition of juvenile sea cucumbers under aquaculture conditions.

7.2 Biological significance of results

High densities of juvenile *A. mollis* were found in an isolated area of the Mahurangi Harbour. This area of high density was revisited throughout three years of experimental work for the collection of juveniles for laboratory experimentation. Detailed surveying of the site revealed highly localised distribution of juveniles in a habitat which is distinct from the shallow rocky habitat that has previously been described for juveniles of this species (Sewell, 1990; Mladenov and Gerring, 1991). The juvenile habitat patch, while distinct, lies within an adult population rather than separated from it as reported for several other aspidochirote sea cucumber species (Mosher, 1980; Yingst, 1982; Hamel and Mercier, 1996; Mercier et al., 1999; Mercier et al., 2000a; Yamana et al., 2006). Instead of associating with macroalgae or other complex structure in shallower water than adults, juvenile *A. mollis* associates with fine grained sediment with high organic content in areas overlapping with adult habitat (Wiedemeyer, 1994; Battaglene et al., 1999; Mercier et al., 2000a; Yamana et al., 2006). Juvenile habitat does not appear to be defined by any obvious potential settlement substrate, or abiotic factors which may otherwise favour juvenile survival and growth (Bulteel et al., 1992; Hamel and Mercier, 1996; Mercier et al., 2000a). Instead, the associated sediment characteristics within this habitat appear optimal for detritivorous feeding by juvenile *A. mollis,* with high organic content and fine grain size. These results tend to support previous research on the sea cucumber *H. scabra* which found an association of juveniles with sediment containing an optimal (comparatively high) organic content prior to adult migration to lower TOM and coarser grain sediments (Lawrence, 1982; Lopez, 1987; Mercier et al., 2000a). Spatial distribution data for *A. mollis* showed that juveniles progressively move into the more widely distributed adult population from a localised settlement or nursery area, a process also suggested by Mercier et al. (2000a) for *H. scabra*. Recruitment of *A. mollis* within the harbour thus appears to be primarily dependent on the maintenance of this discrete patch, or patches, of suitable habitat capable of supporting high densities of juveniles. Identifying and conserving juvenile habitat patches, such as the area surveyed in the Mahurangi Harbour, will be of importance in maintaining fishery stocks for this species. It is important that this also be taken into account in other fished sea cucumber species where juvenile habitat and distribution is unknown.

The research results indicated that habitat selection based on sediment types and facies is unlikely to be the cause of the observed patchy distribution of juveniles. Displaced juveniles survived and grew, often better than the control juveniles held in the natal juvenile habitat. Even where grain size was significantly larger and sediments had significantly lower organic content than the natal sites the juveniles grew rapidly. Along with exhibiting fast growth, juvenile *A. mollis* in the displacement experiment revealed they had a great ability to adjust their feeding so they can efficiently consume a variety of novel diet sediments. A combination of laboratory experimentation and field sampling revealed that juvenile *A. mollis* combine selective feeding for organically rich particles with an ability to relocate in response to the organic content of underlying sediments. Organically selective feeding allows *A. mollis* to optimise exploitation of sediments which are a poor nutrient source, and is an effective mechanism allowing juvenile *A. mollis* to adapt to novel sediments and feed efficiently on them. Selective movement based on the organic content of underlying sediment appeared to be limited above a certain threshold of approximately 3% TOM. Beyond this point the juvenile sea cucumbers no longer discerned between sediment patches and did not adjust feeding rates. The organic content measured in surface sediments within the Mahurangi Harbour at the surveyed juvenile and non-juvenile sites varied between approximately 4% and 7.5%. The TOM values recorded amongst studied areas in the Mahurangi Harbour are thus all above the observed threshold for selective movement. In natural habitats of juvenile sea cucumbers the TOM of sediments is unlikely to vary to the same degree and at the short scales tested within the laboratory. In fact TOM analysis prior to the caging experiment in the Mahurangi Harbour revealed variation of no more than 0.4% TOM in the sediments when measured over several metres in juvenile habitat. Protein content, microbial biomass and breakdown products of microalgae can however vary significantly in surface sediments at much smaller scales (Danovaro et al., 2001).

Avoidance of low TOM areas by juvenile sea cucumbers appears to confirm suggestions that low organic matter benthic sediments beyond the edge of the organically enriched footprints of suspended aquaculture units will serve to inhibit the outward migration of seeded stock (Massin and Doumen, 1986; Slater, 2006). The results also lend support to previous work showing significant retention of translocated tagged adult *A. mollis* within a farm footprint over several months (J. Stenton-Dozey, N. Davey, pers. comm.). The results of the current research provides further evidence of a feed and search strategy in aspidochirotes whereby faster horizontal movement occurs in the absence of an acceptable food source. The rate of juvenile *A. mollis* movement showed an overall decrease as TOM content of sediments increased, with distance moved nearly double the rate in low TOM patches of sediment as opposed to high. Similarly, Da Silva et al. (1986) observed that the temperate aspidochirote *P. californicus* ceased forward movement under a surfeit of sediment deposition. Several tropical and temperate species have been shown to exhibit random movement over evenly distributed horizontal substrate within natural habitat boundaries, and *H. scabra* has also been shown to move faster over unfavourable substrate when compared to suitable diet sediment (Hammond, 1982; Mercier et al., 2000a; Cieciel, 2004).

Juvenile *A. mollis* feeding was further characterised by a lack of selectivity for grain size. Small grain sizes exhibited the highest carbon and nitrogen content in measured sediment fractions and small grains are generally associated with both higher organic content and higher digestibility due to their surface rich nature and associated organic outer layer (Mayer et al., 1985). For juvenile *A. mollis* simply limiting their feeding to the uppermost layers of the sediment may be an effective enough mechanism to consume a sufficient amount of smaller grains (Yingst, 1982). When no grain size selection can be made by the animals, it may be an advantage to juvenile *A. mollis* feed on sediment patches with the smallest grain size. For the development of an artificial diet a lack of grain size selectivity by juvenile sea cucumbers means granulated diets of varying sizes can be used as long as they are suitably enriched. In addition larger particle diets or even small pellet diets may improve food intake and reduce any nutrient losses to leaching. It would be interesting to see where the upper limit of grain size lies for *A. mollis* feeding given that they have been observed in coarse shell habitats (Fenwick and Horning, 1980). Organic selectivity, on the other hand, will require that formulated diets are appropriately uniform and relatively stable to avoid preferential feeding on specific fractions.

7.3 Significance of results for development of nursery diets

Juvenile *A. mollis* exhibited remarkable adaptability to different diets. In the Mahurangi Harbour they were able to adapt to novel natural sediments, in the laboratory they fed and grew well on marine-derived waste diets and showed an ability to effectively digest a broad range of artificial protein sources. Their ability to adapt to various diets is similar to that reported for the successfully cultured aspidochirote *A. japonicus* from temperate waters (Chen, 2004; Yuan et al., 2006; Zhou et al., 2006; Liu et al., 2009). While initial results from surveying juvenile habitat indicated that juvenile *A. mollis* would require organically rich sediments with fine grain size, the results of displacement and feeding experiments in this thesis showed that juveniles are capable of survival and economically viable growth on a broad variety of natural sediments and marine waste diets. The results of the displacement experiment clearly showed that sea cucumbers will grow well where sediment supply is sufficient for maintaining the density of sea cucumbers present and available nutrient exceeds ca. 3% TOM,. At low sea cucumber densities their selective feeding on organic particles makes nutrient density of diet unimportant provided the food supply is sufficient. Organic selectively concentrates organic matter and ensures juveniles consume enough to survive and grow. Where sedimentation rates or primary production are not sufficient to replace the TOM (selectively) removed by sea cucumber feeding, sediments will become depleted below an acceptable organic content threshold. In a natural situation the sea cucumbers may then simply move away from the depleted area, as was observed in selective movement experiments over time, but in a holding situation (e.g. Chapter 5 natural diet) they simply fail to grow. Similarly if sea cucumber biomass increases the demand for the available organic matter increases and growth can become limited (e.g. Chapter 3, six to nine month growth performance).

Essentially the success of any future *A. mollis* artificial diet for aquaculture will depend on the total organic fraction, the bioavailability of that organic fraction and the amount of diet supplied. In natural habitats where organic matter levels are moderately elevated, diets appear to be suited to juvenile digestion. Where particulate organic matter supply is also high (i.e., under mussel farms) growth rates are high and correspondingly high densities of sea cucumbers can be supported (e.g. up to 810 g m^{-2} under high density farms, mussel waste high treatment Chapter 5). Culture situations demand maintenance of high stocking densities to ensure optimal use of limited nursery space. Increasing organic content of sediment/diet or increasing the sediment/diet supply to biomass ratio will provide sufficient total organic input to support increasing sea cucumber biomass. Increasing total sediment/diet supply, e.g. sedimentation or feeding rate, is not likely to be economically viable and tank or pond surface area limitations may reduce the accessibility of diets.

Increasing organic matter is the most viable option for supplying food for intensive culturing of juvenile sea cucumbers. However, there is likely to be an upper limit of *A. mollis* digestive capacity regarding organic matter, as natural diets were found to seldom exceed 10 - 15% TOM (Slater, 2006). When presented with *Sargassum polycystum* (ca. 23% TOM) consumption rates were low and large amounts of unconsumed diet collected in the tanks, while mussel waste (15 - 25% TOM) did not. Providing a diet in excess of approximately 25% TOM may not necessarily reduce survival rates but will require frequent tank cleaning and high water flow to ensure animals are not exposed to excessive biological oxygen demand at the sediment surface. In addition, excessively high organic matter may reduce consumption rates and may also reduce digestive efficiencies for rate limited enzymatic digestion (Yuan et al., 2006; Zhou et al., 2006). Increasing organic matter content of sediments also appears to reduce selective efficiency of feeding in juveniles of *A. mollis* (L. Zamora pers. comm.). Results of this study indicate a lower limit of approximately 3% TOM for *A. mollis* diets and an upper limit of practicable organic enrichment of approximately 25% TOM for natural or artificial diets.

A starting point for the development of effective artificial diets is determining the digestibility of a range of potential diet constituents in a systematic manner (Chapter 6). The digestibility of the five carbohydrate sources were tested and were found to be relatively low in all cases, suggesting that *A. mollis* may not be well equipped with the digestive capacity to utilise carbohydrate nutrient sources. The diets formulated with the same proximate composition and fed at approximately the same rate as highly successful fresh mussel waste diets did not induce juvenile growth. The aforementioned ability of juvenile *A. mollis* to adapt to various diets does not extend to formulated diets with high levels of artificial sources of carbohydrates. Carageenans were the best digested carbohydrate despite previous work showing poor digestibility of complex algal-derived diets (Yingst, 1976; Lawrence, 1982). Wheat starch was the best digested botanical carbohydrate source as has been shown for other marine species fed terrestrial carbohydrate sources (Davis and Arnold, 1993; Glass and Stark, 1995; Cousin et al., 1996; Stone, 2003). Cheaper bulk carbohydrate sources such as maize were still moderately digested and should not be completely disregarded as potential energy nutrient sources. In comparison, the broad range of artificial protein sources tested were generally well digested, which is logical given the need to efficiently utilise scarce protein in natural sediment diets (Mayer et al., 1986; Fabiano and Danovaro, 1994). In particular, a low cost meat meal protein source achieved relatively high digestibility (77.2 %). This is particularly promising for economically viable artificial diet development using cheaper terrestrial protein sources.

Aspidochirote sea cucumbers have a broad range of endogenous enzymes, including active proteases and amylases (Féral, 1989; Fu et al., 2005). However, their digestive efficiency is agreed to be comparatively low and generally digestion is thought to not extend to structural carbohydrates (Yingst, 1976; Lawrence, 1982; Yuan et al., 2006). Their gut morphology fits well with a plug-flow digestive model with minimal mixing and relying on enzymatic digestion which may be rate limited (Penry, 1989; Fu et al., 2005). Results of the digestibility research indicate that *A. mollis* also has active proteases and recovers highly soluble protein breakdown products rapidly. Carbohydrase activity is generally present in sea cucumber enzyme suites but may be specific to the readily available exopolymers present in natural sediments rather than the bulk carboyhdrates provided (Lawrence, 1982; Féral, 1989;

Fu et al., 2005). Carbohydrates at high concentrations as provided in the current research may also overwhelm the enzymatic digestive capacity of the sea cucumber gut. Profiling digestive enzyme presence and activity levels would provide very useful underpinning information for the future optimisation of diets.

In other aspidochirote sea cucumber species, the digestibility of whole diets has been shown to improve with increasing protein content and growth also improves with increasing diet protein content (Huiling et al., 2004; Pitt and Duy, 2004; Giraspy and Ivy, 2008). The protein digestibility results of the current study appear to be in agreement with this previous work, however, it remains unresolved why excellent growth is obtained on natural sediment diets with much lower protein content than those used in feeding assays. Growth was poor with the current formulated diets containing only 5% protein and it appears that juvenile *A. mollis* artificial diets are likely to require higher artificial protein levels if viable growth is to be achieved (Giraspy and Ivy, 2008; Okorie Eme et al., 2008). In addition, lipid digestibility and requirements are yet be determined as they may prove to be an important source of energy given that overall carbohydrate digestive capabilities appear to be relatively poor. The protein:energy requirements of sea cucumbers must also be examined as a further step toward developing an effective artificial diet.

The dried and artificial nature of the constituents and a lack of bacterial or fungal decomposition of artificial diets may underlie the lack of growth performance by sea cucumbers on the experimental artificial diet. Yuan et al. (2006) found that oven-dried bivalve waste produced negative growth in juvenile *A. japonicus*, while Zhou et al. (including Yuan) (2006) showed that excellent growth is achieved for the same sea cucumber species using fresh bivalve faeces (from the same species mix) under the same culture conditions. Drying rendered the diet ineffective in inducing growth despite juveniles exhibiting high consumption rates for both dry and fresh forms. Similarly, Maxwell et al. (2009) fed oven dried abalone waste or dried algae flakes to *A. mollis* and calculated energy budgets indicating diets requiring > 30% TOM to allow juvenile *A. mollis* growth, despite *A. mollis* growing rapidly when fed natural fresh diets with less than 5% TOM in this study. There are many nutrients which may be lost as a result of drying. For example, Yuan et al. (2006) suggested that bacteria, vitamins or fatty acids were lost from otherwise effective diets during the drying process. Irrespective of what is lost in the drying process or is lacking in formulated artificial diets, its absence significantly reduces the growth performance of those diets for juvenile sea cucumbers. Until this factor is identified, it is recommended that fresh or frozen marine-sourced diets be used possibly with protein supplementation to ensure juvenile sea cucumber growth.

Developing effective artificial diets may require alternative methods to improve digestibility and match formulation to the digestive capacity of sea cucumbers in order to improve growth performance. Future research must recognise that bulk carbohydrates or proteins in artificial feeds differ significantly from the bacterial exopolymers, bacterial protein and carbohydrate, microalgae and amorphous detrital aggregates which make up the majority of labile organic matter in the natural sediment diet of deposit-feeders such as *A. mollis* (Decho and Moriarty, 1990; Fabiano and Danovaro, 1994). With this in mind it is recommended that future research quantifies the effect of marine bacterial digestion or fermentation of diets, prior to feeding on diet growth performance. Fermentation to increase bacterial and/or fungal nutrient content and exopolymer content may make significantly cheaper energy sources, such as bulk carbohydrates, more labile and more digestible (Yingst, 1976; Lopez, 1987). A lack of bacterial content and activity may be the most likely factor causing artificial and dried diets to be ineffective. Bacterial content correlates very closely to available protein and lipid in sediments and bacterial exopolymers can form a significant and well absorbed element in a sediment diet (Moriarty, 1982; Mayer et al., 1986; Decho and Moriarty, 1990). Furthermore bacterial activity is equivalent to a first digestion step prior to deposit-feeding. While bacterial biomass itself is only a small part of organically available carbon, bacterial activity is extremely important in making significantly larger amounts of refractive organic content labile as amorphous organic material which is available to deposit feeders (Yingst, 1976; Lopez, 1987; Decho and Moriarty, 1990; Fabiano and Danovaro, 1994).

7.4 Significance of results for future culture and polyculture

Juvenile growth rates measured over a year indicate that *A. mollis* is a commercially viable culture species. Compared to other cultured species of aspidochirote sea cucumber, juvenile *A. mollis* exhibited good growth rates when held at relatively high densities with enriched diets, and when held at low densities with a variety of natural sediment diets (Ramofafia et al., 1997; Chen, 2003; 2004; Zhou et al., 2006; Paltzat et al., 2008). However, as with other cultured sea cucumber species, growth rates were found to be variable and quickly became density limited. Therefore, sea cucumber feeding regimes and/or density of stocking will need to be adapted as juveniles grow in a commercial culture situation (Ramofafia et al., 1997; Battaglene et al., 1999). There were few problems with tank (up to 3 months) or cage (up to 12 months) holding of juvenile *A. mollis* for extended periods of time. None of the bacterial infection problems reported by Archer (1999) for this species or other potential diseases and pests were experienced during the current research, however, this can not be assumed to continue to be the case when culture is further intensified (Ito and Kitamura, 1997; Chen, 2003; Becker et al., 2004). High density tank holding (540 to 700 g m^{-2}) of juvenile *A. mollis* presented few problems as the species appears to be robust in most holding conditions. Sustained high water temperatures above 23°C during the hottest summer month resulted in mortality and poor growth performance and mortalities amongst tank-held juveniles. Reduced growth in the field experiments appeared to be associated with higher summer water temperatures. These conditions are unlikely to prevail in larger tank-holding or pond-holding situations where water temperature can be managed more effectively.

The preference for organically rich diet substrate in juvenile *A. mollis* translates into an ability to adapt to the novel polyculture diet of mussel waste. The high growth and survival data presented in Chapter 5 of this thesis support previous work showing sea cucumber growth and survival in experimental polyculture systems (Purcell et al., 2006a; Slater and Carton, 2007; Zheng et al., 2009). Survival is also high in comparison to the suggested co-culture with shrimp in ponds, possibly due to the inherent lack of physical interaction between mussel and juvenile sea cucumbers (Bell et al., 2007). This appears to reiterate the importance of species selection in polyculture, but also indicates that physical separation of sea cucumbers from shrimp or other promising co-culture species may be advantageous in future work.

Most importantly for future polyculture efforts, excellent growth can be expected from juvenile *A. mollis* released underneath mussel farms at seeding densities similar to, or even higher than, those used in pond culture of *A. japonicus* (approximately 700 g m^{-2})(Chang and Yu, 2004; Chen, 2004). If juvenile survival can otherwise be expected at a candidate mussel farm (i.e., predation is expected to be low and the hydrodynamic regime is acceptable) then juveniles should be released to the farm as early as is practicable to reduce nursery holding costs (Tanaka, 2000). This may also help to improve juvenile survival by reducing behavioural impacts of nursery holding (Purcell, 2004). If there is concern regarding potential predators, nursery type caging or bag holding of juveniles < 10 g within mussel farm impact footprints may be an economically viable nursery holding method prior to full release at farms. Such a nursery method, if successful, has the potential to greatly reduce infrastructure costs, feeding costs and even transport costs. Stocking rates can be accurately adapted to the biodeposit supply at individual farms, although current pond culture methods in China seem to favour high initial stocking rates in which case survival rates determine end stocking densities (Chen, 2004).

Stock retention will be an important factor in developing polyculture of *A. mollis* both economically and within the existing legislative framework for aquaculture in New Zealand. Stock integrity is very unlikely to be guaranteed because wild stock will actively move to the farm impact footprint, which may require legislative adjustment to allow harvesting outside the existing quota framework for *A. mollis* fishery. The boundary effect of low TOM areas at the edge of the farm footprint, combined with a dietary preference for high organic content and previous field research showing high retention rates with a mussel farm footprint support the use of ranching techniques (Slater, 2006; Stenton-Dozey, 2007). Avoidance behaviour and reduced movement within the farm's organic footprint are likely to strongly limit *A. mollis* migration from the polyculture site. With the successful application of hatchery techniques to this species, the biological knowledge gathered regarding juvenile *A. mollis* and the excellent performance of polyculture diets tested in this thesis, there are few knowledge boundaries remaining to the polyculture of *A. mollis* with green-lipped mussels (Stenton-Dozey and Heath, 2009).

7.5 Future research directions

Future research to determine the causes of the underlying highly localised juvenile distribution within the Mahurangi Harbour will need to examine the influence of natural settlement substrate for larvae, patterns of larval supply as a result of hydrodynamics, or predatory effects which may dictate the supply of post-settlement juveniles. Above all it is important that future research attempts to find much earlier post-settlement juveniles, preferably on settlement substrate. This would reveal a great deal regarding not only settlement surfaces but also potential migration to soft-sediment in juvenile *A. mollis* and allow a more complete description of the life cycle and recruitment process (Mercier et al., 2000b; Mercier et al., 2000a). Yamana et al. (2006) found the same highly localised concentration of juvenile *A. japonicus* also at mid-harbour in Hirao Bay, Japan, it is possible that the observed distribution simply represents a concentration of entrained larvae in mid-harbour as found for other species in the Mahurangi Harbour (Martin and Foster, 1986).

Field observations revealed predation by the asteroid *Coscinasterias muricata* in the Mahurangi Harbour. This seastar is more abundant than the predatory seastar *Luidia varia* that has previously been observed attacking adult *A. mollis* (Sewell, 1990) and it is possible that high densities simply reflect early predation (Cameron and Fankboner, 1989; Eckert, 2007). The ability of juveniles to selectively move to favourable feeding areas as they grow and the behaviour of juveniles in response to organic gradients are also worthy of more detailed investigation. It is also possible that previously described cryptic behaviour in response to high incident light or a lack of substrate structure excludes juveniles from shallow areas in the harbour where diet sediment characteristics are otherwise favourable (Slater, 2006).

The potential to improve digestibility of artificial diet constituents using fermentation is worthy of further investigation. It is important that future research determines the factor which causes a significant reduction in the growth performance of diets after drying. This may require research into the range and activities of digestive enzymes utilised by *A. mollis*. Assessment of a wider range of potential dietary ingredients that may be suitable for inclusion in formulated diets can be achieved used the methods developed through this current research. From these initial results it appears that focussing on low-cost protein sources will be particularly important in developing cost effective formulated feeds. Nursery diet development also still requires the determination of lipid digestibility. Determining changes in consumption rate and an optimal level of diet organic enrichment where nutrient density is best suited to both digestive capacity and feeding behaviour will also be important in optimising artificial diets.

Future research building on previous hatchery developments should focus on achieving large scale hatchery and nursery maintenance of juveniles (Morgan, 2008; 2009; Stenton-Dozey and Heath, 2009). A reliable supply of hatchery- produced juveniles will allow larger-scale releases of juvenile sea cucumbers to mussels farms, which is an important step toward proving the potential viability of commercial scale ranching or culture. The use of existing mussel farms as nursery environments is worthy of investigation as it may help avoid costly nursery space and feeding. Methods will need to be developed for protecting small juvenile sea cucumbers from natural predators present beneath mussel farms until they reach a size at which they can be safely released. Early growth rates and survival rates in such a system will need to be determined. On a larger scale the use of potential sedimentary (low TOM) barriers around aquaculture units will need to be examined to confirm its potential for retaining seed stock. Impracticalities regarding stock identification will need to be overcome through acceptable and practical tagging techniques for released juvenile stock (Purcell et al., 2006b; Purcell and Simutoga, 2008). High natural variability in coloration provides the potential to use selectively bred extreme examples of coloration as a convenient identifier of released stock. Alternatively tagging requirements may need to be circumvented by reliable quantification of average standing wild stock at farms and wild stock inclusion in polyculture harvests.

References

Ahlgren, M.O., 1998. Consumption and assimilation of salmon net pen fouling debris by the red sea cucumber *Parastichopus californicus*: Implications for polyculture. Journal of the World Aquaculture Society 29, 133-139.

Agudo, N.N., 2006. Sandfish Hatchery Techniques. CGIAR 45 pp.

An, Z., Dong, Y., Dong, S., 2007. Temperature effects on growth-ration relationships of juvenile sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture 272, 644-648.

Archer, J.E., 1996. Aspects of the Reproductive and Larval Biology and Ecology of the Temperate Holothurian *Stichopus mollis* (Hutton), Ph.D. thesis. School of Biological Sciences, University of Auckland, Auckland, pp. 178.

Asha, P.S., Muthiah, P., 2006. Effects of single and combined microalgae on larval growth, development and survival of the commercial sea cucumber *Holothuria spinifera* Theel. Aquaculture Research 37, 113-118.

Asha, P.S., Muthiah, P., 2007. Growth of the hatchery-produced juveniles of commercial sea cucumber *Holothuria* (Theelothuria) *spinifera* Theel. Aquaculture Research 38, 1082-1087.

Bakus, G.J., 1973. The biology and ecology of tropical holothurians. In: Jones, O.A., Endean, R. (Eds.), Biology and Geology of Coral Reefs 2, Biology 1. Academic Press, New York, pp. 325-367.

Battaglene, S.C., 1999. Culture of tropical sea cucumbers for stock restoration and enhancement. NAGA 22, 4-11.

Battaglene, S.C., Bell, J.D., 1999. Potential of the tropical Indo-Pacific sea cucumber, *Holothuria scabra*, for stock enhancement. Stock Enhancement and Sea Ranching, 478-490

Battaglene, S.C., S.C., Seymour, J.E., Ramofafia, C., 1999. Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. Aquaculture 178, 293-322.

Battaglene, S.C., Seymour, J.E., Ramofafia, C., Lane, I., 2002. Spawning induction of three tropical sea cucumbers, *Holothuria scabra*, *H-fuscogilva* and *Actinopyga mauritiana*. Aquaculture 207, 29-47.

Becker, P., Gillan, D., Lanterbecq, D., Jangoux, M., Rasolofonirina, R., Rakotovao, J., Eeckhaut, I., 2004. The skin ulceration disease in cultivated juveniles of *Holothuria scabra* (Holothuroidea, Echinodermata). Aquaculture 242, 13-30.

Bell, J.D., Agudo, N.N., Purcell, S.W., Blazer, P., Simutoga, M., Pham, D., Patrona, L.D., 2007. Grow-out of sandfish *Holothuria scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable. Aquaculture 273, 509-519.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification Canadian Journal of Biochemistry and Physiology 37, 911-917.

Bulteel, P., Jangoux, M., Coulon, P., 1992. Biometry, bathymetric distribution, and reproductive cycle of the holothuroid *Holothuria tubulosa* (Echinodermata) from Mediterranean seagrass beds. Marine Ecology 13, 53-62.

Bureau, D.P., Harris, A.M., Cho, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout *Oncorhynchus mykiss*. Aquaculture 180, 345-358.

Byers, S.C., Mills, E.L., Stewart, P.L., 1978. A comparison of methods of determining carbon in marine sediments, with suggestions for a standard method. Hydrobiologia 58, 43-47.

Cameron, J.L., 1986. Observations on the ecology of the juvenile life stage of the California sea-cucumber *Parastichopus californicus* (Stimpson). American Zoologist 26, A41.

Cameron, J.L., Fankboner, P.V., 1984. Tentacle structure and feeding processes in life stages of the commercial sea cucumber *Parastichopus californicus* (Stimpson). Journal of Experimental Marine Biology and Ecology 81, 193-209.

Cameron, J.L., Fankboner, P.V., 1989. Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson) (Echinodermata:Holothuroidea). II. Observations on the ecology of development, recruitment, and the juvenile life stage. Journal of Experimental Marine Biology and Ecology 127, 43-67.

Chang, Y., Yu, C., 2004. Pond culture of sea cucumbers, *A. japonicus*, in Dalian. In: Lovatelli, A., Conand, C., Purcell, S.W., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber aquaculture and management: FAO Fisheries Technical Paper FAO, Rome, pp. 269-272.

Chen, J., 2003. Overview of sea cucumber farming and sea ranching practices in China. SPC beche-de-mer Information Bulletin 18, 19-23.

Chen, J., 2004. Present status and prospects of the sea cucumber industry in China. In: Lovatelli, A., Conand, C., Purcell, S.W., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber aquaculture and management: FAO Fisheries Technical Paper FAO, Rome, pp. 25-38.

Choe, S., 1963. Biology of the Japanese Common Sea Cucumber *Stichopus japonicus* Selenka. Pusan National University, Pusan.

Cieciel, K.D., 2004. Movement of the Giant Red Sea Cucumber *Parastichopus californicus* University of Alaska, Fairbanks, pp. 142.

Colman, J.A., 1972. Food of snapper *Chrysophrys auratus* (Forster) in the Hauraki Gulf, New Zealand. New Zealand Journal of Marine and Freshwater Research 6, 221-239.

Conand, C., 1993. Ecology and reproductive biology of *Stichopus variegatus* an Indo-Pacific coral reef sea cucumber (Echinodermata: Holothuroidea). Bulletin of Marine Science 52, 970-981.

Conand, C., 2000. Present status of world sea cucumber resources and utilisation: An international overview, Advances in Sea Cucumber Aquaculture and Management, Dalian, Liaoning Province, China.

Conand, C., 2006. Sea cucumber biology: taxonomy; distribution; biology; conservation status. In: Bruckner, A.W. (Ed.), The Proceedings of the CITES workshop on the conservation of sea cucumbers in the families Holothuriidae and Stichopodidae. NOAA, pp. 244.

Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnology and Oceanography 11, 338-345.

Cousin, M., Cuzon, G., Guillaume, J., 1996. Digestibility of starch in *Penaeus vannamei*: In vivo and in vitro study on eight samples of various origin. Aquaculture 140, 361-372.

Crozier, W.J., 1918. The amount of bottom material ingested by holothurians (stichopus). Journal of Experimental Zoology 26, 379-389.

Da Silva, J., Cameron, J.L., Fankboner, P.V., 1986. Movement and orientation patterns in the commercial sea cucumber *Parastichopus californicus* (Stimpson) (Holothuroidea: Aspidochirotida). Marine Behaviour and Physiology 12, 133-147.

Dalzell, P., Adams, T.J.H., Polunin, N.V.C., 1996. Coastal fisheries in the Pacific Islands. Oceanography and Marine Biology 34, 395-531.

Dance, S.K., Lane, L., Bell, J.D., 2003. Variation in short-term survival of cultured sandfish (*Holothuria scabra*) released in mangrove-seagrass and coral reef flat habitats in Solomon Islands. Aquaculture 220, 495-505.

Danovaro, R., Armeni, M., Dell'Anno, A., Fabiano, M., Manini, E., Marrale, D., Pusceddu, A., Vannucci, S., 2001. Small-scale distribution of bacteria, enzymatic activities and organic matter in coastal sediments. Microbial Ecology 42, 177-185.

Davis, D.A., Arnold, C.R., 1993. Evaluation of 5 carboyhdrate sources for *Penaeus vannamei*. Aquaculture 114, 285-292.

Decho, A.W., Moriarty, D.J.W., 1990. Bacterial exopolymer utilization by a harpacticoid copepod: A methodology and results. Limnology and Oceanography 35, 1039-1049.

Dong, Y.W., Dong, S.L., 2006. Growth and oxygen consumption of the juvenile sea cucumber *Apostichopus japonicus* (Selenka) at constant and fluctuating water temperatures. Aquaculture Research 37, 1327-1333.

Eckert, G.L., 2007. Spatial patchiness in the sea cucumber *Pachythyone rubra* in the California Channel Islands. Journal of Experimental Marine Biology and Ecology 348, 121-132.

Ellis, J., Ysebaert, T., Hume, T., Norkko, A., Bult, T., Herman, P., Thrush, S., Oldman, J., 2006. Predicting macrofaunal species distributions in estuarine gradients using logistic regression and classification systems. Marine Ecology Progress Series 316, 69-83.

Entrambasaguas, L., Pérez-Ruzafa, Á., García-Charton, J.A., Stobart, B., Bacallado, J.J., 2008. Abundance, spatial distribution and habitat relationships of echinoderms in the Cabo Verde Archipelago (eastern Atlantic). Marine and Freshwater Research 59, 477-488.

Fabiano, M., Danovaro, R., 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia 277, 71-84.

FAO, 2003. Sea Cucumber (*Stichopus japonicus*) Culture in China, Training Manual on Breeding and Culture of Scallop and Sea Cucumber in China.

Fenwick, G.D., Horning, D.S.J., 1980. Echinodermata of The Snares Islands southern New Zealand. New Zealand Journal of Marine and Freshwater Research 14, 437-445.

Féral, J.-P., 1989. Activity of the principal digestive enzymes in the detritivorous apodous holothuroid *Leptosynapta galliennei* and two other shallow-water holothuroids. Marine Biology 101, 367-379.

Ferdouse, F., 2004. World markets and trade flows of sea cucumber/beche-de-mer. In: Lovatelli, A., Conand, C., Purcell, S.W., Uthicke, S., Hamel, J.F., Mercier, A. (Eds.), Advances in Sea Cucumber Aquaculture and Management. FAO, Rome, pp. 101-117.

Fern, C.M., 2006. An Investigation of Growth of *Stichopus mollis* in Sea Cages Under a Fin Fish Farm Operation in the Marlborough Sounds, New Zealand. Bay of Plenty Polytechnic, Tauranga, New Zealand, pp. 39.

Forrest, B.M., 1991. Oyster Farm Impacts on the Benthic Environment: A Study in Mahurangi Harbour, School of Biological Sciences. University of Auckland, Auckland, pp. 157.

Francour, P., 1997. Predation on holothurians: A literature review. Invertebrate Biology 116, 52-60.

Fu, X.-y., Xue, C.-h., Miao, B.-c., Li, Z.-j., Gao, X., Yang, W.-g., 2005. Characterization of proteases from the digestive tract of sea cucumber (*Stichopus japonicus*): High alkaline protease activity. Aquaculture 246, 321-329.

Gao, F., Yang, H., Xu, Q., Wang, F., Liu, G., German, D.P., 2008. Phenotypic plasticity of gut structure and function during periods of inactivity in *Apostichopus japonicus*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 150, 255-262.

Gibbs, M., Funnell, G., Pickmere, S., Norkko, A., Hewitt, J., 2005. Benthic nutrient fluxes along an estuarine gradient: influence of the pinnid bivalve *Atrina zelandica* in summer. Marine Ecology Progress Series 288, 151-164.

Giraspy, D.A.B., 2008. Ground-breaking hatchery technology paves the way for sea cucumber culture in Australia, Hatchery International. Capamara Communications, Victoria BC, Canada pp. 28-30.

Giraspy, D.A.B., Ivy, G., 2008. The influence of commercial diets on growth and survival in the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand, 1986) (Echinodermata: Holothuroidea). SPC beche-de-mer Information Bulletin 28, 46-52.

Glass, H.J., Stark, J.R., 1995. Carbohydrate digestion in the European lobster *Homarus gammarus* (L). Journal of Crustacean Biology 15, 424-433.

Godfriaux, B.L., 1969. Food and feeding habits of snapper. New Zealand Journal of Marine and Freshwater Research 3, 518-544.

Gosselin, L.A., Qian, P.Y., 1997. Juvenile mortality in benthic invertebrates. Marine Ecology Progress Series 146, 265-282.

Graham, D.H., 1939. Food and fishes of Otago Harbour and adjacent sea. Transactions of the Royal Society of New Zealand 68, 421-436.

Green, M.O., Hewitt, J., Thrush, S.F., 1998. Seabed drag coefficient over natural beds of horse mussels (*Atrina zelandica*). Journal of Marine Research 56, 613–637.

Hamel, J.F., Mercier, A., 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Canadian Journal of Fisheries and Aquatic Sciences 53, 253-271.

Hamel, J.F., Conand, C., Pawson, D.L., Mercier, A., 2001. The sea cucumber *Holothuria scabra* (Holothuroidea : Echinodermata): Its biology and exploitation as beche-de-mer, Advances in Marine Biology, Vol 41. Academic Press Ltd, London, pp. 129-223.

Hammond, L.S., 1982. Patterns of feeding and activity in deposit-feeding holothurians and echinoids (Echinodermata) from a shallow black-reef lagoon, Discovery Bay, Jamaica. Bulletin of Marine Science 32, 549-571.

Hargrave, B.T., 1976. The central role of invertebrate faeces in sediment decomposition. Blackwell, Oxford, 301-321 pp.

Hari, B., Madhusoodana Kurup, B., Vargese, J.T., Schrama, J.W., Verdegem, M.C.J., 2004. Effects of carbohydrate addition on production in extensive shrimp culture systems. Aquaculture 241, 179-194.

Harrison, G., 1974. Mahurangi Harbour Marine Studies. Report for the Auckland Regional Authority. Harrison Grierson and Partners, pp. 65.

Hartstein, N.D., 2003. Supply and Dispersal of Mussel Farm Debris and its Impacts on Benthic Habitats in Contrasting Hydrodynamic Regimes. Ph.D. thesis. School of Biological Sciences, University of Auckland, Auckland, pp. 181.

Hartstein, N.D., Stevens, C.L., 2005. Deposition beneath longline mussel farms. Aquacultural engineering 33, 192-213.

Hatton, S., Hayden, B.J., James, M.R., 2005. The effects of food concentration and quality on the feeding rates of three size classes of the Greenshell™ Mussel, Perna canaliculus. Hydrobiologia 548, 23-32.

Haukson, E., 1979. Feeding biology of *Stichopus tremulus,* a deposit feeding holothurian. Sarsia 64, 155–159.

Hearn, A., Martinez, P., Toral-Granda, M.V., Murillo, J.C., Polovina, J., 2005. Population dynamics of the exploited sea cucumber *Isostichopus fuscus* in the western Galapagos Islands, Ecuador. Fisheries Oceanography 14, 377-385.

Hudson, I.R., Wigham, B.D., Solan, M., Rosenberg, R., 2005. Feeding behaviour of deep-sea dwelling holothurians: Inferences from a laboratory investigation of shallow fjordic species. Journal of Marine Systems 57, 201-218.

Huiling, S., Mengqing, L., Jingping, Y., Bijuan, C., 2004. Nutrient requirements and growth of the sea cucumber, *Apostichopus japonicus*. In: Lovatelli, A., Conand, C., Purcell, S.W., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber culture and management. FAO.

Imai, T., Inaba, D., 1950. On the artificial breeding of Japanese Sea-Cucumber, *Stichopus japonicus* Selenka. Bull. Inst. Agricult. Res. Tohoku Univ., 2.

Inui, M., Itsubo, M., Iso, S., 1991. Creation of a new non-feeding aquaculture system in enclosed coastal seas. Marine Pollution Bulletin 23, 321-325.

Isgoren-Emiroglu, D., Gunay, D., 2007. The effect of sea cucumber *Holothuria tubulosa* G. 1788 on nutrient and organic matter contents of bottom sediment of oligotrophic and hypereutrophic shores. Fresenius Environmental Bulletin 16, 290-294.

Ito, S., Kitamura, H., 1997. Induction of larval metamorphosis in the sea cucumber *Stichopus japonicus* by periphitic diatoms. Hydrobiologia 358, 281-284.

James, D.B., 2005. Information on juvenile holothurians. SPC beche-de-mer Information Bulletin 21, 26-27.

James, D.B., Rajapandian, M.E., Baskar, B.K., Gopinathan, C.P., 1988. Successful induced spawning and rearing of the holothurian *Holothuria* (metriatyla) *scabra* Jaeger at Tuticorin. Marine Fisheries Information Service Technical and Extension Series.

James, D.B., Ghandi, A.D., Palaniswarmy, N., Rodrigo, J.X., 1994. Hatchery techniques and culture of the sea cucumber *Holothuria scabra*. Central Marine Fisheries Research Institute Special 57, 1-47.

James, M.R., Weatherhead, M.A., Ross, A.H., 2001. Size-specific clearance, excretion, and respiration rates, and phytoplankton selectivity for the mussel *Perna canaliculus* at low levels of natural food. New Zealand Journal of Marine and Freshwater Research, 73-86.

Kang, K.H., Kwon, J.Y., Kim, Y.M., 2003. A beneficial coculture: charm abalone *Haliotis discus* hannai and sea cucumber *Stichopus japonicus*. Aquaculture 216, 87-93.

Kautsky, N., Evans, S., 1987. Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic ecosystem. Marine Ecology Progress Series 38, 201-212.

Kiang, M.J., 1998. Recycling of secondary resources by dry extrusion. In: Chang, Y.K., Wang, S.S. (Eds.), International Symposium on Animal and Aquaculture Feedstuffs by Extrusion Technology/International Seminar on Advanced Extrusion Technology in Food Applications. Technomic Publ Co Inc, Sao Paulo, Brazil, pp. 203-207.

Kinch, J., 2002. The beche-de-mer fishery in the Milne Bay Province of Papua New Guinea. A report to the National Fisheries Authority, Port Moresby, Papua New Guinea & CSIRO, Queensland, Australia.

Kinch, J., 2005. The commercial use of *Thelenota rubralineata* in the Solomon Islands. Beche de Mer Bulletin 21, 3-4.

Lawrence, J.M., 1982. Digestion. In: Jangoux, M., Lawrence, J.M. (Eds.), Echinoderm Nutrition. A.A. Balkema, Rotterdam, pp. 283-316.

Lechowicz, M.J., 1982. The sampling characteristics of electivity indices. Oecologia 52, 22-30.

Levin, V.S., 1979. A composition of feeding particles of aspidochirote holothurians from the upper sublittoral zone of Indo-West Pacific. Biologiya Morya-Marine Biology, 20-27.

Liu, Y., Dong, S., Tian, X., Wang, F., Gao, Q., 2009. Effects of dietary sea mud and yellow soil on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture 286, 266-270.

Lopez, G.R., 1987. Ecology of deposit-feeding animals in marine sediments. Quarterly Review of Biology 62, 235-260.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnology and Oceanography 12, 343-346.

Martin, A.T., Foster, B.A., 1986. Distribution of barnacle larvae in Mahurangi Harbour, North Auckland. New Zealand Journal of Marine and Freshwater Research 20, 67-76.

Massin, C., 1982. Food and Feeding Mechanisms: Holothuroidea. In: Jangoux, M., Lawrence, J.M. (Eds.), Echinoderm Nutrition. A. A. Balkema, Rotterdam., pp. 43-55.

Massin, C., Doumen, C., 1986. Distribution and feeding of epibenthic holothuroids on the reef flat of Laing Island (Papua New Guinea). Marine Ecology Progress Series 31, 185-195.

Mattsson, J., Linden, O., 1983. Benthic macrofauna succession under mussels, *Mytilus edulis* L.(Bivalvia), cultured on hanging long-lines. Sarsia 68, 97-102.

Maxwell, K., 2006. The Role of the Sea Cucumber, *Stichopus mollis* (Hutton) in a Semi-recirculating Polyculture System. Victoria University of Wellington, Wellington, pp. 115.

Maxwell, K., Gardner, J.P.A., Heath, P., 2009. The effect of diet on the energy budget of the brown sea cucumber, *Stichopus mollis* (Hutton). Journal of the World Aquaculture Society 40, 159-170.

Mayer, L.M., Schick, L.L., Setchell, F.W., 1986. Measurement of protein in nearshore marine-sediments. Marine Ecology-Progress Series 30, 159-165.

Mayer, L.M., Rahaim, P.T., Guerin, W., Macko, S.A., Watling, L., Anderson, F.E., 1985. Biological and granulometric controls on sedimentary organic matter of an intertidal mudflat. Estuarine, Coastal and Shelf Science 20, 491-503.

MedeirosBergen, D.E., Miles, E., 1997. Recruitment in the holothurian *Cucumaria frondosa* in the Gulf of Maine. Invertebrate Reproduction & Development 31, 123-133.

Mercier, A., Battaglene, S.C., Hamel, J.F., 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology 239, 125-156.

Mercier, A., Battaglene, S.C., Hamel, J.F., 2000a. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. Journal of Experimental Marine Biology and Ecology 249, 89-110.

Mercier, A., Battaglene, S.C., Hamel, J.F., 2000b. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. Hydrobiologia 440, 81-100.

Mladenov, P.V., Gerring, P., 1991. Resource Evaluation of the Sea Cucumber (*Stichopus mollis*) in Fiordland, New Zealand. Marine Science and Aquaculture Research Centre, University of Otago, 34 pp.

Mladenov, P.V., Campbell, A., 1998. Resource evaluation of the sea cucumber (*Stichopus mollis*) in Fiordland, New Zealand., Proceedings of the 9th International Echinoderm Conference, San Francisco, pp. 481-487.

Moraes, G., Norhcote, P.C., Kalinin, V.I., Avilov, S.A., Silchenko, A.S., Dmitrenok, P.S., Stonik, V.A., Levin, V.S., 2004. Structure of the major triterpene glycoside from the sea cucumber *Stichopus mollis* and evidence to reclassify this species into the new genus Australostichopus. Biochemical Systematics and Ecology 32, 637-650.

Morgan, A.D., 2004. Variation in Reproduction and Development of the Temperate Sea Cucumber *Stichopus mollis*, PhD - School of Biological Sciences. University of Auckland, Auckland, pp. 179.

Morgan, A.D., 2009. Spawning of the temperate sea cucumber, *Australostichopus mollis* (Levin). Journal of the World Aquaculture Society 40, 363-373.

Morgan, A.D., Archer, J.E., 1999. Aspects of sea cucumber industry research and development in the South Pacific. SPC beche-de-mer Information Bulletin 12, 4-8.

Moriarty, D.J.W., 1982. Feeding of *Holoturia atra* and *Stichopus chloronotus* on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. Australian Journal of Marine and Freshwater Research 33, 255-263.

Morrison, M.A., 2000. Mahurangi Harbour Habitat Map, NIWA Information Series no. 13. National Institute of Water and Atmospheric Research, Wellington, NZ, pp. 1 Map, colour.

Morton, J.E., Miller, M.C., 1973. The New Zealand Sea Shore. Collins, London.

Mosher, C., 1980. Distribution of *Holothuria - Arenicola* Semper in the Bahamas with observations on habitat, behaviour and feeding activity (Echinodermata, Holothuroidea). Bulletin of Marine Science 30, 1-12.

Nealson, K.H., 1997. Sediment bacteria: Who's there, what are they doing, and what's new? Annual Review of Earth and Planetary Sciences 25, 403-434.

Okorie Eme, O., Su Hong, K., Sugeun, G., Seunghyung, L., Jun Young, B., Kyungmin, H., Sungchul, C.B., 2008. Preliminary study of the optimum dietary ascorbic acid level in sea cucumber, *Apostichopus japonicus* (Selenka). Journal of the World Aquaculture Society 39, 758-765.

Osman, R.W., Whitlatch, R.B., Malatesta, R.J., 1992. Potential role of micropredators in determining recruitment into a marine community. Marine Ecology Progress Series 83, 35-43.

Paltzat, D.L., Pearce, C.M., Barnes, P.A., McKinley, R.S., 2008. Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). Aquaculture 275, 124-137.

Pawson, D.L., 1970. The marine fauna of New Zealand: Sea Cucumbers (Echinodermata: Holthuroidea), Bulletin (New Zealand Department of Scientific and Industrial Research), pp. 69.

Penry, D.L., 1989. Tests of kinematic models for deposit-feeders' guts: patterns of sediment processing by *Parastichopus californicus* (Stimpson) (Holothuroidea) and *Amphicteis scaphobranchiata* Moore (Polychaeta). Journal of Experimental Marine Biology and Ecology 128, 127-146.

Petry, H., Rapp, W., 1971. Zur Problematik der Chromoxidbestimmung in Verdauungsversuche. Zeitschrift der Tierphysiologie, Tierernährnung und Futtermittelkunde 27, 181-189.

Pitt, R., Duy, N.D.Q., 2003. How to produce 100 tonnes of sandfish. SPC beche-de-mer Information Bulletin, 15-17.

Pitt, R., Duy, N.D.Q., 2004. Breeding and rearing of the sea cucumber *Holothuria scabra* in Vietnam. In: Lovatelli, A., Conand, C., Purcell, S.W., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber aquaculture and management. FAO.

Pitt, R., Duy, N.D.Q., Duy, T.V., Long, H.T.C., 2004. Sandfish (*Holothuria scabra*) with shrimp (*Penaeus monodon*) co-culture tank trials. SPC beche-de-mer Information Bulletin, 12-22.

Purcell, S.W., 2004. Criteria for release strategies and evaluating the restocking of sea cucumbers. In: Lovatelli, A., Conand, C., Purcell, S.W., Uthicke, S., Hamel, J.-F., Mercier, A. (Eds.), Advances in sea cucumber aquaculture and management. FAO, Dalian, pp. 439.

Purcell, S.W., Kirby, D.S., 2006. Restocking the sea cucumber *Holothuria scabra*: Sizing no-take zones through individual-based movement modelling. Fisheries Research 80, 53-61.

Purcell, S.W., Simutoga, M., 2008. Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumbers in the wild. Reviews in Fisheries Science 16, 204-214.

Purcell, S.W., Patrois, J., Fraisse, N., 2006a. Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). Aquaculture Research 37, 515-522.

Purcell, S.W., Blockmans, B.F., Agudo, N.N., 2006b. Transportation methods for restocking of juvenile sea cucumber, *Holothuria scabra*. Aquaculture 251, 238-244.

Qin, C., Dong, S., Tan, F., Tian, X., Wang, W., Dong, Y., Gao, Q., 2009. Optimization of stocking density for the sea cucumber, *Apostichopus japonicus* Selenka, under feed-supplement and non-feed-supplement regimes in pond culture. Journal of Ocean University of China (English Edition) 8, 296-302.

Raj, L.K., 1997. Photo-identification of *Stichopus mollis*. SPC Coastal Fisheries Porgramme.

Raj, L.K., 1998. Reproductive Biology and the Use of Photoidentification to Study Growth in *Stichopus mollis* (Echinodermata: Holothuroidea) in Doubtful Sound, Fiordland, New Zealand. University of Otago.

Ramofafia, C., Foyle, T.P., Bell, J.D., 1997. Growth of juvenile *Actinopyga mauritiana* (Holothuroidea) in captivity. Aquaculture 152, 119-128.

Ricker, W.E., 1979. Growth rates and models. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology. Academic Press, New York.

Roberts, D., 1979. Deposit-feeding mechanisms and resource partitioning in tropical holothurians. Journal of Experimental Marine Biology and Ecology 37, 43-56.

Roberts, D., Bryce, C., 1982. Further observations on tentacular feeding mechanisms in holothurians. Journal of Experimental Marine Biology and Ecology 59, 151-163.

Roberts, D., Gebruk, A., Levin, V., Manship, B.A.D., 2000. Feeding and digestive strategies in deposit-feeding holothurians. Oceanography and Marine Biology 38, 257-310.

Sewell, M.A., 1987. The Reproductive Biology of *Stichopus mollis* (Hutton), Department of Zoology. University of Auckland, Auckland, pp. 98.

Sewell, M.A., 1990. Aspects of the ecology of *Stichopus mollis* (Echinodermata: Holothuroidea) in north-eastern New Zealand. New Zealand Journal of Marine and Freshwater Research 24, 97-103.

Sewell, M.A., 1992. Reproduction of the temperate aspidochirote *Stichopus mollis* (Echinodermata: Holothuroidea) in New Zealand. Ophelia 3, 103-121.

Shears, N.T., Babcock, R., 2007. Quantitative description of mainland New Zealand's shallow subtidal reef communities. Science for Conservation 280, 126.

Shiel, G., 2004. Field observations of juvenile sea cucumbers. SPC beche-de-mer Information Bulletin 20, 6-11.

Sibuet, M., 1984. Quantitative distribution of echinoderms (Holothuroidea, Asteroidea, Ophiuroidea, Echinoidea) in relation to organic matter in the sediment in deep sea basins of the Atlantic Ocean. In: Keegan, B.F., O'Connor, B.D.S. (Eds.), International Echinoderm Conference. CRC, Galway, pp. 99-108.

Singer, J.K., Anderson, J.B., Ledbetter, M.T., McCave, I.N., Jones, K.P.N., Wright, R., 1988. An assessment of analytical techniques for the size analysis of fine-grained sediments. Journal of Sedimentary Petrology 58, 534-543.

Skewes, T.D., Dennis, D.M., C., B., 2000. Survey of *Holothuria scabra* (sandfish) on Warrior Reef, Torres Strait, January, 2000: CSIRO Division of Marine Research Final Report. . CSIRO Division of Marine Research, Cleveland, Qld. (Australia).

Slater, M.J., 2006. Polyculture of the Sea Cucumber *Stichopus mollis* with Green-lipped Mussels, Leigh Marine Laboratory. MSc. Thesis. University of Auckland, Auckland, pp. 112.

Slater, M.J., Carton, A.G., 2007. Survivorship and growth of the sea cucumber *Australostichopus* (Stichopus) *mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. Aquaculture 272, 389-398.

Slater, M.J., Carton, A.G., 2009. Effect of sea cucumber (*Australostichopus mollis*) grazing on coastal sediments impacted by mussel farm deposition. Marine Pollution Bulletin 58, 1123-1129.

Slater, M.J., Jeffs, A.G., Carton, A.G., 2009. The use of the waste from green-lipped mussels as a food source for juvenile sea cucumber, *Australostichopus mollis*. Aquaculture 292, 219-224.

Sloan, N.A., von Bodungen, B., 1980. Distribution and feeding of the sea cucumber *Isostichopus badionatus* in relation to shelter and sediment criteria of the Bermuda Platform. Marine Ecology Progress Series 2, 14-28.

Sperazza, M., Moore, J.N., Hendrix, M.S., 2004. High-resolution particle size analysis of naturally occurring very fine-grained sediment through laser diffractometry. Journal of Sedimentary Research 74, 736-743.

Stenton-Dozey, J., 2007. Finding hidden treasure in aquaculture waste. Water & Atmosphere 15, 10-11.

Stenton-Dozey, J., Heath, P., 2009. A first for New Zealand: culturing our endemic sea cucumber for overseas markets. Water & Atmosphere 17, 20-21.

Stone, D.A.J., 2003. Dietary carbohydrate utilization by fish. Reviews in Fisheries Science 11, 337-369.

Tanaka, M., 2000. Diminution of sea cucumber *Stichopus japonicus* juveniles released on artificial reefs. Bulletin of Ishikawa Prefecture Fisheries Resource Center 2, 19-29.

Tanaka, Y., 1958. Feeding and digestive processes of *Stichopus japonicus*. Bulletin Faculty Fish Hokkaido University 9, 14-28.

Taylor, K.A.C.C., 1995. A modification of the phenol/sulfuric acid assay for total carbohydrates giving more comparable absorbances. Applied Biochemistry and Biotechnology 53, 207-214.

Toral-Granda, M.V., Martínez, P.C., 2000. Population density and fishery impacts on the sea cucumber (*Stichopus fuscus*) in the Galápagos Islands, Advances in Sea Cucumber Aquaculture and Management (ASCAM) Conference, Dalian, Liaoning Province, China.

Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M., 2008. Sea cucumbers - A global review of fisheries and trade. FAO, Rome, 317 pp.

Tuwo, A., 2000. Status of sea cucumber fisheries and farming in Indonesia, Advances in Sea Cucumber Aquaculture and Management (ASCAM) Conference, Dalian, Liaoning Province, China.

Uthicke, S., 1993. Distribution patterns and growth of two reef flat holothurians, *Holothuria atra* and *Stichopus chloronotus*. In: David, B., Guille, A., Féral, J.-P., Roux, M. (Eds.), Eight International Echinoderm Conference. Balkema, Dijon, France, pp. 569-576.

Uthicke, S., 1994. Distribution patterns and growth of two reef flat holothurians, *Holothuria atra* and *Stichopus chloronotus*, Balkema, Rotterdam, 569-576 pp.

Uthicke, S., 1999. Sediment bioturbation and impact of feeding activity of *Holothuria* (Halodeima) *atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. Bulletin of Marine Science 64, 129-141.

Uthicke, S., 2000. Overfishing of holothurians: Lessons from the Great Barrier Reef, Advances in Sea Cucumber Aquaculture and Management (ASCAM) Conference, Dalian, Liaoning Province, China.

Uthicke, S., 2001. Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology 265, 153-170.

Uthicke, S., Karez, R., 1999. Sediment patch selectivity in tropical sea cucumbers (Holothurioidea: Aspidochirotida) analysed with multiple choice experiments. Journal of Experimental Marine Biology and Ecology 236, 69-87.

Uthicke, S., Benzie, J.A.H., 2001. Effect of bêche-de-mer fishing on densities and size structure of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations on the Great Barrier Reef Coral Reefs 19, 271-276.

Uthicke, S., Conand, C., 2005. Local examples of beche-de-mer overfishing: An initial summary and request for information. SPC beche-de-mer Information Bulletin 21, 9-14.

Uthicke, S., Welch, D., Benzie, J.A.H., 2004. Slow growth and lack of recovery in overfished holothurians on the Great Barrier Reef: Evidence from DNA fingerprints and repeated large-scale surveys. Conservation Biology 18, 1395-1404.

Vanderploeg, H.A., Scavia, D., 1979. Calculation and use of selectivity coefficients of feeding: zooplankton grazing. Ecological Modelling 7, 135-149.

Ward-Rainey, N., Rainey, F.A., Stackebrandt, E., 1996. A study of the bacterial flora associated with *Holothuria atra*. Journal of Experimental Marine Biology and Ecology 203, 11-26.

Webb, B.F., 1973. Fish populations of the Avon-Heathcote Estuary. New Zealand Journal of Marine and Freshwater Research 7, 223-234.

Webb, K.L., D'Elia, C.F., Dupaul, W.D., 1977. Biomass and nutrition flux measurements on *Holothuria atra* populations on windward reef flats at Enewetak, Marshall Islands. In: Taylor, D.L. (Ed.), Third International Coral Reef Symposium, Miami, Florida, pp. 410-441.

Welschmeyer, N.A., Lorenzen, C.J., 1985. Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific Gyres. Limnology and oceanography 30, 1-21.

Wiedemeyer, W.L., 1994. Biology of small juveniles of the tropical holothurian *Actinopyga echinites*: growth, mortality, and habitat preferences. Marine Biology 120, 81-93.

Wing, S.R., McLeod, R.J., Clark, K.L., Frew, R.D., 2008. Plasticity in the diet of two echinoderm species across an ecotone: microbial recycling of forest litter and bottom-up forcing of population structure. Marine Ecology Progress Series 360, 115-123.

Wu, R.S.S., 1995. The environmental impact of marine fish culture: Towards a sustainable future. Marine Pollution Bulletin 31, 159-166.

Yamamoto, N., Lopez, G., 1985. Bacterial abundance in relation to surface area and organic content of marine sediments. Journal of Experimental Marine Biology and Ecology 90, 209-220.

Yamana, Y., Hamano, T., Miki, K., 2006. Distribution of the Japanese sea cucumber *Apostichopus japonicus* in the intertidal zone of Hirao Bay, eastern Yamaguchi Prefecture, Japan - Suitable environmental factors for juvenile habitats. Journal of National Fisheries University 54, 111-120.

Yanagisawa, T., 1998. Aspects of the biology and culture of the sea cucumber. In: De Silva, S. (Ed.), Tropical Mariculture. Academic Press, London, pp. 291-308.

Yaqing, C., Changqing, Y., Xin, S., 2000. Sea cucumber (*Apostichopus japonicus*) pond polyculture in Dalian, Liaoning Province, China, Advances in Sea Cucumber Aquaculture and Management (ASCAM) Conference, Dalian, Liaoning Province, China.

Yingst, J.Y., 1974. The Utilization of Organic Detritus and Associated Microorganisms by *Parastichopus parvimensis*, a Benthic Deposit-feeding Holothurian. University of California, Los Angeles, pp. 154.

Yingst, J.Y., 1976. The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. Journal of Experimental Marine Biology and Ecology 23, 55-69.

Yingst, J.Y., 1982. Factors influencing rates of sediment ingestion by *Parastichopus parvimensis* (Clark), an epibenthic deposit-feeding holothurian. Estuarine Coastal and Shelf Science 14, 119-134.

Young, C.M., Chia, F.S., 1982. Factors controlling spatial distribution of the sea cucumber *Psolus chitionoides*—settling and post-settling behaviour. Marine Biology 69, 195-205.

Yuan, X., Yang, H., Zhou, Y., Mao, Y., Zhang, T., Liu, Y., 2006. The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture 256, 457-467.

Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall, New Jersey.

Zheng, Z.M., Dong, S.L., Tian, X.L., Wang, F., Gao, O.F., Bai, P.F., 2009. Sediment-water fluxes of nutrients and dissolved organic carbon in extensive sea cucumber culture ponds. Clean-Soil Air Water 37, 218-224.

Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Xu, X., Zhang, F., 2006. Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. Aquaculture 256, 510-520.

Zor, T., Selinger, Z., 1996. Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. Analytical Biochemistry 236, 302-308.