Co-culture of invertebrates with sablefish (*Anoplopoma fimbria*) in IMTA in British Columbia: Use of laboratory feeding trials to assess the organic extractive potential of various candidate species

by

Lindsay Catherine Orr
B.Sc., University of Victoria, 2009

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Supervisory Committee

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Abstract

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One advantage of Integrated Multi-Trophic Aquaculture (IMTA) is the potential for bioremediation by organic-extractive organisms. In British Columbia, a number of marine invertebrate species are being considered for use in open-water IMTA with sablefish (*Anoplopoma fimbria*). These include both filter-feeding bivalves (*e.g.* cockles, mussels, oysters, scallops) which would consume the finer suspended particulates from the finfish culture component and deposit/detrital feeders (*e.g.* sea cucumbers, sea urchins, prawns) which would feed on the heavier-settleable solids. The following candidate species were tested for their ability to consume sablefish faeces and uneaten sablefish feed in laboratory feeding trials: green sea urchin (*Strongylocentrotus droebachiensis*), basket cockle (*Clinocardium nuttallii*), blue mussel (*Mytilus edulis*), spot prawn (*Pandalus platyceros*), and California sea cucumber (*Parastichopus californicus*). Whether they can remove organic material from aquaculture wastes was tested by measuring ingestion rate or clearance rate and absorption efficiency when they were fed a diet of sablefish waste, relative to those fed a natural control diet. Egestion rates in the candidate species were quantified to estimate the potential amount of waste that may be lost from the organic-extractive component. Biophysical properties including shape, size, and settling velocity were measured in faecal pellets egested by the candidate species to provide input data for models to assess dispersal of faeces from IMTA sites. Results from the laboratory feeding trials demonstrate that all candidate species are capable of consuming wastes from sablefish aquaculture and absorbing the organic material. The relative merits and drawbacks of each candidate species are discussed with respect to the results and within the broader context of IMTA. The general conclusion is that, in order to achieve efficient removal of organic material and successful bioremediation, deposit feeders should be included in the organic-extractive component, whether alone or in conjunction with suspension feeders.

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1. Chapter 1 - Introduction

Globally, significant expansion of the aquaculture industry is predicted as wild stocks continue to collapse, fishing rates are at their maximum, and the demand for seafood rises (Corbin, 2007; Neori et al., 2004, 2007; Troell et al., 2009). However, the intensive monoculture approach to farming finfish is ecologically imbalanced and, therefore, unsustainable in the long-term (Neori et al., 2007). The intensive monoculture approach means that top predators are held in relatively small enclosures at high densities. Thus, they are removed from the natural ecosystem and normal interactions with other species or lower trophic levels no longer occur. The input of manufactured feed is required to maintain farmed finfish at acceptable growth rates and survivorship levels to produce a marketable product. Concern exists over the potentially harmful effects of intensive, open-water finfish aquaculture on the marine environment. These include alterations to the underlying sediments and surrounding water column through nutrient loading and the accumulation of organic wastes such as uneaten feed and finfish faeces (Buschmann et al., 2006; Holmer and Kristensen, 1992; Papageorgiou et al., 2010; Reid et al., 2009). In order to achieve long-term sustainability and meet world seafood demand, the aquaculture industry must strive to reduce its impact on the natural environment (Troell et al., 2003).

Recently, interest has developed in the potential for Integrated Multi-Trophic Aquaculture (IMTA) to reduce some impacts of finfish aquaculture, while providing economic benefits through product diversification (Buschmann et al., 2009; Chavez-Crooker and Obreque-Contreras, 2010; Neori et al., 2007; Troell et al., 2009). In IMTA systems, waste produced by upper trophic levels (*e.g.* fed finfish) would be consumed by inorganic-extractive seaweed species and organic-extractive invertebrate species (both suspension- and deposit-feeding organisms) at lower trophic levels. Thus, excess energy and materials are internalized and converted into commercially-useful products instead of impacting the ecosystem. Adding to this, Barrington et al. (2010) found that IMTA could improve social acceptability of the aquaculture industry. Although IMTA has potential for economic, societal, and environmental benefits, research is still required to address several issues, including potential risk, the degree of bioremediation, feasibility, and technological challenges (Troell et al., 2009). Understanding how each species in an IMTA system interacts with the others is key to understanding the scale of organic extraction at each level and in turn the trophic transfer efficiency of the system as a whole.

The primary source of organic loading in aquaculture is faeces. This excess organic matter can lead to eutrophication, the development of anoxic conditions, and eventually lead to a decrease in biodiversity in the local benthic environment surrounding aquaculture sites (Brown et al., 1987; Holmer and Kristensen, 1992; Kutti et al., 2007; Papageorgiou et al., 2010). However, in an IMTA system, undigested material in the faeces is also available for consumption by species occupying lower trophic levels. Depending on their biophysical properties, the repackaging and transport of undigested materials as faecal pellets by lower trophic level species may serve to localize the impact of suspended organic matter (Wotton and Malmqvist, 2001). Understanding energy and material transfer between trophic levels via faecal material will be essential to the development of effective IMTA. Also, knowledge of biophysical properties in finfish and invertebrate faeces will be required, as both will have implications for the dispersal of nutrients within the IMTA system and the natural marine environment.

In current plans for open-water IMTA, culture of suspension-feeding bivalves is proposed for the removal of finer particles of uneaten feed and finfish faeces (referred to as “waste” from here on) from the water column. Suspension-feeding organisms can play an important role in the transfer of nutrients between pelagic and benthic ecosystems, removing small, organic particles suspended in the water column via filtration and initiating their sedimentation through deposition of biodeposits (Kautsky and Evans, 1987). In British Columbia (BC), two candidate suspension-feeding species for incorporation in IMTA include the basket cockle (*Clinocardium nuttallii*) and the blue mussel (*Mytilus edulis*). Historically, *C. nuttallii* has not been commercially harvested, but it does show promise for use in aquaculture as it is a native species to the BC coast, has a relatively high growth rate in colder waters, and can be successfully reared during early life stages in the laboratory (Liu et al., 2009). Mussels have been examined far more extensively in terms of their potential as an aquaculture species in general and as an organic-extractive organism in particular. Studies have shown that those held adjacent to open-water net pens display enhanced growth rates relative to those cultured away from the influence of intensive aquaculture (Cheshuk et al., 2003; Lander et al., 2004; Sara et al., 2009; Stirling and Okumus, 1995). Based on this, some studies have concluded that mussels represent a good candidate species for use in IMTA. As an example, Lander et al. (2004) reported that blue mussels showed increased feeding rates in response to periodic elevations in the level of suspended particulate materials, which were correlated with feeding times of salmon in a pilot open-water IMTA system in the Bay of Fundy, New Brunswick. In addition, mussels grown adjacent to the salmon pens in New

Brunswick had a larger mean shell length than the control populations held away from the site (Lander, 2004). In the Tyrrhenian Sea, Italy, Sara et al. (2009) observed significantly increased growth in *Mytilus galloprovincialis* cultured downstream of open-water pens containing seabass and seabream, compared to those cultured upstream (all within 1000 m of the pens). Consistent with this, the authors measured higher levels of chlorophyll *a*, protein to carbohydrate ratios, and total suspended organic matter downstream of the finfish aquaculture site (Sara et al. 2009). These results suggest that organic effluents from open-water net pens provide an additional food source downstream of the pens which can be subsequently captured and assimilated by suspension-feeding bivalves, resulting in increased growth. Although some correlational evidence suggests that bivalves utilize particulate organic wastes from finfish aquaculture, few studies have attempted to directly measure consumption rates by bivalves in an IMTA field setting. Stable isotopes and fatty acid signatures have been used to trace dietary source in some cases (Mazzola and Sara, 2001; Navarrete-Mier et al., 2010) and absorption efficiency has been quantified over a limited temporal scale (Reid et al., 2010). Redmond et al. (2010) demonstrated that both the stable isotope $\delta^{13}C$ and fatty acid signatures worked well to trace the assimilation of salmon feed pellets into the digestive gland tissue of *M. edulis*, whereas $\delta^{15}N$ was successful at tracing assimilation of the pellets into both digestive gland and mantle tissue of the mussels. Expanding upon this, Navarrete-Mier et al. (2010) used $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios to compare dietary source between *M. galloprovincialis* and the oyster *Ostrea edulis* grown at increasing distances from a finfish aquaculture site in Alicante, Spain. While the authors did not detect a significant difference in length, weight, or stable isotope composition between bivalves cultured at each station, they did find a significant overall increase in length for all mussels over the course of the study, meaning that the mussels did grow, but at a similar rate. In this case, the stable isotope composition in bivalve tissues did not resemble that of the fish feed.

Local hydrodynamic regime also plays a role in nutrient dispersal and bioavailability to lower trophic levels. Water circulation and particle movement are both spatially and temporally complex; furthermore, local oceanographic characteristics are highly site-specific (Cross, 2004; Page et al., 2004). In general, the effluents expelled from sites experiencing strong currents will be rapidly diluted, whereas those from sites in enclosed regions will remain in the surrounding water column for a greater period of time (Cross, 2004). Reid et al. (2010) stress the importance of understanding plume dynamics to the design of IMTA. When they placed *Mytilus trossulus* and *M. edulis* in close proximity to an Atlantic salmon (*Salmo salar*) farm, the absorption efficiencies of the mussels were lower than expected based on data from laboratory feeding trials. Additional examination of water samples revealed that periods of silt influx, characterized by relatively low organic content, occurred over the course of the study. Dispersal models will be required for the optimal design of IMTA field experiments and systems and these models will be sensitive to biophysical properties of the faecal pellets of interest (Reid et al., 2009). Faecal dispersal characteristics will vary depending on many factors, including species, diet, collection time, settling velocity, and site-specific environmental conditions.

The integration of deposit-feeding or grazing benthic marine invertebrates into IMTA is proposed to reduce the accumulation of heavier-settleable organic materials on the sea floor underneath open-water finfish pens. In BC, three candidate species include the green sea urchin (*Strongylocentrotus droebachiensis*), the spot prawn (*Pandalus* *platyceros*), and the California sea cucumber (*Parastichopus californicus*). Feeding activity by *S. droebachiensis* is known to significantly impact the benthic environment and can influence nutrient cycling (Sauchyn and Scheibling, 2009a, 2009b). Although sea urchins are best known as grazers of macroalgae (Lawrence, 1975), they also feed on other materials including detritus (Kirchhoff et al., 2008) and as such may be able to live off the organic waste produced by intensive finfish aquaculture. The gonads from sea urchins (termed roe or uni) are harvested and sold for human consumption and opportunities for aquaculture are developing in response to a global decline in wild stocks (Andrew et al., 2002b). Subsequent research on sea urchin culture has revealed that growth and gonad development are affected by the type and quality of diet (*e.g.* Cook and Kelly, 2009; Daggett et al., 2010). While there have not been any published studies investigating the feasibility of *S. droebachiensis* culture in IMTA with finfish, they have been grown on an experimental scale with oysters (Switzer et al., 2011). Also, the related sea urchin species *Paracentrotus lividus* has been grown successfully in close proximity to open-water Atlantic salmon net pens on the north-west coast of Scotland (Cook and Kelly, 2007). In that study, juvenile (< 60 mm test diameter) *P. lividus* suspended 0 m away from net pens had a higher survivorship, and were the only individuals to develop gonads after twelve months, relative to those suspended 50 m and 2.5 km away. Adults (60–70 mm test diameter) suspended at both the 0 and 50 m stations had acceptable gonad appearance after three months, although supplemental feeding with macroalgae further enhanced their condition. Importantly, *P. lividus* assimilated fatty acids found in the salmon feed into gonadal tissue, confirming that sea urchins can feed on organic waste from finfish aquaculture can produce a commercially acceptable product based on gonad appearance.

The spot prawn, *P. platyceros*, is the larger of two commercially important Pandalids native to Northwest North America. No published studies have tested the co-culture of this benthic-feeding scavenger in an IMTA system, but previous research has investigated the potential for its use in aquaculture. Kelly et al. (1977) reported reasonable survivorship of cultured prawns under some conditions, but low growth rates which led them to suggest that growth may be enhanced through polyculture techniques. Rensel and Prentice (1980) also noted the potential for the culture of *P. platyceros* as a companion crop alongside Pacific salmon open-water aquaculture. However, proper site selection would be necessary for the successful culture of *P. platyceros* as the authors observed mortalities in response to environmental conditions including fluctuations in temperature and phytoplankton blooms. Feeding experiments on a related species, *Pandalus borealis*, have shown that some fatty acids commonly found in salmon feed pellets can be assimilated into muscle tissue in the laboratory (Olsen et al., 2009).

The sea cucumber *P. californicus* is commercially important, harvested for its longitudinal muscles which are sold for human consumption. Like many Holothuroids, this species is a deposit-feeding organism. Deposit feeders obtain food by ingesting marine sediment and absorbing the organic fraction (Lopez and Levinton, 1987; Roberts et al., 2000). While the organic fraction of natural marine sediment can be very low, enrichment near aquaculture sites can increase its nutritive value. Sea cucumber feeding activity can inhibit growth of microalgae and reduce accumulation of detritus and organic carbon in marine sediments (Michio et al., 2003; Slater and Carton, 2009). Therefore, it stands to reason that sea cucumber co-culture can reduce impacts to the benthos underneath intensive or semi-intensive aquaculture sites. Ahlgren (1998) found that *P. californicus* was effective at clearing fouling organic debris from salmon net pens via ingestion of the material. Furthermore, *P. californicus* has a documented high survivorship, high absorption efficiency, a resulting positive growth rate, and increased muscle development in polyculture trials where aquaculture wastes were available as its primary food source (Ahlgren, 1998; Paltzat et al., 2008). Sea cucumbers have been tested for polyculture with various shellfish, including scallops, oysters (Zhou et al., 2006), mussels (Slater and Carton, 2007), and abalone (Maxwell et al., 2009). Feeding trials have confirmed that sea cucumbers will actively utilize aquaculture waste as a food source, meeting their nutritional demands and resulting in high specific growth rates.

The primary objective of this thesis was to determine whether candidate invertebrate species can remove organic material from sablefish (*Anoplopoma fimbria*) aquaculture waste and to estimate their trophic transfer efficiencies. Parameters were measured in the laboratory to test the hypothesis that candidate species will increase bioremediation by removing organic material from the fish waste by using it as a source of food. These were all measured relative to a control diet that the invertebrate is known to consume. In Chapter 2, the ingestion rate, absorption efficiency, and oxygen consumption rate were measured in *S. droebachiensis* fed a diet consisting of sablefish waste relative to those fed a natural kelp diet. The expected organic egestion was also estimated. In Chapter 3, the clearance rate, absorption efficiency, and egestion rate were measured in *C. nuttallii* and *M. edulis* fed the sablefish waste diet, compared to those fed monocultures of the microalga *Isochrysis* sp. (Tahitian strain; TISO). In Chapter 4, the ingestion rate, absorption efficiency, and egestion rate were measured in *P. platyceros* and *P. californicus* fed the fish waste diet or natural control diets (krill and sediment, respectively). The shape, size, and settling velocity of invertebrate faecal pellets were also determined for both dietary treatments for both species. The results are summarized and their implications are discussed within a broader IMTA context.

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2. Chapter 2 - Ingestion rate, absorption efficiency, oxygen consumption, and faecal production in green sea urchins (*Strongylocentrotus droebachiensis*) fed waste from sablefish (*Anoplopoma fimbria*) culture

Introduction

Various species of echinoids are harvested for their gonads which are sold for human consumption in many countries (Andrew et al., 2002). Interest in sea-urchin aquaculture has been developing over the last two decades in response to a global decline in wild stocks as harvesting pressure increases to meet demands (Andrew et al., 2002). The green sea urchin (*Strongylocentrotus droebachiensis*) forms the basis of lucrative fisheries in Canada, Norway, and the USA (Johnson et al., 2012; Miller and Nolan, 2008; Sivertsen et al., 2008) and, due to its excellent gonad quality, is a species of interest for possible aquaculture development (Hagen, 1998; Pearce et al., 2002a, 2002b, 2002c; Siikavuopio et al., 2007b, 2008). In British Columbia (BC), Canada, many shellfish growers are interested in culturing sea urchins (either *S. droebachiensis* or *S. franciscanus*) in conjunction with oysters, clams, or mussels as urchins naturally settle on aquaculture gear and may provide a means of biofouling mitigation through their grazing activity, as shown in Lodeiros and Garcia (2004) and Ross et al. (2004). *Strongylocentrotus droebachiensis* has been grown experimentally with Pacific oysters (*Crassostrea gigas*) with high urchin survivorship (Switzer et al., 2011). Additionally, the green sea urchin has garnered research interest as a candidate species for organic extraction in open-water Integrated Multi-Trophic Aquaculture (IMTA) both in BC and New Brunswick (NB), Canada (Barrington et al., 2009; Chopin, 2006).

In IMTA, species from multiple trophic levels are co-cultured. Both inorganic extractive (seaweeds) and organic extractive (invertebrates) species are strategically placed for extensive culture next to intensively cultured finfish. The lower trophic levels are meant to recapture waste materials lost in the form of finfish faeces and uneaten feed particulates (Chopin et al., 2007; Neori et al., 2004; Troell et al., 2009). The organic extractive component may include both suspension-feeding organisms (such as bivalves) and deposit-feeding or grazing organisms (such as sea cucumbers or sea urchins). In this design, suspension-feeding organisms would theoretically consume the finer suspended particulates from the finfish culture while deposit-feeding or grazing animals would feed upon the heavier, settleable solids. IMTA has a number of potential advantages over finfish monoculture including: economic gain through the harvest and sale of additional species, bioremediation of the pelagic and benthic environments through a reduction in inorganic and organic inputs, and increased social acceptability due to its reduced environmental impact. Removal of excess inorganic/organic material from the marine environment may reduce potentially harmful impacts such as eutrophication and the development of anoxic benthic conditions that have been associated with typical intensive and semi-intensive aquaculture (Buschmann et al., 2006; Holmer and Kristensen, 1992; Papageorgiou et al., 2010; Reid et al., 2009).

In Canada, *S. droebachiensis* is presently being considered for organic extraction of the heavier, settleable solid wastes produced by the culture of sablefish (*Anoplopoma fimbria*) in BC and Atlantic salmon (*Salmo salar*) in NB. The green sea urchin is well known as an ecologically-important herbivore, feeding primarily on macroalgae (Lawrence, 1975; Miller and Mann, 1973). Enhanced, out-of-season gonad production can be achieved in laboratory-grown *S. droebachiensis* fed a diet of kelp (Hagen, 1998).

Despite the green sea urchin's preference for kelp and other fleshy macroalgae, it is a generalist species known to feed on a variety of other items including coralline algae, benthic detritus, carrion, and various sessile and mobile invertebrate species (CAB International, 2006; Kirchhoff et al., 2008). No published research, however, has examined whether *S. droebachiensis* is able to consume and live on the settleable organic waste produced by intensive finfish aquaculture.

If *S. droebachiensis* is to be successfully co-cultured as an organic extractive species in IMTA with *A. fimbria*, its ability to feed on sablefish waste (*i.e.* faeces and uneaten feed) must be assessed. Furthermore, the ability of *S. droebachiensis* to remove excess organic material from sablefish waste must be confirmed. In this study, ingestion rate, absorption efficiency, and oxygen uptake were investigated in adult green sea urchins fed a diet of sablefish waste to test the hypothesis that sea urchins can remove organic material from it by feeding upon and digesting it (relative to a natural control diet of kelp). Properties of the faeces egested by *S. droebachiensis* (*i.e.* organic egestion and faecal organic content) were also quantified and it is anticipated that these properties will be used as parameters in models for the optimization of IMTA design, specifically the organic extractive component. Additionally, the shape, size, and settling velocity of urchin faecal pellets were also measured. These biophysical properties can be used as model inputs to predict the potential for dispersal or deposition in the area surrounding IMTA facilities on a site-specific basis.

Methods

Collection and Maintenance of Experimental Animals

On April 20, 2011, *S. droebachiensis* were collected by SCUBA divers from the subtidal zone at Five Fingers Islands, BC (49° 13’ 52.19” N, 123° 54’ 58.21” W). They were transported inside an insulated cooler to the Pacific Biological Station (Nanaimo, BC) and held outdoors in uncovered, flow-through tanks for a minimum of 1 month. The tanks were supplied continuously with ambient, sand-filtered and UV-treated seawater. Sea urchins were fed a diet of frozen bull kelp (*Nereocystis luetkeana*) *ad-libitum* 3 d per week. During this holding period, water temperature and salinity ranged between 9 and 12 °C and 26 and 30, respectively.

Preparation of Experimental Diets

The diets tested were sablefish waste and giant kelp (*Macrocystis pyrifera*), a preferred natural diet, which had been previously frozen at −20 °C. The fish waste material was collected from adult *A. fimbria* (mean±SE wet weight: 1.28±0.02 kg, *n*=100) held in a flow-through outdoor tank (volume: 10500 L) at the Pacific Biological Station. The tank was continuously supplied with sand-filtered and UV-treated seawater and the temperature ranged between 7 and 11 °C. Three days per week, the fish were fed 1500 g of Taplow Feed (Vancouver, BC, Canada) pellets specially formulated for sablefish. Following feeding, the tank was flushed to clear it of faeces and uneaten feed pellets. Fish waste for all experimental trials was collected 16 h after feeding. Waste material that had settled in the tank was collected by flushing it into a 100-L collection container that was placed below the waste water out-flow. Waste material was left to settle for 1 h, after which the surface water was siphoned away. Water content was standardized in the remaining 1-L slurry of fish waste by centrifuging 50-mL aliquots at 1500 x g for 10 min at 10 °C. This procedure was shown to significantly improve accuracy when measuring portions of the wet diet (Orr, unpublished data). Prior to feeding, the diet was ground with a mortar and pestle to ensure uniform mixing and particle size. Proximate and caloric content analysis was performed by SGS Canada Inc. (Vancouver, BC) on samples of both diets, that had been dried previously to a constant weight at 60 °C, using either in-house laboratory tests or Association of Official Analytical Chemists methods (03-01-SLM-FD-0001, 03-01-SLM-FD-0005, 03-01-SLM-FD-0009, 03-01-SLM-FD-0021, 03-01-SLM-FD-0022; AOAC 954.02).

Experimental Apparatus for Feeding Trials

One laboratory feeding trial was performed to measure ingestion rate and absorption efficiency and to estimate organic egestion. A second trial was conducted to measure shape, size, and settling velocity of egested sea-urchin faecal pellets. For each trial, sea urchins were randomly selected from the outdoor tanks and placed individually inside feeding chambers (L x W x H: 20 x 16.5 x 11 cm) contained within one of two seawater tables (L x W x H: 1.5 x 1.0 x 0.3 m) which were filled with 10 °C seawater to a depth of 10 cm. Sea urchins within each chamber were supplied with 1-µm cartridge-filtered and UV-treated seawater (salinity: 28) at a flow rate of approximately 290 ml min^{-1}. Three outlets (diameter: 1 cm) located on the opposite side of the chamber from the inflow, allowed the effluent to exit and were covered with 100-µm mesh to prevent loss of feed and faecal material. Feeding chambers were made of opaque plastic, to let in light while minimizing outside disturbance, and covered with a solid lid. Sea urchins were maintained at 10 °C and exposed to a simulated natural photoperiod using overhead fluorescent lights (25 lx; measurement taken at the bottom of a closed chamber). A temperature of 10 °C is within the optimal range for production of *S. droebachiensis* (Pearce et al., 2005; Siikavuopio et al., 2006). Each feeding chamber was randomly assigned to either the sablefish waste or kelp (*M. pyrifera*) treatments. Diets were added to six additional chambers (*n*=3 for the fish waste diet and *n*=3 for the kelp diet) containing no animal; these autogenic controls were used to measure changes in diet weight unrelated to sea-urchin feeding activity. Sea urchins were left to acclimate to the feeding chambers for 3 d prior to initiating both feeding trials. On the first day, individuals were fed the fish waste or control kelp diet (depending on treatment designation) *ad-libitum*. For the following 2 d of the acclimation period urchins were starved to standardize hunger levels and to ensure that subsequent faecal production was from the experimental meal type (Lawrence and Klinger, 2001). Chambers were thoroughly cleaned between feeding trials.

Ingestion Rate, Absorption Efficiency, and Organic Egestion

On September 7, 2011, 24 sea urchins, with a live weight of 54.4±2.5 g (mean±SE, *n*=24), were randomly selected from the communal holding tanks and placed in the experimental set up as described above (one urchin per chamber). After the 3-d acclimation period, each sea urchin was fed a known amount (4.20±0.08 g, mean±SE, *n*=24) of the wet sablefish waste (*n*=12) or thawed wet *M. pyrifera* (*n*=12) and allowed to feed for 24 h. Following this, all uneaten feed material was removed via gentle suction (being diligent to not include urchin faeces, which were easily distinguishable from the uneaten feed). After which, faeces were removed and discarded. Forty-eight hours after addition of food to the chambers (*i.e.* 24 h after the removal of all uneaten feed and any faecal pellets present at that time) all urchin faecal material was collected from each chamber via gentle suction and immediately stored at −80 °C. Uneaten feed was centrifuged at 1500 x g for 10 min at 10 °C. The supernatant was removed by careful pipetting, while the pellet was rinsed with distilled water to remove salts and spun down a second time. The supernatant was removed again and the pellet dried to a constant weight, at 60 °C for 24 h, and weighed. Dry-weight ingestion rate was calculated per individual per day by subtracting the dry weight of the uneaten feed from the total dry weight of feed added to the chamber, using values from autogenic controls to correct for changes in diet weight without urchins present. Total dry weight of added feed was estimated from conversion ratios obtained by regression analysis of wet to dry weights for both diets ($R^2=0.994$, $P<0.0001$ for fish waste; $R^2=0.978$, $P<0.0001$ for *M. pyrifera*). Wet-weight ingestion rates were determined by applying the same conversion ratios to dry-weight ingestion rates.

To determine organic content, thawed faeces were vacuum filtered onto pre-ashed, pre-weighed Whatman™ GF/C filters and rinsed with distilled water to remove salts. Filtered samples were dried to a constant weight at 60 °C for 24 h then weighed prior to ashing in a muffle furnace at 450 °C for 3–4 h (Conover, 1966; Reid, 2010). Ash-free dry weight (AFDW) was the difference in weight between dried and ashed samples, and is assumed to represent organic content. Diet samples were processed following the same procedure in triplicate. Absorption efficiency (AE) was calculated using the Conover ratio (1966):

$$AE = (F - E) / [(1 - E) \times F] \times 100$$

where F denotes the organic fraction measured in the diet and E denotes the organic fraction measured in the faeces. In this study, absorption efficiency is defined as the percentage of organic material absorbed by the sea urchin as material passes through the digestive system.

This method assumes that loss of inorganic material from the diet during digestion is negligible; therefore, the inorganic portion of the diet is used as an inert tracer to measure net absorption (Reid et al., 2010).

Because total faecal collection was not possible for this feeding trial, the expected organic egestion was determined by calculating the organic ingestion rate (dietary organic fraction x dry weight ingestion rate) and using AE to estimate the amount of organic material that would pass through the digestive system without being absorbed [organic ingestion rate x (1 – (AE/100))].

Shape, Size, and Settling Velocity of Sea Urchin Faecal Pellets

On May 8, 2011, 12 sea urchins with a live weight of 41.1±3.8 g (mean±SE, *n*=12) were randomly selected from the communal holding tanks, placed in the experimental set up as described above (one urchin per chamber) and allowed to acclimate for 3 d. Sea urchins were fed 4.52±0.20 g (mean±SE, *n*=12) of the wet fish waste (*n*=6) or thawed wet *M. pyrifera* (*n*=6) and allowed to feed for 24 h. Following this, all uneaten feed and faeces were removed via gentle suction. Faecal material was then carefully collected 24 h after feeding was halted, via gentle suction so as not to disrupt pellet integrity, transferred to 50-mL centrifuge tubes, and held on ice. Each tube was carefully inverted and its contents poured into a zooplankton counter consisting of three rows overlaid with a 1.00-

mm^2 grid. Three randomly chosen faecal pellets from each sea urchin were photographed with a digital Canon EOS Rebel xsi camera (Canon Canada Inc. Mississauga, Ontario, Canada) mounted on a Nikon dissecting microscope (Nikon Canada Inc., Mississauga, Ontario, Canada). The length and width of each pellet was measured using the digital imaging software ImageJ (version 1.45h). Shape was calculated as the ratio of width to length and size was described using equivalent circular diameter (length x width)$^{0.5}$ (Sauchyn and Scheibling, 2009b). Settling velocity was measured for each pellet, after it was photographed, by gently releasing it immediately below the surface in a cylindrical settling column (height: 45 cm, diameter: 10 cm) at a water temperature and salinity of 22±1 °C and 28, respectively. Two marks were placed 10 cm apart on the side of the settling column with the upper mark located 7 cm below the surface (Callier et al., 2006). The time for each pellet to descend between them was recorded. For shape, size and settling velocity, mean values for each urchin were generated based on the 3 representative fecal pellets and were used in all subsequent analyses.

Oxygen Consumption Rate

On August 24, 2011, 16 sea urchins with a live weight of 35.5±1.5 g (mean±SE, *n*=16) were randomly selected from the communal holding tank and divided equally into two seawater tables (L x W x H: 1.5 x 1.0 x 0.3 m). These were continuously supplied with 10 °C seawater, which was 1-µm cartridge filtered and UV treated, and held under constant illumination provided by overhead fluorescent lighting. The animals in each seawater table were fed either the fish waste or *M. pyrifera* diet *ad-libitum* throughout the entire 52-d experiment. Seawater tables were cleaned daily by siphoning away settled faecal material and uneaten food. Sea urchins were allowed to acclimate to these conditions for a minimum of 7 d prior to measuring oxygen consumption.

For each trial, an urchin was removed from one of the feeding tanks and oxygen consumption was measured (0-d, “fed”), following which the urchin was isolated from the general population and held without food for 2 d. Oxygen consumption was again measured for the same animal (2-d, “unfed”). To measure oxygen consumption, a sea urchin was placed in a sealed 1.9-L glass respirometry chamber filled 1-um cartridge filtered and UV-treated seawater and held in an incubator at 10 °C. The incubator was kept dark in order to minimize stress to the animal and thereby help stabilize its metabolic rate. After a 1-h acclimation period, the draw down in oxygen due to respiration was measured for an additional 2 h (during this time, oxygen levels remained above 5 mg L^{-1}). Oxygen concentration was measured every 15 s using a NeoFox oxygen sensing system (Ocean Optics, Dunedin, Florida, USA). Mass specific oxygen uptake was calculated by multiplying the slope of the oxygen depletion curve by the volume of water inside the chamber and dividing by the live sea urchin weight. Each urchin was only tested once (after 0 and 2 d of starvation).

Statistical Analysis

Mean wet-weight ingestion rate, absorption efficiency, organic egestion, faecal pellet shape, pellet size, and pellet settling velocity in the two different dietary treatments were initially compared using a one-way analysis of co-variance (ANCOVA), with sea-urchin live weight included as the covariate. There was no significant effect of live weight on these test variables when it was included as a co-variate and it was subsequently removed from the models. Further testing on these variables was done with one-way analysis of variance (ANOVA). The live weight co-variate was significant in the ANCOVA on dry-weight ingestion rate, so it was left in the model. Wet-weight ingestion rate, dry-weight ingestion rate, absorption efficiency, dietary organic content, organic egestion, faecal pellet shape, and pellet settling velocity met the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05) while faecal pellet size data were log-transformed to meet these assumptions. Faecal organic content data did not meet these assumptions after various transformation attempts, so this variable was compared between the two diet treatments using a Wilcoxon rank-sum test. During sample collection, some samples of uneaten feed and sea urchin faeces were lost, which resulted in a reduced sample size for some of the variables (wet and dry-weight ingestion rates n=12, fish waste and kelp n=9; absorption efficiency and faecal organic content n=10 fish waste, and n=8 kelp; organic egestion n=10 fishwaste, and n=6 kelp).

Oxygen consumption was compared using a partially-nested mixed model ANOVA (Gueorguieva and Krystal, 2004; Krueger and Tian, 2004; Sall et al., 2007; Wolfinger, 1997). The model tested for fixed main effects (diet, starvation period) and a random subject effect (sea urchin, nested within diet) on oxygen consumption rates. The nested factor was used as an error term in the calculation of the F statistic. The data were log transformed to meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (confirmed by examining the residuals plotted against predicted values).

Results

Proximate Analysis of the Experimental Diets

The results of the proximate analyses for the fish waste and kelp diets are presented in Table 2.1. There was higher protein, fat, and energy content in the fish waste diet compared to the kelp, while carbohydrate levels were similar.

Ingestion Rate, Absorption Efficiency, and Organic Egestion

When measured as wet-weight, ingestion rate was significantly greater in *S. droebachiensis* fed the *M. pyrifera* diet than in those fed the fish waste diet (Table 2.2, Fig. 2.1a). There was no significant difference between the two treatments in either the dry-weight ingestion rate or absorption efficiency (Table 2.2, Fig. 2.1a, b). In addition, there was no significant difference in organic content of the diets or in the organic egestion for individuals fed the two diets (Tables 2.2, 2.3).

Shape, Size, and Settling Velocity of Sea Urchin Faecal Pellets

Faecal pellets from both treatments were easily discernible based on their colour, shape and texture; samples from *S. droebachiensis* fed the fish waste diet were beige to brown, while those from individuals fed the *M. pyrifera* diet were bright green, noticeably more globular, and softer in texture (Fig. 2.2). The summary data for the lengths and widths measured are presented in Table 2.4. Faecal pellets egested by *S. droebachiensis* fed the fish waste diet had a significantly rounder shape, smaller size, and greater settling velocity than those egested by urchins fed a diet of *M. pyrifera* (Table 2.2; Fig. 2.3a-c).

Oxygen Consumption Rate

There was a significant effect of both diet and starvation period on oxygen consumption rates in *S. droebachiensis*, but no significant interaction between the two factors and no significant effect of nesting sea urchin in dietary treatment (Table 2.5). Oxygen consumption rate was significantly greater in fish-waste fed urchins than in kelp-fed individuals and significantly greater in fed than unfed sea urchins (Fig. 2.4).

Table 2.1. Proximate composition (g/100 g) and energy content (Calories/100 g or kJ/100 g) in samples of the experimental sablefish (*Anoplopoma fimbria*) waste and kelp (*Macrocystis pyrifera*) diets previously dried to a constant weight.

	Fish waste	***Macrocystis pyrifera***
Protein (N x 6.25)	10.9	8.4
Fat	5.0	0.8
Ash	21.5	28.4
Energy (Calories)	316	269
Energy (kJ)	1323	1126
Carbohydrate	56.9	57.1

Table 2.2. Results from one-way ANOVAs or one-way ANCOVAs testing for an effect of diet on wet and dry-weight ingestion rate, absorption efficiency, dietary organic content, organic egestion, and faecal pellet shape, equivalent circular diameter, and settling velocity in green sea urchins (*Strongylocentrotus droebachiensis*).

Source of variation	**df**	**MS**	***F***	***P***
Wet-weight ingestion rate				
Diet	1	16.0040	38.9361	<0.0001
Error	19	0.4110		
Dry-weight ingestion rate				
Diet	1	0.0049	0.4513	0.5108
Sea-urchin live weight	1	0.0530	4.8343	0.0420
Diet x Sea-urchin live weight	1	0.0400	3.6543	0.0729
Error	17	0.0110		
Absorption efficiency				
Diet	1	<0.0001	0.0010	0.9753
Error	16	0.0440		
Dietary organic content				
Diet	1	0.0008	5.2390	0.0840
Error	4	0.0001		
Organic egestion				
Diet	1	0.0104	0.5885	0.4557
Error	14	0.0176		
Faecal pellet shape				
Diet	1	0.2028	44.8012	<0.0001
Error	10	0.0453		
Faecal pellet circular diameter				
Diet	1	0.0913	40.6948	<0.0001
Error	10	0.0022		
Faecal pellet settling velocity				
Diet	1	935.6268	127.7734	<0.0001
Error	10	73.2255		

Table 2.3. Organic content (g ash-free dry weight g^{-1} dry weight) in diets and faecal samples and organic egestion (g ash-free dry weight $individual^{-1}$) for green sea urchins (*Strongylocentrotus droebachiensis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste or kelp (*Macrocystis pyrifera*).

	n	**Mean±SE**	**Minimum**	**Maximum**
Fish waste				
Dietary organic content	3	0.868±0.005	0.861	0.877
Faecal organic content	10	0.794±0.011	0.745	0.851
Organic egestion	10	0.238±0.041	0.110	0.463
M. pyrifera				
Dietary organic content	3	0.846±0.009	0.834	0.862
Faecal organic content	8	0.739±0.040	0.504	0.833
Organic egestion	6	0.291±0.057	0.115	0.485

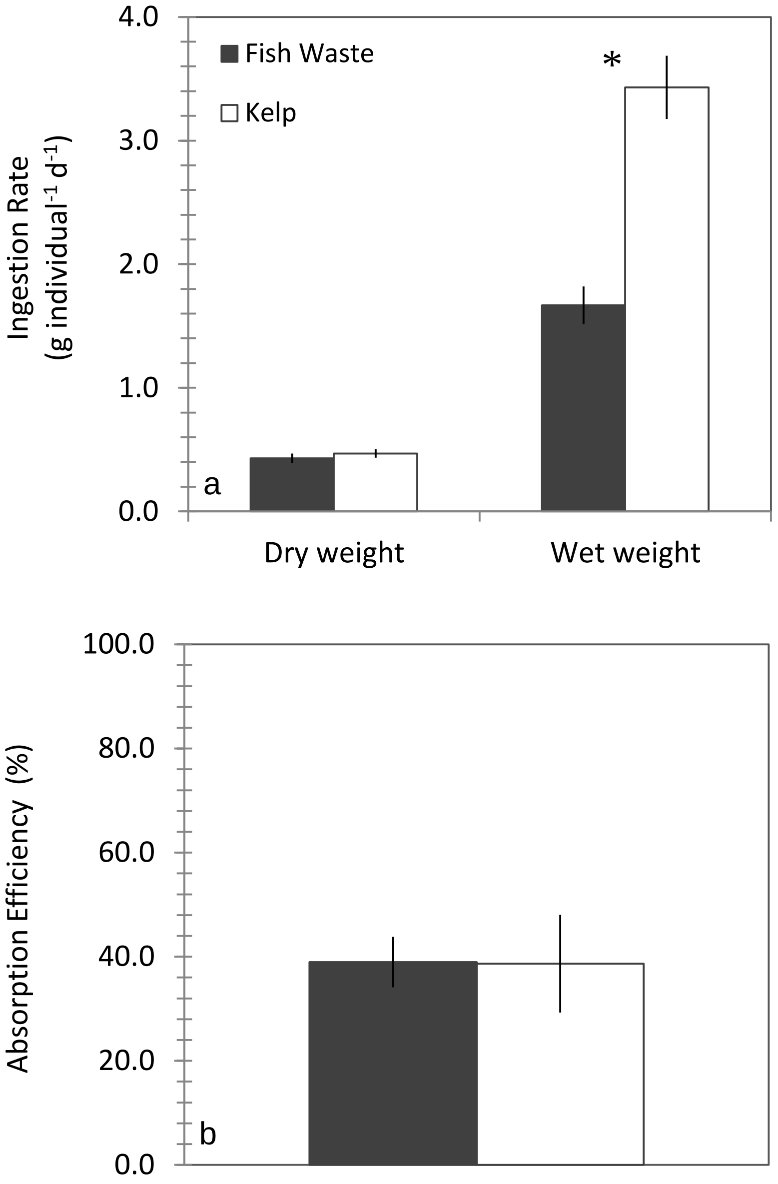

Figure 2.1. (a) Wet and dry-weight ingestion rates (g individual^{-1} d^{-1}) and (b) absorption efficiency (%) in green sea urchins (*Stronglyocentrotus droebachiensis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=12 for a and *n*=10 for b) or kelp (*Macrocystis pyrifera*) (*n*=9 for a and *n*=8 for b). Data are mean±SE. *=significant difference (P<0.05).

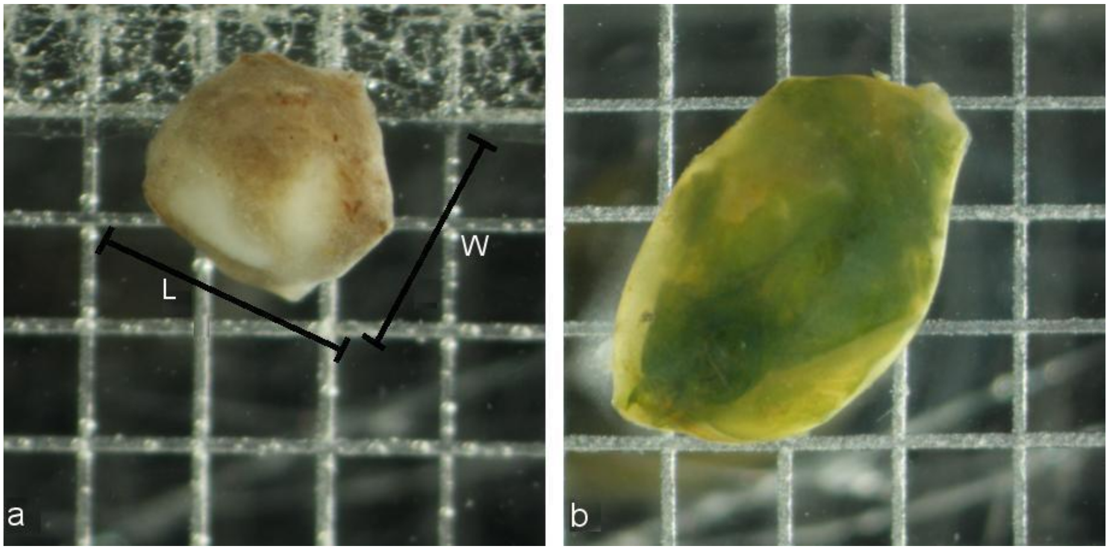

Figure 2.2. Individual faecal pellets from green sea urchins (*Strongylocentrotus droebachiensis*) fed (a) sablefish (*Anoplopoma fimbria*) waste and (b) kelp (*Macrocystis pyrifera*). Squares in the grids are each 1.0 mm^2. Black bars demonstrate how length (L) and width (W) were measured.

Table 2.4. Mean, minimum, and maximum values for length (mm) and width (mm) of faecal pellets egested by green sea urchins (*Strongylocentrotus droebachiensis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste or kelp (*Macrocystis pyrifera*).

	n	Mean±SE	Minimum	Maximum
Fish waste				
Length	6	1.68±0.05	1.46	1.84
Width	6	1.46±0.06	1.27	1.70
M. pyrifera				
Length	6	3.21±0.33	2.70	4.85
Width	6	1.78±0.08	1.56	2.00

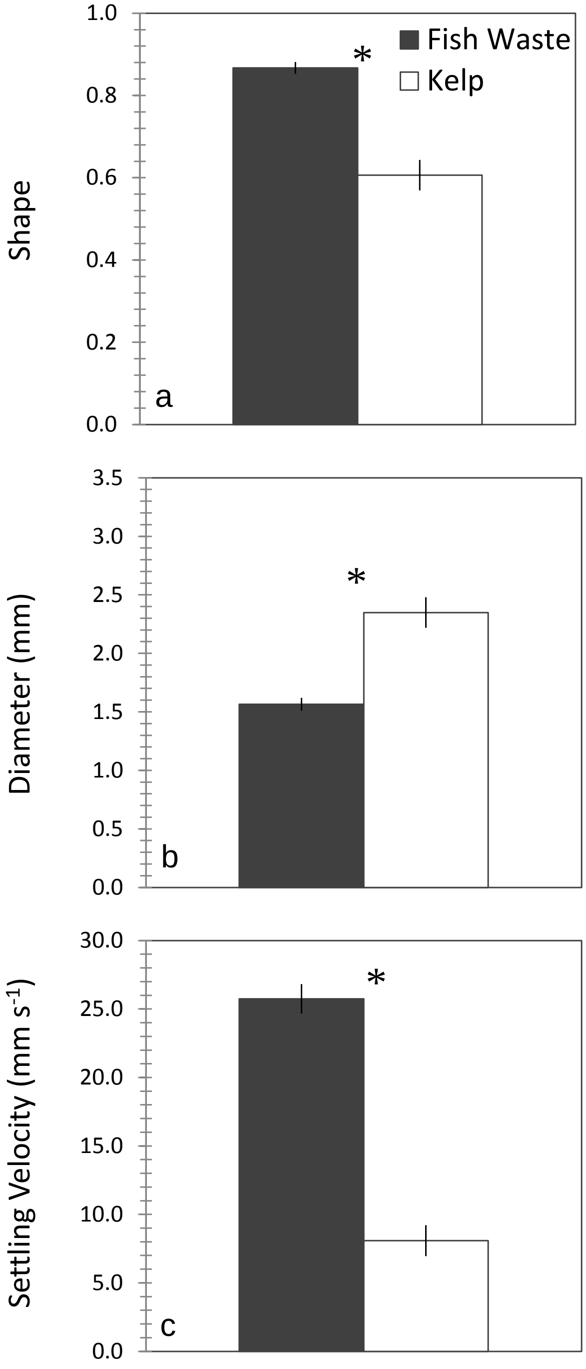

Figure 2.3. Biophysical properties of faecal pellets from green sea urchins (*Strongylocentrotus droebachiensis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=6) or kelp *Macrocystis pyrifera* (*n*=6): (a) pellet shape (width/length), (b) pellet equivalent circular diameter [(length x width)$^{0.5}$ in mm], and (c) pellet settling velocity (mm s^{-1}). Data are mean±SE. *=significant difference (*P*<0.05).

Table 2.5. Results from the partially-nested mixed model ANOVA testing for main effects of diet and starvation period and random effect of sea urchin nested within diet on oxygen consumption rate in green sea urchins (*Strongylocentrotus droebachiensis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste or kelp (*Macrocystis pyrifera*).

Source of variation	**df**	**MS**	***F***	***P***
Oxygen consumption				
Diet	1	0.2875	30.5055	<0.0001
Sea urchin [Diet]	14	0.0094	1.7783	0.1467
Starvation period	1	0.4690	88.4994	<0.0001
Diet x Starvation period	1	0.0020	0.3738	0.5507
Error	14	0.0053	9.8833	

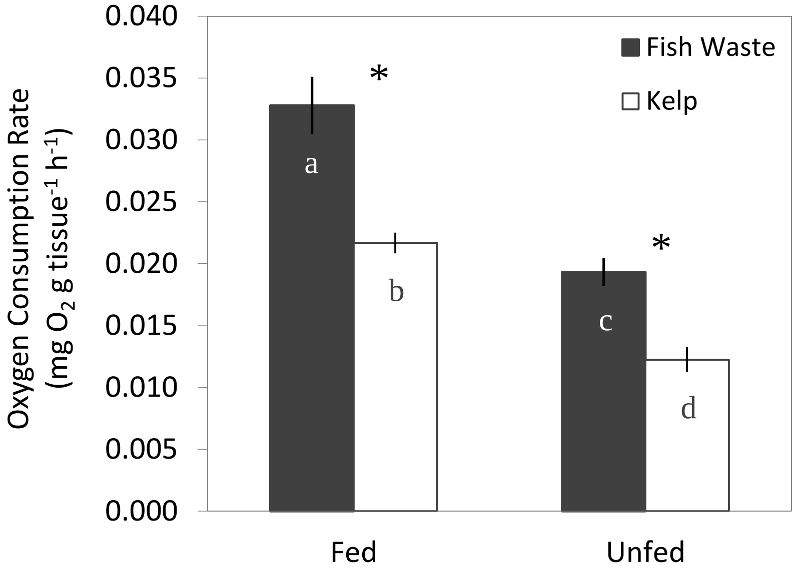

Figure 2.4. Oxygen consumption rate (mg O_2 g urchin-1 h-1) measured in: (a) green sea urchins (*Strongylocentrotus droebachiensis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=8) or kelp *Macrocystis pyrifera* (n=8) ad-libitum and green sea urchins 1 h after removal from the feeding tank (fed; *n*=8) and again after a 2-d starvation period (unfed; *n*=8). Data are mean±SE. *=significant difference (P<0.05).

Discussion

Results of this study show that *S. droebachiensis* is capable of ingesting and absorbing organics from sablefish waste at rates comparable to those fed a preferred natural food source (kelp), despite differences in the physical and biochemical nature of the two diets. During feeding trials, sea urchins were observed actively grazing on the diets and both faecal production and a rise in metabolic activity following periods of feeding on the two diets confirm that material was digested. Dry-weight ingestion rate and absorption efficiency with the sablefish waste diet were almost identical to those with kelp and, given that the former had higher levels of protein, fat, and energy, it is probable that urchins would be able to extract sufficient nutrition from the sablefish waste diet to have high survival and growth rates over time. Further studies are required to determine whether or not sea urchins can subsist on a diet of finfish waste long term. The potential impacts of this diet on the flavour and colour of urchin gonads (two qualities that are extremely important in the marketing of urchins) will also need to be determined, as fish-based protein has previously been shown to produce off flavours in echinoid roe (Pearce et al., 2002a; Siikavuopio et al., 2007a).

There is evidence that sea urchins can be grown long term with finfish as juvenile *Psammechinus lividus* have been experimentally cultured with Atlantic salmon (*S. salar*) for 12 months on a Scottish fish farm without the addition of extra feed (Cook and Kelly, 2007). In that study, juvenile *P. lividus* suspended at net pens had a higher survivorship (98.2 %) than those held 50 m and 2.5 km away (74.1 and 56.5 %, respectively), with the former developing gonads of suitable marketable colour after 12 months, while the latter did not. In addition, the fatty acids assimilated in the gonadal tissue of those urchins held at the net pens were the same as those commonly found in the salmon feed being used, indicating that urchins were ingesting fish feed from the farm. All *P. lividus* in the study exhibited positive specific growth rates [0.8±0.14 % d^{-1} at 0 m, 0.43±0.15 % d^{-1} at 50 m, and 0.6±0.15 % d^{-1} at 2.5 km (mean±SE)], but with a significant difference between 0 m and 50 m only. These results have implications for the use of sea urchins in IMTA, confirming that, if placed within the exiting waste plume, at least some echinoid species can successfully utilize organic effluents from finfish aquaculture to produce a commercially acceptable product in terms of gonad colouration. In a properly designed IMTA system, organic waste deposited by the finfish component will be the most abundant food source available to the urchins. The marketability of sea-urchin gonads, somatic growth, and survivorship will all depend in part on the quality of this diet (Daggett et al., 2010; Meidel and Scheibling, 1999; Pearce et al., 2002a, 2004) and gonad quality (especially flavour) testing will be required on urchins feeding long-term on finfish waste.

When ingestion rate was expressed on a wet-weight basis, *S. droebachiensis* ingested nearly twice as much of the kelp as the sablefish waste. However, when ingestion rate was expressed on a dry-weight basis, there was no significant difference across dietary treatments. This difference is likely a result of variable moisture content in the fresh diets, which was higher in the kelp than in the sablefish waste (Orr, unpublished data). Similar dry-weight ingestion rates have been reported in the literature for green sea urchins fed formulated diets. For example, Christiansen and Siikavuopio (2007) measured values between 0.3 and 0.4 g individual^{-1} d^{-1} for *S. droebachiensis* in laboratory trials while Siikavuopio et al. (2007a) reported dry-weight ingestion rates ranging between 0.2 and 0.3 g individual^{-1} d^{-1} in the winter and 0.5 and 0.6 g $\text{individual}^{-1}d^{-1}$ in the summer under laboratory conditions. Dry-weight values are most useful for measuring nutrient intake. Based upon the results for dry-weight ingestion rate, in conjunction with measurements for dietary organic content and absorption efficiency, it is clear that *S. droebachiensis* removed organic material from both diets equally. The results for wet-weight ingestion rates and measurements of the proximate composition revealed differences in the physical and biochemical nature of the two diets. These included higher moisture content in the freshly prepared kelp diet, and lower protein, fat, and energy levels in dried samples of the kelp, as compared to freshly prepared and dried samples of the sablefish waste, respectively. Differences in diet palatability or ease of handling may have affected wet-weight ingestion rate, as the textures of the two diets were qualitatively different. For example, sea urchins would have to capture and hold on to pieces of the kelp fronds (Lyons and Scheibling, 2007b), whereas the fish waste diet settled at the bottom of the chambers and the sea urchins would need only to crawl on top of it to capture the food. However, these factors were not measured specifically and determining an underlying mechanism was beyond the scope of the present study. Feeding activity in sea urchins can also be influenced by diet quality. For example, they can increase ingestion rate to maximize energy or nutrient intake (Lyons and Scheibling, 2007a). Various studies have reported greater wet-weight ingestion rates when sea urchins are fed diets of lower nutritional quality or caloric value (Azad et al., 2011; Daggett et al., 2010; Larson et al., 1980; Lyons and Scheibling, 2007a). Generally, the explanation given is that sea urchins adjust their feeding behaviour to compensate for lower nutrient intake. Ingestion rate will be an important determinant of the ability for *S. droebachiensis* to recapture energy lost as fish faeces and uneaten feed in IMTA, as it is a limiting factor for the physical removal of deposited waste.

Although diet had no significant effect on dry-weight ingestion rate in *S. droebachiensis*, sea-urchin live weight had a significant effect when it was included as a co-variate in the analysis, showing a slight positive trend between live weight in sea urchins and ingestion rate. Siikavuopio et al. (2008) also found a trend of increasing ingestion rate (g individual^{-1}d^{-1}) with increasing sea-urchin live weight. Siikavuopio et al. (2007a), however, found higher ingestion rates (g individual^{-1}d^{-1}) in smaller sea urchins when individuals were divided into small (30–40 mm), medium (40–50 mm), and large (50–60 mm) size classes based on test diameter. However, test diameter does not necessarily follow the same trend as live wet weight, as the latter variable can be greatly influenced by gonad development (Christiansen and Siikavuopio, 2997; Siikavuopio et al., 2007a). In the present study, efforts were made to keep variation in size to a minimum since the primary purpose was testing for the effect of diet. However, the fact that a significant positive effect of live weight was detected in spite of these efforts indicates that sea-urchin live weight has a positive relationship with ingestion rate for *S. droebachiensis*. Therefore, at an operational facility, the capacity for organic waste recapture by sea urchins within an IMTA system may increase per individual over the course of a production cycle as individuals grow. This may be offset by a reduction in urchin numbers over time due to mortality and/or density thinning. Further research is required to elucidate an optimal size at which the sea urchins should be harvested to maximize their bioremediative function.

There was no significant difference in the absorption efficiency of sea urchins fed the sablefish waste or kelp diets. Although numerous studies have addressed the relationship between diet, ingestion rate, somatic growth, and gonad maturation or production in green sea urchins (for examples see Christiansen and Siikavuopio, 2007;

Pearce et al., 2002a, 2002b; Siikavuopio et al., 2007b) information describing absorption efficiency in this species is relatively scarce. Nevertheless, the values measured in this study do fall within the wide range that has been reported in two published works. For example, Larson et al. (1980) found values ranging between 26 and 77 % in *S. droebachiensis* fed a variety of macroalgae and Thompson (1983) measured values between 60 and 90 % in those fed a mixture of blue mussel (*Mytilus edulis*) tissue and kelp *Alaria esculenta*. Organic absorption efficiency is not a true measure of absolute food value, but it is still a useful metric for assessing the relative food value in comparative feeding experiments, when coupled with measurements of ingestion rate (Larson et al., 1980). Results from the present study indicate that sea urchins can absorb organic material from sablefish waste since the absorption efficiency was positive. The fact that similar results were observed in sea urchins fed a preferred diet of kelp is promising, as it suggests that sablefish waste is an acceptable diet for the sea urchins.

Once a meal is ingested, it is absorbed across the gut wall and the nutrients obtained are allocated to growth, storage in the body, or metabolic activity (Cox and Secor, 2007). The process of feeding and digestion is associated with a general increase in metabolic rate that is referred to as specific dynamic action (SDA) and is manifested as elevated rates of oxygen consumption (Secor, 2009). Oxygen consumption rates were significantly greater in sea urchins fed the sablefish waste than the kelp and significantly higher in fed than unfed sea urchins (*i.e.* following a 2-d starvation period). However, there was no significant interaction between diet and feeding. Lilly (1979) found that oxygen consumption in *S. droebachiensis* increased during feeding, but that the magnitude of increase was greater in individuals fed the kelp *N. luetkeana* than those given the eelgrass *Zostera marina*. The authors attributed greater oxygen consumption rate to a greater food absorption rate in sea urchins fed *N. luetkeana* and suggest that in green sea urchins, the energetic cost of digesting a meal may be linked to their digestive capabilities. In addition, the amount of food consumed has been shown to affect SDA in echinoids (McGaw and Twitchit, 2012). It is unlikely that this is the case in the present study, since there was no difference in dry-weight ingestion rates or absorption efficiencies between the two diets. The lack of interaction between diet and starvation period suggests that the energetic costs of digesting either a fishwaste or kelp diet are similar and that the differences observed for fed animals are attributable to the differences in oxygen uptake between the two diets for starved animals. Although the exact reason for the differences in oxygen uptake between the two diets for starved animals are difficult to ascertain, one possible explanation may be difference in locomotor activity. Green sea urchins continued to produce fecal pellets well past 2-d post feeding. In urchins fed the kelp diet, fecal pellets floated away, whereas those fed the fishwaste diet were observed using their tube feet to continuously remove faecal pellets that settled on their aboral surface. It is likely that in a natural setting where fecal pellets do not settle on the urchin, there would be minimal difference in oxygen uptake between the two diets. If ingestion rate, absorption efficiency, and the metabolic rate associated with feeding on each of the two diets are similar, differences in the overall energy budget will be attributable to differences in the energetic content of each diet. In this case, the fishwaste diet actually had a slightly higher energetic content than the kelp diet. Although many factors contribute to the growth of an organism, the current results suggest that from an energetic perspective, green sea urchins are likely able to grow and even thrive on a diet composed primarily of sablefish waste. However, longer feeding trials are required to examine specific nutritional requirements and the effect of long-term consumption of finfish waste on urchin survival, growth, and gonad quality.

Faecal pellets are an important aspect of marine food webs, since they are readily transported by currents and contain undigested organic material (Wotton and Malmqvist, 2001). By feeding on sablefish faeces and uneaten feed, *S. droebachiensis* has the potential to impact the benthic environment by repackaging waste material in compact aggregates (Sauchyn and Scheibling, 2009a, 2009b). Passage through the urchin digestive system may reduce the tendency for sablefish waste to resuspend in the water column and travel with the currents. While no quantitative analysis was performed on the physical nature of the sablefish waste, no discrete pellets were observed during its collection. Instead, the material existed as a slurry prior to ingestion by the sea urchins. Faecal pellets egested by *S. droebachiensis* fed sablefish waste were rounder, smaller, and had a significantly greater settling velocity than those fed kelp. This study provides the first measurements of settling velocity for faecal pellets from sea urchins fed a diet of sablefish waste. However, Sauchyn and Scheibling (2009b) measured comparable settling velocities (between 5 and 25 mm s^{-1}) when they tested various macroalgal diets with *S. droebachiensis*. Shape and size can both affect settling velocity; however, faster faecal-pellet settling rates with the sablefish diet were likely due to greater density (Reid et al., 2009; Sauchyn and Scheibling, 2009a). Dispersal of faecal pellets from an IMTA setting will depend upon site-specific characteristics (*e.g.* current speed, flow patterns, temperature) as well as inherent biophysical properties of the faeces. Predictive models are commonly employed to estimate the organic load and dispersal from open-water finfish aquaculture (Reid et al., 2009). The results of the current study emphasize the importance of including the effects of organic extractive species when extending these models to IMTA.

This study confirms that *S. droebachiensis* will actively ingest and absorb organic material from the solid waste of intensive sablefish culture. This is an important first step in the assessment of the sea urchin as a candidate for co-culture in IMTA. The results from feeding trials suggest that the green sea urchin has the potential to remove up to 40 % of the organic material from the wastes released from sablefish net pens, and this percentage may increase further if sea urchins were to re-ingest the material by consuming their own faeces. This may reduce the development of anoxic conditions in the sediment directly through removal of excess nutrients. Sea urchins could also impact the benthic environment indirectly if they were to ingest benthic microbes responsible for generating anaerobic conditions. In addition, foraging activity of benthic-reared sea urchins would increase bioturbation, which could also act to prevent the build-up of anoxic sediments. The degree of bioremediation by *S. droebachiensis* (and other benthic deposit feeders) under farm sites, however, will depend greatly on the fraction of their diet that is made up of farm-derived wastes. The greater this fraction, the greater the level of bioremediation. If non-farm materials (*e.g.* drift algae, naturally-settled seston, dislodged biofouling from the culture gear) make up a substantial proportion of the diet, then urchins may only be exasperating the problem of increased organic loading. However, if placed directly underneath the net pens on the seafloor it is likely that the bulk of the urchin's diet will be farm-produced particulates. Additional investigations should be carried out in the field with sea urchins on commercial fish farms to evaluate the fraction of their diet that is made up of farm-derived material and long-term growth and gonad quality when sablefish waste is the primary diet.

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3. Chapter 3 - Clearance rate, absorption efficiency, and faecal production in basket cockles (*Clinocardium nuttallii*) and blue mussels (*Mytilus edulis*) fed waste from sablefish (*Anoplopoma fimbria*) culture

Introduction

The basket cockle (*Clinocardium nuttallii*) and the blue mussel (*Mytilus edulis*) are two bivalve species being considered for use in Integrated Multi-Trophic Aquaculture (IMTA) in British Columbia (BC), Canada. The former is not currently cultured in the province – although there is growing interest in its potential for aquaculture – while production of the latter reached 1.1 metric tons, worth CAD $4.8 million (combined with scallops) in BC in 2010 (BC Ministry of Agriculture). Native populations of *C. nuttallii* are commonly found along the Pacific coast of North America, ranging as far north as the Bering Sea, Alaska and as far south as San Diego, California (Quayle, 1960). Basket cockles occupy the low intertidal or shallow subtidal zones of fine to medium grain-sized sandy habitats (Quayle, 1963). Although not targeted by commercial fisheries, *C. nuttallii* is a traditional food source for First Nations of the region and exhibits a relatively fast growth rate given the colder waters in which it occurs (Liu et al., 2010, 2011). Recent research on the feasibility and development of culture techniques has demonstrated that larvae and juveniles can be reliably produced under hatchery conditions (Liu et al., 2008a, b, 2009, 2010, 2011).

In contrast to *C. nuttallii*, the culture of marine mussels, including *M. edulis*, is well established and they are produced in many countries (FAO 2012; Wallace, 1980). Members of the *Mytilus* species complex (*M. galloprovincialis*, *M. trossulus*, and *M.*

edulis) are found globally in temperate intertidal and subtidal zones (Shields et al., 2008). These species tend to be extremely successful colonizers and efficient filter feeders (Seed et al., 2000). The suitability of *C. nuttallii* and *M. edulis* for culture make them excellent candidate species for commercial-scale IMTA.

IMTA is a system of aquaculture designed to mitigate the inorganic and organic effluents from fed monoculture. This is accomplished by strategic co-culture of seaweeds (which utilize the dissolved inorganics) and various invertebrate species (which take up the organic particulates) with finfish. By capturing wastes (*i.e.* dissolved nutrients, uneaten feed pellet fines, and fish faeces) lost from intensive finfish aquaculture, inorganic and organic extractive species may be able to reduce the potentially harmful impacts associated with nutrification (Buschmann et al., 2006; Holmer and Kristensen, 1992; Mazzola and Sara, 2001; Modica et al., 2006). Elevated levels of suspended particulate organic material (POM) have been detected downstream from intensive open-water finfish aquaculture operations, particularly during periods of feeding (Lander et al., 2004; Modica et al., 2006; Sara et al., 2009; Stirling and Okumus, 1995). Elevated POM levels may result in changes to the biochemistry of the water column, leading to increases in the occurrence of algal blooms (Modica et al., 2006; Sara et al., 2009). When present at high densities, suspension-feeding bivalves can facilitate the removal and vertical transfer of small suspended particles by repackaging them into larger aggregates in the form of faeces and pseudofaeces (cumulatively termed biodeposits) (Giles et al., 2006). In addition, if bivalves efficiently absorb the organic material from particles in the water column, they may reduce organic loading to benthic sediments.

The extent of nutrient transfer due to suspension feeding depends upon the quality and quantity of biodeposits. These are, in turn, related to the amount of ingested material as well as the fraction of material absorbed across the gut wall (Giles and Pilditch, 2004; Reid et al., 2010). For example, higher rates of ingestion will lead to increased clearance of particles from the water column. However, if bivalves only absorb a small portion of the organics ingested and a certain percentage of their diet is derived from non-farm sources, greater levels of biodeposits will be released into the system and more organic material will be deposited on the seafloor. Vertical nutrient transfer also depends upon the dispersal characteristics of the biodeposits. These include inherent biophysical properties such as particle shape, size, and settling velocity, as well as site-specific factors related to local hydrodynamic conditions (Callier et al., 2006; Giles and Pilditch, 2004). Accordingly, these parameters must be taken into account for any candidate species when assessing environmental impact and efficiency of design for commercial-scale IMTA.

Current plans for IMTA in BC include the co-culture of suspension-feeding organisms in proximity to sablefish (*Anoplopoma fimbria*) as part of the organic-extractive component. As organic extractive organisms, *C. nuttallii* and *M. edulis* would ingest finfish wastes, such as fish faeces and uneaten feed pellet fines, suspended in the water column (as opposed to heavier, settable solids that would fall directly under or close to the farm) and direct the absorbed energy into growth. While promising, evidence supporting this idea remains largely indirect. Several studies have found that *Mytilus* spp. grown in conjunction with finfish for a year or more reached significantly larger sizes than conspecifics grown away from the influence of the farms (Lander et al., 2004; Sara et al., 2009; Stirling and Okumus, 1995; Wallace, 1980). This augmented growth is usually attributed to increased nutritional benefits to the bivalves from feeding on the aquaculture waste in addition to natural seston. However, other studies have found no significant effect of finfish on the growth of bivalves (Cheshuk et al., 2003; Navarrete-Mier et al., 2010;). Although clearance rate and absorption efficiency have been directly measured in *M. edulis* fed Atlantic salmon waste (MacDonald et al., 2011; Reid et al., 2010) they have not been examined in *C. nuttallii* and, to date, no studies have tested bivalves fed a diet of sablefish waste. If *C. nuttallii* and *M. edulis* are to provide a net benefit in IMTA with sablefish, they must be efficient at capturing and absorbing the organic matter from *A. fimbria* waste.

As suspension feeders, *C. nuttallii* and *M. edulis* must remove POM from the water column in order to survive. It is therefore hypothesized that they will ingest and absorb wastes produced by sablefish aquaculture, thus reducing the transfer of organic wastes to the benthos. To test this hypothesis, clearance rate (CR) and absorption efficiency (AE) were tested for individuals of both species fed sablefish waste or a control phytoplankton diet in separate laboratory feeding trials. Also, the quantity (egestion rate) and quality (organic content) of faeces egested by *C. nuttallii* and *M. edulis* fed the two diets were measured. These parameters will be important to estimate potential organic loads from the bivalves in an IMTA setting. Finally, the shape, size, and settling velocity of faecal pellets produced by *C. nuttallii* and *M. edulis* were quantified. These parameters are important for predicting dispersal and deposition of faecal pellets. Together, these measurements provide reference values that can be used for comparisons *in situ*, where environmental and hydrodynamic conditions will vary greatly across location and time.

Methods

Collection and Maintenance of Experimental Animals

Clinocardium nuttallii were collected from subtidal grow-out trays at the Pacific Biological Station (Nanaimo, BC, 49° 12' 26.4594",-123° 57' 35.0274") in March 2011. These were immediately transferred indoors to seawater tables (L x W x H: 1.5 x 1.0 x 0.3 m) supplied continuously with ambient, sand-filtered, UV-treated seawater and held for a minimum of 1 month prior to experimentation. *Mytilus edulis* were collected from a shellfish lease site at King Islets, BC (50° 9' 59.8242",-125° 8' 31.3872") in September 2011 and were transported in an insulated cooler to the Pacific Biological Station within 24 h. Mussels were held in outdoor tanks supplied continuously with ambient, sand-filtered, UV-treated seawater for a minimum of 1 month prior to experimentation. During the holding period, all bivalves were fed a monoculture diet of the microalgae *Isochrysis* sp. (Tahitian strain: TISO) or *Chaetoceros muelleri* (CM) (depending on availability) *ad-libitum* 3 d per week. Water temperature and salinity during the holding periods ranged between 9 and 12 °C and 26 and 30, respectively.

Preparation of Experimental Diets

Solid fish waste materials were used as an experimental diet and collected from adult sablefish. The fish waste was collected and prepared as described in Chapter 1. A monoculture of TISO (a microalgal species commonly used in shellfish culture) was used for the control diet. The algae were harvested from 250-L columns, under constant illumination from full-spectrum fluorescent light bulbs, at the early-stationary phase.

Experimental Set Up for Feeding Trials

Each species of bivalve was tested in separate laboratory feeding trials. Bivalve shells were scrubbed manually to remove any biofouling organisms and placed individually into separate feeding chambers (L x W x H: 20 x 16.5 x 11 cm) contained within one of four seawater tables (L x W x H: 1.0 x 1.0 x 0.3 m). Each chamber was supplied with 1-µm cartridge-filtered and UV-treated seawater (temperature: 10°C, salinity: 28) at a flow rate of approximately 290 ml min^{-1}. An air stone was added to each chamber to increase water circulation. On the opposite side of the chamber from the inflow there were three outlets (diameter: 1 cm) covered with 100-µm mesh to allow for effluents to exit while preventing loss of faecal material. Each seawater table served as a common water bath in which the chambers were submerged (depth: 10 cm) and these were maintained at a temperature of 10 °C and exposed to a simulated natural photoperiod (25 lx; measurement taken at the bottom of a closed chamber) using overhead fluorescent lights.

Feeding Trials and Sample Collection

On July 17, 2011, *C. nuttallii* with a shell length of 35.55±0.29 mm (mean±SE, *n*=48) were randomly selected for the first feeding trial. On September 25, 2011, *M. edulis* with a shell length of 62.26±0.52 mm (mean±SE, *n*=48) were randomly selected for the second feeding trial. Feeding chambers were randomly assigned to one of the two dietary treatments: sablefish waste (*n*=24 for *C. nuttallii* and *n*=23 for *M. edulis*) or TISO (*n*=24 for *C. nuttallii* and *n*=25 for *M. edulis*). The bivalves were allowed to acclimate for 3 d prior to each feeding trial. On the first day, the shellfish were fed the fish waste or TISO (depending on treatment designation) *ad-libitum* to acclimate individuals to both diets. For the following 2 d of the acclimation period, bivalves were starved to standardize hunger levels and to ensure that any faeces produced were the result of the experimental feeding. At the end of the 3-d acclimation period, all faeces produced were removed prior to starting feeding trials.

At the beginning of each feeding trial, seawater flows to the experimental chambers were turned off and the bivalves were left in static conditions with air bubbling for 2 h. At the beginning of the 2-h feeding period, sablefish waste or a monoculture of TISO was added to each chamber to obtain chamber concentrations of 236.5±35.3 mg L^{-1} (mean±SE, *n*=12) in the fish waste treatment and 202.3±36.3 (mean±SE, *n*=12) in the TISO treatment for *C. nuttallii* and 27.7±6.6 mg L^{-1} (mean±SE, *n*=10) in the fish waste treatment and 37.2±4.3 mg L^{-1} (mean±SE, *n*=7) in the TISO treatment for *M. edulis* (see clearance rate in Methods section for explanation of sample size). These concentrations were determined by back-calculation from representative water samples that were collected immediately after adding the feed to the chambers. The water samples were collected from chambers later used to determine clearance rate, taken from the middle of each chamber just beneath the surface, held on ice, and processed within 6 h of collection. To calculate the concentration of the diet (mg L^{-1}), dry weight was determined as described below for the bivalve faecal samples. For the fish waste diet, some material tended to settle out within the first few seconds after addition to the chamber, so the water sample was assumed to be representative of the diet that was available suspended in the water column. The algal cells were more buoyant, however, and no material was observed settled at the bottom of the chambers in the TISO treatment. At the conclusion of the 2-h feeding period, any particulate material settled on the bottom was siphoned away and water flow was restored to the chambers. No faeces or pseudofaeces were observed in the feeding chambers at this time. Air stones, which had been present in the chambers the entire acclimation and feeding time, to ensure that small feed particles remained suspended, were now removed to allow all bivalve biodeposits to settle undisturbed on the bottom.

After another 24 h had passed (that is, 24 h after diets were removed and water flow restored, but 26 h after the feeding trial began and feed was first added), all bivalve faecal material was collected from each chamber via gentle suction. No pseudofaeces production was observed at this point for either species in any chamber. Faecal samples collected from 24 chambers containing *C. nuttallii* (*n*=12 fish waste and *n*=8 TISO; see Statistical Analysis section for explanation of sample size) and 24 chambers containing *M. edulis* (*n*=11 fish waste and *n*=13 TISO) were used to determine organic content, absorption efficiency, and total faecal egestion. Faecal samples collected from the remaining 24 chambers containing *C. nuttallii* (*n*=12 fish waste and *n*=12 TISO) and 13 chambers containing *M. edulis* (*n*=9 fish waste and *n*=4 TISO) were used to measure shape, size, and settling velocity of individual faecal pellets. Faeces from *M. edulis* lacked cohesiveness, especially from those fed TISO, and pellets tended to disintegrate upon collection, despite extremely careful collection techniques. Therefore, sample size was reduced for these parameters of *M. edulis* faecal pellets.

Clearance Rate, Absorption Efficiency, and Egestion Rate

Bivalve faeces were stored at −80 °C immediately after collection and were thawed prior to further analysis. Water samples used to determine diet concentration were processed on the same day as the feeding trials, so samples were never frozen. To determine the total dry weight and organic content, samples were vacuum-filtered onto pre-ashed, pre-weighed 42.5-mm Whatman^{TM} GF/C filters and rinsed with 0.5 M ammonium formate to remove salts. Filtered samples were dried to a constant weight at 60 °C for 24 h then weighed prior to ashing at 450 °C for 3–4 h in a muffle furnace (Conover, 1966; Petersen et al., 2004; Reid et al., 2010). The ratio of ash-free dry weight (AFDW) to total sample dry weight was used to determine organic content. Samples of fresh fish waste (*n*=6) and TISO (*n*=3) collected at the time of feed preparation, to determine diet organic content, and water samples collected at the beginning of the 2 h feeding period, to determine feed concentration, were treated using the same process. During processing of the water samples for diet concentration for *M. edulis*, some filters were damaged in transit, and therefore sample size was reduced for the determination of both diet concentration and clearance rate (*n*=10 fish waste and *n*=7 TIS0).

Clearance rate (CR) over the 2-h feeding period was calculated using the biodeposition method [as described in: Bayne, (2004); Hawkins et al., (1996); Iglesias et al., (1998); Petersen et al., (2004)]:

$$CR = (IRR + IER) / PIM$$

where IRR is the inorganic concentration measured in the pseudofaeces (none produced in the present study), IER is the inorganic concentration measured in the faeces, and PIM is the concentration of inorganic matter in the diet. This method assumes that the inorganic content is similar in the diet, in particles retained by the gill, in the pseudofaeces (if present), and in the faeces after digestion; therefore, it uses inorganic content as an inert tracer (Iglesias et al., 1998). Previous studies have provided examples where the biodeposition method gave comparable results to other commonly used methods for determining clearance rate (Bayne et al., 2004; Iglesias et al., 1998).

Absorption efficiency (AE) was calculated using the Conover ratio (1966):

$$AE = (F - E) / [(1 - E) \times F] \times 100$$

where F is the organic fraction measured in the diet and E is the organic fraction measured in the faeces. The Conover method assumes that absorption of inorganic material across the gut wall is negligible and therefore uses the inorganic portion of the diet as an inert tracer to measure net absorption (Reid et al., 2010). Dry weight of the faecal material collected from the bivalves was used to determine egestion rate (g ind^{-1} d^{-1}). That is, the dry weight of the faecal samples represented all the material egested by the bivalves in the 24-h period after removal of the feed.

Shape, Size, and Settling Velocity of Bivalve Faecal Pellets

Faecal material was carefully collected 24 h after the bivalves were fed (as described above) via gentle suction so as not to disrupt pellet integrity, transferred to 50-mL centrifuge tubes, and held on ice. No pseudofaeces were observed in any of the chambers for either feeding trial. Each tube was carefully inverted and its contents poured into a zooplankton counter consisting of three rows overlaid with a 1.00-mm^2 grid. Three randomly chosen faecal pellets from each individual bivalve were photographed with a digital Cannon EOS Rebel xsi camera (Canon Canada Inc. Mississauga, Ontario, Canada) mounted on a Nikon dissecting microscope (Nikon Canada Inc., Mississauga, Ontario, Canada). The length and width of each pellet was measured using the digital imaging software ImageJ (version 1.45h). Shape was calculated as the ratio of width to length and size was determined as planar area of the pellet image (length x width). Settling velocity was measured for each pellet, after it was photographed, by gently releasing it immediately below the surface in a cylindrical settling column (height: 45 cm, diameter: 10 cm) at a water temperature and salinity of 22±1 °C and 28, respectively. Two marks were placed 10 cm apart on the side of the settling column with the upper mark located 7 cm below the surface (Callier et al., 2006). The time for each pellet to descend between them was recorded. Once shape, size, and settling velocity were determined for each pellet, a mean was calculated from the three values for each individual bivalve and this value was used in subsequent statistical analyses.

Statistical Analysis

Mean absorption efficiency and faecal pellet shape, size, and settling velocity for *C. nuttallii* in the two different dietary treatments were initially compared using a one-way analysis of co-variance (ANCOVA) with shell length included as the covariate. There was no significant effect of shell length on any of these test variables when it was included as a co-variate, so it was removed from the models and results were analyzed using one-way ANOVAs. Settling velocity data were log-transformed to meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05). The shell length co-variate was significant in the ANCOVA on egestion rate of *C. nuttallii*, so it was left in the model. Faecal organic content data did not meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05) after various transformation attempts, so this variable was compared between the two dietary treatments using a Wilcoxon rank-sum test. During analysis of faecal organic content for *C. nuttallii*, it became clear that four data points were below the limit of resolution as determined from blank controls (unpublished data) after ashing. Therefore, sample size was reduced ($n=8$) for faecal organic content, clearance rate, and absorption efficiency in the TISO treatment for *C. nuttallii*. Calculations were attempted to determine clearance rate for *C. nuttallii*, but these were unsuccessful due to an analytical error that occurred while processing water samples for determination of diet concentration, which resulted in the overestimation of diet concentrations.

Mean clearance rate, absorption efficiency, egestion rate, faecal pellet shape, and pellet size of *M. edulis* in the two different dietary treatments were initially compared using a one-way analysis of co-variance (ANCOVA) with shell length included as the covariate. There was no significant effect of body size on any of these test variables when it was included as a co-variate, so it was removed from the models and results were analyzed with one-way ANOVAs. The shell length co-variable was significant in the ANCOVA on settling velocity of faeces from *M. edulis*, so it was left in the model. Clearance rate and settling velocity data was log-transformed to meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05). Faecal organic content data did not meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05) after various transformation attempts, so this variable was compared between the two dietary treatments using a Wilcoxon rank-sum test. Organic content of the diets was compared using a one-way ANOVA.

Results

Clearance Rate, Absorption Efficiency, and Egestion Rate

There was no statistically significant difference in clearance rate in *M. edulis* fed TISO or sablefish waste (Table 3.1, Fig. 3.2a). The absorption efficiency for *C. nuttallii* (Fig. 3.1a) and *M. edulis* (Fig. 3.2b) fed TISO was significantly greater than those fed sablefish waste (Table 3.1). While no statistical comparisons were made between species, as they were run as separate trials, it is noteworthy that the absorption efficiencies were nearly identical for *C. nuttallii* and *M. edulis* for both diets. Conversely, egestion rate was significantly greater in *C. nuttallii* fed sablefish waste than in those given TISO, yet there was no significant difference in the egestion rates of *M. edulis* (Table 3.1, Fig. 3.1b, 3.2c). The dietary organic content was significantly higher in TISO than the sablefish waste (Table 3.1, 3.2) and significantly higher in the faeces from both *C. nuttallii* (Z=2.276, P=0.0228) and *M. edulis* (Z=−3.187, P=0.0014) fed TISO than in those fed sablefish waste.

Shape, Size, and Settling Velocity of Bivalve Faecal Pellets

Faecal pellets from both dietary treatments were easily discernible based on colour; samples from the bivalves fed TISO were bright green, while those from the fish waste treatment were beige to brown (Fig. 3.3). Faecal pellets were generally cylindrical in appearance, with those from *M. edulis* exhibiting a longitudinal groove along one side

(Fig. 3.3). Length and width data for faecal pellets from both treatments and feeding trials are summarized in Table 3.3.

Again, no direct statistical comparisons were made between data from both species. Nevertheless, similar trends were observed in the shape, size, and settling velocity of faecal pellets from both dietary treatments in both species (Fig. 3.4a-c, 3.5a-c). There was no significant difference in the shape or size of faecal pellets for *C. nuttallii* or *M. edulis* (Table 3.4, Fig. 3.4a-b, 3.5a-b). Settling velocity was significantly greater in faecal pellets from bivalves fed the fish waste diet in both *C. nuttallii* and *M. edulis* (Table 3.4, Fig. 3.4c, 3.5c).

Table 3.1. Results from one-way ANOVAs or one-way ANCOVAs testing for an effect of diet on clearance rate, absorption efficiency, egestion rate, and dietary organic content for both basket cockles (*Clinocardium nuttallii*) and blue mussels (*Mytilus edulis*).

	Source of variation	**df**	**MS**	***F***	***P***
C. nuttallii	**Absorption efficiency**				
	Diet	1	0.5763	23.2216	0.0001
	Error	18	0.0248		
	Egestion rate				
	Diet	1	0.0001	110.1420	<0.0001
	Shell length	1	<0.0001	13.2702	0.0016
	Diet x Shell length	1	<0.0001	2.3093	0.1443
	Error	20	1.050×10^{-6}		
M. edulis	**Clearance rate**				
	Diet	1	0.2722	3.1796	0.0948
	Error	15	0.0856		
	Absorption efficiency				
	Diet	1	0.9778	53.2858	<0.0001
	Error	22	0.0183		
	Egestion rate				
	Diet	1	<0.0001	0.5249	0.4764
	Error	22	<0.0001		
Diets	**Dietary organic content**				
	Diet	1	0.0127	112.5059	0.0004
	Error	4	0.0001		

Table 3.2. Organic content (OC) (g ash-free dry weight g^{-1} dry weight) in diets and faecal samples from basket cockles (*Clinocardium nuttallii*) and blue mussels (*Mytilus edulis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste or *Isochrysis* sp. (TISO).

	n	**Mean±SE**	**Minimum**	**Maximum**
Diet OC				
Fish waste	6	0.86±0.01	0.86	0.88
TISO	3	0.96±0.01	0.95	0.97
Faecal OC				
C. nuttallii				
Fish waste	12	0.81±0.01	0.73	0.86
TISO	8	0.84±0.05	0.47	0.93
M. edulis				
Fish waste	11	0.80±0.02	0.62	0.86
TISO	13	0.87±0.01	0.72	0.91

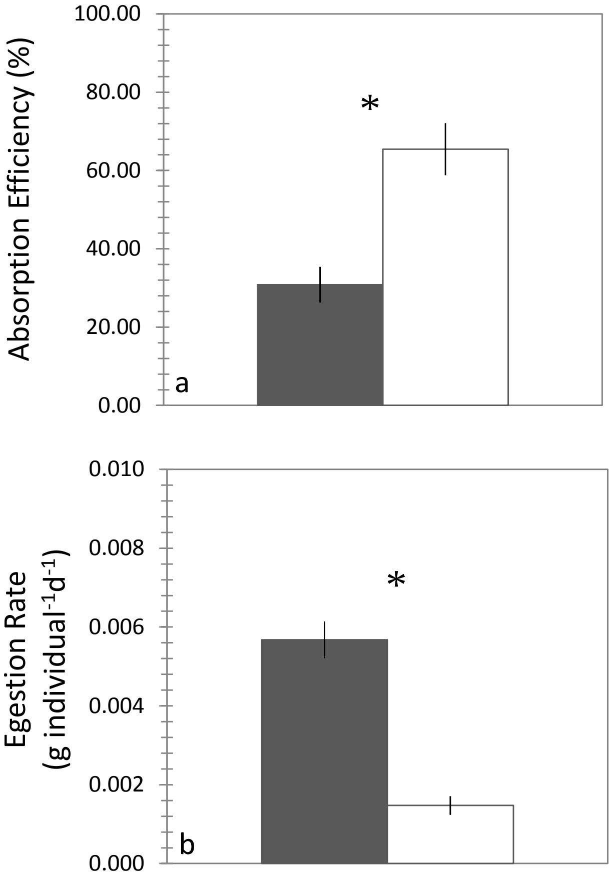

Figure 3.1. (a) Absorption efficiency (%) and (b) egestion rate (g dry weight individual^{-1} d^{-1}) in basket cockles (*Clinocardium nuttallii*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=12 for a and b) or *Isochrysis* sp. (TISO) (*n*=8 for a and *n*=12 for b). Data are mean±SE.*=significant difference (P<0.05).

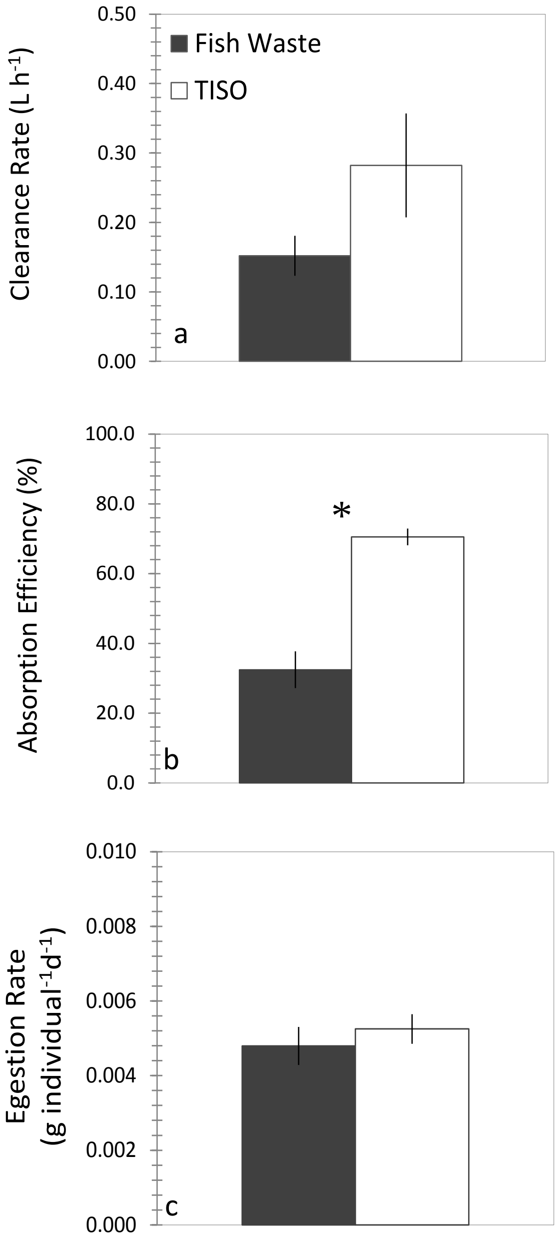

Figure 3.2. (a) Clearance rate (L h^{-1}), (b) absorption efficiency (%), and (c) egestion rate (g dry weight individual^{-1} d^{-1}) in blue mussels (*Mytilus edulis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=11) or *Isochrysis* sp. (TISO) (*n*=13). Data are mean ±SE.*=significant difference ($P<0.05$).

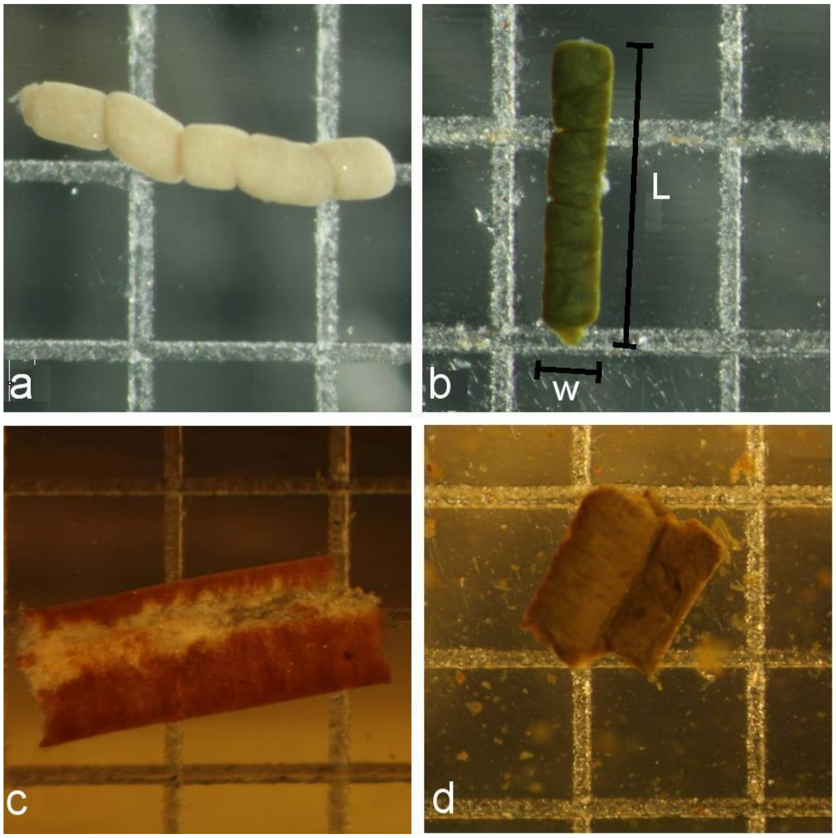

Figure 3.3. Individual faecal pellets from basket cockles (*Clinocardium nuttallii*) fed (a) sablefish (*Anoplopoma fimbria*) waste or (b) *Isochrysis* sp. (TISO) and blue mussels (*Mytilus edulis*) fed (c) sablefish waste or (d) TISO. Squares in the grids of each photograph are 1.00 mm^2. Black bars in figure b demonstrate how length (l) and width (w) were measured for each pellet.

Table 3.3. Mean, minimum, and maximum values for length (mm) and width (mm) of faecal pellets egested by basket cockles (*Clinocardium nuttallii*) and blue mussels (*Mytilus edulis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste or *Isochrysis* sp. (TISO).

	n	**Mean±SE**	**Minimum**	**Maximum**
Length				
C. nuttallii				
Fish waste	12	1.84±0.28	0.87	2.94
TISO	12	1.46±0.22	0.56	2.59
M. edulis				
Fish waste	9	2.20±0.73	0.77	5.73
TISO	4	1.11±0.56	0.76	1.72
Width				
C. nuttallii				
Fish waste	12	0.30±0.03	0.22	0.41
TISO	12	0.30±0.03	0.19	0.41
M. edulis				
Fish waste	9	0.70±0.23	0.36	1.01
TISO	4	0.60±0.30	0.35	0.97

Table 3.4. Results from one-way ANOVAs or one-way ANCOVAs testing for an effect of diet on the shape, size, and settling velocity of faecal pellets egested by basket cockles (*Clinocardium nuttallii*) and blue mussels (*Mytilus edulis*).

	Source of variation	**df**	**MS**	***F***	***P***
C. nuttallii	**Shape**				
	Diet	1	0.0081	0.9257	0.3464
	Error	22	0.0087		
	Size				
	Diet	1	0.0925	3.6441	0.0694
	Error	22	0.0254		
	Settling velocity				
	Diet	1	0.8177	70.8550	<0.0001
	Error	22	0.0115		
M. edulis	**Shape**				
	Diet	1	0.0549	1.1730	0.3020
	Error	11	0.0468		
	Size				
	Diet	1	1.5210	3.4308	0.0910
	Error	11	0.4433		
	Settling velocity				
	Diet	1	0.4863	40.415	<0.0001
	Shell length	1	0.1602	13.3115	0.0053
	Diet x Shell length	1	0.0050	0.4195	0.5334
	Error	9	0.0120		

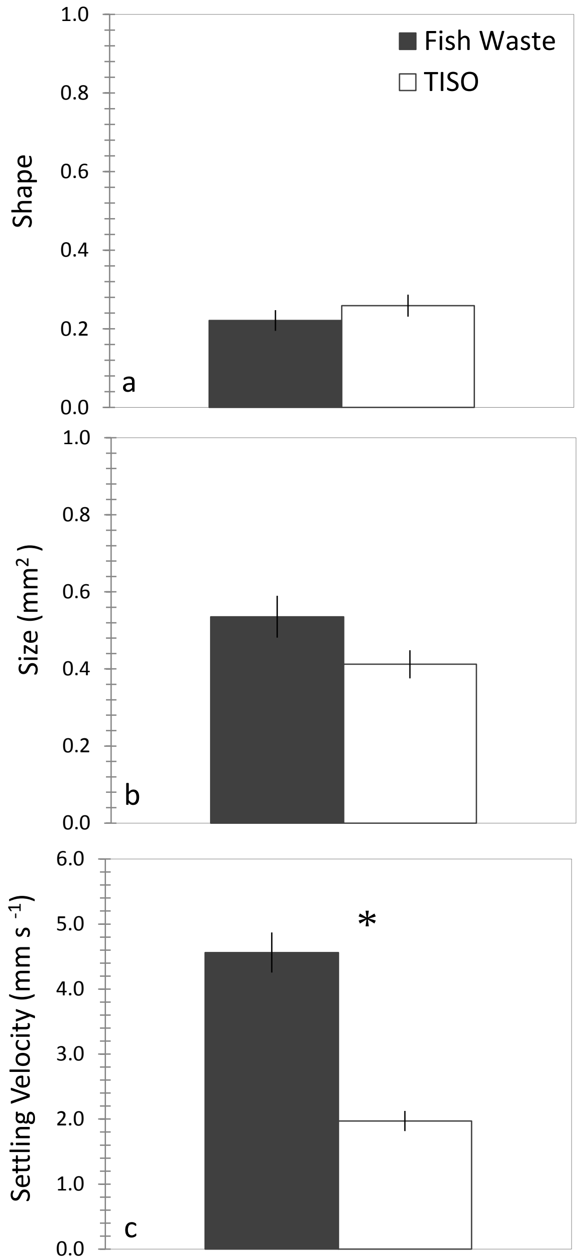

Figure 3.4. Biophysical properties of faecal pellets from basket cockles (*Clinocardium nuttallii*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=12) or *Isochrysis* sp. (TISO) (*n*=12): (a) pellet shape (width/length), (b) pellet size (mm^2), and (c) pellet settling velocity (mm s^{-1}). Data are mean±SE. *=significant difference (P<0.05).

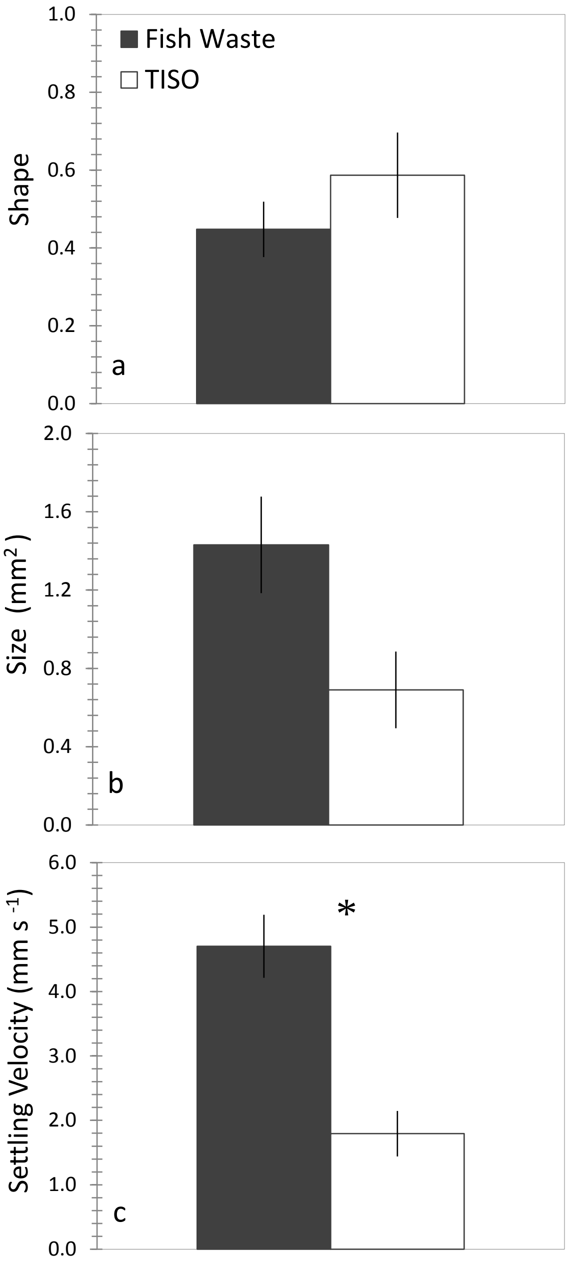

Figure 3.5. Biophysical properties of faecal pellets from blue mussels (*Mytilus edulis*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=9) or *Isochrysis* sp. (TISO) (*n*=4): (a) pellet shape (width/length), (b) pellet size (mm^2), and (c) pellet settling velocity (mm s^{-1}). Data are mean±SE. *=significant difference (P<0.05).

Discussion

Clinocardium nuttallii and *M. edulis* are candidate species for integration with sablefish in IMTA in BC. It is anticipated that suspension feeding activity by the bivalves will remove fine particulate organic material suspended in the water column near fish farms. Pulses of excess organic material released from the finfish component of the farm, in the form of uneaten feed pellet fines and faeces, are expected to enhance growth and survival by acting as a supplement to the natural diet. Results from the current study demonstrate that the blue mussel, *M. edulis,* will actively feed upon sablefish waste in the water column, and that *C. nuttallii* and *M. edulis* are also able to absorb organic matter in the waste.

Information on feeding activity in *C. nuttallii* is relatively scarce in the literature (but see Bernard and Noakes, 1990; Liu et al., 2008a, 2011; Meyhofer, 1985). In contrast, feeding activity has been well-studied in *M. edulis* and other bivalve species (*e.g.* Bernard and Noakes, 1990; Grant and Bacher, 1998; Hawkins et al., 1996; Iglesias et al., 1998; MacDonald et al., 2011; Okumus and Stirling, 1994; Riisgard et al., 2011; Riisgard and Larsen, 1995; Troost et al., 2009). In the present study, there was no difference in clearance rate for *M. edulis* fed the microalgal diet and those fed the sablefish waste. The regulation of clearance rate in bivalves is species-dependent and may be modulated by a number of different factors (Gardner et al., 2002). In nature, bivalves occur in several types of coastal environments, where the seston characteristics fluctuate rapidly over space and time (Gardner, 2000). As a result, many species regulate their feeding behaviour and digestive physiology to maximize nutrient uptake under variable conditions (Gardner, 2002; Hawkins et al., 1996). In mussels, clearance rate varies in a positive linear manner with seston organic content (Gardner, 2002; Hawkins et al., 1996) and so higher clearance rates should have been expected in *M. edulis* fed the microalgal diet, which had a significantly higher organic content. A lack of significant difference in clearance rate between the two diets could have been due to the reduced sample size, since the p-value was still relatively low (*P*=0.0948). The difference may have been statistically significant if all samples were successfully processed. Clearance rates of *M. edulis* in the present study (0.15–0.29 L h^{-1}) are lower than those reported in others [*e.g.* 1.05–2.55 L h^{-1} by Bayne et al. (1987), 1.27–1.76 L h^{-1} by Okumus and Stirling (1994), average 2 L h^{-1} by MacDonald et al. (2011)]. However, mussels are known to exhibit high physiological plasticity (Hawkins et al., 1996) and the range of reported clearance rates for bivalve species in the literature is wide and variable and can be attributed to differences in experimental set up and the measurement methods used (Riisgard, 2001). The biodeposition method used here potentially underestimates clearance rate if the retention efficiency differs between the bivalve gill and the filter used to sample seston in the water column (Petersen et al., 2004), although this was not accounted for in the present study.

Absorption efficiency was significantly higher in *C. nuttallii* fed the microalgal diet than those fed sablefish waste. Similarly, absorption efficiency was also higher in *M. edulis* fed TISO. Absorption efficiency in mussels has a strong positive correlation with the organic content of the seston (Hawkins et al., 1996) and this may explain the higher absorption efficiencies measured in both *C. nuttallii* and *M. edulis* fed microalgae, which had a significantly higher organic content than sablefish waste. Despite the higher values from the TISO treatment, positive absorption efficiencies in bivalves fed sablefish waste indicate that they both absorbed organic material from their diet. It is known from previous studies that bivalves can absorb organic material over a wide range of efficiencies (Bayne et al., 1987; Gardner, 2002; Hawkins et al., 1996). MacDonald et al. (2011) measured comparable values to the present study (32.4) for blue mussels fed fish wastes (24.8–38.3 %), although higher values have been reported by Liutkus et al. (2012) (86 %) and Reid et al. (2010) (90 %). It also appears that absorption efficiency may be lower for animals placed *in situ* than for those held in the laboratory (Reid et al., 2010).

For *C. nuttallii*, absorption efficiency was higher in those fed TISO. Therefore, more material was absorbed from the microalgal diet, which may have resulted in the lower egestion rate in cockles fed TISO compared to those fed sablefish waste. For *M. edulis*, there was no significant difference in clearance rate, but absorption efficiency was higher in those fed TISO. Although this indicates that more material was absorbed, similar egestion rates were also found in mussels fed both diets. If clearance rate results were in fact influenced by the reduced sample size compared to egestion rate and absorption efficiency, this could explain the apparent lack of consistency in the data. While higher or equal egestion rates in bivalves fed the sablefish waste compared to those given TISO may seem discouraging from an IMTA standpoint, it should be noted that the faecal organic content was significantly higher in bivalves fed TISO than in those given sablefish waste. Slightly higher egestion rates and lower faecal organic content values have been reported for various bivalves in the literature [*e.g.* 0.017−0.086 g individual^{-1} d^{-1} egestion rate and 12.5−28.4 % organic content for *M. edulis* (Callier et al., 2006), 24.8−30.4 % organic content for *M. edulis* (Kautsky and Evans, 1987)]. It is possible that egestion rates were underestimated for the bivalves in the present study since there would have been a lag between the time the bivalves began feeding and the time they began egesting material after it passed through the gut. This may also reflect differences between field and laboratory conditions. In the field, shellfish are able to feed continuously, whereas bivalves in the present study were starved for 2 d and then only allowed to feed for 2 h. Also in field-based studies, faecal organic content is measured in animals feeding upon natural seston which typically has a much lower organic content (Bayne et al., 1987; Hawkins et al., 1996; Widdows et al., 1979).

The reduction of organic loading attributable to IMTA will depend not only on ingestion rate and absorption efficiency of the organic extractive components, but also on the dispersal characteristics of the waste particles produced by the invertebrate extractive species. Egestion by bivalves can act to increase the vertical transfer of small suspended particles that are high in organic content (Kautsky and Evans, 1987). In the present study, there was no significant difference in the shape or size of faecal pellets egested by *C. nuttallii* or *M. edulis* fed either diet; however, in both species, settling velocities of faecal pellets were significantly greater in bivalves fed sablefish waste. Higher settling velocities in faeces produced on the fish waste diet were likely a result of greater density in the fish waste material, which may be related to its lower organic content (Giles and Pilditch, 2004). Bivalve faecal pellets may act to localize the impact of finfish farms by reducing the dilution or advection of particulate waste materials. If the bivalves were co-cultured with deposit-feeding species in IMTA, this localization would increase the efficiency of waste recapture by making organic material more available to the deposit feeders. Faecal pellets are an important aspect of marine food webs, being readily transported and containing undigested organic material (Wotton and Malmqvist, 2001). In the present study, the solid waste material collected from the sablefish was a slurry of large to fine particulate material. No quantitative analysis was performed on the physical nature of the sablefish waste, but no discrete pellets were present at the time of collection. Dispersal of faecal material from open-water aquaculture depends upon several factors, including the biophysical properties of the faecal pellets. Sablefish waste material was re-packaged into a discrete pellet as it passed through the digestive system of *C. nuttallii* and *M. edulis*. This change in its physical nature may increase its sinking velocity and thus limit dispersal away from open-water net pens (Giles and Pilditch, 2004). Predictive models that estimate the organic load exiting from open-water aquaculture (Reid et al., 2009) should include inputs specific to the extractive trophic levels to optimize their inclusion in IMTA.

Arguably, the most important aspect of organic extractive suspension feeders in IMTA is their potential for bioremediation through the removal of particulate organic wastes. Hydrodynamic properties will, to a large extent, determine the zone that is influenced by these particulate organic wastes in the water column (Cross, 2004; Troell and Norberg, 1998). To date, there have been conflicting reports on the success of bivalve integration into experimental-scale IMTA. Some authors have reported significantly higher growth for bivalves co-cultured at finfish farms (Handa et al., 2012; Lander et al., 2004; Sara et al., 2009; Stirling and Okumus, 1995). In many cases, this augmented growth was observed with a corresponding elevation in particulate organic matter. Lander et al. (2004) observed elevated concentrations of organic particulates that occurred in pulses; these were associated with feeding of the finfish component. In agreement with this, Handa et al. (2012) reported that mussel growth was significantly correlated with feed use and total particulate matter at salmon farms. When available, particulate fish farm waste likely provides a supplemental food source during the winter, when ambient food supply is naturally low (Handa et al., 2012; Stirling and Okumus, 1995; Wallace, 1980). Despite these promising results, other studies have found no significant effect of fish farming, either on bivalve growth or on the biochemical properties of the surrounding water column (Navarrete-Mier et al., 2010; Cheshuk et al., 2003), bringing into question whether a nutrient plume is present at all. If bivalves in IMTA are not feeding upon farmed fish waste then they may actually enhance organic loading to the benthos by incorporating the organics from natural seston into the system and increasing its vertical transfer to the seafloor. In their study, Mazzola and Sara (2001) concluded that organic waste from a fish farm was more important as a food resource for deep-water species based on stable carbon isotope analysis. In concordance with these conflicting reports, the present study reinforces the importance of ensuring that fish waste is made available to the suspension-feeding component of an IMTA system and re-affirms the importance of proper site selection and system design.

Evidence from laboratory feeding trials in the present study suggests that *C. nuttallii* can absorb organic material from sablefish waste, and suggests that *M. edulis* will actively filter the waste particles from the water column and absorb the organic material. Under the right hydrodynamic conditions, suspension feeding bivalves may act to reduce organic enrichment in the water column surrounding a properly designed IMTA system. Both *C. nuttallii* and *M. edulis* could be used for organic extraction, but additional co-culture of a deposit-feeding species may be required to fully optimize bioremediation through feeding upon settled bivalve faeces and the heavier settleable solids from the finfish component. IMTA is one method that could improve the environmental sustainability of finfish aquaculture, given that proper care is taken to optimize its design.

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4. Chapter 4 - Ingestion rate, absorption efficiency, and faecal production in spot prawns (*Pandalus platyceros*) and California sea cucumbers (*Parastichopus californicus*) fed waste from sablefish (*Anoplopoma fimbria*) culture

Introduction

Integrated multi-trophic aquaculture (IMTA) has a long history that begins with polyculture in China, where practices have been established for at least 1000 years, mostly in freshwater ponds to grow carp (Lin, 1982). This type of culture involves growing multiple species occupying different ecological niches together so that synergistic interactions are maximized and resources are used efficiently (Milstein, 1992). The major benefits of the IMTA system are increased food resources, improved environmental conditions, and maximized space usage, all of which may serve to increase production yields (Lin, 1982; Milstein, 1992). Within the last decade there has been a surge in research focussing on improving the environmental performance of intensive and semi-intensive open-water IMTA (Cheshuk et al., 2003; Mazzola and Sara, 2001; Neori et al., 2004, 2007; Paltzat et al., 2008; Sara et al., 2009; Slater and Carton, 2009; Troell et al., 2003, 2009). IMTA systems combine species so that waste by-products from upper trophic levels (*e.g.* finfish that require feeding) are recycled by species at lower trophic levels, including various seaweeds and invertebrates. Seaweeds represent the inorganic extractive component which use dissolved inorganics, such as nitrate or phosphate, as nutrients. Suspension- and deposit-feeding invertebrates comprise the organic extractive component, consuming solid wastes that remain suspended in the water column or that settle on the seafloor (Troell et al., 2009). In addition to this environmental bioremediation, IMTA can have economic benefits through diversifying production and may improve the social acceptability of the aquaculture industry (Barrington et al., 2010; Nobre et al., 2010; Ridler et al., 2007; Whitmarsh et al., 2006).

The spot prawn (*Pandalus platyceros*) is an opportunistic predator and scavenger that feeds on benthic organisms or detritus during daylight hours (Bergstrom, 2000). It lives primarily in rocky subtidal habitats, but is known to undertake vertical migrations in the water column at night when it feeds on plankton (Bergstrom, 2000; Butler, 1964; Marliave and Roth, 1995). Pandalid shrimp are found exclusively in the northern hemisphere (Komai, 1999). Native to the northeast Pacific Ocean, *P. platyceros* ranges from Alaska to California off the coast of western North America, and populations are also present around Japan (Butler, 1964). An important fishery exists in British Columbia (BC), Canada for Pandalid shrimp, including *P. platyceros*, which is the largest species to occur in the region (Boutillier and Sloan, 1987; Butler, 1964; Drouineau et al., 2012). In 2009, the fishery had a landed value of CAD $ 1.5 million from a harvest of 750 metric tons (DFO, 2011b).

Marine crustacean farming in general has increased significantly in Asia and Central America since the 1980s, following the development of improved culture techniques (Forster and Beard, 1974; Laubier and Laubier, 1993). The vast majority of this production comes from the harvest of Penaeid shrimp and the aquaculture of Pandalids like *P. platyceros* has yet to be established. Due to its economic importance, however, an interest in developing aquaculture for the spot prawn has persisted for quite some time (Hunt et al., 1995; King, 1997). Kelly et al. (1977) were able to grow *P. platyceros* in the laboratory from wild-caught brood stock, but the prawns did not reach a marketable size, leading the authors to conclude that a polyculture system may be more appropriate for commercial production of the species, since it may be more economically viable and there would be no need for additional prawn feed. Further to this, Rensel and Prentice (1980) tested *P. platyceros* as a companion crop with farmed salmon to diversify production and increase returns. They found that spot prawns were able to survive without the addition of supplemental feed if environmental conditions were favourable.

The California sea cucumber (*Parastichopus californicus*) is a deposit-feeding holothuroid that consumes epibenthic detritus (Cameron and Fankboner, 1984). It is commonly found in sheltered rocky intertidal or subtidal areas along the northeast Pacific Ocean, ranging from Alaska to California (Kozloff, 1983). Sea cucumber fisheries play an important role in worldwide seafood production, with a history that dates back at least 1000 years (Conand and Byrne, 1993). Currently, there is substantial interest in developing aquaculture for new sea cucumber species to fill unexploited niches in the global market (Eriksson et al., 2012; Sicuro and Levine, 2011). In the Pacific Northwest, a valuable, but limited, fishery exists for *P. californicus* (Bruckner, 2005; DFO, 2011a). In British Columbia, this fishery brought in a reported landed value of CAD $ 2.65 million and a harvest of 623 metric tons in 2007 (DFO, 2011a). Additionally, *P. californicus* has been included in polyculture systems on an experimental basis. Ahlgren (1998) grew California sea cucumbers in open-water net pens with pink salmon (*Oncorhynchus keta*), Paltzat et al. (2008) co-cultured *P. californicus* with Pacific oysters (*Crassostrea gigas*), and natural occurrences of juveniles settling on oyster culture gear have been recorded (Cheng and Hillier, 2011).

Both *P. platyceros* and *P. californicus* are candidate species for integration with sablefish (*Anoplopoma fimbria*) culture in BC. They are being considered as organic-extractive components as both are expected to feed upon the heavier settleable solid wastes, in the form of uneaten feed pellets and faeces, thus potentially reducing the transfer of organic material to the benthic environment. In order for *P. platyceros* or *P. californicus* to be considered successful organic extractive species, they must have suitable survivorship and growth rates and be able to produce a commercially acceptable product when feeding on cultured fish waste. In part, this means that nutritional demands must be met for each species when their diet is composed primarily of sablefish wastes. Conversely, if bioremediation of the culture system is to be achieved, both species must efficiently remove organic material from the sablefish wastes via their feeding activity. To assess their ability to remove organic material from a diet of sablefish waste, ingestion rate and absorption efficiency were measured in adult *P. platyceros* and *P. californicus* relative to those fed a control diet in laboratory feeding trials. Because no organism absorbs ingested material with 100% efficiency, organic-extractive species will also have an ecological footprint, though theoretically reduced in comparison to the fed trophic level. During feeding trials, egestion rates were also measured to estimate the quantity of solid waste materials that may be released by *P. platyceros* or *P. californicus* when they are included in an IMTA system as the organic extractive-component. Finally, the shape, size, and settling velocity of faecal pellets egested by both species were measured in order to predict the dispersal properties of this solid waste. The biophysical properties of the invertebrate faeces will also be important for assessing the overall efficiency of IMTA systems, since these properties will aid in determining the spatial extent of faecal dispersal.

Methods

Collection and Maintenance of Experimental Animals

On February 27, 2011, male *P. platyceros* were collected with baited traps in Howe Sound, BC. They were transported inside an insulated cooler to the Pacific Biological Station (Nanaimo, BC) within 24 h and held outdoors in uncovered tanks for a minimum of 1 month. The tanks were continuously supplied with flow-through, ambient, sand-filtered and UV-treated seawater. Prawns were fed a diet of frozen krill *ad-libitum* three days per week. On June 15, 2011, *P. californicus* were collected from trays hanging at an experimental aquaculture site at the Pacific Biological Station. They were immediately transferred to outdoor tanks continuously supplied with flow-through, ambient, sand-filtered and UV-treated seawater where they were held for a minimum of 1 month. Sea cucumbers were fed *ad-libitum* a diet of natural sediment, collected from the shoreline near the Station. During the holding period for both species, water temperature and salinity ranged between 7 and 12°C and 26 and 30, respectively.

Preparation of Experimental Diets

The solid fish-waste materials used in the present experiments were collected from adult sablefish. The fish were maintained and the waste collected as described in chapter one. The control diet used in feeding trials with *P. platyceros* was commercially-bought krill which was frozen at −20°C. The control diet used for *P. californicus* was natural sediments collected from the shoreline nearby the Station and stored at −20°C.

Experimental Setup for Feeding Trials

Two laboratory feeding trials were conducted. On July 6, 2011, 24 *P. platyceros* with a live weight of 26.73±0.61 g (mean±SE, *n*=24) were used in the first feeding trial. On August 23, 2011, 24 *P. californicus* with a live weight and body size index of 43.70±6.31 and 25.25±3.03 (mean±SE, *n*=21), respectively, were used in the second feeding trial. Sea cucumber body size index was calculated as described by Yingst (1982) and Paltzat et al. (2008).

At the beginning of each feeding trial, the animals were randomly selected from the communal holding tank and transferred individually into separate feeding chambers (L x W x H: 20 x 16.5 x 11 cm) held within one of two seawater tables (L x W x H: 1.5 x 1.0 x 0.3 m). Each chamber was individually supplied with 1-µm cartridge-filtered and UV-treated, seawater (salinity: 28) at a flow rate of approximately 290 ml min^{-1}. Effluent flowed through three outlets (diameter: 1 cm), located on the opposite side (from the inflow) of the chamber, which were covered with 100-µm mesh to prevent loss of feed particles and faecal material. The chambers were submerged in a common water bath (depth: 10 cm), maintained at a temperature of 10°C, and exposed to a simulated natural photoperiod using overhead fluorescent lights (25 lx; measurement taken at the bottom of a closed chamber).

Each feeding chamber was assigned at random to either sablefish waste material or a natural control diet (krill for *P. platyceros* and natural sediment for *P. californicus*). In both trials, six chambers were left empty (*i.e.* no prawns or sea cucumbers added) to serve as autogenic controls. These accounted for changes in diet weight unrelated to feeding activity (*n*=3 for fish waste diet and *n*=3 for control diet, in both trials). Animals were left to acclimate to the feeding chambers for 3 d prior to initiating each feeding trial. On the first day, individuals were fed the fish waste or natural control diet (depending on treatment designation) *ad-libitum*. For the following 2 d of the acclimation period individuals were starved to standardize hunger levels and to ensure that subsequent faecal production was from the diets fed at the beginning of the feeding trial.

At the start of the feeding trials, prawns were fed a known amount (2.83±0.78 g, mean±SE, *n*=24) of the wet fish waste (*n*=12) or thawed wet krill diet (*n*=12) and allowed to feed for a period of 1 h. Sea cucumbers were fed a known amount (4.03±0.01 g mean±SE, *n*=21) of the wet fish waste (*n*=11) or sediment diet (*n*=10) and allowed to feed for a period of 24 h. Following each feeding period, uneaten feed was collected from experimental chambers and autogenic controls. Faecal samples were collected from prawns 24 h after the removal of the feed. Faecal samples were collected from sea cucumbers at the same time as the uneaten feed. These samples were analyzed to determine either: ingestion rate, organic content, absorption efficiency, and total faecal egestion by the animal, or the shape, size, and settling velocity for the individual faecal pellets. Between trials, feeding chambers were removed and thoroughly cleaned.

Ingestion Rate, Absorption Efficiency, and Egestion Rate

Uneaten feed material was removed via gentle suction (being diligent to exclude any invertebrate faeces, which were easily distinguishable from the food) from 12 chambers containing prawns (*n*=6 fishwaste and *n*=6 krill) and from 9 chambers containing sea cucumbers (*n*=5 fishwaste and *n*=4 sediment). One data point was lost from the prawn fish waste treatment during sample processing, and one data point was lost from the sea cucumber fish waste treatment when one individual ingested all of the diet offered before the end of the designated feeding period. Samples were rinsed with distilled water to remove salts, and dried at 60°C for 24 h to constant weight before weighing. Uneaten krill was rinsed using a 100 µm sieve. However, the sediment and fish waste diets were made of finer particulate material, so rinsing with a sieve was not possible. These samples were therefore rinsed once by addition of 50 mL distilled water and centrifuged at 1500 x g for 10 minutes at 10 °C. Following this, the top half of the supernatant was carefully removed with a Pasteur pipette and the remainder of the distilled water was removed during the drying process. Dry-weight ingestion rates were calculated per individual per day by subtracting the actual dry weight measured of the uneaten feed from the estimated total dry weight of feed added to the chamber, using values from autogenic controls to correct for changes in diet weight without animals present. Total dry weight of added feed was estimated from regression analysis of wet and dry weights for each diet ($y=0.258x-0.158$, $R^2=0.994$, $P<0.0001$ for the fish waste; $y=0.179x-0.015$, $R^2=0.996$, $P<0.0001$ for the krill; $y=0.847x-0.067$, $R^2=0.999$, $P<0.0001$ for the natural sediment). Wet-weight ingestion rates were determined by applying the same conversion to dry-weight ingestion rates.

Total faecal material was collected individually from 12 chambers containing prawns ($n=6$ fishwaste and $n=6$ krill) and 9 chambers containing sea cucumbers ($n=5$ fishwaste and $n=4$ sediment). Two data points were lost from both prawn treatments during sample processing and one data point was lost from the sea cucumber sediment treatment during sample processing. Samples were immediately stored at −80°C. Thawed samples were vacuum-filtered onto pre-ashed, pre-weighed 42.5-mm Whatman™ GF/C filters and rinsed with distilled water to remove salts. Filtered samples were dried at 60°C for 24 h to constant weight, weighed, placed in a muffle furnace at 450°C for a minimum of 3 h, and re-weighed. Ash-free dry weight (AFDW) was calculated as the difference in weight between dried and ashed samples and expressed as a fraction of total sample dry weight to calculate organic content. Three samples of each diet type, collected at the time of feed preparation, were processed following the same procedure to determine organic content.

Absorption efficiency (AE), defined as the percent of organic material absorbed from the total ingested material as it passes through the digestive system, was calculated using the Conover ratio (1966):

$$AE = (F - E) / [(1 - E) \times F] \times 100$$

where F denotes the organic content fraction of the diet and E denotes the organic content fraction of the faeces. The Conover method assumes that absorption of inorganic material across the gut wall is negligible and therefore uses the inorganic portion of the diet as an inert tracer to measure net absorption (Reid et al., 2010).

Total faecal sample dry weights were used to determine egestion rate on a per-individual per-day basis.

Shape, Size and Settling Velocity of Prawn and Sea Cucumber Faecal Pellets

Faecal pellets were collected from 9 chambers containing prawns (*n*=5 fishwaste and *n*=4 krill) and 12 chambers containing sea cucumbers (*n*=6 fishwaste and *n*=6 sediment). One prawn from the fish waste treatment and two prawns from the krill treatment had not egested any faecal pellets at the time of faecal sample collection. Samples were collected via gentle suction, being careful to maintain pellet integrity.

Faecal pellets from *P. platyceros* were transferred to 50-mL centrifuge tubes held on ice. Three randomly chosen faecal pellets from each experimental prawn were photographed with a digital Canon EOS Rebel xsi camera (Canon Canada Inc., Mississauga, Canada) mounted on a Nikon dissecting microscope (Nikon Canada Inc., Mississauga, Canada). Faeces from *P. californicus* were photographed before removal from the feeding chamber, since they were too large for use with the microscope. Three randomly chosen faecal pellets from each experimental sea cucumber were photographed with a ruler placed beside them on the bottom.

The length and width of each pellet was measured using the digital imaging software ImageJ (version 1.45h). Shape was calculated as the ratio of pellet width to pellet length (Sauchyn and Scheibling, 2009). Size was calculated as the planar area of the pellet (length x width).

Settling velocity was measured for each pellet after it was photographed, by gently releasing them immediately below the surface in a cylindrical settling column (height: 45 cm, diameter: 10 cm) at a water temperature and salinity of 22±1°C and 28, respectively. Two marks were placed 10 cm apart on the side of the settling column with the upper mark located 7 cm below the surface (Callier et al., 2006). The time for each pellet to descend between them was recorded.

For shape, size and settling velocity, mean values for each individual were generated based on the three representative fecal pellets and these means were used in all subsequent analyses.

Statistical Analysis

Mean dry-weight ingestion rate, wet-weight ingestion rate, absorption efficiency, egestion rate, faecal organic content, and faecal pellet shape, pellet size, and pellet settling velocity in *P. platyceros* in the two different dietary treatments were initially compared using a one-way analysis of co-variance (ANCOVA), with prawn live weight included as the covariate. There was no significant effect of live weight on any of the these test variables when it was included as a co-variate, so it was removed from the models and results were analyzed with simple one-way ANOVAs. Organic content of the diet was compared using a one-way ANOVA. Data for wet-weight ingestion rate, egestion rate, and faecal pellet size were log-transformed to meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05).

Mean dry weight ingestion rate, wet-weight ingestion rate, absorption efficiency, egestion rate, faecal organic content, and faecal pellet shape, pellet size, and pellet settling velocity in *P. californicus* in the two different dietary treatments were initially compared using a one-way analysis of co-variance (ANCOVA), with body size index included as the covariate. There was no significant effect of body size on most of the test variables when it was included as a co-variate and for these it was removed from the models and results were analyzed with simple one-way ANOVAs. The body size co-variable was significant in the ANCOVA on faecal pellet size, so it was left in the model. The data for pellet size and settling velocity were log-transformed to meet the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance

(Levene's test, α=0.05). Organic content of the diet was compared using a one-way ANOVA.

Results

Ingestion Rate, Absorption Efficiency, and Egestion Rate

Dry-weight ingestion rate was significantly greater in *P. platyceros* fed the krill diet than those fed sablefish waste, but the reverse was true for wet-weight ingestion rate (Table 4.1, Fig. 4.1a). Absorption efficiency was significantly greater in prawns fed the krill diet than in those fed sablefish waste (Table 4.1, Fig. 4.1b), but egestion rate was significantly greater in *P. platyceros* fed a diet of sablefish waste than those fed a diet of krill (Table 4.1, Fig. 4.1c). The organic content of the krill diet was significantly higher than that of the sablefish waste, but the reverse was true for faecal organic content (Tables 4.1, 4.2).

Diet had no significant effect on dry-weight ingestion rate in *P. californicus*, but wet-weight ingestion rate was significantly higher in those fed sablefish waste (Table 4.1, Fig. 4.2a). Absorption efficiency was significantly greater in *P. californicus* fed sablefish waste than those fed natural sediment, but egestion rate was significantly greater in those fed the natural sediment (Table 4.1, Fig. 4.2b, 4.2c). In addition, the organic content of sablefish waste and faeces from individuals fed sablefish waste was higher than that of sediment or faeces from individuals fed sediment (Tables 4.1, 4.2).

Shape, Size, and Settling Velocity of Prawn and Sea Cucumber Faecal Pellets

Faecal pellets from each treatment were easily discernible based on their colour; samples from *P. platyceros* and *P. californicus* fed the fish waste diet were beige to brown, while those egested by *P. platyceros* fed the krill diet were red to brown, and those egested by

P. californicus fed the sediment diet were grey and resembled the sediment prior to feeding (Fig. 4.3). Length and width data for faecal pellets from each treatment and feeding trial are summarized in Table 4.3.

There was no significant difference in the shape (Table 4.4, Fig. 4.4a) or size (Fig. 4.4b) of faecal pellets from *P. platyceros* in the two dietary treatments. However, faecal pellets from *P. platyceros* fed the fish waste diet had a significantly greater settling velocity than those fed the frozen krill (Table 4.4, Fig. 4.4c). There was no significant difference in size of faecal pellets from *P. californicus* in the two dietary treatments when the variation due to body size index was taken into account (Table 4.4, Fig. 4.5b). However, faecal pellets from those sea cucumbers fed the fish waste diet had a significantly more elongate shape than those from individuals fed the natural sediment (Table 4.4, Fig. 4.5a). Finally, settling velocity was significantly greater in faecal pellets from *P. californicus* fed the sediment diet (Table 4.4, Fig. 4.5c).

Table 4.1. Results from one-way ANOVAs testing for an effect of diet on dry-weight ingestion rate, wet-wet ingestion rate, absorption efficiency, egestion rate, and dietary and faecal organic content for both spot prawns (*Pandalus platyceros*) and California sea cucumbers (*Parastichopus californicus*).

	Source of variation	**df**	**MS**	***F***	***P***
P. platyceros	**Dry-weight ingestion rate**				
	Diet	1	0.0113	6.4895	0.0313
	Error	9	0.0016		
	Wet-weight ingestion rate				
	Diet	1	0.8967	130.7650	<0.0001
	Error	9	0.0069		
	Absorption efficiency				
	Diet	1	1.5482	47.7050	0.0005
	Error	6	0.0325		
	Egestion rate				
	Diet	1	2.2500	32.3637	0.0013
	Error	6	0.0695		
	Dietary organic content				
	Diet	1	0.0047	21.0057	0.0102
	Error	4	0.0002		
	Faecal organic content				
	Diet	1	0.1962	13.5404	0.0103
	Error	6	0.0145		
P. californicus	**Dry-weight ingestion rate**				
	Diet	1	0.5436	3.6738	0.1037
	Error	6	0.1480		
	Wet-weight ingestion rate				
	Diet	1	1.2348	9.9203	0.0198
	Error	6	0.1245		
	Absorption efficiency				
	Diet	1	0.0912	20.8167	0.0038
	Error	6	0.0044		
	Egestion rate				
	Diet	1	0.2444	13.8675	0.0098
	Error	6	0.0176		

Dietary organic content				
Diet	1	1.0885	31179.1100	<0.0001
Error	4	<0.0001		
Faecal organic content				
Diet	1	1.1052	4214.3370	<0.0001
Error	6	0.0003		

Table 4.2. Organic content (g ash-free dry weight g^{-1} dry weight) in diets and faecal samples from spot prawns (*Pandalus platyceros*) fed a diet of sablefish (*Anopoploma fimbria*) waste or krill and California sea cucumbers (*Parastichopus californicus*) fed a diet of sablefish waste or natural sediments.

	N	Mean±SE	Minimum	Maximum
P. platyceros				
Dietary organic content				
Fish waste	3	0.824 ± 0.006	0.817	0.825
Krill	3	0.880 ± 0.012	0.861	0.901
Faecal organic content				
Fish waste	4	0.826 ± 0.011	0.779	0.860
Krill	4	0.513 ± 0.059	0.294	0.692

		Mean±SE	Minimum	Maximum
P. californicus				
Dietary organic content				
Fish waste	3	0.868 ± 0.005	0.861	0.877
Sediment	3	0.016 ± 0.001	0.013	0.017
Faecal organic content				
Fish waste	5	0.781 ± 0.005	0.758	0.808
Sediment	3	0.012 ± 0.001	0.011	0.134

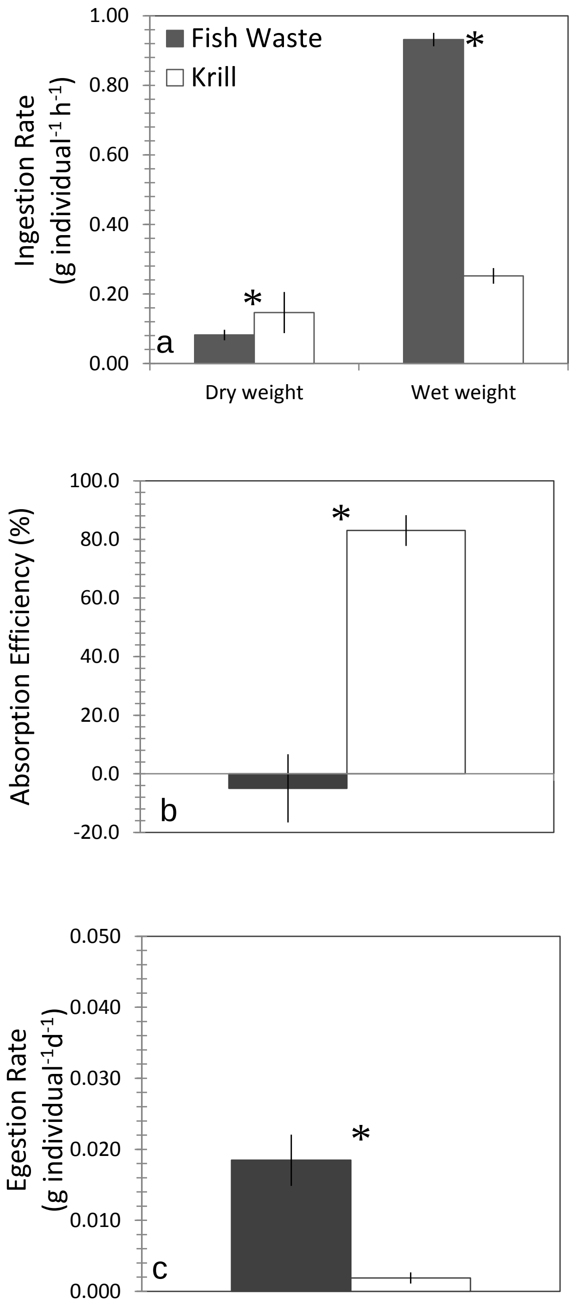

Figure 4.1. (a) Ingestion rate (g individual^{-1} h^{-1}), (b) absorption efficiency (%), and (c) egestion rate (g individual d^{-1}) in spot prawns (*Pandalus platyceros*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=5 for a and *n*=4 for b and c) or frozen krill (*n*=6 for a and *n*=4 for b and c). Data are mean±SE.*=significant difference (*P*<0.05).

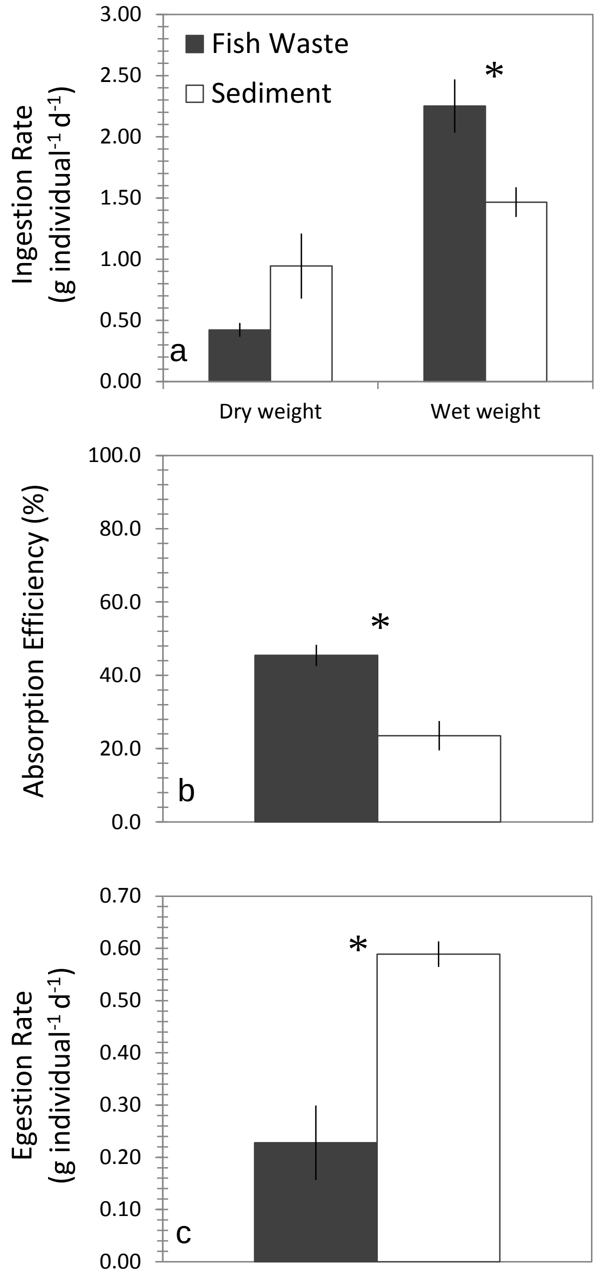

Figure 4.2. (a) Ingestion rate (g individual^{-1} d^{-1})), (b) absorption efficiency (%), and (c) egestion rate (g individual^{-1} d^{-1}) in California sea cucumbers (*Parastichopus californicus*) fed a diet of sablefish (*Anoplopoma fimbria*) waste (*n*=4 for a and *n*=5 for b and c) or natural sediment (*n*=4 for a and *n*=3 for b and c). Data are mean±SE.*=significant difference ($P<0.05$) detected.

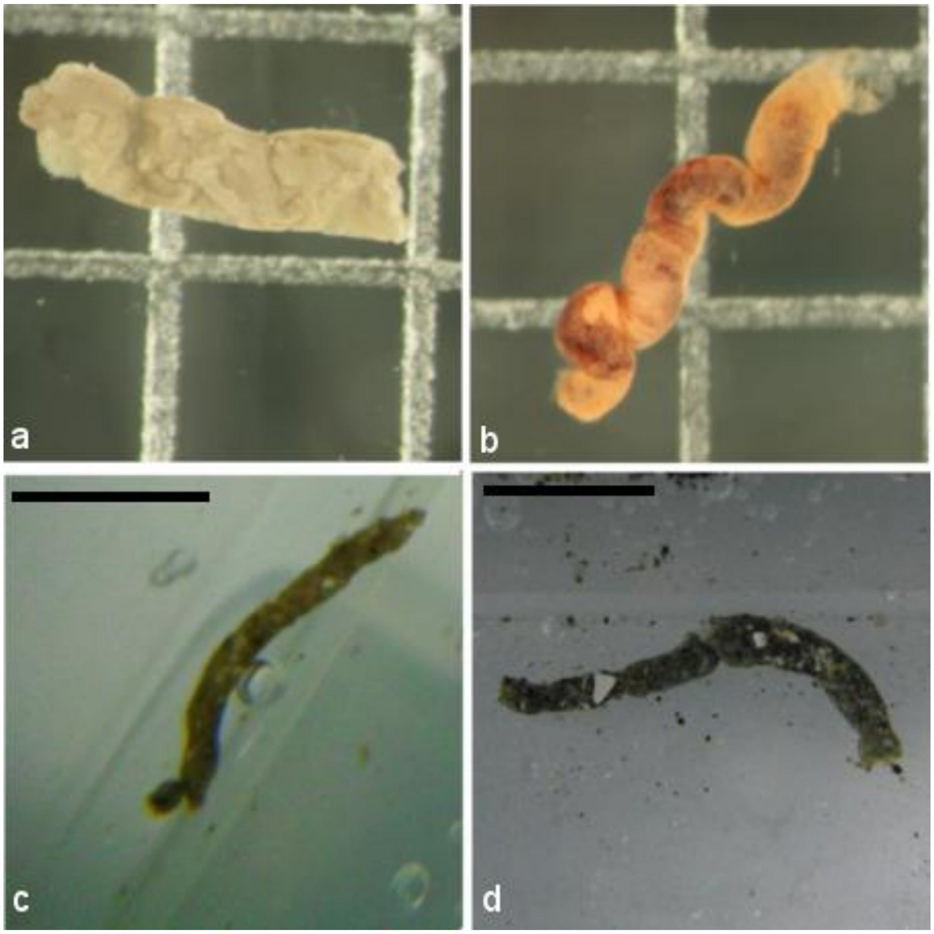

Figure 4.3. Individual faecal pellets from spot prawns (*Pandalus platyceros*) fed (a) sablefish (*Anopoploma fimbria*) waste or (b) krill (squares in the grid=1.00 mm^2) and California sea cucumbers (*Parastichopus californicus*) fed (c) sablefish waste or (d) natural sediment (scale bars=10 mm).

Table 4.3. Mean, minimum, and maximum values for length (mm) and width (mm) of faecal pellets egested by spot prawns (*Pandalus platyceros*) and California sea cucumbers (*Parastichopus californicus*) fed a diet of sablefish (*Anopoploma fimbria*) waste or a control diet (krill and natural sediments, respectively).

	n	Mean±SE	Minimum	Maximum
P. platyceros				
Length				
Fish waste	5	2.29±0.38	1.62	3.65
Krill	4	1.93±0.34	0.33	0.52
Width				
Fish waste	5	0.42±0.04	1.10	2.78
Krill	4	0.33±0.04	0.23	0.40
P. californicus				
Length				
Fish waste	6	14.49±1.37	10.64	20.40
Sediment	6	9.23±1.95	4.86	16.61
Width				
Fish waste	6	1.70±0.21	0.85	2.26
Sediment	6	2.16±0.31	1.44	3.35

Table 4.4. (a) Results from one-way ANOVAs or one-way ANCOVAs testing for an effect of diet on the shape, size (planar area), and settling velocity of faecal pellets egested by both spot prawns (*Pandalus platyceros*) and California sea cucumbers (*Parastichopus californicus*).

	Source of variation	**df**	**MS**	***F***	***P***
P. platyceros	**Shape**				
	Diet	1	0.0026	0.2103	0.6605
	Error	7	0.0122		
	Size				
	Diet	1	0.0589	2.4776	0.1595
	Error	7	0.0238		
	Settling velocity				
	Diet	1	52.4448	9.6114	0.0173
	Error	7	5.4565		
P. californicus	**Shape**				
	Diet	1	0.1220	9.1955	0.0126
	Error	10	0.0133		
	Size				
	Diet	1	0.2114	4.6618	0.0629
	Body Size Index	1	0.2881	6.3538	0.0358
	Diet x Body Size Index	1	0.0013	0.0286	0.8699
	Error	8	0.0453		
	Settling velocity				
	Diet	1	0.5419	49.6450	<0.0001
	Error	10	0.0109		

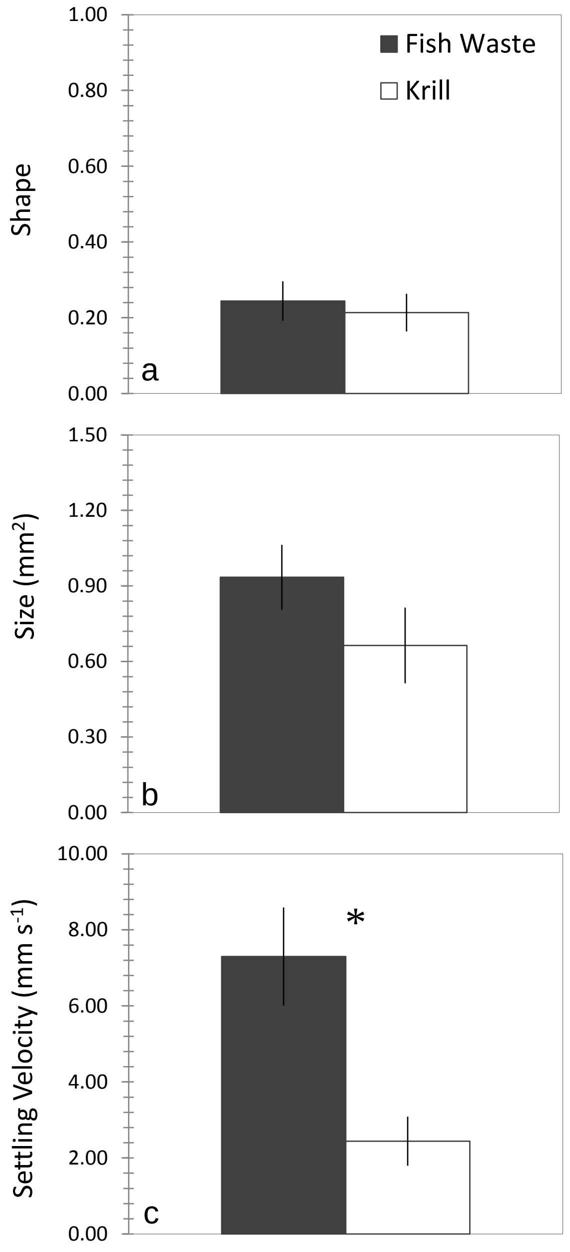

Figure 4.4. Biophysical properties of faecal pellets from spot prawns (*Pandalus platyceros*) fed a diet of sablefish waste (*Anopoploma fimbria*) (*n*=5) or frozen krill (*n*=4): (a) pellet shape (width/length), (b) pellet size (mm^2 area), and (c) pellet settling velocity ($mm\ s^{-1}$). Data are mean±SE. *=significant difference ($P<0.05$).

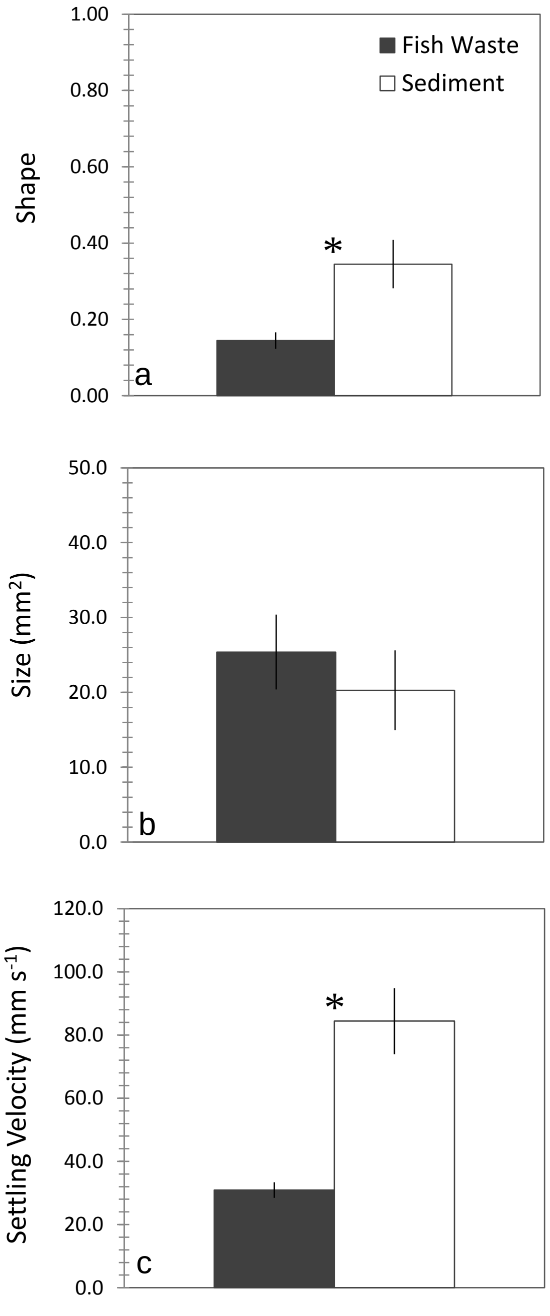

Figure 4.5. Biophysical properties of faecal pellets from California sea cucumbers (*Parastichopus californicus*) fed a diet of sablefish (*Anopoploma fimbria*) waste (*n*=6) or natural sediment (*n*=6): (a) pellet shape (width/length), (b) pellet size (mm^2 area), and (c) pellet settling velocity (mm s^{-1}). Data are mean±SE. *=significant difference (*P*<0.05).

Discussion

Both *P. platyceros* and *P. californicus* are candidate species being considered for integration with sablefish as part of the organic extractive-component of IMTA in BC, Canada. It is expected that these benthic feeders will consume uneaten feed pellets and fish faeces that accumulate on the seafloor beneath open-water net pens, thereby reducing organic enrichment of the sediments. However, if either *P. platyceros* or *P. californicus* are to be cultured at viable densities commercially in an IMTA system, their nutritional demands must be met when they feed on a diet that consists primarily of mariculture waste. In addition, they must be able to absorb organic material from this waste in order to achieve bioremediation. The results from laboratory feeding trials confirm that both *P. platyceros* and *P. californicus* will actively ingest settleable solid sablefish waste, but only *P. californicus* was able to absorb organics from the material. Positive measurements for both ingestion rate and absorption efficiency in sea cucumbers fed either diet indicate a net gain of organics to the animal and a net removal of organics from the dietary materials, suggesting that they have bioremediative potential.

Pandalus platyceros ingested significantly more of the krill diet than the sablefish waste on a dry-weight basis, but ingested significantly more of the sablefish waste when ingestion rates were expressed on a wet-weight basis. This difference is likely due to variation in water content between the two diets, which was higher in the krill, as shown from the regression analyses. Because dry-weight measurements are more useful for comparing nutrient intake, they are more important to consider when analyzing the removal of organic material from the diets. The organic content of krill was significantly higher than that of sablefish waste, and prawns were more efficient at absorbing organic material from the krill diet. Therefore, prawns ingested a greater absolute amount of organic material when fed krill. In fact, the negative values for absorption efficiency measured in prawns fed sablefish waste indicate that prawn feeding would actually increase organic loading in IMTA. Also, based on these results, it is very unlikely that prawns are meeting their nutritional demands when feeding upon this diet, although proximate or caloric content was not quantified. This would translate to poor growth and survivorship in cultured individuals. Few studies have investigated feeding activity in *P. platyceros*, especially with experiments conducted in the laboratory. Hunt et al. (1995) measured the effect of different feeding rates on growth rate in *P. platyceros* in polyculture, but no attempt was made to measure ingestion rate or absorption efficiency for the prawns. Assimilation of nitrogen has been estimated in *P. platyceros*, with values ranging between 82 to 97.4 % for various formulated diets (Forster and Gabbott, 1971). Overall, Forster and Gabbott (1971) found that *P. platyceros* assimilated nutrients less efficiently than the prawn *Palaemon serratus* in a series of experiments, but they were unable to offer an explanation for this result. Whyte and Boutillier (1991) have used fatty acid analyses to test for differences in dietary use by *P. platyceros* from various field sites and Olsen et al. (2009) monitored fatty acid profile and levels in a related prawn *Pandalus borealis* fed Atlantic salmon feed to test their potential for use as tracers of fish farm waste in the natural benthic food web. Further work confirmed that fatty acids commonly found in fish farm waste are assimilated into the tissues of *P. borealis* caught within the vicinity of Atlantic salmon farms, indicating that prawns will make use of this nutrient resource when it is available (Olsen et al., 2012). The present study suggests that, although *P. platyceros* will feed upon the settleable-solid wastes produced by adult sablefish, it is unlikely to result in the net removal of organic material, or efficient bioremediation.

For *P. californicus*, there was no significant difference when ingestion rate was expressed on a dry-weight basis, but sea cucumbers ingested significantly more of the fish waste diet on a wet-weight basis. Again, discrepancy between the results for wet and dry-weight ingestion rates are likely due to differences in dietary water content. The organic content of the sablefish waste was significantly higher than that of the natural sediment, and absorption efficiency was significantly higher in sea cucumbers fed the sablefish waste than those fed sediment. This means that sea cucumbers removed more organic material from the sablefish waste than the natural sediment. Positive values measured indicate that sea cucumbers were able to absorb a net increase of organic material by feeding on sablefish waste. These results are in agreement with a previous study investigating the ability of *P. californicus* to reduce biofouling on open-water net pens used for growing Pacific salmon (Ahlgren, 1998). In that study, sea cucumbers were observed feeding on fouling debris on the nets. This fouling had a significantly higher organic content than natural marine sediments collected from a reference site and sea cucumbers absorbed organics from the fouling debris three times more efficiently than from the natural sediment. In the present study, organic content of the diets likely influenced the absorption efficiency in the sea cucumbers. In some *Parastichopus* sp., lower absorption efficiencies are observed when individuals feed on sediments of a lower organic content (Yingst, 1982). Absorption efficiency measured in *P. californicus* fed sablefish waste during the present study was higher than the reported 24.3 % for sea cucumbers consuming fouling debris on salmon net pens (Ahlgren, 1998). This might be attributed to differences in diet quality, since the organic content measured in the sablefish waste was nearly twice as high as levels reported for the fouling debris (Ahlgren, 1998). The proximate composition of different diets may also have some affect, but this was not tested in the current study. Paltzat et al. (2008) reported a wide range of absorption efficiencies (14.17 – 57.87 %) for *P. californicus* grown underneath oyster culture rafts over the course of a year; variations are likely due to seasonal affects on the quality of the diet and digestive capabilities. Deposit-feeding sea cucumbers do not appear to selectively ingest sediments based on grain size, but there is substantial evidence that some species, including *P. californicus*, selectively ingest organic-rich or nutrient-rich material (Hammond, 1983; Paltzat et al., 2008; Slater et al., 2011; Yingst, 1982). The results of the present study are an important step towards developing IMTA with *P. californicus*, directly confirming that the sea cucumber will actively ingest and absorb organic material from the sablefish waste.

Organic enrichment in sediments underlying fish farms is well documented, with known detrimental impacts to the local benthic environment. Often sediments underlying fish farms have decreased species diversity or a complete absence of macrofauna (Brown et al., 1987; Holmer and Kristensen, 1992). They show decreased levels of dissolved oxygen, increased levels of particulate organic matter, and increased sediment metabolism that result from anaerobic decomposition (Brown et al., 1987; Holmer and Kristensen, 1992; Papageorgiou et al., 2010). Elevated levels of phosphorus are also present, reflecting decomposition of excess nutrients (Kutti et al., 2007; Papageorgiou et al., 2010). In general, impacts are mediated when nutrient loading does not surpass the assimilative capacity of the local environment (Kutti et al., 2007). Addition of deposit feeding species may help to increase the assimilative capacity in an IMTA system with sablefish. Michio et al. (2003) found that the sea cucumber *Stichopus japonicus* inhibited anaerobic processes in sediment and decreased the concentration of chlorophyll a, phaeophytin, and organic matter. Slater and Carton (2009) also found reduced levels of organic carbon and phytopigments in sediments impacted by mussel farming when they were grazed upon by the sea cucumber *Australostichopus mollis*. Based on this information, and on the results from the present study, *P. californicus* could be used as an organic extractive-species in IMTA with sablefish (or perhaps other finfish species). *Parastichopus californicus* had higher absorption efficiencies when fed sablefish waste, both statistically in comparison to sea cucumbers fed a natural diet and qualitatively in comparison to *P. platyceros* fed sablefish waste. Furthermore, *P. californicus* has shown better survivorship and has required less maintenance than *P. platyceros* in previous polyculture field trials (Paltzat et al., 2008; Rensel and Prentice, 1980). Finally, there is evidence that a diet of salmon biofouling material can enhance muscle growth in *P. californicus* (Ahlgren, 1998) and related sea cucumber species can feed, survive, and grow on a diet of mariculture waste (Maxwell et al., 2009; Slater and Carton, 2007; Slater et al., 2009; Zhou et al., 2006). Therefore, *P. californicus* may be a better candidate for integration with sablefish than *P. platyceros.*

The degree of impact on the benthic environment at a given mariculture site depends on many factors, including oceanographic conditions. If fish farms are placed in an exposed location, strong currents are more likely to re-suspend and dilute farm wastes (Cross, 2004; Kutti et al., 2007). The quantity and quality of waste, as well as its dispersal characteristics, are strong determinants of deposition rate underneath fish farms (Reid et al., 2009). Because no animal absorbs nutrients with 100 % efficiency, some organic material will still be lost from the organic-extractive deposit feeders in IMTA.

Egestion rate was significantly higher in *P. platyceros* fed a diet of sablefish waste compared to those fed krill, corresponding well with the results for absorption efficiency. The faecal organic content was also significantly higher in prawns from the sablefish waste treatment, further suggesting that prawns may increase organic loading. Although shape and size of faecal pellets were unaffected by diet, faeces egested from *P. platyceros* fed sablefish waste had significantly higher settling velocities than from prawns fed krill. Altogether, this means that prawns integrated with sablefish would be expected to release greater quantities of waste with a higher organic content and a higher settling velocity. Higher settling velocities lead to smaller, more localized deposition of particulate matter with less dilution of waste material (Liutkus et al., 2012). Although this would reduce the total area impacted by organic waste from IMTA, it would also concentrate organic deposition in the area that is affected. Measurements of egestion rate and faecal characteristics in the present study are the first of their kind for *P. platyceros*, and they could be used to predict deposition of waste from prawns on a site-specific basis.

Egestion rate was significantly lower in *P. californicus* fed a diet of sablefish waste compared to those fed a diet of natural sediment, also corresponding well with the results for absorption efficiency. The relatively low egestion rates with sablefish waste are promising, since this means sea cucumbers in IMTA would be expected to egest quantitatively fewer wastes relative to those fed a natural diet. Maxwell et al. (2009) found egestion rates ranging between 0.1 and 0.6 mg organic matter g^{-1} dry weight h^{-1} in a related sea cucumber *Stichopus mollis*. In that study no effect of diet was found, likely because no natural sediments were tested. Therefore, the organic contents of all diets, which consisted of a commercial abalone feed or abalone faeces, were artificially high. In this study, faecal organic content from the sea cucumbers fed sablefish waste was significantly higher than those fed the sediment, which had a very low organic content to begin with when compared to the sablefish waste. Because of this, higher levels of organic material in faeces produced by sea cucumbers fed sablefish waste are not surprising. The organic content of natural marine sediments, not impacted by fish farming, is generally low (Lopez and Levinton, 1987). Diet did not have a significant effect on the size of faecal pellets egested by *P. californicus*, but faecal pellets were more elongate and had a lower settling velocity from sea cucumbers fed sablefish waste. Faecal pellets with lower settling velocities can be expected to disperse more widely and deposit over a greater area (Liutkus et al., 2012). Although this potentially creates more scope for fish farm-environment interactions, it also allows for greater dilution of wastes, thus lessening benthic impacts at the farm site. The present study is the first to describe dispersal characteristics in faecal pellets egested by *P. californicus* fed a fish waste diet and, together with measurements for faecal quantity and quality, deposition of wastes released by the sea cucumber integrated with sablefish can be estimated at IMTA sites.

IMTA is one method that can increase the sustainability of intensive marine finfish farming. The organic-extractive component is expected to include both suspension-feeding species to remove smaller particulate organic wastes from the water column and deposit-feeding ones to reduce organic enrichment of the benthic sediments below open-water net pens due to the heavier settleable solids. The present study examines preliminary results to assess the potential for integrating the deposit feeders *P. platyceros* and *P. californicus* with sablefish in BC, Canada. Presented are the first measurements for ingestion rate, absorption efficiency, egestion rate, and faecal characteristics in either the spot prawn or the California sea cucumber after feeding on the settleable solid wastes (faeces and uneaten feed pellets) from adult sablefish. The results show that both species will actively ingest sablefish waste products when they are available as a food source. However, only *P. californicus* absorbed organic material from this waste. *Parastichopus californicus* may be a more feasible candidate for IMTA integration than *P. platyceros* based on these results, and from previous studies.

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5. Chapter 5 - General Conclusion

The ability to extract organic material from sablefish (*Anoplopoma fimbria*) waste (*i.e.* faeces and uneaten feed) by five invertebrate species proposed for Integrated Multi-Trophic Aquaculture (IMTA) in British Columbia was assessed through laboratory feeding trials. Chapter 2 examined feeding activity in the green sea urchin (*Strongylocentrotus droebachiensis*) fed a diet of sablefish waste relative to those given a control preferred diet of kelp *Macrocystis pyrifera*. There was no significant difference in dry-weight ingestion rate or absorption efficiency for sea urchins fed either diet. Oxygen consumption rates were significantly greater in *S. droebachiensis* 2 d after feeding on sablefish waste than the kelp diet, indicating higher metabolic rates with the former. The faecal pellets egested by sea urchins fed sablefish waste were rounder, smaller, and had greater settling velocities than those fed kelp. Overall, *S. droebachiensis* was able to actively ingest and absorb the organic material from sablefish waste, with an average absorption efficiency that was comparable to the kelp control. Therefore, *S. droebachiensis* is probably a good candidate species for organic extraction in IMTA. Longer-duration feeding experiments with *S. droebachiensis* are recommended in order to determine the long-term effect of sablefish waste on urchin survivorship, growth, and gonad quality (especially flavour).

Chapter 3 examined feeding activity in the basket cockle (*Clinocardium nuttallii*) and the blue mussel (*Mytilus edulis*) fed a diet of suspended sablefish waste relative to those fed a monoculture of the microalga *Isochrysis* sp. (Tahitian strain; TISO). There was no difference in clearance rate in *M. edulis* fed TISO or those fed sablefish waste.

The absorption efficiency was significantly greater in both *C. nuttallii* and *M. edulis* fed TISO. Egestion rates were significantly greater in *C. nuttallii* fed the sablefish waste, but there was no significant difference in egestion rate for *M. edulis* from either dietary treatment. Both bivalve species had similar results in terms of the biophysical properties of egested faecal pellets. There was no significant difference in the shape or size of faecal pellets egested by bivalves fed either diet; however, the settling velocity was significantly greater in faecal pellets egested by bivalves fed the sablefish waste. At present, there is no other literature addressing culture of basket cockles in IMTA. There is debate as to whether or not mussels have been a success in IMTA, since there are conflicting reports about augmented growth in mussels cultured near fish farms, and if a nutrient-enriched plume of wastes from the farm is even present. Because bivalves can increase the vertical transfer of nutrients, co-culture of deposit-feeding species in conjunction with suspension feeders may be necessary for efficient bioremediation through organic extraction, especially if the bivalves are feeding upon natural seston in addition to fish waste particles.

Chapter 4 examined feeding activity in the spot prawn (*Pandalus platyceros*) and the California sea cucumber (*Parastichopus californicus*) fed a diet of sablefish waste relative to those fed natural control diets of krill and sediment, respectively. For *P. platyceros*, dry-weight ingestion rates were significantly greater in prawns fed krill than those fed the sablefish waste diet. There was a significant difference in the absorption efficiency of prawns, with negative values in those fed fishwaste. Egestion rates were significantly greater in those fed the sablefish waste. For *P. californicus*, dry-weight ingestion rates were significantly greater in sea cucumbers fed the sediment diet, but absorption efficiency was significantly greater in those fed sablefish waste. Egestion rates were also significantly greater in sea cucumbers fed the sediment diet. There was no significant difference in the shape or size of faecal pellets egested by prawns fed either diet, but settling velocity was significantly greater in those fed the sablefish waste. Faecal pellets egested by *P. californicus* fed the sablefish waste were significantly longer and larger, but had lower settling velocities than those egested by sea cucumbers fed natural sediment. Unlike the other control diets, the sediment probably had a greater density than the sablefish waste. These results show that both *P. platyceros* and *P. californicus* can also actively ingest and absorb the organic material from sablefish waste, with absorption efficiencies comparable to or greater than those fed control diets. Of the two species, *P. californicus* represents the better candidate species for organic extraction in IMTA, since previous studies on its co-culture have resulted in better survivorship than studies using *P. platyceros*. Therefore, sea cucumbers may be generally more suited for use in aquaculture.

To date, some field and laboratory studies in the literature have provided evidence that wastes produced by intensive finfish aquaculture can be re-captured and utilized by organic-extractive organisms. Most studies have focussed on the culture of suspension-feeding mussel or deposit-feeding sea cucumber species, although some data exists for the sea urchin *P. lividus*, and others are being considered for use. Despite this research, few details are known about the trophic transfer efficiency between intensive and extensively cultured species. Ingestion rate, clearance rate, and absorption efficiency measured for candidate species in laboratory feeding experiments will help to bridge this gap in knowledge. Biophysical properties of egested faecal pellets will also have implications for nutrient transfer within an IMTA system and the larger marine environment. Overall, the results presented in this thesis confirm that five candidate invertebrate species for organic extraction in IMTA in BC, Canada, can actively ingest and absorb the organic material from sablefish wastes. However, it is recommended that further research efforts should focus primarily on the green sea urchin (*S. droebachiensis*) and the California sea cucumber (*P. californicus*).