Quantification of particulate size class ingestion, egestion, and water clearance rates by the orange-footed sea cucumber, *Cucumaria frondosa*, and implications for Integrated Multi-Trophic Aquaculture (IMTA)

by

Kurt Simmons

B.Sc. (Hon), Cape Breton University, 2012

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Supervisor: Bruce A. MacDonald, Ph.D., Dept. of Biology, UNB Saint John
Shawn M.C. Robinson, Ph. D., St. Andrews Biological Station

Examining Board: Thierry B.R. Chopin, Ph.D., Dept. of Biology, UNB Saint John
Keith Dewar, Ph.D., Dept. of Business, UNB Saint John

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Abstract

The sea cucumber *Cucumaria frondosa* is a candidate for Integrated Multi-Trophic Aquaculture (IMTA) farms as a species to extract organic effluent. Ingestion, egestion, and water clearance rates, and size of particulates ingested were assessed for *C. frondosa*, through field and laboratory experiments. Sea cucumbers in the field had a clearance rate of 7.2 L·individual⁻¹·day⁻¹, significantly different from those feeding in the laboratory (3.6 L·individual⁻¹·day⁻¹). Ingestion rates of *C. frondosa* feeding in the field (38 mg·individual⁻¹·day⁻¹; SD=18) and in the laboratory (30 mg·individual⁻¹·day⁻¹) were not significantly different. Nor were egestion rates in the field (13 mg·individual⁻¹·day⁻¹) or laboratory (11 mg·individual⁻¹·day⁻¹).

There was little reduction in particulate concentrations in laboratory experiments, making it unclear which sizes of particulates sea cucumbers are primarily ingesting, *C. frondosa* is unlikely to remove large quantities of waste effluent from IMTA farms, although it will likely remain a potential candidate due to its marketability and ability to efficiently absorb waste.
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List of Symbols, Nomenclature or Abbreviations

~ Approximately
° Degrees
′ Minute (1/60 of a degree)
″ Second (1/3600 of a degree)
> Greater than
≥ Greater than or equal to
< Less than
# Number
% Percent
∙ Multiplication
α Alpha
µm Micrometre
AE Absorption efficiency
ANOVA Analysis of variance
Cm Centimetre
ER Egestion rate
CR Clearance Rate
G Gram
H Hour
IMTA Integrated Multi-Trophic Aquaculture
IRR Inorganic rejection rate
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>IER</td>
<td>Inorganic egestion rate</td>
</tr>
<tr>
<td>IR</td>
<td>Ingestion rate</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>M</td>
<td>Metre</td>
</tr>
<tr>
<td>Min</td>
<td>Minute (time)</td>
</tr>
<tr>
<td>ML</td>
<td>Millilitre</td>
</tr>
<tr>
<td>n</td>
<td>Sample size</td>
</tr>
<tr>
<td>PIM</td>
<td>Particulate inorganic material (in diet)</td>
</tr>
<tr>
<td>S</td>
<td>Second (time)</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>ΔSF</td>
<td>Change in size frequency distribution</td>
</tr>
<tr>
<td>SFF</td>
<td>Final size frequency distribution</td>
</tr>
<tr>
<td>SFI</td>
<td>Initial size frequency distribution</td>
</tr>
<tr>
<td>TPM</td>
<td>Total particulate matter of diet</td>
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1. General Introduction

1.1 Aquaculture and *Cucumaria frondosa*

By the year 2020, aquaculture is expected to account for 60% of all seafood supply (Food and Agriculture Organization of the United Nations., 2016). While most global capture fishery landings have leveled off and some have collapsed, aquaculture has become the fastest growing sector of global food production (Food and Agriculture Organization of the United Nations., 2016). Canada’s aquaculture industry has experienced a four-fold increase from 1990-2002. Production declined in the early 2000s before recovering and stabilizing through the 2010s, making it the fourth largest producer of Atlantic salmon in the world (Fisheries and Oceans Canada, 2013). If the total current levels of fish consumption continue to rise, global aquaculture will need to provide an additional 23 million tons of farmed fish (an increase of approximately 17%; Nierenberg and Spoden, 2012).

Intensive monoculture practices, while expanding rapidly, present challenges for long-term sustainability. One of the primary concerns with finfish aquaculture is associated with the concentration of waste effluents containing faeces and uneaten feed. An increase in nutrients within an aquatic or marine ecosystem may lead to eutrophic algal blooms and increased bacterial activity resulting in hypoxia and anoxia (Nixon, 1995; Chávez-Crooker and Obreque-Contreras, 2010). In addition to loss of crops (be it finfish, shellfish, or seaweed), these effects may themselves lead to physical/chemical changes in the benthic environment, as the majority of organic waste solids will ultimately settle to the benthos (Brooks and Mahnken, 2003; Edgar et al., 2010), which may also lead to
changes within the benthic trophic structure (Sanz-Lázaro and Marín, 2006). Intensive
culturing can also potentially deplete wild stocks, as less valuable fish species (the
reduction fisheries) are often fished for the production of certain aquafeeds (Naylor et al.,
2009). There are essentially three distinct but non-mutually exclusive options to reduce
waste effluent associated with aquaculture: culture fewer products thereby reducing total
effluent, physically remove effluent from the farm, or convert effluent to a more usable
(form organisms or for sites themselves) form.

Recently, there has been interest in Integrated Multi-Trophic Aquaculture (IMTA) as a
potential strategy to convert portions of waste effluents to a useable secondary product
through conversion, thereby reducing and utilizing excess particulate matter and
potentially augmenting growth of extractive species (Chopin et al., 2007). IMTA is
characterized by the co-culturing of one or more species that occupy different (and
complimentary) trophic levels. These species would act as organic or inorganic extractive
species, ingesting organic and inorganic effluents and converting them to harvestable
biomass. The inclusion of inorganic extractive species (likely kelps) at aquaculture sites
could potentially help to clear water of excess soluble nitrogen and phosphorus (Chopin
et al., 2007), while other species ingest organic particulate matter (MacDonald et al.,
2011). This provides a greater diversity of products for farmers, with feed supplemented
at little or no cost by waste material of those organisms at a higher trophic level, and
nutrient losses of one species providing a nutrient source for others (Chopin et al., 2007).
These economic and environmental advantages may also improve the social acceptability
of IMTA (Troell et al., 2009). Research is nevertheless ongoing, as there are still many
existing issues with IMTA regarding the logistics required to achieve bioremediation,
how much bioremediation takes place, risks associated with the farming of multiple
species, and the development of appropriate new technologies. Even so, IMTA is thought
to have great potential as a means to improve the sustainability of seafood production and
reduce the impact on the environment (Barrington et al., 2009). IMTA may have
potential as a way of increasing the efficiency, acceptability, and sustainability of seafood
production. There is evidence that mussels held near salmon net pens have grown faster
than those held further away from aquaculture sites (Sara et al., 2009; Lander et al.,
2012). Increased growth of IMTA species may be related to increased chlorophyll α and
organic material from salmon pens that is able to be captured and assimilated (Sara et al,
2009).

Other studies show no significant effect of proximity to fish farms on species growth
(Cheshsuk et al., 2003; Stirling and Okumus, 1995). Similarly, bivalves do not
necessarily intercept and assimilate organic wastes from fish farms, in which case the
settling faeces and pseudofaeces derived from natural seston (matter suspended in the
water column) may be adding to the deposition of organic material rather than reducing it
(Cranford et al., 2013). In highly dynamic areas such as the Bay of Fundy, mussels may
not have the opportunity to intercept a large fraction of particulate wastes, especially
when positioned in the upper water column in low densities as is currently the case
(Cranford et al., 2013). Thus, it is unlikely that filter-feeders suspended in the water
column will have a significant effect on waste effluents and organic loading at an
industrial scale (Fisheries and Oceans Canada, 2013). Cranford et al. (2013) therefore
suggest that a benthic component of IMTA that is able to effectively capture and
assimilate particles associated with vertical fluxes of fish net-pens will be necessary for IMTA to operate effectively.

Particulate plume dynamics from fish cages are highly variable and difficult to predict, as they will be a function of hydrographic characteristics and husbandry practices at a given site. Local currents and other hydrodynamic conditions will influence the dispersal and settlement of waste products. Approximately 15% of fish feed consumed may be egested as faecal matter, which may result in 2025 kg of faeces released per day at the maximum production level over a full production cycle at a large salmon farm (Fisheries and Oceans Canada, 2013). Bio-deposition-related effects are generally localized to between 25 – 50 m of the fish farm (Brooks and Mahnken, 2003) as the majority of heavier particulate material settles close to the cage site, though this will be variable depending on the depth and current regimes of the area. This suggests that in order for a species to effectively remove larger particulates, they will need to be situated on the benthos or in a mid-water structure near the cage. As such, one option for biomitigation appears to be the inclusion of benthic organisms such as sea cucumbers at open-water aquaculture sites.

The orange-footed sea cucumber, *Cucumaria frondosa*, a benthic dendrochirotic (suspension-feeding) echinoderm, is the largest and most common native sea cucumber species on the east coast of Canada. It is often found in dense populations on rocky substrates with a wide distribution across the North Atlantic ranging from New England, along Eastern Canada, Greenland, and extending into Scandinavia (Jordan, 1972; Hamel and Mercier, 1996; Therkildsen and Petersen, 2006). Their range of depths is broad, being most abundant in sub-tidal zones and also found in greater depths up to 30 m
(Jordan, 1972). This species is quite long lived, taking at least 10 years for individuals to reach full size (Hamel and Mercier, 1996; So et al., 2010). For this reason, *C. frondosa* fisheries are particularly vulnerable to over-exploitation. Feeding habits are seasonal, with feeding occurring mainly in the spring and summer, which is thought to be primarily induced by the availability of a higher quality diet, as well as day length and temperature (Singh et al., 1999). Diet seems to be composed of phytoplankton cells, though eggs and larvae from other species have been found in the gut as well, suggesting they may be capable of ingesting a wide size range of diets (Hamel and Mercier, 1998). *Cucumaria frondosa* is somewhat unique among sea cucumbers as it is a passive suspension feeder. Most species of sea cucumbers are deposit feeders (Aspidochirotes), ingesting sediment or debris found at the sediment surface and digesting the organic portion of this material. *Cucumaria frondosa* feeds by extending its ten tentacles into the water column so particles can adhere to the mucus on the surface of the tentacles, which are then inserted to the mouth for ingestion. While feeding varies seasonally, it can also vary on a shorter time scale, with feeding activity being correlated with increasing chloropigment concentration and decreasing current speed (Singh et al., 1999). One study conversely found that feeding rate increases with current speed up to 55 cm/s, and decreased steadily at higher speeds (Holtz and MacDonald, 2009).

Both deposit feeding and suspension feeding sea cucumbers may be potentially extractive organisms in IMTA. Deposit-feeding cultured species of sea cucumbers are capable of ingesting and utilizing fish feed and faeces as well as bivalve biodeposits. These sea cucumbers have been co-cultured with a variety of bivalves, where they experience high survival and growth rates (Ahlgren, 1998; Kang et al., 2003; Zhoue et
When exposed to assemblages of sablefish (*Anoplopoma fimbria*) waste and natural sediment, the deposit feeding California sea cucumber (*Parastichopus californicus*) had higher absorption efficiencies and wet-weight ingestion rates than when feeding on sablefish waste alone (Orr, 2012; Hannah et al., 2013). Less work has been done with suspension feeding sea cucumbers and it is unknown how effective they may be extracting organic material in an aquaculture setting. When feeding on natural diets, *C. frondosa* are efficient at absorbing organic material and, when challenged with diets of aquaculture waste of higher organic content, absorption efficiencies were equal or enhanced (Nelson et al., 2012a). Preliminary work has found that *C. frondosa* show high variability in their individual feeding behaviour (Hamer, 2010; Nelson et al., 2012a). In a laboratory setting it has also historically been difficult to reliably induce “full” (that is, with tentacles fully extended) feeding activity, with an appropriate current and volume exchange rate, aeration, and food concentration needed to maintain typical feeding behaviour (Hamel and Mercier, 1998; Hamer, 2010). This difficulty in measuring reliable feeding rates for *C. frondosa* has hampered our ability to estimate their extractive capability in the field at aquaculture sites.

In Asian and European countries, there are lucrative markets for *Cucumaria frondosa* sold as “bêche-de-mer” or “trepang” (Therkildsen and Petersen, 2006). A dried consumer grade product currently ranges from about $100-$130 USD per pound, although this translates to quite a large volume of individuals, as they shrink considerably when dried. Historically, Asian markets have considered it a lower grade product in comparison to other species of sea cucumbers (which can be double the price), and thus fisheries would
target a high volume of the low-value product (Bruckner, 2005). In Atlantic Canada, there was no fishery present until 1996, though there has been a fishery in the Gulf of Maine as early as 1988 (Fisheries and Oceans Canada, 2009). Landings in the Gulf of Maine peaked in 2004 at just under 4000 metric tonnes, frequently fluctuating and having declined substantially in recent years to less than 500 tonnes (Nelson et al., 2012b; Maine Department of Marine Resources, 2015). This is not unusual for sea cucumber fisheries, as peak harvests will often lead to subsequent declines, with populations having difficulty recovering from deleterious effects of fishing (Fisheries and Oceans Canada, 2009).

*Cucumaria frondosa* is thought to have aquaculture and hatchery potential (Nelson et al., 2012b; Gianasi et al., 2016), which may help supplement or replace potentially vulnerable fisheries. The price per pound of *C. frondosa* has more than tripled since peak harvest in 2004 (Gianasi et al., 2016), and prices may continue to increase as higher-value nutraceutical and biochemical products such as the anti-cancer drug Frondoside A (Attoub et al., 2013; Ma et al., 2013) are developed. This market value, coupled with their potential ability to ingest a wide size range of waste particles has led to interest in incorporating the native orange-footed sea cucumber into IMTA sites.

However, we will need to establish the quantity of particulate waste material they are capable of removing. *Cucumaria frondosa* is being assessed for use in IMTA as an organic extractive species in New Brunswick salmon farming operations. In order for the inclusion of *C. frondosa* in IMTA to be beneficial, the species must provide either a marketable product (i.e. biomass) or bio-mitigation activity that reduces the organic loading of the farm site. The species is thought to be marketable and is known to be capable of ingesting and absorbing aquaculture waste (Nelson et al., 2012a; Nelson et al.
In order for its bio-mitigation potential to be determined, the quantity of material the sea cucumber would be ingesting and the quantity of waste it would be egesting must be known. In this thesis, results from field and laboratory experiments provide quantification of water clearance/ingestion/egestion rates of *C. frondosa* (or any suspension-feeding sea cucumber) for the first time and provide a novel technique for these measurements.

Suspension feeders may also have an impact on the benthic ecosystem and while potentially clearing the water of particulates, they also produce biodeposits. These organisms may be important mediators in benthic-pelagic coupling, wherein nutrients are cycled between the pelagic and benthic zones (Commito and Boncavage, 1989). Suspended particles and nutrients are taken in by the organism, digested, aggregated, and deposited onto the benthos. This material may support food webs based on biodeposits, affecting the community structure by providing detritus to other benthic invertebrates (Gergs et al., 2009). It is therefore important to consider not only how much material potential extractive species are clearing from the water, but how much they may be depositing on the benthos.

### 1.2 Objectives

The primary objective of this thesis was to estimate deposition rates, ingestion rates, and water clearance rates of the sea cucumber *Cucumaria frondosa*. This was accomplished through a combination of experiments where sea cucumbers were exposed to natural particles in the field and salmon feed and faeces in the laboratory and is discussed in Chapter 1.
The secondary objective of this thesis was to attempt to quantify the sizes of particulates on which *Cucumaria frondosa* feeds, discussed in Chapter 2.

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2. Quantification of clearance, ingestion, and egestion rates of the orange-footed sea cucumber, *Cucumaria frondosa*

### 2.1 Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is a strategy wherein species of multiple trophic levels are co-cultured adjacent to one another such that the nutrient loss (faeces) of one trophic level become nutrient inputs (feed) for other trophic levels. In eastern Canada, pilot-scale farmed IMTA species include salmon (*Salmo salar*), blue mussel (*Mytilus edulis*), and kelps (*Saccharina latissimi* and *Alaria esculenta*). Early conceptual models envisioned that mussels feed upon small organic particulate matter in the water column, while kelps absorb dissolved inorganics such as nitrate and phosphate (Chopin et al., 2007). There has been increasing interest in IMTA techniques, as it may provide certain advantages for farmers: a diversification of crops, an economical diet for lower trophic levels in the form of egested faeces and waste feed of higher trophic species, a means of potentially mitigating environmental impacts through the reduction and utilization of waste effluents, and an increased social acceptability of aquaculture practices (Chopin et al., 2007; Barrington et al., 2009, Troell et al., 2003). Interest in IMTA systems also comes at a time when aquaculture food production is increasing rapidly, having surpassed beef production in 2012 and wild fishery catches in 2014 (Food and Agriculture Organization of the United Nations., 2016).

This recent increase in farmed seafood comes largely from intensive monoculture practices. High densities of monoculture species are held together, creating large volumes of faecal waste material. Faeces and waste feed can lead to high volumes of
waste effluent produced by a farm, giving rise to concerns over the effect such effluents will have on the marine pelagic and benthic environment near aquaculture sites. An accumulation of these organic wastes can lead to increased bacterial activity, resulting in hypoxia and anoxia (Nixon, 1995; Chávez-Crooker and Obreque-Contreras, 2010). In addition to loss of crops (be it finfish, shellfish, or seaweed), this physical/chemical change in the benthic environment may also lead to changes within the trophic structure (Sanz-Lázaro and Marín, 2006), as the majority waste effluent will ultimately settle to the benthos (Brooks and Mahnken, 2003; Edgar et al., 2010). Intensive monoculture approaches are therefore not likely to be environmentally sustainable due to the associated ecological imbalance (Neori et al., 2007).

The orange-footed sea cucumber, *Cucumaria frondosa*, is one species that is being considered as a benthic organic extractive species within IMTA systems. A benthic dendrochirotic echinoderm, it is the largest and most common of our native sea cucumber species. It has a wide distribution across the North Atlantic ranging from New England, along Eastern Canada, Greenland, and extending into Scandinavia, where they form dense beds most often on rocky substrates (Jordan, 1972; Hamel and Mercier, 1996; Therkildsen and Petersen, 2006). They also have a broad range of depths, found in depths up to 30m, as well as in sub-tidal zones. (Jordan, 1972). It is also somewhat unique among sea cucumbers as it employs a passive suspension feeding strategy. Most species of sea cucumbers are deposit feeders – ingesting sediment or debris atop the sediment and digesting the organic portion of its contents. *Cucumaria frondosa*, however, feeds by extending its ten tentacles into the water column and are dependent on ambient water currents to move seston across these tentacles so that it may adhere to the mucus and be
captured where the tentacle will subsequently be brought to the mouth and stripped of captured particulates (Figure 2.1). This strategy has led to some difficulty from a research perspective, as individuals will retract their tentacles when stressed (either through captivity or disturbance), thereby ceasing feeding activity (Hamel and Mercier, 1998; Hamer, 2010). Nevertheless, the species is thought to have potential as a culture species (Nelson et al., 2012b; Pietrak et al., 2014), with an existing Asian market as a seafood product (Therkildsen and Petersen, 2006; Gianasi et al., 2016b) and an emerging market in higher-value nutraceutical and biochemical products (Attoub et al., 2013; Ma et al., 2013).

For IMTA species to be effective at biomitigation and reduce the impact of waste effluent, their diet must be composed primarily of waste effluent. For example, in order for mussels to reduce the net organic load at open-water IMTA sites, >15% of their diet must be comprised of particulates from farm sites (Reid et al., 2013). In addition, in order to achieve a net reduction in organic loading, extractive species must be efficient at intercepting and absorbing this waste. The efficiency with which they are able to intercept waste organic particulates will be influenced by their containment and location within an aquaculture site to some degree, though their feeding behaviour and activity will largely determine the volume of material that is ingested. For C. frondosa, this can be applied to the mass of particulates ingested as well as the volume of the water column that would have been “cleared” of these particulates (Coughlan, 1969). The amount of clearance will be a dependent on the amount of material ingested as well as the concentration of this material within the water column at a given time. Likewise, the absorption efficiency will determine the quantity of organics that is egested by the
species themselves. While some work has been done on the absorption efficiency of *C. frondosa* (Nelson et al., 2012a), the quantity of food that may be ingested over a given timescale is not known (and, by extension, neither is the volume of water this would relate to, nor the quantity of faecal organic loading being deposited by the sea cucumbers themselves).

To assess the potential of *C. frondosa* as an organic extractive species, ingestion rates, water clearance rates, and egestion rates were measured and calculated during field and laboratory feeding trials. Adult specimens from natural populations were deployed in Brandy Cove in the Bay of Fundy, and allowed to feed, before being retrieved and collecting faeces. A laboratory trial was also run, where sea cucumbers were allowed to feed on an assemblage of salmon feed and faeces particulates simulating particualtes at an open-water aquaculture site. This was done using the biodeposition method, wherein biodeposits (faeces) are collected as a metric for determining the total particulate ingestion of an organism. This allows for the determination of the volume of water that must have been cleared. Here, it is assumed that absorption of ingested inorganic matter is negligible (Prins et al., 1991; Iglesias et al., 1992) and the amount of inorganic matter of biodeposits is therefore representative of the total amount of inorganic matter filtered by *C. frondosa*. With this rate of biodeposition of inorganics (mg·day$^{-1}$), along with the concentration of inorganics in the water column (mg·l$^{-1}$), the volume of water cleared by sea cucumbers in one day was calculated using standard techniques (Prins et al., 1991). The clearance rate was then compared to the total amount of material in the diet assemblage the sea cucumbers had been feeding upon in order to calculate the ingestion
rate which allowed for the calculation of egestion rate using a known absorption efficiency for the species (Nelson et al., 2012a).

2.2 Objectives

The primary objective in this chapter was to estimate deposition rates, ingestion rates, and water clearance rates of the sea cucumber *Cucumaria frondosa*. The secondary objective was to test the hypothesis:

\[ H_0 = \text{Ingestion/egestion/water clearance rates will not differ between field and laboratory conditions.} \]

\[ H_a = \text{Ingestion/egestion/water clearance rates will differ between field and laboratory conditions.} \]

2.3 Methods

2.3.1 Species collection

Adult orange-footed sea cucumbers (*Cucumaria frondosa*) were collected from natural populations in the Passamaquoddy Bay within the Bay of Fundy, in south-west New Brunswick in the summers of 2013 and 2014. Sea cucumbers were collected as part of a biodiversity survey undertaken by the St. Andrews Biological Station. The survey took place in the eastern Passamaquoddy Bay (45°06'51"N, 66°04'62"W to 45°08'15"N, 66°03.97'W) using an otter trawl with a mesh size 42 mm on the cod end and towed at a depth of approximately 60 m. This collection method has been used successfully in the past and has shown no demonstrable impact on the sea cucumbers (personal
In order to assess clearance, ingestion, and egestion rates of *C. frondosa*, preliminary feeding trials were conducted in the summer of 2013. These took place at the Navy Island salmon farm site (45°01’51″N, 67°00’18″W) and a reference site 1000 m away (45°02’03″N, 67°01’01″W). Aluminum frames holding four tubular sediment traps made from 4 inch (10 cm) diameter PVC pipe were deployed at these sites. They were hung by rope from either salmon cages or by buoy at a depth of approximately 15m. Traps had an aspect ratio of 8:1 to allow for the settlement and retention of material in turbulent mixing waters (such sediment traps generally need an aspect ratio of >4:1; Hargrave, 1979). Traps had a detachable and sealable bottom section to allow for the transport and analysis of water samples, and frames were encased in a 10-gauge wire housing to provide stability and protection during deployment and retrieval (Figure 2.2).

Three of the four sediment traps on each frame contained a sea cucumber situated near the top of the trap, resting on a square mesh staging with a 2.5 cm pore size to allow settling material to pass through easily (Figure 2.2). A mesh covering extended over the top of the trap to prevent escape. Sea cucumbers were positioned such that their mouths were extending into the water column to allow them to feed, while their anuses pointed towards the bottom of the trap to allow for the collection and retention of egested faeces. One trap per frame was left empty to allow for the collection of naturally settling material. The amount of faeces collected could then be calculated using the bio-
deposition method (Prins et al., 1991; Iglesias et al., 1992). The difference in settled material between traps containing sea cucumber (collecting a combination of naturally settling material and egested material from sea cucumbers) and control traps with no sea cucumbers (which collected only naturally settling material) was assumed to be an estimate of total egested material.

2.3.3 Gut-passage time

Experiments were conducted in June 2014 in order to estimate gut-passage time in *C. frondosa*. Using a plastic pipette, individuals were fed fluorescent 10 µm latex micro-spheres. This was done by ejecting the micro-spheres onto the tentacles and into the mouths of sea cucumbers, before deploying them off the wharf at the St. Andrews Biological Station (45°04′57.57″N, 67°05′13.72″W). It was assumed that these micro-spheres move through the gut at the same rate as ingested food, and can therefore be used to track gut-passage time (Figure 2.3). Individuals were contained in covered funnels which were connected at the base of the funnel via rubber tubing to Teledyne Isco samplers (Figure 2.4), automated samplers that collect and store up to 24 water samples at a specified time interval. Samples were taken every 4 hours for 5-7 days. Water samples were filtered onto Whatman glass microfiber GF/C filters and examined with a dissecting microscope under UV light, with each filtered sample being associated with a specific time after deployment. The first sample in which micro-spheres were present in faeces were an indication of the minimum gut-passage time of *C. frondosa*. Sea cucumbers would often escape these funnels or cover the rubber tubing, therefore 3
successful trials were used for gut-passage time estimates. Water temperature, salinity, would be helpful including variability over time.

2.3.4 Field trials

In July 2014, specimens were starved in 1 µm filtered seawater for 5 days (based on gut-passage time estimations) to ensure their guts were empty of faeces. Individuals were haphazardly selected from holding tanks, and then deployed in Brandy Cove (45°05'02.88''N, 67°05'13.72''W) in a large weighted funnel (an inverted Bigfoot Systems concrete footing form with a plastic egg crate staging and a mesh covering) suspended in the water column by buoy at a depth of approximately 10m and allowed to feed for 2 days before being retrieved (Figure 2.5). Preliminary trials with these containment devices were monitored by underwater video during 72-hour time periods off the wharf at the St. Andrews Biological Station (45°04'57.57''N, 67°05'13.72''W), which determined that sea cucumbers would actively feed within 12 hours of acclimation in holding conditions. Five deployment trials were conducted over a period of approximately 3 months, with 3-4 individuals contained within a funnel at a time. However, because sea cucumbers were selected haphazardly from a group of 30-50 individuals, some may have been involved in trials more than once. Holding conditions did not allow for the separation of individuals that had previously been used in experiments. Individuals could not have been disposed of after each experiment, as they were needed for other experiments at SABS. Samples of the adjacent water column (3-5 per trial) were also taken during this process to determine the concentration of organic and inorganic particulates in the water upon which sea cucumbers were feeding. Upon retrieval, sea
cucumbers were placed in individual plastic rectangular 1.9 L containers with filtered seawater and brought back to the lab. These sealed containers were kept in a raceway flume and submerged in ambient seawater to keep them at a constant ambient temperature. Sea cucumbers remained in these containers for another 5 days to allow all the faeces produced during the feeding trial to be expelled. Sea cucumbers were removed and the total volume of water from these containers was filtered to ensure all faeces would be collected. No disintegration of faeces was noticed, though it is unlikely that disintegration would occur to such an extent as to not be captured during filtration.

2.3.5 Laboratory trials

Unfortunately, aquaculture site licensing issues prevented sea cucumbers from being deployed at a salmon farm site as they had been during preliminary feeding trials in the summer of 2014. Therefore, laboratory trials were conducted to simulate a similar diet assemblage of salmon feed and faeces. Laboratory trials followed the same starvation protocols as field trials. Individuals were held within a 48 L cylindroconical incubation tank supplied with approximately 3 L·min⁻¹ of water siphoned from a nearby salmon rearing tank at the St. Andrews Biological Station (Figure 2.6). Salmon within this tank were fed twice daily with Skretting Optiline 500™ commercial feed. Diet for these sea cucumbers therefore consisted of an assemblage of salmon feed and faeces. Five separate trials were conducted with 4-5 individuals per trial. There were 24 individual measurements in total. Again, because sea cucumbers were selected haphazardly, some individuals may have been involved in trials more than once. As in field trials, sea cucumbers were allowed to feed for 2 days before being retrieved and subsequently held
for an additional 5 days in filtered seawater in order to collect faeces. Samples of water from the salmon rearing tank were taken during feeding with an automated ISCO™ sampler.

2.3.6 Water samples

During field and laboratory trials, it was necessary to collect water samples in order to determine the concentration of particulate matter and inorganic matter in the diet *Cucumaria frondosa* had been feeding on. In the field, these were obtained through use of a Niskin bottle during the deployment of sea cucumbers at the beginning of a trial and during their retrieval at the end of a trial. The mean concentration value for each of these trials was used to calculate the respective feeding rates associated with them. Water samples in the laboratory were taken with an automated ISCO™ sampler were taken approximately once per hour for the duration of the feeding period.

2.3.7 Determining particulate and inorganic content

For feeding trials, water samples containing deposited faeces and those collected from the water column were filtered onto pre-ashed, pre-weighed glass microfiber filters (Whatman® GF/C) and rinsed with a 3% solution of ammonium formate to remove any salt residue. Filters were dried at 80°C for at least 12 hours and weighed. Total particulate matter was determined as the weight remaining after drying. Filters were then placed in aluminum weigh pans and ashed in a muffle furnace at 475°C for 5 hours (Nelson et al., 2012a). Total inorganic material was determined as the weight of material remaining after ignition.
2.3.8 Clearance, ingestion, and egestion rates

Total inorganic deposition of the faecal load was measured directly by weight loss-on-ignition. Clearance rate is a function of the quantity of inorganics produced by the animal (mg·day\(^{-1}\)) divided by the concentration of inorganics in the water upon which the organisms had fed (mg·L\(^{-1}\)). This was calculated using the biodeposition method (Bayne, 2004; Hawkins et al., 1996; Iglesias et al., 1998; Petersen et al., 2004).

\[
CR = \frac{(IRR + IER)}{PIM} \quad \text{(Equation 2.1)}
\]

Where IRR (inorganic rejection rate) is the inorganic concentration measured in the pseudofaeces (none produced in the present study), IER (inorganic egestion rate) is the inorganic concentration measured in the faeces, and PIM (particulate inorganic matter) is the concentration of inorganic matter in the diet.

By knowing how much inorganic material is in a liter of water and the quantity of inorganics deposited by the sea cucumber, it is possible to calculate how much water was cleared. During each deployment trial of sea cucumbers in the field, 3-5 water samples were taken at a depth of 10m. The mean value of these water samples for each respective deployment was used to calculate the clearance rate. During field experiments, preliminary work using underwater video revealed that *C. frondosa* took approximately 12 hours after transfer before resuming normal feeding activity. As they were deployed for two days, it was assumed that this acclimation period resulted in approximately 1.5 days of total feeding, though these estimations were not conducted with a rigorous methodology and are not associated with a variance across individuals or over time. Measured clearance rates were therefore divided by 1.5 in order to give a per day measurement. In laboratory experiments, sea cucumbers did not show the same reduction
in feeding (likely from not having experienced the same stress during deployment), therefore measured clearance rates were divided by 2 to reflect a full 2 days of feeding.

The amount of material ingested \((g \cdot \text{day}^{-1})\) was calculated as:

\[
IR(g \cdot \text{day}^{-1}) = CR(L \cdot \text{day}^{-1}) \cdot TPM (g \cdot L^{-1})
\]  
(Equation 2.2)

Where \(IR\) is the ingestion rate of a sea cucumber in one day, \(CR\) is the clearance rate, and \(TPM\) is the total particulate matter (both organic and inorganic) concentration of the diet. TPM was calculated based on the samples of natural seston collected during deployment and retrieval. As seston levels may vary temporally (1.4-8.6mg/L), the mean TPM for each respective trial (generally based on 3 water samples taken at the beginning and end of each trial, for a total of 6 samples per trial) was used in the calculation of ingestion rates.

Egestion rates were calculated by multiplying ingestion rates by the inverse of known absorption efficiencies of \(C. frondosa\). Absorption efficiencies are the percentage of ingested organic matter absorbed during passage through the digestive system (Conover, 1966). The inverse of this ratio would therefore be the amount egested by an organism (for example, an individual with an absorption efficiency of 75% would be expected to egest 25% of the food it had previously ingested). Absorption efficiencies can vary with organic content Nelson et al. (2012a). Therefore, absorption efficiencies used in the calculation of egestion rates were taken from Nelson et al. (2012a), where diets of comparable organic content were calculated. This study by Nelson et al. (2012a) dealt generally with diets of relatively high and uniform organic content such that the relationship of organic content to absorption efficiency was linear. However, there is evidence that when diets of low organic content are included, there is a logarithmic
relationship that plateaus somewhat at higher organic contents (Hawkins et al., 1996), which may explain the linear relationship of Nelson et al. (2012a). There are few studies that have looked at the relationship between diet organic content and absorption efficiencies of sea cucumbers – particularly suspension feeding sea cucumbers at lower organic content. One study measured the absorption efficiency of the deposit feeding sea cucumber *Parastichopus parvimensis* feeding on sediment of low organic content, though no high organic content sediments were measured, and thus a logarithmic relationship with sea cucumbers could not be confirmed (Yingst, 1976). Few studies have measured absorption efficiencies with diets low in organic content, making comparisons among species difficult. It was assumed that due to their similar physiologies, absorption efficiencies of *C. frondosa* are more comparable to other sea cucumber species (although most are deposit rather than suspension feeders) than they are to bivalves and other more distantly related species. Absorption efficiency data of sea cucumbers associated with lower organic content (Yingst, 1976) was therefore added to those of Nelson et al. (2012a) to create a hypothetical logarithmic relationship for *C. frondosa* feeding upon a lower organic content diet. This relationship was used to estimate egestion rates (Figure 2.7).

\[
ER(g \cdot day^{-1}) = IR(g \cdot day^{-1}) \cdot AE
\]  

(Equation 2.3)

where ER is the egestion rate if *C. frondosa* and AE is the Absorption Efficiency at a given diet organic content.
2.3.9 Statistical analyses

In each trial data were tested for assumptions of normality (Shapiro-Wilk test, $\alpha=0.05$) and homogeneity of variance (Levene’s test, $\alpha=0.05$).

Mean concentrations of inorganic material in sediment traps containing sea cucumbers and in control traps without sea cucumbers were) met assumptions of normality and were compared using independent samples t-tests ($\alpha=0.05$).

Concentration of total particulate concentration, inorganic concentration, and organic content (of diet in the field and in the laboratory) did not meet assumptions of normality, even after log-transformation. Mann-Whitney U Tests were therefore conducted comparing concentration of total particulate concentration, inorganic concentration, and organic content between field and laboratory conditions.

Clearance, ingestion, and egestion rates did not meet assumptions of normality. For the purpose of transforming data in order to meet assumptions of normality, clearance rates were converted from L·individual$^{-1}$·day$^{-1}$ to mL·individual$^{-1}$·day$^{-1}$. This was done in order to for all data values to be $\geq 1$ such that they could be log-transformed.

Log-transformed data met the assumptions of normality and for homogeneity of variance. Log-transformed clearance, ingestion, and egestion rates were compared between the two treatments (field and laboratory conditions) using an independent samples t-test (IBM SPSS Statistics 23).
2.4 Results

2.4.1 Preliminary feeding trials

Concentration of inorganic material in sediment traps containing sea cucumbers were not greater than concentrations in control sediment traps, indicating that sea cucumbers would not feed readily within the confines of sediment traps. Control traps often had higher levels of deposition than treatment traps, suggesting that sea cucumbers were not depositing material at a detectable level (or at least not at a level higher than the settlement of natural seston). Instead, they likely acted as a plug, blocking the settlement of natural seston while not depositing material themselves. Clearance rates calculated using the bio-deposition method (Prins et al, 1991; Iglesias et al, 1992) were often negative, indicating that no ingestion or egestion had taken place (Table 2.1). There was no significant difference in inorganic concentration between control and treatment traps at the salmon farm site (p=0.49, df=2.6, t=-0.80) or the reference site (p=0.26, df=2.5, t=1.5). In most cases, concentrations were lower in traps containing sea cucumbers, suggesting that they were blocking the settlement of material rather than increasing settled material through defecation (Figure 2.8). While sometimes traps containing sea cucumbers did accumulate more settled material than their respective control traps (Figure 2.8), it is not likely that sea cucumbers would reliably or consistently feed within sediment traps (perhaps from the confined space, sharp and irritating mesh material, or motion of traps in the current). Subsequent underwater video monitoring of sediment trap frames confirmed that little, if any, feeding had taken place within them and the technique was not appropriate for quantifying clearance, ingestion, or egestion rates.
2.4.2 Gut-passage time

Estimations of gut-passage time were obtained from field experiments and used to calculate clearance, ingestion, and egestion rates. Experiments were based on 3 trials (Table 2.2). Gut-passage time ranged from 64-96 hours. Water samples were taken every 4 hours therefore limiting the estimation of defecation to within a 4 hour window. Sea cucumbers had a mean gut-passage time of 79 hours (SD = 15.14) based on the last 4 hour window. As limited trials were conducted and because sea cucumbers can show high individual variability (Hamer, 2010), 96 hours (the longest gut-passage time observed) was used as a conservative estimate when all sea cucumbers had defecated. In field experiments, sea cucumbers were therefore starved in the laboratory for at least 96 hours to ensure their guts were empty of faeces and no faecal material collected had come from feeding during husbandry in the lab.

2.4.3 Particulate concentration in water

Total particulate matter (TPM) (both organic and inorganic) in the water column in the field had a mean concentration of 5.6 mg·L⁻¹ and ranged from 3.2 mg·L⁻¹ to 6.6mg·L⁻¹ (SD = 1.4 mg·L⁻¹). Inorganic matter was similarly variable, ranging from 2.7 mg·L⁻¹ to 4.6 mg·L⁻¹, with a total mean concentration of 4.0 mg·L⁻¹ (SD = 0.81 mg·L⁻¹). Organic content was generally more stable, with a mean content of 29% (SD = 7.0). Results are summarized in Table 2.3.

Particulates from the laboratory salmon tank had a similar organic content of 28% (SD = 5.7), not significantly different from those in the field (p=0.47, U=9.0, W=24). Total particulate and inorganic concentrations from the salmon rearing tank were generally
higher than those in the field. Particulate matter in the tank had a mean concentration of 9.4 mg·L\(^{-1}\), ranging from 6.5 mg·L\(^{-1}\) to 15 mg·L\(^{-1}\) (SD = 3.3 mg·L\(^{-1}\)), significantly different from concentrations in the field (p=.01, U<0.00, w=15). Inorganic concentrations were also higher with a mean value of 6.9 mg·L\(^{-1}\), ranging from 5.7 mg·L\(^{-1}\) to 12 mg·L\(^{-1}\) (SD = 2.7 mg·L\(^{-1}\)), which was significantly different from the field (p=0.04, U=3.0, W=18). Results are summarized in Table 2.4.

In both the field and in the laboratory, the concentration of diet varied considerably day to day. The organic content of the diet also varied depending on the day, though this was less pronounced in the laboratory with water supplied from a salmon rearing tank.

2.4.4 Clearance, ingestion, and egestion rates

Clearance rates for sea cucumbers feeding in the field (n=19) had a mean rate of 7.2 L·individual\(^{-1}\)·day\(^{-1}\) (SD=3.6) while those in the laboratory had a mean rate of 3.6 L·individual\(^{-1}\)·day\(^{-1}\) (SD=1.9) (Table 2.5, Figure 2.9). There was a significant difference between mean clearance rates of individuals feeding in the field and those feeding on assemblages of salmon feed and faeces in the laboratory (p<0.001, df=40, t=5.1).

Mean ingestion rates of *C. frondosa* feeding in the field (38 mg·individual\(^{-1}\)·day\(^{-1}\); SD=0.02, n=19) and in the laboratory (30 mg·individual\(^{-1}\)·day\(^{-1}\); SD=0.01, n=24) were not significantly different (p<0.001, df=40, t=-7.0; Table 2.6, Figure 2.10).

Log-transformed mean egestion rates were not statistically significantly different (p=0.08, df=40, t=1.8) between sea cucumbers feeding in the field (13.1 mg·individual\(^{-1}\)·day\(^{-1}\); SD=0.01, n=19) and in the laboratory (10.6 mg·individual\(^{-1}\)·day\(^{-1}\); SD=0.01, n=24). The organic content of diet in the field and in salmon tank outflow was 29% and 28%,
respectively. Therefore, the absorption of efficiency of *Cucumaria frondosa* was estimated to be 55% using the equation in Figure 2.7. Using this estimate, sea cucumbers in the field egested approximately 13.1 mg·individual$^{-1}$·day$^{-1}$ (SD=0.01). Rates of egestion in the laboratory had a mean value of 10.6 mg·individual$^{-1}$·day$^{-1}$ (SD=0.01) (Table 2.7, Figure 2.10).

2.5 Discussion

Water clearance rates, ingestion rates, and egestion rates of *Cucumaria frondosa* were all higher when feeding on a natural diet in the field than when feeding on an assemblage of waste particles in the salmon tank in the lab, though only water clearance rates were statistically significant. It is likely then that these differences are a consequence of the conditions in which the sea cucumbers were held more than an effect of the diet itself. *Cucumaria frondosa* feeds most actively when exposed to large volumes of high-flow water, with an optimum rate of tentacle insertion (an indicator of food intake) at a water velocity of 55 cm·s$^{-1}$ (Holtz and MacDonald, 2009). When deployed in the field, sea cucumbers were exposed to dynamic natural conditions, able to feed more actively and extend their tentacles into the water column when conditions were favorable. In the laboratory however, the flow-through system devised to hold the sea cucumbers could not maintain the same high-flow, high-volume water supply, potentially leading to less favourable conditions. The flow may have been further slowed through the blockage of the outflow by the sea cucumbers themselves, whose pliable bodies had a tendency to plug the pipes of their holding tanks if they were given the opportunity. Feeding activity may have also been higher in field experiments due to the geometry of the containers.
themselves. *Cucumaria frondosa* lives on rocky substrates (Jordan, 1972), where the irregular surface likely provides many anchor points for the sea cucumber’s many tube feet, allowing for stability. Sea cucumbers will retract their tentacles and stop feeding when disturbed or stressed. Therefore, the large funnels that housed sea cucumbers in the field, with their indentations and ribbed reinforcements, would have provided a more suitable substrate than the smooth surface of the incubation tank which housed sea cucumbers during laboratory experiments. Laboratory experiments likely did not provide sea cucumbers with ideal or even adequate feeding conditions and would not be a good representation of ingestion/egestion/clearance rates. While feeding activity is thought to be primarily associated with food availability (Singh et al., 1999), it is also possible that individuals were not kept at optimal temperatures or salinities, which may have affected metabolism.

While the values obtained from field experiments are likely accurate, they are also lower than those of other taxa (Kryger and Riisgard, 1988; Horgan and Mills, 1997; Orr, 2012). Although these species all utilize different feeding strategies, comparisons between levels of ingestion, egestion, and clearance will still be relevant to their potential inclusion in aquaculture sites. *Cucumaria frondosa* cleared approximately 7.2 L of water in one day. Blue mussels (*Mytilus edulis*), which would likely be farmed alongside sea cucumbers as an organic extractive species at IMTA sites, have shown considerably higher water clearance rates despite being comparatively smaller than *C. frondosa*. One study measured the clearance rates of blue mussels exposed to varying concentrations of fish food and microalgae, comparable to what they may be exposed to at an IMTA site. Under these conditions, *M. edulis* cleared an average of 2 L per hour, or 48 L per day.
(MacDonald et al., 2011). Petersen et al. (2004) used the bio-deposition method to measure clearance rates in mussels in two locations (not associated with aquaculture sites) in the field, where clearance rates were 3.2 and 2.2 L per hour, or 77 and 53 L per day. Another study found that, when exposed to a diet assemblage of fish waste in the laboratory, mussels cleared about 0.15 L per hour (or 3.6 L per day), suggesting that clearance rates can be highly variable, at least between diets (Orr, 2012) The comparatively higher water clearance rate of bivalves also holds true for other bivalve species – for example, the hard clam (Mercenaria mercenaria; Bricelj and Malouf, 1984), the zebra mussel (Dreissena polymorpha; Horgan and Mills, 1997), the basket cockle (Clinocardium nuttali; Orr, 2012), and the pacific oyster (Crassostrea gigas; Smaal and Zurburg, 1997). Each of these commercial important bivalves had clearance rates several times higher than that of C. frondosa. This could be due to the different feeding strategies of the two species, with mussels actively pumping water and particulates across their gill and efficiently capturing particulates, while sea cucumbers feed more passively and opportunistically ingest particulates that adhere to their tentacles.

In the field, C. frondosa ingested approximately 40 mg per day. Orr (2012), fed California sea cucumbers (Parastichopus californicus, a species being considered for implementation into IMTA sites on the west coast of Canada) diet assemblages consisting of sablefish (Anoplopoma fimbria) waste (an organic content of 87%) and natural sediment (organic content of 1.6%). The California sea cucumbers ingested approximately 40 mg·day⁻¹ and 90 mg·day⁻¹, respectively – potentially double that of C. frondosa, though similar at the lower end of this range. The green sea urchin (Strongylocentrotus droebachiensis) is another benthic species being considered as an
organic extractive species at IMTA sites both on the east and west coast of Canada. When feeding on diets of fish waste, the urchins ingested approximately 40 mg per day (Orr, 2012). Comparatively higher rates of ingestion are regularly seen in a variety of other species of sea cucumbers (1500 mg·g⁻¹·day⁻¹; Yuan et al., 2006), sea urchins (300 mg·day⁻¹; Fernandez and Boudouresque, 2000), and bivalves (1400 mg·day⁻¹ MacDonald and Ward, 1994).

In the field, Cucumaria frondosa egested approximately 17 mg·day⁻¹. For comparison, California sea cucumber egested about 220 mg per day (Orr, 2012). When feeding on an assemblage of fish waste, blue mussels egested approximately 4.4 mg·day⁻¹, less than C. frondosa (Orr, 2012). However, the Orr (2012) study also measured the clearance rates of Mytilus edulis as being much lower than in other studies, suggesting there may be less feeding activity. In cases where reported mussel clearance and ingestion rates are higher (MacDonald et al., 2011), egestion rates may be comparable to C. frondosa due to the relatively high absorption efficiency (and thus relatively low egestion compared to ingestion) of blue mussels (Reid et al., 2010). While not measured directly in the study, the egestion rate of the green sea urchin can be inferred through the measured ingestion rate and absorption efficiency as being approximately 270 g·day⁻¹ (Orr, 2012). Should C. frondosa be implemented into IMTA, farmers would certainly be targeting a species with a lower egestion rate and contributing less to nutrient loading at the site. Ideally, this would be a function of a high rate of ingestion with a high absorption efficiency. While the egestion of C. frondosa is low in this case, it is likely due to a reduced feeding activity rather than an extremely high absorption efficiency (Hawkins et al., 1996; Nelson et al., 2012a). Egesta is likely proportional to ingested
material, allowing for estimations of faecal loading around the farm which can be adjusted with changes in feeding rates. However, farmers’ decision to implement a species into IMTA will be an economic one based on the market value of the species itself, as well as the value of its organic extraction abilities. As there is currently no definition about what amount of conversion of waste effluent is necessary to be considered to be an effective IMTA species, the rate of ingestion/clearance/absorption a farmer would hope to see is unclear. It may be that for a species to be a worthwhile addition to IMTA, it would not necessarily need to rid the farm of the majority of its organic loading burden. Rather, a relatively small (perhaps 5%) net reduction of organic loading could be sufficient to avoid eutrophication. Depending on the net reduction goal, stocking density, and effective placement within an IMTA site, *C. frondosa* may be capable of accomplishing such a reduction. The effectiveness of an IMTA species will be dependent on the amount of ingestion/absorption of material, as well as whether the organism is reducing net organic loading by ingesting more organic material from the farm than they do from outside the farm.

Comparatively lower feeding activity of *C. frondosa* than other potential IMTA species could be due to a number of factors. In some cases, the bio-deposition method of measuring water clearance can give significantly lower estimates than rates measured by other methods (Petersen et al., 2004). One explanation for this could be due to an overestimation of the particulate concentration in the seston available to the organism, thus leading to an underestimation of clearance rate (Petersen et al., 2004). When measuring the seston upon which the sea cucumbers were feeding, water samples were analyzed using filters with an advertised retention efficiency of approximately 1.2 µm
according to Millipore Sigma. As more material is captured on this glass fiber matrix, particles even smaller than this limit may be retained. If sea cucumbers are not able to capture and ingest these small particulates, the amount of diet available to them will therefore be overestimated. When using the bio-deposition method to measure clearance rates in bivalves, which feed by actively drawing water through their siphons and capturing particulates on a filter-like gill that may have low retention efficiencies for very small particulates, this can lead to overestimation. It is not known whether the particulate-trapping mucus on the tentacles of *C. frondosa* would have the same concern with retention efficiencies. Furthermore, the bio-deposition method has been reliable in other studies and is generally considered to be a reliable methodology that is particularly useful for in situ measurements (Iglesias et al, 1998; Navaro and Velasco, 2003). The data in the present study likely represent accurate estimate of feeding rates in the conditions experienced by these sea cucumbers. While unrestricted and optimally feeding sea cucumbers in the wild may indeed have higher rates of feeding activity than those I measured, these data likely provide a minimum estimate of feeding rates. They are likely accurate with respect to *C. frondosa* in captivity in any case, and future husbandry/containment/farming activity should take this into account.

Lower estimates of feeding activity than other potential IMTA species could also be related to the manner in which ingestion and egestion rates were estimated. One of the advantages of using egested material (as in the bio-deposition method) to calculate clearance and ingestion rates is that egestion rates will have consequently been measured directly, provided all egested material is collected and weighed. As there was relatively little material ingested by individual sea cucumbers and it could not be assumed that all
had been collected, the entirety of the water sample containing egested material was filtered to ensure all of the faeces were collected. Water samples, however, would also contain mucus shed from the sea cucumbers, as well sloughing of skin and tube feet. This excess organic material could lead to to an overestimation of the total egested material. The amount of inorganic matter was not affected to the same degree by this excess material (due to its high organic content) and was still able to be measured directly. Clearance rates (calculated by dividing the faecal inorganic production by the concentration of inorganics in the diet) were therefore also not affected and should be an accurate representation of feeding activity. Ingestion rate was calculated by multiplying the clearance rate by the total concentration of material (both organic and inorganic) in the water. Organic sloughing prevented a reliable measurement of egestion rates, making it necessarily to estimate these rates based on absorption efficiency.

Egestion rates were originally calculated by measuring the total dry weight of material settled on the filter (before ignition in the muffle furnace), but organic material was found to be much higher in deposited material than in the diet upon which they had been feeding. Faecal material can sometimes have organic content higher than the diet if the organism is a selective feeder and ingesting only those particulates with the highest organic content. With the observed presence of skin, mucus, and tube feet it was assumed that in this case the high organic content was a result of sloughing rather than selective feeding. Assuming calculated ingestion rates are accurate, the amount of faeces produced will be the portion of ingested material that is not absorbed by *C. frondosa*. As absorption efficiency was not measured in this study, this value was estimated based on data from Nelson et al. (2012a), which showed the relationship of absorption efficiency to
corresponding organic content of diets. However, Nelson et al. (2012a) dealt with diets of higher organic content than the present study, so a hypothetical logarithmic relationship for *C. frondosa* feeding upon a lower organic content diet was developed. Values for absorption at lower organic contents were based on a different species of sea cucumber (*Parastichopus parvimensis*; Yingst, 1976). Absorption efficiencies can vary with diet organic content (Yingst, 1976; Hawkins et al, 1996) between species and taxa (Reid et al, 2010; Orr, 2012; Nelson et al, 2012). Few studies have measured absorption efficiencies with diets of low organic content, making comparisons between species and taxa difficult. Yingst (1976) measured absorption efficiencies of the deposit-feeding sea cucumber *Parastichopus parvimensis* which, due to its different feeding strategy, may have an absorption efficiency that varies considerably from *C. frondosa*. It could be that the absorption efficiency of *C. frondosa* is more similar to distantly related taxa. However, with the paucity of data associated with absorption efficiencies at low organic contents, it was assumed that the more similar physiologies between *C. frondosa* and *P. parvimensis* (compared to more distantly related taxa) would provide the best estimated. Deposit-feeding sea cucumbers can have lower absorption efficiencies than other species/taxa (Orr, 2012). Therefore, variation between *C. frondosa* and *P. parvimensis* would most likely lead to an underestimation of absorption efficiency, and thus an overestimation of egestion rate.

This method of calculating egestion based on reported absorption efficiencies therefore only provides an estimate of egestion rates. It is also not known whether a logarithmic relationship would exist between organic content and absorption efficiency for *C. frondosa* if measured directly. However, this does appear to be the case when the
data of Yingst (1976) and Nelson et al. (2012a) are combined (Figure 2.7). While these two sets of data could also be analyzed using two set of linear regressions rather than a singular logarithmic relationship, the limited number of values associated with Yingst (1976) data would lead to a similar slope, and thus similar absorption efficiencies. Furthermore, several studies indicate that logarithmic relationships are common when comparing absorption efficiency to organic content (Cranford et al., 1995; Hawkins et al., 1996; Reid et al., 2010).

Quantifications of ingestion/egestion/clearance rates in the present study are likely reliable reflections of the conditions experienced by these sea cucumbers in my study. While they may not represent sea cucumbers feeding at their most optimal conditions, they also do not represent a failure of quantification methodology. Future studies may benefit from these relatively novel methods, while improving the holding conditions and environment to encourage optimal feeding.

Feeding activity of *C. frondosa* in my study could have also been inhibited by stress. When disturbed, sea cucumbers will often retract their tentacles and cease feeding when their tentacles are disturbed. Other times, when conditions are not optimal, they will instead extend only the tips of their tentacles slightly from the mouth and not fully engage in feeding (personal observations). When contained somewhere without adequate space/current/substrate/stability, *C. frondosa* will not fully extend their tentacles and feed optimally. This has been a common problem during laboratory experiments in particular (Sutterlin and Waddy, 1976; Singh et al., 1998; Holtz and MacDonald, 2009; Hamer, 2010; Nelson et al., 2012a). For example, when attempting to measure retention efficiencies, Hamer (2010) needed to adjust volume exchange rates, aeration and food
concentration over the course of a month before sea cucumbers would extend their tentacles and feed normally. Despite these adjustments, feeding rates in the Hamer (2010) study were too low to effectively measure retention efficiencies. While sea cucumbers in the present study would extend their tentacles in the laboratory without these continual adjustments, often they would not appear to be fully active. During the design and testing of equipment to contain sea cucumbers in the field, preliminary underwater camera work was conducted. It appeared that sea cucumbers would feed fully after approximately 6 hours after deployment. However, these were only estimates of 3-5 separate trials and there was no systematic measurement or statistical analysis of feeding activity based on this underwater camera work. It could be that sea cucumbers did not feed optimally in this scenario either, likely because of the relative instability of being suspended above the benthos. The organic sloughing of visible skin and mucus found in the water samples used for faecal sampling could be a stress response associated with this handling and containment, as epithelial sloughing will occur in bivalves (Potter et al., 1997; Molloy et al., 2013) and sea cucumbers (Gianasi et al., 2016a) when exposed to environmental stressors. Should C. frondosa be implemented in IMTA, infrastructure and containment should be constructed such that they are able to feed optimally.

Preliminary work estimating the gut-passage time of C. frondosa was also important in the quantification of clearance, ingestion, and egestion rates. The bio-deposition method requires that all faecal material be collected in order to calculate clearance rate. Therefore, if sea cucumbers were not contained for the full duration of their gut-passage time, then some faeces would be left in the gut and not collected. This is unlikely to be the case, as the 5-day holding period used (from preliminary experiments
suggesting a 3-5 day gut-passage time) likely gave sea cucumbers ample time to evacuate any ingested material. This is comparable to other echinoderms, with urchin gut-passage times ranging from 3-4 days (Klinger et al., 1994; Thompson and Riddle, 2005). Gut-passage time can also vary between locations, likely due to temperature. Tropical urchins had a gut-passage time of 7 hours (Lares and McClintock, 1991), compared to 63 hours in the Bay of Fundy (LeGault and Hunt, 2016). It is somewhat surprising that *C. frondosa* could take up to 5 days (though this was the maximum length of passage and was therefore used to represent the most conservative estimate) to egest all faecal material, given that 4-day gut-passage times in urchins mentioned above have taken place in cold Antarctic waters, where it would be expected to be slowest (Miller et al, 2014).

*Cucumaria frondosa* also show high individual variability (Hamer, 2010) and a full 5-day gut-passage time may only occur in some individuals. Gut-passage time was also important in determining the time period in which sea cucumbers were starved to ensure their guts were empty and any faeces produced was the result of feeding trials. While starvation may have been a stressor for the sea cucumbers (and this reduced feeding activity), it was necessary to control the time period during which faeces was produced. Starvation has also been used in other studies involving sea cucumbers (Dong et al., 2008; Liu et al., 2009). Prolonged periods of feeding inactivity are also common for *C. frondosa*, as feeding behaviour varies with seasons and tides (Hamel and Mercier, 1998; Singh et al, 1999). Starvation may have also had the effect of making sea cucumbers hungrier and resulted in subsequently higher feeding rates.

If the observations in the present study are accurate, low water clearance and ingestion rates of *C. frondosa* suggest that the species may have limited potential as an
organic extractive species, especially when compared to bivalves and other echinoderms. These results may be best viewed as minimum estimates of feeding activity rather than a complete picture of the extractive potential of *C. frondosa*. While feeding activity can often be reduced in the laboratory (Sutterlin and Waddy, 1976; Holtz and MacDonald, 2009; Hamer, 2010; Nelson et al., 2012a) and ingestion rates are difficult to measure in the field, the data given by the present study provide an estimation of at least the lower limit of clearance and ingestion rates. *Cucumaria frondosa* also feeds mainly during the spring and summer (Hamel and Mercier, 1998; Singh et al, 1999), which could result in lower bio-mitigation during the fall and winter. Nevertheless, ingestion and clearance rates would almost certainly be higher when individuals are less stressed in more optimal field conditions. Lander et al. (unpublished data) have recently improved holding conditions such that *C. frondosa* has shown ingestion rates 10x higher than in the present study. Whether this probable increase in feeding activity would lead to a significant reduction of organic load is unknown. There are also logistical constraints on the number of individuals that can be housed within a farm. As a simple (if arbitrary) model, consider a cage containing 30,000 salmon producing 200 kg of faeces per day. Using what currently appears to be the maximum ingestion rate of Lander et al. (unpublished data), it would take approximately 500,000 sea cucumbers to remove 10% of salmon faeces. Under a cage with a surface area of 796m², this would require a stocking density of over 600 individuals per m². It would require an increase in ingestion of another order of magnitude in order to achieve a logistically possible stocking density. Though improvements in holding conditions and knowledge of sea cucumber behaviour and biology will continue to improve, such an increase in activity seems overly ambitious.
As it is also unclear what level of extraction would be necessary for sea cucumbers to be a viable IMTA species, even if these data represent the minimum amount of feeding activity that would be exhibited by sea cucumbers, this does not necessarily disqualify *C. frondosa* from IMTA implementation.

Low egestion rates, on the other hand, may be promising from an IMTA perspective. Observed ratios of egesta to ingesta were approximately 35%, allowing for a prediction of the quantity of material sea cucumbers would be contributing to benthic loading. When feeding readily on high quality diets, sea cucumbers are relatively efficient at absorbing organic material, with absorption efficiencies of particles at IMTA sites being comparable to those in natural field conditions (Nelson et al., 2012a). A high absorption efficiency and a low egestion rate means that the contribution of sea cucumbers to the organic loading at an IMTA site may be minimal. Depending on the settling velocity of this faeces, the total deposition of the aquaculture site may be reduced. When fed sablefish waste, the California sea cucumber (*Parastichopus californicus*) produced faeces with a lower settling velocity than other cucumbers feeding on natural sediment (Orr, 2012). A lower settling velocity leads to a wider dispersion of egested material (Liutkus et al., 2012), allowing for a more dilute waste product and a lower total deposition rate at a given site. This also allows for more farm-environment interactions, as effluent is spread over a wider area (Orr, 2012).

The degree to which sea cucumbers may contribute to bioremediation will depend on the proportion of their diet that is comprised of waste effluent. Though feeding activity and clearance/ingestion rates may be low, if the diet of *C. frondosa* is made up of mostly farm waste that they can efficiently absorb, then there will be a net reduction in
organic load. If their diet comprises mostly natural seston and they are depositing onto the benthos, they will be contributing to an increase in organic loading. A physiological model by Reid et al. (2013) predicted that a mussel’s diet would need to comprise of 10-20% salmon aquaculture waste to have a net extractive effect. While this estimation may be different for sea cucumbers (depending on the quality of the diet and the associated absorption efficiency), it may actually be easier for them to achieve a net decrease in organic loading. At IMTA sites, mussels are situated floating adjacent to (or in the future, perhaps directly underneath) salmon cages and feeding on material that is being dispersed laterally away from the cage, generally feeding on fine particulates with low settling velocities. In some cases the effect of waste effluent adjacent to farms in minimal and episodic (Brager et al., 2015). Should farmers choose to implement sea cucumbers into an aquaculture site, they would be situated on the benthos or in mid-water cages. Here, they would be exposed to the vertical flux of heavier particulates that settle beneath the cages, an important step in bioremediation (Department of Fisheries and Oceans Canada, 2013; Cranford et al., 2013). In cases where heavier settling solids make up the majority of particles under a salmon cage, they will likely also make up the majority of a sea cucumber’s diet and thus lead to a net decrease in organic loading. This would depend on where precisely sea cucumbers are situated on the benthos, whether it is directly beneath a cage or adjacent to it and away from the vertical flux of particles.

While *Cucumaria frondosa* may not have the ability to clear extremely large volumes of particulates from the water, they may still lead to a reduction in organic material at IMTA sites, especially if technology and behavioural/physiological knowledge continues to develop. Their potential as an IMTA extractive species will
therefore depend on a number of factors. Specifically, on the east coast of Canada, this will depend on the respective bioremediation and economic potential of *C. frondosa* and the other benthic species being considered for IMTA, such as the green sea urchin *Strongylocentrotus droebachiensis*. Strongylocentrous *droebachiensis* has been found to have ingestion rates many times higher than those of *C. frondosa* measured in this study (Orr, 2012). It could be that the limited space on an IMTA site may be better filled with the more voracious sea urchin which will likely have the larger total decrease in waste effluent. This may also be hampered by the potential marketability of sea urchins in an IMTA context, as fish-based protein can result in off-flavouring in urchin roe (Siikavuopio et al., 2007). *Cucumaria frondosa* may have the economic viability to make up for its (potentially) limited use as an organic extractive species, though it is also not without its challenges. It has a relatively low market price and would thus be a high-volume low-value crop (Nelson et al., 2012b; Pietrak et al., 2014). Given their relatively low individual clearance/ingestion rates and their tendency to form dense assemblages (Jordan, 1972; Singh et al., 1999), this is likely how they would be farmed regardless of market price. More valuable species of sea cucumbers have historically been overexploited due to high market demand, leading to declining stocks (Nelson et al., 2012b; Gianasi et al., 2016b). This has led to interest in underutilized cold-water species (Hamel and Mercier, 2008) and the potential for the creation of unexploited niche markets (Orr, 2012; Gianasi, et al., 2016b). *C. frondosa* in particular may be marketable as food, as well as in pharmaceutical and nutraceutical industries (Bordbar et al., 2013). Thus, their inclusion in IMTA sites will depend on whether their value derives from having a significant impact on organic material as a powerful organic extractive species,
or whether it is derived from being an economically feasible crop that yields a small but reliable decrease in organic loading.
2.6 Literature cited


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MacDonald, B. A., Robinson, S.M.C., and Barrington, K.A. (2011). Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multi-trophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. Aquaculture, 314, 244-251.


2.7 Tables

Table 2.1: Mean (and standard deviations) clearance rates of sea cucumbers (mg·individual$^{-1}$·day$^{-1}$) during 3 preliminary feeding trials wherein sea cucumber (Cucumaria frondosa) were housed in sediment traps attached to structural frames (3 sea cucumbers per frame) at a salmon farm site and reference site approximately 1 km away.

<table>
<thead>
<tr>
<th>Farm site</th>
<th>Mean clearance rates (L·individual$^{-1}$·day$^{-1}$)</th>
<th>Standard deviation (L·individual$^{-1}$·day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frame 1: -0.00</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Frame 2: 0.03</td>
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</tr>
<tr>
<td></td>
<td>Frame 3: -0.07</td>
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</tr>
<tr>
<td>Reference site</td>
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</tr>
<tr>
<td></td>
<td>Frame 2: -0.18</td>
<td>0.16</td>
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<td></td>
<td>Frame 3: -0.54</td>
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</tr>
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</table>

Table 2.2: Results of preliminary trials used to estimate gut-passage time of the sea cucumber Cucumaria frondosa. Sea cucumbers were contained in funnels off the wharf of the St. Andrews Biological station and water samples were taken at 4-hour intervals using an automated sampler.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Gut passage time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64-68</td>
</tr>
<tr>
<td>2</td>
<td>92-96</td>
</tr>
<tr>
<td>3</td>
<td>68-72</td>
</tr>
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</table>
Table 2.3: Mean (and standard deviations) levels of total particulate matter, total inorganic matter, and organic content of the water upon which the sea cucumber *Cucumaria frondosa* fed during each feeding trial in the field. These values were used in the calculation of clearance and ingestion rates for during each respective trial. Masses are dry weights.

<table>
<thead>
<tr>
<th>Trial number</th>
<th>n</th>
<th>Mean total particulate matter (mg·L⁻¹)</th>
<th>SD of total particulate matter (mg·L⁻¹)</th>
<th>Mean total inorganic matter (mg·L⁻¹)</th>
<th>SD of total inorganic matter (mg·L⁻¹)</th>
<th>Mean organic content (%)</th>
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<td>1.3</td>
<td>4.2</td>
<td>1.1</td>
<td>32.3</td>
</tr>
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</table>

Table 2.4: Mean (and standard deviations) levels of total particulate matter, total inorganic matter, and organic content of the water upon which the sea cucumber *Cucumaria frondosa* fed during each feeding trial in the laboratory. These values were used in the calculation of clearance and ingestion rates for during each respective trial. Masses are dry weights.

<table>
<thead>
<tr>
<th>Trial number</th>
<th>n</th>
<th>Mean total particulate matter (mg·L⁻¹)</th>
<th>SD of total particulate matter (mg·L⁻¹)</th>
<th>Mean total inorganic matter (mg·L⁻¹)</th>
<th>SD of total inorganic matter (mg·L⁻¹)</th>
<th>Mean organic content (%)</th>
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<td>5.7</td>
<td>3.3</td>
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Table 2.5: Summary of descriptive statistics of clearance rates (L·individual⁻¹·day⁻¹) of the sea cucumber *Cucumaria frondosa* feeding on particulate assemblages from a salmon (Salmo salar) rearing tank in the laboratory and natural seston in the field (Brandy Cove, New Brunswick; 45°05′02.88″N, 67°05′13.72″).

<table>
<thead>
<tr>
<th>Trial number</th>
<th>n</th>
<th>Mean total particulate matter (mg·L⁻¹)</th>
<th>SD of total particulate matter (mg·L⁻¹)</th>
<th>Mean total inorganic matter (mg·L⁻¹)</th>
<th>SD of total inorganic matter (mg·L⁻¹)</th>
<th>Mean organic content (%)</th>
<th>SD of mean organic content (%)</th>
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<tbody>
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<td>9.8</td>
<td>4.8</td>
<td>7.2</td>
<td>2.8</td>
<td>25.9</td>
<td>0.07</td>
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</tbody>
</table>

<table>
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<th></th>
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<th>Mean total particle clearance rate (L·individual⁻¹·day⁻¹)</th>
<th>SD of mean total particle clearance rate (L·individual⁻¹·day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field clearance</td>
<td>19</td>
<td>7.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Laboratory clearance</td>
<td>24</td>
<td>3.6</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Table 2.6: Summary of descriptive statistics of ingestion rates (mg-individual\(^{-1}\)·day\(^{-1}\)) of the sea cucumber *Cucumaria frondosa* feeding on particulate assemblages from a salmon (Salmo salar) rearing tank in the laboratory and natural seston in the field (Brandy Cove, New Brunswick; 45°05′02.88″N, 67°05′13.72″W).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean total particle ingestion rate (mg-individual(^{-1})·day(^{-1}))</th>
<th>SD of mean total particle ingestion rate (mg-individual(^{-1})·day(^{-1}))</th>
<th>Std. Error of mean total particle ingestion rate (mg-individual(^{-1})·day(^{-1}))</th>
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<td>19</td>
<td>38</td>
<td>18</td>
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<tr>
<td>Laboratory ingestion</td>
<td>24</td>
<td>30</td>
<td>12</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 2.7: Summary of descriptive statistics of egestion rates (mg-individual\(^{-1}\)·day\(^{-1}\)) of the sea cucumber *Cucumaria frondosa* feeding on particulate assemblages from a salmon rearing tank in the laboratory and natural seston in the field.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean total particle egestion rate (mg-individual(^{-1})·day(^{-1}))</th>
<th>SD of mean total particle egestion rate (mg-individual(^{-1})·day(^{-1}))</th>
</tr>
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<tbody>
<tr>
<td>Field egestion</td>
<td>19</td>
<td>13</td>
<td>6.3</td>
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<tr>
<td>Laboratory egestion</td>
<td>24</td>
<td>11</td>
<td>4.3</td>
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</tbody>
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Figure 2.1: (A) Feeding behaviour of the sea cucumber *Cucumaria frondosa*: inserting a tentacle into its open mouth and (B) closing mouth and removing food from tentacle.
Figure 2.2: (A) PVC pipe sediment traps attached to an aluminum frame. Traps are 10.2 cm in diameter and approximately 85 cm long. (B) Frame with sediment traps being lowered off a salmon cage. (C) Modified frame with a yellow 10 gauge wire mesh protective encasement being retrieved.
Figure 2.3: Fluorescent microspheres in faeces of the sea cucumber *Cucumaria frondosa*

after egestion and filtration onto GF/C filter and exposure to UV light
Figure 2.4: (A) Bottom half containing sample bottles (left) and top half containing control module (right) of Teledyne ISCO sampler. (B) ISCO samplers (rear) connected via rubber tubing to sea cucumber containment funnels.
Figure 2.5: (A) Top view of sampler used in field trials with mesh covering removed for visibility. (B) Side view of sampler used in field trials.

Figure 2.6: Incubation tank housing sea cucumbers (*Cucumaria frondosa*) being supplied with water from nearby salmon (*Salmo salar*) tank.
Figure 2.7: Relationship between diet organic content and absorption efficiency used for estimations of Absorption Efficiency used in feeding experiments. Data taken primarily from Nelson et al. (2012a; circles) for the sea cucumber *C. frondosa* and supplemented with *Parastichopus parvimensis* absorption data from Yingst (1976; triangles).
Figure 2.8: Mean (±1SE) concentration of inorganics in sediment traps on 3 separate frames from one set of deployments at a salmon (*Salmo salar*) cage site near Navy Island and at a reference site approximately 1 km away. Each frame consists of 3 sediment traps – 3 traps containing sea cucumbers, *Cucumaria frondosa* (for a total of n=9) and 1 not containing sea cucumbers (n=3), which served as a control to measure natural sedimentation rates. Dark bars represent control traps while light bars represent the average concentration of the 3 traps containing sea cucumbers on each frame.
Figure 2.9: Clearance rates (L·individual$^{-1}$·day$^{-1}$) of the sea cucumber *Cucumaria frondosa* in field (Brandy Cove, New Brunswick; 45°05′02.88″N, 67°05′13.72″; n=19) and in the lab (n=24) Data are mean ±SE. Presence of an asterisk indicates significant difference.
Figure 2.10: Ingestion and egestion rates (mg·individual⁻¹·day⁻¹) of the sea cucumber *Cucumaria frondosa* in and field (Brandy Cove, New Brunswick; 45°05′02.88″N, 67°05′13.72″; n=19) and in the lab (n=24). Data are mean ±SE.
3. Particulate size classes ingested by the orange-footed sea cucumber, *Cucumaria frondosa*

3.1 Introduction

Aquaculture is the fastest growing food production sector, having surpassed beef production in 2012 (Food and Agriculture Organization of the United Nations, 2014) and wild fishery catches for food in 2014 (Food and Agriculture Organization of the United Nations., 2016). Canada’s aquaculture industry in particular is experiencing rapid growth in recent years, with Atlantic salmon monoculture accounting for the majority of production (Fisheries and Oceans Canada, 2013). There is concern however, that rapidly expanding intensive monoculture production is ultimately not sustainable. High densities of fish held together produce high volumes of waste effluent in the form of faeces and uneaten feed which can lead to organic loading. Much of this waste will settle to the benthos near aquaculture sites which can lead to deleterious effects within the trophic structure stemming from physical and chemical properties of the benthos (Brooks and Mahnken, 2003; Edgar et al., 2010). Such an increase in organics may lead to bacterial blooms resulting in hypoxia or anoxia and the loss of crops (Nixon, 1995; Chávez-Crooker and Obreque-Contreras, 2010).

In an effort to mitigate these effluents, there has been recent interest in Integrated Multi-Trophic Aquaculture (IMTA). Here, one or more species occupying different trophic levels are co-cultured such that excess particulates from higher trophic levels
(faecal and feed waste) can be ingested and converted to a useable secondary product (biomass) by species at lower trophic levels. In eastern Canada, IMTA farms would likely include the Atlantic salmon (*Salmo salar*), blue mussel (*Mytilus edulis*), and kelps (*Saccharina latissima* and *Alaria esculenta* Chopinet et al., 2007), while other supplementary species are also being considered (e.g. oysters, scallops, urchins, and sea cucumbers) Mussels feed upon organic particulate matter in the water column, while kelps can absorb dissolved inorganics such as nitrate and phosphate. IMTA allows for a diversification in crops for farmers as well as an economical means of supplementing feed with waste material from organisms at a higher trophic level. These economic and environmental advantages may also improve the social acceptability of IMTA and open-water aquaculture in general (Troell et al., 2009) Organic extractive species will be an important aspect of the implementation of IMTA.

Most previous research focusing on bivalves as candidate species. There is some evidence that mussels experience increased growth rates associated with proximity to salmon net-pens with effluent from these cages accounting for a significant portion of their diet (Sara et al., 2009; Lander et al., 2012). Other studies show no significant effect of proximity to fish farms on species growth (Cheshuk et al., 2003; Stirling and Okumus, 1995), nor do they necessarily intercept and assimilate organic wastes from fish farms (in which case the settling faeces and psedofaeces derived from natural seston may be adding to the deposition of organic material rather than reducing it (Cranford et al, 2013). In highly dynamic areas such as the Bay of Fundy, mussels may not have the opportunity to intercept a large fraction of particulate wastes when positioned in the upper water column (Cranford et al., 2013). In some cases the concentration of waste effluent adjacent to
farms is minimal and episodic (Brager et al., 2015). Cranford et al. (2013) similarly suggest that the size-class distribution of particulates that mussels would have access to while in the upper water column would be insufficient in removing a large fraction of particulate waste (Fisheries and Oceans Canada, 2013; Cranford et al., 2013). This is due in part to the dominance of vertical fluxes in the settlement of organic material at IMTA sites. Fish faeces have a high settling velocity, and remain in suspension for a limited amount of time (Liutkus et al., 2012; Filgueira et al., 2017). It is estimated that >75% of organic effluents settle within 500 m of an aquaculture site in 250 m of water (Bannister et al., 2016). Farm sites in the Bay of Fundy would be considerably shallower (at about 30 m) and would therefore likely have effluents settle even closer to the farm (Brooks and Mahnken, 2003). The rapid settlement of faeces may be exacerbated by a reduction of flow from on-site structures, biofouling, and turbulence (Brager et al., 2015). It is therefore unlikely that IMTA can significantly reduce organic loading through use of suspended filter-feeders alone.

As much of the waste effluent at aquaculture sites settles to the benthos, benthic organic extractive species are an attractive (and likely necessary) option in order to ensure ingestion of waste particles. The orange-footed sea cucumber, *Cucumaria frondosa*, is one species that is being considered as a benthic organic extractive species within IMTA systems. A benthic dendrochirotic (suspension-feeding) echinoderm, it is the largest and most common of our native sea cucumber species. It is also somewhat unique among sea cucumbers in that it employs a passive suspension feeding strategy. Most species of sea cucumbers are deposit feeders (aspirochirote) – ingesting sediment or debris atop the sediment and digesting the organic portion. *Cucumaria frondosa*,
however, feeds by extending its ten tentacles into the water column and are dependent on ambient water currents to move seston across these tentacles so that it may adhere to the mucus and be captured after which the tentacle will subsequently be brought to the mouth and stripped of captured particulates (Hamel and Mercier, 1998; Singh et al., 1998; Figure 2.1). This strategy has led to some difficulty from a research perspective, as individuals will retract their tentacles when stressed (either through captivity or disturbance), thereby ceasing feeding activity (Hamel and Mercier, 1998; Hamer, 2010). The species is thought to have potential as a culture species (Nelson et al., 2012; Pietrak et al., 2014) Though not as valuable as some other species of sea cucumber, there is existing Asian market as seafood product (Therkildsen and Petersen, 2006; Gianasi et al., 2016) and an emerging market in higher-value nutraceutical and biochemical products (Attoub et al., 2013; Ma et al., 2013).

*Cucumaria frondosa* may represent a potential organic extractive species for IMTA that, despite relatively low feeding/clearance rates (Chapter 2), may be able to capture and assimilate heavier settling salmon faeces not accessible to mussels in the upper water column. While larger particulates may make up a significant portion of a mussel’s diet (>110 µm; Newell et al., 1989), under the current proposed system of IMTA, they are not likely to be capable of ingesting the majority of settling material due to their position within the farm and their exposure time to effluent plumes (Cranford et al., 2013). Conversely, while sea cucumbers would be situated on or near the benthos (presumably with more access to heavier settling waste effluent), it is not known what size of particulates they are capable of ingesting, or what proportion of their diet is made up of different particle sizes. *Cucumaria frondosa* is reported to be non-selective in its feeding,
with a large proportion of its diet comprising of small phytoplanktonic cells (Hamel and Mercier, 1998). When held in the lab, they are reported to feed well on the alga *Isochrysis galbana*, which has a diameter of 5 µm (Singh et al., 1998). In a recent study they displayed high specific growth rates when fed a diet of much larger fish eggs (1000 ± 500 µm; Gianasi et al., 2016). A variety of ingested material has been found in their stomachs – phytoplankton cells, larvae, eggs, and small crustaceans ranging from 25-350 µm (Hamel and Mercier, 1998), suggesting opportunistic feeding.

### 3.2 Objectives

The objective of this study was to attempt to quantify the sizes of particulates on which *Cucumaria frondosa* feeds.

### 3.3 Methods

#### 3.3.1 Species collection

Adult orange-footed sea cucumbers (*Cucumaria frondosa*) were collected from natural populations in the Passamaquoddy Bay within the Bay of Fundy, in South-west New Brunswick in the summer of 2014. Sea cucumbers were collected as part of a biodiversity survey undertaken by the St. Andrews Biological Station. The survey took place in the eastern Passamaquoddy Bay (45°06′85″N, 66°94′62″W to 45°08′15″N, 66°93′97″W) using an otter trawl with a mesh size on the cod end of 42 mm at a depth of approximately 60 m. They were subsequently held at the St. Andrews Biological Station in St Andrew, New Brunswick, Canada.
3.3.2 Laboratory experiments

Experiments were conducted at the St. Andrews Biological Station using an annular flume in the summer and fall of 2014. The flume was filled with 636 L of seawater at ambient temperature and supplied with air stones (Figure 3.1). The current velocity of the flume was set to approximately 50 cm/s, a speed found to be optimal for sea cucumber feeding (Holtz and MacDonald, 2009). To stimulate feeding, 3 ml (an amount determined through preliminary work to be an appropriate concentration to stimulate feeding without excessively clouding the water in the flume) of Reed Mariculture Shellfish Diet ® (an algae diet consisting of 30% *Isochrysis*, 20% *Pavlova*, 20% *Tetraselmis*, and 30% *Thalassiosire weissflogii*), as *Cucumaria frondosa* has demonstrated an ability to detect food in the water, reacting by extending its tentacles and feeding (Hamel and Mercier, 1998). Skretting Nutra XP Microdiet (1.14 g) was also added to the flume. This commercial diet consists of small pellets approximately 500 µm in diameter. The mass of 1.14g was selected somewhat arbitrarily as an approximation of 1% of a sea cucumber’s wet weight, an early estimation of what an organism may eat in one day. As the purpose of this experiment was to measure the depletion of particulates in the water rather than the rate of ingestion of *C. frondosa*, the addition of the microdiet served to add larger particulates into the water so that their depletion could be measured. Both of these diets were added in each experiment whether or not sea cucumbers were present, in order to maintain similar particle concentrations between subsequent experiments.

Experiments were each 24 hours long and were conducted back-to-back. The flume would be first run for 24 hours without the presence of sea cucumbers, cleaned, and then run for another 24 hours with sea cucumbers present. Before each experiment, sea
cucumbers were starved in 1 µm filtered seawater for 5 days (based on gut-passage time estimations in Chapter 2) to ensure their guts were empty of faeces. This was done so that sea cucumbers would not release faeces from previous ingestion into the flume. Two sea cucumbers were then placed within a funnel sitting in a shallow depression in the flume where they could anchor their tube feet to remain stable to feed. Should specimens feed during the experiment and subsequently defecate, it was thought that faeces would settle to the funnel bottom, which led to a collecting basin sheltered from the current to avoid resuspension of faeces. A ring of copper wire surrounded the entrance to the collecting basin so that individuals would not plug the hole. However, it is possible that some faeces was re-suspended, as the funnel housing the sea cucumber was not deep enough to be a true sediment trap. This could mean that sea cucumbers are contributing to the particulate concentration within the flume.

3.3.4 Methodology Development

In all experiments, water samples were taken intermittently and analyzed in a Beckman-Coulter Multisizer 4™ Coulter-Counter ®. The Multisizer allows for the accurate measurement of particle size distributions by moving them through an aperture tube and sizing them via electrical zone sensing. The machine was originally fitted with a 280 µm aperture tube, which could measure particles 5.6 µm -167 µm in diameter (60% of the diameter of the aperture tube). The relatively small aperture of this tube would sometimes become blocked by the larger particles that had been introduced into the water (although the diets were sieved beforehand, larger particulates could have been introduced through aggregation of flocs, from the sea cucumbers themselves, or from
ambient seawater) and the readings may not have been accurate and the data will not be used here. Interest in sea cucumbers as an IMTA candidate species is partially associated with their potential to ingest larger particulates therefore it was decided that a larger aperture tube was needed to obtain meaningful results. A 2000 µm aperture tube was used for subsequent analyses, enabling particles 200-1200 µm in diameter to be sized and counted into 400 separate size categories. Water samples were collected from the flume and measured manually over the course of the day. The flume was left running through the night, after which more samples were taken the following morning. As measurement times of particle size distributions using the Multisizer could vary between samples and with maintenance of the machine and other duties, the time between water samples was not uniform. Irregular timing of water samples due to Multisizer and flume maintenance across experiments meant that sample sizes were unequal across different variables. Over a 24 h period, 20-30 water samples were collected. Thus, 20-30 particle size distributions were generated - each consisting of particles comprising 400 logarithmically increasing size classes between 200-1200 µm for each 24 h flume experiment. Each sample removed approximately 300 mL of water, a negligible amount of the full 636 L volume. There were 3 separate 24 h experiments with no sea cucumbers present (as it was necessary to monitor the change in particle size distributions in the flume over time that may be caused by the action of the flume itself), and 3 with sea cucumbers present within the flume.
3.3.5 Analysis of data

This experiment attempted to quantify the reduction of particulate size classes ingested by sea cucumbers over time. Average size distributions were created for the beginning, middle, and end of each 24 h experiment. In order to ensure that enough water samples had been taken to create an average distribution, three 90 minute time periods were selected (the first 90 minutes, the last 90 minutes, and a 90 minute period from the middle of the experiment – because water samples were not taken uniformly, this time period could range from 200-450 minutes after the commencement of the experiment). Smaller particles are more numerous (Figure 3.2), but each particle has a substantially smaller volume than larger ones. The total volume ($\mu m^3$) of all particulates was therefore summed in order to assess the total amount of potential matter available to sea cucumbers. The mean total volumes of all particulates (averaged across the 3 experiments in which sea cucumbers were present and the 3 in which they were absent) were calculated for these time periods.

As sea cucumbers may be selectively removing a certain size of particulates, and because part of their potential as an organic extractive species comes from their potential to remove large particulates, changes in size distributions were also examined. Due in part to the large number of narrow size classes measured by the Multisizer, there may not have been particles detected in all the size classes, making comparison of size distributions between water samples difficult. Particle counts were therefore grouped into three broader size classes (200-211 $\mu m$, 212-280 $\mu m$, and 281-1200 $\mu m$). These size classes were selected so as to include an approximately equal number of particulates at the beginning of an experiment as the number of small particulates vastly outnumbered
larger ones in most water samples (Figure 3.2). If each of these three size classes had an equal range of sizes, the smallest would far outnumber the largest in terms of number of particulates. Again, because water samples were not taken uniformly throughout and between experiments, the number of samples averaged (across the 3 experiments in which sea cucumbers were present and the 3 in which they were absent) to calculate each particle distributions varied (Figure 3.3, Figure 3.4).

Separately, average size frequency distributions were calculated from the 90 min beginning and ending time intervals for each 24 h experiment without grouping the 400 size categories calculated by the Multisizer software into broader ranges. For each experiment, the initial average size frequency distribution (SFI) was compared to the final size frequency distribution (SFF) to calculate a percent change in size frequencies (ΔSF). If this value was negative it would indicate that particulates of a given size had been created, whereas if it was positive it would indicate that particle count had been reduced. These changes were calculated for each pair of experiments when sea cucumbers were present,

\[ SFI_{\text{present}} - SFF_{\text{present}} = \Delta SF_{\text{present}} \]  
(Equation 3.1)

and when they were absent.

\[ SFI_{\text{absent}} - SFF_{\text{absent}} = \Delta SF_{\text{absent}} \]  
(Equation 3.2)

The change from when sea cucumbers were absent was subtracted from the change from when they were present:

\[ \Delta SF_{\text{present}} - \Delta SF_{\text{absent}} = \Delta SF_{\text{difference}} \]  
(Equation 3.3)

This was done for each of the three pairs of experiments. The differences in percent changes (\( \Delta SF_{\text{difference}} \)) obtained for each were then averaged. If sea cucumbers are
actively removing particulates from the water, the $\Delta SF$ of a given size class will be greater when they are present than when they are absent. Therefore, if $\Delta SF_{difference}$ is positive, it should indicate that particulates have been removed by sea cucumbers (while a negative value would suggest that particles are being created by them).

3.3.6 Statistical analyses

Total particulates volumes were tested for assumptions of normality (Shapiro-Wilk test, $\alpha=0.05$) and homogeneity of variance (Levene’s test, $\alpha=0.05$). While the assumption of homogeneity of variance was met, the data did not meet the assumptions of normality after various transformation attempts. Despite not meeting the assumptions of normality, it was determined that, due to the fact that one of the factors in the experiment was nested (different flume runs being nested within treatments with and without sea cucumbers present), that a nested ANOVA be most appropriate. The nested ANOVA used total particulate volume ($\mu m^3$) a response variable; treatment (sea cucumbers present or absent), size (small, medium, and large particulates), and time (beginning, middle, and end of a flume run) as fixed factors; and individual flumes runs as a random factor nested within the fixed treatment variable. In the nested model, not all factors were tested against the total error term. The error term each factor or interaction was tested against are as follows: the “Treatment” factor was tested against “Run”, “Size”, “Run”, “RunXTime” and “TreatmentXSize” were tested against the interaction of the factors “RunXSize”, “Time” against “RunXTime”, “SizeXTime”, “RunXSize” and “TreatmentXSizeXTime” against “RunXSizeXTime”, and “RunXSizeXTime” against the residual error.
3.4 Results

There was no significant difference in total volume of particulates with respect to the presence or absence of sea cucumbers within the flume (F=0.11, p=0.795; Table 3.1, Figure 3.3). There was no clear reduction in the total volume of particulates across time or when compared to experiments in which sea cucumbers were absent, as would be expected if sea cucumbers were actively feeding and removing an appreciable number of particulates from the flume. In fact, there was a general increase in particle volume over time in both treatments, particularly in the middle time period (Figure 3.3). There was also no consistent reduction in particle numbers when sea cucumbers were present when compared to when they were absent across time (Figure 3.4). There was a significant difference (F=9.29, p=0.000; Table 3.1) in total particulate volume between time periods. There were significant differences in volume with respect to the interactions between particulate size and time (F=4.54, p=0.011, Table 3.1), size and individual flume runs (F=7.86, p<0.001, Table 3.1), and between time and flume run (F=2.16, p=0.09, Table 3.1). No other interactions between factors were associated with a significant difference in volume (Table 3.1).

Sea cucumber treatment did not have a significant effect on numbers of particulates (F=0, p=0.952; Table 3.2, Figure 3.4). There was no clear reduction of number of particulates of a given size category across time when sea cucumbers were present, indicating that there was no significant removal taking place. There was no consistent pattern of one sea cucumber treatment being associated with a higher number of
particulates (Figure 3.4). There was a significant difference in particle numbers for both sea cucumber treatments with respect to both time (F=163.7, p<0.001; Table 3.2) and particle size (F=114, p<0.001; Table 3.2). There were significant interactions between all of the variables tested (Table 3.2).

The difference in particle number change - the number of particulates of a given size over time with respect to the sea cucumber treatment – was generally around 0, with little pattern positively or negatively (Figure 3.5). There was a noticeable difference in particle number change among the smallest size categories measured (approximately 206-208 µm), which would suggest that sea cucumbers had removed these particulates.

3.5 Discussion

Total particulate volume and number of particulates of different size categories (200-1200 µm) with sea cucumbers present were not significantly different from when sea cucumbers were absent. Sea cucumber treatment was consistently non-significant, while the changes in particulates over time and across size categories were consistently significant, as were the interactions between variables. Particulates varied between individual flume runs, likely reflecting differences in particulate concentration in ambient seawater in each run. Particles also varied across time within individual flume runs, suggesting that flocculation and fragmentation of particulates may be occurring. There was no clear pattern of change across size categories (with the specific size ranges of the categories designated somewhat arbitrarily). It could be that sea cucumbers were feeding on a wide breadth of smaller particle sizes and not causing a significant reduction in any
of the larger particle sizes, although if this were the case, they likely would have had an effect on the overall volume of particulates. It could also be that sea cucumbers did remove particulates effectively, but also contributed to particulate concentration through sloughing of skin, mucus, or faeces (though there was no obvious sloughing present in the flume). Faeces of different species have different consistencies (Orr, 2012), and some may be more susceptible to disintegration and the creation of smaller particulates. It is possible that some faeces were resuspended, as the funnel housing the sea cucumber was not deep enough to be a true sediment trap (Hargrave, 1979). While sea cucumbers were observed to feed actively during these flume experiments, they likely did not feed enough to cause a detectable change in particulates (200-1200 µm) in the 636 L flume. Water clearance rate estimates of sea cucumbers feeding in the laboratory (Chapter 2) suggest that sea cucumbers can clear about 3.2 L of water per day. Individuals fed actively (but not always fully) within the flume, though with two sea cucumbers feeding over a 24 hour period, this would amount to about 1% of the flume’s volume. The volume of this flume was likely too large to be effective for this experiment. Estimates of ingestion rates suggest that sea cucumbers remove approximately 35-45 mg of food a day. Even if this represents an underestimation of the minimum amount food ingested by an individual sea cucumber, to detect this difference in such a large volume of water would require an extremely sensitive methodology and analysis. A longer acclimation period or a longer time within the flume may have led to more active feeding and a potentially detectable difference. However, sea cucumbers did feed within the flume, suggesting that a low ingestion rate, rather than inadequate acclimation time was responsible. Experiments would have had to have been impractically long to allow enough time for sea cucumbers
to ingest a large enough number of particulates to be detectable. They would also have
defecated during this longer timeframe, further complicating analyses.

There was also little difference in particle number when comparing the change over
time of particulate numbers between treatments with and without sea cucumbers.
However, this technique is not particularly sensitive as it compares the difference in
change between the two sea cucumber treatments. It also appears that particulate number
generally increases over time – perhaps a result of the turbulence of the flume breaking
apart larger particulates into smaller ones – meaning that if sea cucumbers are ingesting
material, then the increase over time in particulate numbers will actually be lower than if
they were absent. This would make comparison between the changes in the two
treatments over time less reliable. However, overall there is little evidence that sea
cucumbers fed on particles between 200-1200 µm in diameter at a level sufficient to
detect a change in the flume. Part of the difficulty in detecting a change in particulate
concentration comes from the fact that only two sea cucumbers were present in the flume
at a time. A larger group of sea cucumbers may have been able to remove more material,
but unfortunately the area of the flume in which sea cucumber could be safely contained
and protected was only large enough to contain two individuals. Had more individuals
been introduced to other areas of the flume, the turbulence and movement of paddles
would have caused them to be tossed about and damaged. A design with more sea
cucumber per volume of water may be better able to detect changes in particulate
concentrations associated with feeding.

It is also possible that although sea cucumbers fed within the flume during the
experiment, they were not feeding optimally. When stressed, individuals will retract their
tentacles and cease feeding activity (Hamel and Mercier, 1998; Hamer, 2010). *C. frondosa* has been shown to be a difficult organism to work with experimentally (Singh et al., 1998; Hamer, 2010). Hamer (2010) needed to adjust volume exchange rates, aeration and food concentration over the course of a month before sea cucumbers would extend their tentacles and feed. This was done in an effort to measure the retention efficiency of sea cucumbers. Despite these efforts to induce fully active feeding, retention efficiencies were not significantly different from those of empty containers. Several other studies involving laboratory experiments involving *C. frondosa* indicate a reduction or modification of feeding activity as well (Sutterlin and Waddy, 1976; Holtz and MacDonald, 2009; Nelson et al., 2012). In our study, the relatively fast current speed and shallow depth may have interacted with structures within the flume (air stones, air hoses, structural supports, and the sea cucumber containment system) to create potentially disruptive currents that lead to a stress response in the sea cucumbers. If sea cucumbers did not feed fully or reliably, they likely would not have removed a detectable amount of particulates from the water.

There have been few studies dealing with the diet of *Cucumaria frondosa*, resulting little information on the size range of food it consumes. Some studies that have focused on the feeding behavior of *C. frondosa* have looked at sizes of particulates only incidentally. For example, Singh et al. (1998) examined feeding response of the species to changing food concentrations in the lab, and found that they fed well on *Isochrysis galbana*, an alga with a diameter of approximately 5-7 µm. In another study, *C. frondosa* was exposed to different algal diets in the lab and in the field, including *Isochrysis galbana* as well as a commercial shellfish diet which included algae up to 12 µm in
diameter (Nelson et al, 2012). In most cases if individuals are exposed to a modified or natural food source in the field, there is no recorded particulate size distribution associated with a particular diet (Hamel and Mercier, 1998; Singh et al, 1999, Nelson et al, 2012).

Preferential selection/avoidance of food could affect changes in particulate concentrations. For example, male and female individuals do not eat their own gametes during or after release when spawning (Hamel and Mercier, 1996). Eggs of *C. frondosa* are significantly larger than most species of phytoplankton ingested by the species – approximately 650 µm in diameter (Hamel and Mercier, 1996). This does not necessarily mean that eggs are prohibitively large to be ingested by sea cucumbers, as *C. frondosa* has successfully been fed a diet of fish eggs >1000 µm in diameter (Gianasi et al., 2016). The avoidance of gamete ingestion is likely a function of the positive buoyancy of the gametes as well as a reduced feeding behavior when spawning, rather than an example of food selectivity (Hamel and Mercier, 1996). Hamel and Mercier (1996) suggested that *C. frondosa* are very selective feeders that only eat a few species of phytoplankton among the many present in their habitat, though this was based on the authors’ personal observations and was not tested during their study. Suspension-feeding sea cucumbers possess chemosensory as well as mechanoresponsive tentacles that may be the mechanism for such a selective behavior (Costelloe and Keegan, 1984).

In a subsequent study Hamel and Mercier (1998) suggested that the feeding behavior of *Cucumaria frondosa* is less selective compared to other sea cucumber species such as *Psolus fabricii* (Hamel et al., 1993). From observations of feeding occurring a wide breadth of particulate sizes, as well as a lack of observed changes in particulates of any
one particular size, it appears that *C. frondosa* captures most of suspended particles available to them, both living and nonliving. Analyses of intestinal contents showed that their diet included an abundance of phytoplankton cells, with small crustaceans and a variety of eggs being ingested occasionally as well (Hamel and Mercier, 1998). This varied diet included items with a diameter range of 25-350 µm. The variation in intestinal contents are likely associated with the varying abundances of planktonic cells, eggs, embryos, and nonliving material throughout the year (Hamel and Mercier, 1998). It could be that diet selectivity has more to do with chemosensory stimuli (perhaps organic content) or varying levels of feeding activity during periods where different dietary items are more dominant in the water. Other species of sea cucumbers respond to changes in organic material or preferentially select organic rich particles (though this is more established in deposit-feeding rather than suspension-feeding sea cucumbers) to suit their nutritional needs (Hudson et al, 2003; Liu et al., 2009; Navarro et al., 2013; Paltzat et al, 2008; Yuan et al., 2006; Zamora and Jeffs, 2011). It appears to be capable of ingesting a wide variety of diets, and is likely an opportunistic feeder that has few dietary restrictions. Evidence for the selection of particle or grain size (in the case of deposit-feeding sea cucumber species) is not as clear. Yingst (1976) suggested that holothurians are generally unselective of grain size. The deposit-feeding sea cucumber *Stichopus termolus* feeds primarily on coarse (>100 µm) sediments (Haukson, 1979), as does *Australostichopus mollis* (Slater and Jeffs, 2010). *Holothuria scabra* feeds on fine sediments (Baskar, 1994). The species *Holothuria atra, Holothuria hawaiiensis*, and *Bohadschia vitiensis* alternatively select between course and fine grained sediment in different seasons (Dar and Ahmad, 2006), while *Isostichopus badionotus* is an
unselective feeder with regard to particle size of sediment (Sloan and von Bodungen, 1980). There is little research on particle size selectivity with respect to suspension-feeding sea cucumbers.

A recent study tested the influence of food sources on the condition of *Cucumaria frondosa* (Gianasi et al, 2016). Individuals were fed either diatoms or fish eggs for 3 months. Diatoms are relatively small (4-9 µm), but the fish egg diet used in this study consisted of a cod eggs up to 1500 µm in diameter. Not only was *C. frondosa* capable of ingesting these eggs, but individuals fed with fish eggs showed higher specific growth rates and organ indices than all other treatments (Gianasi et al, 2016). Sea cucumbers were able to capture and ingest eggs up to 1500 µm, though water flow had to be interrupted to ensure that concentrations were maintained in the water long enough for individuals to react and capture them.

Though not part of the methodology of the present study, subsequent work involved the preparation of a macroalgal seaweed diet consisting of a variety of different particulate sizes. These particulates were measured and introduced one at a time onto the tentacles of *Cucumaria frondosa*. This was done manually via expulsion from a plastic pipette. Sea cucumbers were capable of retaining all sizes of particulates, up to and including those over 1 mm in length - these were not spherical particulates and thus were not 1000 µm in diameter (Simmons, unpubl. obs.). This suggests that *C. frondosa* is capable of ingesting particulates of any size, so long as they can fit into its mouth. More specifically, *C. frondosa* is likely to be capable of ingesting any particulate that can be retained by their tentacles, either by adhesion to mucus or ensnared by grasping or pinching. This may be particularly relevant in an IMTA setting, as open cage salmon
farms can change particle aggregation dynamics to favour the formation of larger flocs (Milligan and Law, 2005). In some species, a particle making contact with a tentacle will be grasped and transported to the mouth (Graham and Thompson 2009). In others, entrapment of particulates by adhesive papillae of the tentacles appears to be the primary means of food capture (Costolloe and Keegan, 1984). For suspension-feeding sea cucumbers, the principal feeding mechanism is likely adhesive papillae, with the grasping of particulates accounting for less of the material taken in by the individual (Fankboner, 1978). While sea cucumbers are therefore likely capable of ingesting any particulate than can be transported to the mouth, the capture efficiency of large particulates may be lower. A large, heavy, particulate will have less of its surface area in contact with the adhesive papillae of the tentacle and may be more susceptible to dislodgement by current or disturbance.

Another potentially limiting factor to the ingestion of large particulates may be their encounter rate. In an aquaculture context, large particulates have to be present in great enough concentrations in the water column in order to be reliably captured by an organic extractive species. Large particulates individually have exponentially higher volumes (and thus provide more nourishment), though it is unknown if preferentially targeting these would be effective. Lander et al. (2013) report that the majority of particles from aquaculture sites are small (1-10 µm) and of high organic content (up to 90%). In some cases, these small particulates associated with aquaculture waste effluent can interact with natural suspended particulates to form larger flocs (Milligan and Law, 2005). One study found that these “macroflocs” (with an average size of ~340 µm) can dominate the volume of suspended particulates at an aquaculture site, accounting for 65-80% of
volume concentration (Law et al., 2015). These relatively large flocs will not necessarily be the particulates that settle directly beneath salmon cages, as their settling velocity can be as low as 1 mm s\(^{-1}\), compared to the 40 mm s\(^{-1}\) settling velocity of salmon faeces itself and the 100 mm s\(^{-1}\) of salmon feed (Law et al., 2015). A lower settling velocity will allow a particulate to be carried by currents before settling, though there is also evidence that cage arrays can reduce the current speed around them and potentially allow for the settlement of lighter and larger particulates (Turner et al, 2015). Reid et al. (2009) state that there is limited information on particle size and density of material in an open-water aquaculture setting.

Further work will need to be done in order to estimate which sizes of particulates sea cucumbers (or another potential benthic organic extractive species) are likely to be exposed to on a salmon farm. It is likely that sea cucumbers are capable of ingesting any and all sizes of ambient particulates. Should a particulate be captured (either through adhesion to or grasping by the tentacles), it will likely comprise a portion of the diet of *Cucumaria frondosa*. However, there is currently little information on the capture efficiency of different size classes. While large particulates can make up a significant portion of the total volume of food available to sea cucumbers, their total numbers are also lower. If sea cucumbers are not present in sufficient numbers, densities, or positioning, they may not have the opportunity to ingest larger particulates. Hamel and Mercier (1998) suggest that the diet of *C. frondosa* consist of roughly the same proportions of particulate size and quality as are present in the water column. This may be the case with respect to an aquaculture environment as well. Overall, we have found
little evidence of pronounced size selectivity, large volume of particulates, or the ability to remove specific sizes of particulates.

3.6 Literature cited


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feces egested under simulated IMTA conditions. Journal of Shellfish Research 31, 1, 69-77.


to changing food concentrations in the laboratory. Canadian Journal of Zoology, 76, 1842-1849.


3.7 Tables

Table 3.1: Results from a nested ANOVA of the total volume ($\mu m^3$) of particulates in an annular flume water samples as a dependent variable, with sea cucumber *Cucumaria frondosa* treatment (presence/absence) and time period (beginning/middle/end of a given run) as fixed factors, and run (individual flume trials) as random factors nested with the treatment variable.

<table>
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<th>Source</th>
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<th>Adj MS</th>
<th>F-Value</th>
<th>P-Value</th>
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<td>2.01</td>
<td>1.96</td>
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<tr>
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</tr>
</tbody>
</table>
3.8 Figures

Figure 3.1: Annular flume setup used in laboratory experiments wherein sea cucumbers (Cucumaria frondosa) were allowed to feed with a known volume and concentration of food. Water samples were taken intermittently and analyzed for changes in particulate size distributions. Flume is ~1.7 m in diameter.
Figure 3.2: Example of a typical particulate size frequency distribution of a 200 ml water sample from annular flume experiments. Smaller particulates are generally considerably more abundant than larger ones.
Figure 3.3: Average (± standard error) total volume of all particles in treatments with (n=3) and without (n=3) sea cucumbers (*Cucumaria frondosa*) across different time periods – experiments were each 24 h in length, with mean size distributions taken from water samples at the beginning (the first 90 minutes), middle (a 90 minute period from the middle of the experiment – because water samples were not taken uniformly, this time period could range from 200 to 450 minutes after the beginning of the experiment), and end (the last 90 minutes).
Figure 3.4: Number of particulates comparing treatments with (n=3) and without (n=3) sea cucumbers (*Cucumaria frondosa*) across different size categories over time. Error bars are ± standard error. Categories consist of the mean number of particles over 3 experimental trials. Experiments were each 24 h in length, with mean size distributions taken from water samples at the beginning (the first 90 minutes), middle (a 90 minute period from the middle of the experiment – because water samples were not taken uniformly, this time period could range from 200 to 450 minutes after the beginning of the experiment), and end (the last 90 minutes).
Figure 3.5: Difference in change in particulate count of different size classes (initial average size frequency distribution compared to the final size frequency distribution, calculated for treatments with and without sea cucumbers (Cucumaria frondosa), with the “absent” size frequency distribution subtracted from the “present” size frequency distribution) for each size of particulates.
4. General conclusion

4.1 Conclusion

Ingestion, egestion, water clearance rates, and size of particulates ingested were assessed for the orange-footed sea cucumber, *Cucumaria frondosa*, through field and laboratory experiments. Specifically, feeding rates were assessed with respect to the potential of *C. frondosa* to be included in Integrated Multi-Trophic Aquaculture systems as a benthic organic extractive species.

Chapter 2 examined ingestion, water clearance, and egestion rates of *C. frondosa* in the laboratory and in the field. Preliminary feeding trials using sediment traps did not yield useful data; therefore, holding techniques were modified to allow sea cucumbers to defecate in a more controlled environment. Gut-passage time experiments were conducted to inform experimental methodology and ranged from 64 to 96 hours. An assumption of a 5-day gut passage time was used to reflect a conservative estimate in field and laboratory experiments. Particulate matter in the water column in the laboratory and in the field varied between trials. Sea cucumbers cleared significantly more in the field (7.2 L·individual$^{-1}$·day$^{-1}$, SD=3.6) than in the lab (3.6 L·individual$^{-1}$·day$^{-1}$, SD=1.9). In the field they ingested 38 mg·individual$^{-1}$·day$^{-1}$ (SD=18) and egested 13 mg·individual$^{-1}$·day$^{-1}$ (SD=6.3). In the laboratory they ingested 30 mg·individual$^{-1}$·day$^{-1}$ (SD=12) and egested of 11 mg·individual$^{-1}$·day$^{-1}$ (SD=4.3). There was no significant difference between field and laboratory ingestion or egestion rates. Total particulate matter was significantly different between laboratory and field experiments, as was concentrations of inorganics, though organic content was not significantly different. Egesta/ingesta rations
were approximately 35%, indicating that about one third of ingested material will contribute to benthic loading. Should sea cucumbers be incorporated into IMTA, these likely represent appropriate minimum estimates for the feeding activity and environmental impact. Absorption efficiencies are generally relatively high, so organic loading from sea cucumbers may be low. Feeding rates are also somewhat low, especially compared to other organisms that may be considered as organic extractives species, such as sea urchins or different species of sea cucumbers. This would indicate that the capacity of *Cucumaria frondosa* for organic extraction and bio-remediation may be limited, as would be their own impact on organic loading.

Chapter 3 examined the size of particulates *Cucumaria frondosa* ingests. A laboratory experiment was conducted in which sea cucumbers were allowed to feed within a flume, and the reduction in particulates of different sizes over time was measured. There was little detectable reduction in particulates (be it in a particular size class over time or in the overall volume of particulates), making it unclear which sizes of particulates sea cucumbers are primarily ingesting. This could indicate that they are consuming all particles equally, or that they are not consuming enough to have a measurable impact on concentration. It is more likely the case that they did not have a measurable impact, as there was also no change in total particle numbers. There was also no detectable difference in particulates over time when sea cucumbers were present compared to when they were not. This could be due to the characteristic stress response of *C. frondosa* leading to reduced feeding, coupled with being contained in a large volume of water where it was likely difficult for small number (n=2) of sea cucumbers to impact the particle concentration in such a large volume of water. Other studies, along with my own
unpublished observations, suggest that the species is capable of ingesting a wide breadth of particulate sizes. The proportion of their diet that would be composed of large particulates, remains unknown. It could be that sea cucumbers ingest particulates relatively unselectively (rather than preferentially targeting items of high organic content as some other species of sea cucumbers do), and their diet would therefore be proportional to the concentration of different items in the water they are feeding upon.

*Cucumaria frondosa* is infamously difficult to work with in the laboratory – often it will not feed fully if not exposed to optimal space/current/substrate/stability/aeration/food concentration. Other studies have found reduced feeding behavior in *C. frondosa* when held in confined or artificial environments, often leading to difficulties in the measurement of aspects of their feeding behaviour (Hamel and Mercier, 1998; Singh et al., 1999; Hamer, 2010). However, the present study does not represent a failure to quantify feeding rates. The methodology and analyses used to measure these feeding rates are likely an accurate assessment of feeding rates in the conditions the sea cucumbers experienced, and clearance/ingestion/egestion rates likely represent the minimum of feeding activity *C. frondosa* is capable of. To our knowledge, this is the first time water clearance rate has been quantified for a passive suspension-feeding organism. The methods employed here provide a novel adaptation to the bio-deposition method used for measuring, one that can be used in future studies (be it with passive suspension-feeders or otherwise). Future studies assessing the potential implementation into IMTA sites can use these methods, while improving upon the holding conditions to allow for full and uncompromised feeding. This also highlights the importance of having appropriate and well-researched containment infrastructure, should sea cucumber indeed
be implemented into IMTA in the future. In order to feed, grow, and provide an organic extractive service, sea cucumbers will require an environment and appropriate conditions conducive to optimal feeding activity. However, a reliable means of quantifying the sizes of particulate sea cucumbers ingest remains to be developed, as the methods used in the present study are likely not viable without modifications to the experimental holding conditions.

The present study not only provides novel methods for quantifying feeding in *Cucumaria frondosa* with respect to aquaculture, it is relevant to wild populations as well. There is relatively little data on the biomass of *C. frondosa* available, though a 2005 survey conducted in Frenchman Bay (a productive site for the Maine sea cucumber fishing industry, and of comparable size to the Passamaquoddy Bay in New Brunswick) in central Maine estimated biomass to be approximately 46,000,000 individuals (Chen et al., 2007). Based on estimates of feeding activity (Chapter 2), this population would be ingesting approximately 1725 kg of particulates, egesting 603 kg of waste material, and clearing 331,000 tonnes of water per day. Again, these estimations are likely absolute minimums.

The ingestion and egestion rates of wild populations are particularly relevant in their implications for benthic-pelagic coupling. Benthic-pelagic coupling generally refers to the exchange of nutrients, mass, and energy between the water column and the benthic substrate (Dame, 1993; Griffiths et al., 2017). One important aspect of benthic-pelagic coupling is the deposition of organic and inorganic material as faeces. Deposition rates in beds of suspension-feeding bivalves can be higher than those found in areas subject only to passive physical sedimentation (Dame, 1993). Though not actively pumping water
from the water column, *Cucumaria frondosa* collects naturally settling, laterally moving, and resuspended particulates on its tentacles, digesting them, and depositing them as settling faeces. As the particles collected will have likely come from those that are already settling as well as those moving throughout the water column, *C. frondosa* may be in effect “funneling” particulates onto the benthos, making exposure to the particulate plume (by situating the sea cucumbers appropriately or by managing the plume itself) an important consideration. Additionally, through the process of digestion, particulates will be reduced in organic content, changing both the quantity and the composition of settling material. Despite the importance of organic matter exchange between habitats, the magnitude of biological deposition processes are rarely assessed (Griffiths et al., 2017).

In an aquaculture setting, where settling waste effluent is of particular concern, it will be important to understand processes of benthic-pelagic coupling. Benthic-pelagic interactions are subject not only to the deposition of material, but to bio-resuspension as well. While sea cucumbers will actively be egesting and depositing material on the benthos, the complexity of these processes may vary depending on their diet and faecal characteristics. Though the settling velocity of faeces of *Cucumaria frondosa* was not measured in this study, the faecal settling velocity of the California sea cucumber (*Parastichopus californicus*) feeding on sablefish waste was around 30 mm·s$^{-1}$. The settling velocity of salmon faeces seems to vary somewhat between studies, ranging from 30 mm·s$^{-1}$ to 60 mm·s$^{-1}$. It could be that by ingesting salmon faeces, *C. frondosa* would reduce the settling velocity of ambient particulates and make them more prone to resuspension. This could be advantageous in that a resuspended particulate may be...
ingested and digested once more. Sea cucumbers may act as a force for particulate deposition in one context and particulate resuspension in another.

This may also be the case with larger flocculations of particulates formed through the interaction of “sticky” aquaculture waste effluents with natural seston. The interactions governing benthic-pelagic coupling are complex, though it does seem likely that *C. frondosa* would be capable of ingesting these flocs (with an average size of ~340 µm; Milligan and Law, 2005). It would seem that *C. frondosa* is an opportunistic feeder, capable of ingesting even very large particulates (>1000µm; Gianasi et al., 2016) with a diet breadth limited only by which particulates will readily adhere to their tentacles and are present in the water column when feeding. This may very well be appealing with respect to IMTA, where sea cucumbers could be capable of ingesting large particulates and flocs that are high in organic matter and inaccessible to filter-feeding organisms. This will be dependent on the encounter rate of these large particulates and whether their feeding rate is high enough to provide adequate particulate removal. Flocs may account for a large proportion of the volume of the diet available to *C. frondosa* due to their individually high surface-volume ratios, but if they are not present and able to be captured frequently and in high abundance, then mitigation will be minimal.

Estimations of ingestion rates may also help provide an idea of the scope of which the species is able to mitigate aquaculture waste. Take, for example, a cage containing 30,000 salmon producing 200 kg of faeces per day. With these minimum feeding rates at ~0.04 g of material per day, it would take approximately 5,000,000 sea cucumbers to remove 10% of salmon faeces. Under a cage with a surface area of 796 m², this would require a stocking density of over 6000 individuals per m², certainly not a practical
strategy. While these numbers are somewhat arbitrary estimates, they provide the baseline data needed to inform decisions on the potential utility of *C. frondosa* as an organic extractive species. In this case, sea cucumbers would likely not be an effective organic extractive species, though their potential as a farmed species remains. Recent developments in sea cucumber husbandry have led to consistent feeding in the laboratory. With what is likely optimal feeding, sea cucumbers have ingested 0.45 g per day – a full order of magnitude greater than ingestion rates observed in the present study (Lander et al., unpublished data). It would certainly appear the *C. frondosa* is capable of ingesting significantly more particulates when feeding in a conducive environment (though still at a level that would impractical to implement with the expectation of high organic extraction). This highlights the fact that holding conditions will be extremely important if the inclusion of sea cucumbers in IMTA or in conventional aquaculture operations are to be pursued. Such a significant increase in ingestion would also be coupled with an increase in egestion and thus a higher rate or organic loading from sea cucumbers. If the sea cucumbers are feeding upon aquaculture wastes, their high absorption efficiency should still allow them to have a net reduction of organic loading at aquaculture sites. While the present study did not allow for sea cucumbers to feed optimally, it provides valuable insights into how future work should proceed. Sea cucumbers should be provided with considerable water flow, oxygen, food, and a stable substrate. However, even with these greatly improved feeding rates when held in improved conditions, the potential of *C. frondosa* to remove effluent remains in question. In the scenario described above, sea cucumbers feeding readily would still require a stocking density of 600 individuals per m². While a marked improvement, this would still likely be impractical.
Should they be implemented into IMTA farms, infrastructure will need to be developed to ensure that feeding rates remain consistent while effectively containing individuals within the desired location on a farm. The design of such an open-water aquaculture cage for *Cucumaria frondosa* will be novel and will necessarily allow for access to the water column and currents while providing a stable and appropriate substrate. Their design will also be informed by the optimal placement of sea cucumbers in the water column. Sea cucumbers will be positioned directly on the benthos or on a mid-water structure. Their optimal placement will depend on the concentration and dynamics of aquaculture waste effluent at individual sites. Infrastructure associated with the implementation of sea cucumbers into IMTA extends beyond their containment. It may be necessary to develop the hatchery potential for *C. frondosa*, as regulatory issues and competition with the commercial fishery may hamper the capture of adults for brood stock and aquaculture activity. Juveniles are also cryptic and can experience post-settlement loss, which hatchery development may mitigate (Nelson et al., 2012).

*Cucumaria frondosa* has recently been spawned for the first time in the laboratory (Ross, 2016), suggesting that hatchery potential may be attractive. However, if regulatory and competitive disincentives can be alleviated, wild caught stock may be possible as well. The commercial fishery in the Bay of Fundy is quite active, catches are generally high-volume and it would be relatively cheap to purchase live sea cucumbers, and individuals generally show high survivorship after catch and transport (Sea Cucumber Harvesters Society, personal communication, 2017). While they are considered a high-volume low-value product - a pound of dried sea cucumber currently sells for $100-130 USD, equating to a somewhat high volume of approximately 40 individuals - demand for the
product is consistently high (Sea Cucumber Harvesters Society, personal communication, 2017) and there exists the potential for the creation of new high value nutraceutical markets (Attoub et al., 2013; Ma et al., 2013).

While feeding rates (and by extension, organic extractive capabilities) in the present study were limited, this is not necessarily an indictment of the potential of *C. frondosa* as an effective IMTA species. Implementation of sea cucumbers into IMTA farm will be a combination of their value and capability as an organic extractive species, their own impact on organic loading environment, and their economic return and marketability.

4.2 Literature cited


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Curriculum Vitae

Kurt Simmons

B.Sc. (Hon), Cape Breton University, 2012

Publications: None

Conference Presentations:


