The diet and influence of the spionid polychaete *Marenzelleria* on benthic communities in coastal Newfoundland

by

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ABSTRACT

Spionid polychaetes within the genus *Marenzelleria* are common inhabitants of organically enriched sediments in the Northern hemisphere. The species *M. viridis* has unique ventilation behaviors that create dynamic, fluctuating oxygen conditions in sediments, enhancing sulfate reduction. These behaviours may have negative effects on other macrofauna and positive effects on sulfur bacteria. A *Marenzelleria* species recently sampled in Newfoundland is here identified as *M. viridis*, and its abundance correlates little with abiotic factors and macrofaunal community composition at examined sites. Various types of surrounding sediments (oxic and suboxic as well as *M. viridis* burrow linings) contained surprisingly similar total prokaryotic, sulfate reducing and sulfur oxidizing bacteria numbers. The high abundance of sedimentary prokaryotes, combined with the stable isotopic composition of *M. viridis* tissues and lack of obvious symbionts, suggest that, thanks to its ventilation behaviour, this species may "farm" sulfur bacteria in sediments and use them as a primary food source.

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List of Symbols, Nomenclature and Abbreviations

bp = basepairs

°C = degrees Celsius

Chl a = chlorophyll a

cm = centimeter

CO_2 = Carbon Dioxide

COI = cytochrome oxidase subunit 1

DOC = Dissolved Organic Carbon

g = gram

m = metres

mL = milliliter

mm = millimeter

ng = nanogram

nm = nanometer

Ø = phi

rpm = rotation per minute

sp. = species

SPOM = suspended particulate organic matter

ul = microliter

µm = micrometer

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Chapter One: Introduction

1.1 Polychaete Feeding Modes and the Family Spionidae

Polychaetes, one of the dominant taxonomic groups in marine sedimentary environments, are key links in benthic and pelagic ecosystems as common prey items for fish, birds and other invertebrates and play important roles in biogeochemical cycles (Compton et al. 2013; Jumars et al. 2015). Recently, polychaetes have been classified into several feeding guilds, including microphages (active or passive suspension feeders, mixed mode suspension feeders, surface or subsurface deposit feeders, funnel feeders, food-cachers or scrapers), macrophages (carnivores, scavengers, parasites, herbivores on microphotoautotrophs or macroalgae), omnivores or osmotrophs (vestimentiferan tube worms), with some families capable of switching between feeding modes depending on food conditions (Jumars et al. 2015).

The family Spionidae Grube, 1850 is one of the largest groups of polychaetes, with more than 450 species in 38 taxa, and is the predominant polychaete group in mud-sand sediments from the intertidal zone to the deep sea, where they construct tubes from mucus and sand (Rouse and Pleijel, 2001). Spionids are generally described as passive suspension feeders, relying on particle movement or water currents to bring food within reach of typically long, grooved feeding palps that intercept passing food particles (Dauer et al. 1981; Rouse and Pleijel, 2001; Jumars et al. 2015). Although suspension feeding is observed frequently in spionids, some members of the family may also surface deposit

feed using feeding palps, depending on the availability and quality of food particles in the water column, thus providing an advantage in environments with changing water flow and food supply (Dauer et al. 1981; Jumars et al. 2015). While many polychaetes that surface deposit feed can also suspension feed, there are no species known to subsurface deposit and suspension feed, with the exception of some Magelonidae and *Praxillura masculata* in the Maldanidae family and *Nereis virens* (Herringshaw et al. 2010, Jumars et al. 2015). However, one taxon within the Spionidae, the genus *Marenzelleria* (Figure 1.1), has members which have been reported to surface deposit feed, suspension feed and potentially feed on reduced burrow sediments (Dauer et al. 1981; Essink and Kleef 1988; Miller et al. 1992; Zettler et al. 1996; Urban–Malinga et al. 2013). Because of this potential diversity in feeding modes, *Marenzelleria* species may have variable impacts on benthic ecosystems in different regions.

Figure 1.1: *Marenzelleria* species collected in Neddy Harbour, NL. Specimen length is approxiamately 12 cm

1.2 The genus **Marenzelleria** *and sibling species*

The genus *Marenzelleria* is distributed in the Northern hemisphere and is abundant in organically enriched intertidal and subtidal sediments of fine sand to mud (Hines and Comtois 1985; Sikorski and Bick 2004; Bick 2005; Blank et al. 2006). Within this genus, there are five described species found in the Arctic, both sides of the Atlantic and in the Eastern Pacific (Sikorski and Bick 2004). Species of *Marenzelleria* reported in the Arctic include *M. arctia* Chamberlin, 1920, *M. wireni* Augner, 1913 and *M. neglecta* Sikorski and Bick, 2004 (Sikorski and Bick 2004; Blank et al. 2006). In the Western North Atlantic, *Marenzelleria* species include *M. viridis* Verrill 1873, *M. neglecta* Sikorski and Bick 2004 and *M. bastropi* Bick, 2005, with the latter endemic to the Currituck Sound (Sikorski and Bick 2004; Bick 2005; Blank et al. 2006). Species are difficult to identify based on morphology alone, with three species, *M. viridis*, *M. neglecta,* and *M. arctia,* forming a cryptic sibling species complex (Sikorski and Bick 2004; Blank et al., 2006). Although morphologically identical, the three sibling species differ in burrow morphology, burrow construction, sediment reworking and water transport (Renz and Forster 2014). Other species of *Marenzelleria* with distributions overlapping those of *M. viridis, M. neglecta* and *M. bastropi* were only recently recognized as separate species (Sikorski and Bick 2004; Blank et al., 2006), so it is possible that studies of *"M. viridis*" have also included *M. neglecta* and *M. bastropi*. Most studies on the *Marenzelleria* genus have focused on *M. viridis*, as this species has become a highly successful invader in European seas, along with *M. neglecta* and *M. arctia. Marenzelleria viridis* was first observed in European seas during the 1980's in relatively

low numbers, and since then the population has increased drastically (Essink and Kleef 1988). In the Baltic Sea, *Marenzelleria* now composes up to 80% of species assemblages, with the introduction of the species correlating with decreases in other macrobenthic species (Zettler et al. 1996; Delefosse et al. 2012).

1.3 Physiological Adaptations to Hydrogen Sulfide and Anoxia

As occupants of nearshore organic-matter rich environments, many *Marenzelleria* species are adapted to withstand salinity fluctuations (from 0 – 20) and exposure to anoxic and sulfidic conditions (Delefosse et al. 2012). In particular, *M. viridis* and *M. neglecta* typically inhabit I- or J-shaped burrows up to 40 cm depth in organically enriched, sulfidic intertidal sand-mudflats and are often the first colonizers in polluted sediments (Blank and Bastrop 2008; Norkko et al. 2012; Renz and Forster 2014). As such, both species can be exposed to high levels of ambient hydrogen sulfide. The presence of hydrogen sulfide in sediments is considered to be an important environmental factor for endobenthic animals, due to its toxicity (Bochert et al. 1997). In soft-bodied animals such as polychaetes, hydrogen sulfide diffuses into tissues, and the fraction that is not oxidized accumulates and can bind irreversibly to cytochrome c oxidase, inhibiting 20 different enzymes, and causing sulfohemoglobin formation, mitochondrial polarization, increased free radical production as well as oxidative stress (Bochert et al. 1997; Schiedek 1997; Hance et al. 2008). When exposed to sulfide, most animals show a reduction in metabolic activity (Dubilier et al. 1997). In contrast, *M. viridis* has an enhanced metabolic rate in the presence of sulfide in concentrations up to 250μmol l^{-1}, apparently

gaining energy via the oxidation of hydrogen sulfide to sulfur, even in the absence of oxygen (Schneider 1996). The mitochondria of *M. viridis* may be capable of sulfide oxidation, as in *Arenicola marina* and *Heteromastus filiformis* (Schiedek 1997). Furthermore, *M. viridis* has better survival during hydrogen sulfide exposure than *M. wireni,* is better able to survive longer exposures to hydrogen sulfide even at toxic levels by oxidizing sulfide faster but with a lower energy gain, and is proposed to have an alternative pathway for sulfide oxidation that bypasses cytochrome c oxidase (Bochert et al. 1997; Hahlbeck et al. 2000).

1.4 Ventilation and Irrigation by Marenzelleria viridis

Physiological adaptations of *Marenzelleria viridis* for detoxifying hydrogen sulfide would be beneficial when ambient levels in the sediment are high. Intriguingly, *M. viridis* has a unique, ventilation behavior that increases the exposure of the polychaetes to sulfidic porewater. Typically, polychaetes create ventilation currents through either muscular pumping or ciliary action (Quintana et al. 2011). Ventilation currents created by peristaltic muscular pumping, in which the body forms a seal against the burrow wall to create a current, are usually more forceful than currents created by cilia (Quintana et al. 2011). Unlike polychaetes that use only one mode of ventilation, *M. viridis* creates currents through a combination of muscular pumping and ciliary action (Quintana et al. 2011). The muscular pumping directs anoxic water from deeper sediments upwards, resulting in regular anoxic events at the opening of *M. viridis* burrows lasting from 6-13 minutes (Quintana et al. 2011). Ciliary ventilation, on the other hand, is continuous and

directed towards the posterior of the polychaete, with water drawn from the anterior end by bands of cilia located on arched gills (Figure 1.2), supplying oxygen for respiration (Quintana et al. 2011). Consequently, the dual ventilation behavior creates oscillations from oxic to anoxic conditions in both the burrow and near the sediment surface, and *M. viridis* is therefore regularly exposed to anoxic and sulfidic water (Quintana et al. 2011; Jovanovic et al. 2014). Furthermore, the ciliary ventilation into the burrow forces oxygenated water across the burrow wall and into surrounding sediment, transporting anoxic, sulfidic and nutrient rich porewater from deeper sediments towards the surface sediment-water interface (Quintana et al. 2011). The powerful, deep irrigation created by *M. viridis* increases the availability of organic substrates and electron acceptors for microbes, and flushes the burrows of inhibitory agents, thereby altering microbial community structure (Quintana et al. 2013).

Figure 1.2: Photograph of bright red, arched gills on anterior end of *Marenzelleria* sp. found in Neddy Harbour, NL.

The unique ventilation behavior of *Marenzelleria viridis*, combined with the depth of its burrows, has been proposed to affect microbial activity in occupied sediment, in particular sulfate reduction and sulfur oxidation. In experimental cores containing *M. viridis*, sulfate reduction in sediments below 12-14 cm depth was higher than in control sediments, and total sulfate reduction was more than doubled in cores with *M. viridis* (Kristensen et al. 2011). In other experiments involving *M. viridis,* the presence of the polychaetes had a distinct effect on microbial reactions involving total CO_2, dissolved organic carbon (DOC) and sulfide, with a stimulation of microbial CO_2 production and sulfur and carbon turnover (Quintana et al. 2013). While it is not clear how *M. viridis* causes the microbial response, the deep irrigation behavior likely increases the flux of produced sulfide throughout the sediment column and the sediment water interface (Kristensen et al. 2011; Quintana et al. 2011). In turn, this flux encourages the growth of sulfur-oxidizing bacteria, as observed with the growth of *Beggiatoa* mats on the sediment surface of cores with *M. viridis* (Kristensen et al. 2011, Quintana et al. 2011). Not only does the presence of *M. viridis* stimulate sulfate reducers and sulfide production, but it also affects other anaerobic processes, decreasing denitrification and increasing ammonium production (Bonaglia et al. 2013; Renz and Forster, 2014).

1.5 Goals and Objectives

The physiological adaptations of *Marenzelleria viridis* to anoxic and sulfidic conditions suggest the importance of its unique ventilation behavior and subsequent enhanced sulfide exposure for this species. While the effect of *M. viridis* ventilation on

biogeochemical process has been investigated in recent years, the function of this unique behavior remains unknown. Furthermore, while the presence of *M. viridis* has been documented to stimulate microbial processes in sediments, there have been no direct investigations of the influence of this behavior on microbes or other macrofauna in the sediment. In Chapter 2, I determine the species identity of *Marenzelleria* found in coastal Newfoundland and describe site characteristics and the macrofauna associated with these polychaetes. In Chapter 3, I examine the abundances of prokaryotic microbes in sediments inhabited by *M.* sp., in particular the numbers and proportions of sulfate reducers and sulfur oxidizers, as the activity of these groups is known to be stimulated in the presence of *M. viridis.* Finally, in Chapter 4 I explore the potential food sources of *M.* sp. and consider whether its ventilation behavior may be linked with the enhancement of a chemosynthetically-derived food source. Collectively, this research expands knowledge of *Marenzelleria* genus distribution, adds insight into potential impacts in invaded regions, including Newfoundland, and increases understanding of ecosystem roles of *Marenzelleria* species in benthic communities, such as species displacement as well as effects on biogeochemical cycles.

Chapter Two: Sediment Characteristics and Macrofauna Community Structure of *Marenzelleria* sp. Occupied Sandflats

2.1 Introduction

Soft-sediment habitats cover the majority of the Earth's surface, and are easily modified by both physical forces and infaunal organisms (Reise 2002; Norkko et al. 2012). In intertidal zones, sediments are highly influenced by waves, tides, and faunal activity, creating an extremely dynamic, ephemeral, harsh habitat (McLachlan et al. 1993; Reise 2002). The organisms that inhabit this fluctuating environment must also overcome the challenges of emersion, fluctuating air temperature and salinity, humidity changes and desiccation, and, in many cases, high organic loading and low oxygen concentrations (Raffaelli and Hawkins 1996; Blank et al. 2006). The benthic fauna living in these harsh conditions play important roles in biogeochemical cycles, and are key to the sustainability of intertidal ecosystems, as they recycle nutrients, decompose organic matter, and are an important food source for higher trophic levels, including migrating birds, fish, mobile invertebrates and even large land mammals, serving as an important link between benthic, pelagic, marine and terrestrial ecosystems (Quammen 1984; Compton et al. 2013; Norkko et al. 2012).

The activities of organisms living in coastal marine environments can modify sedimentary fabric and chemistry, and the interplay between community members in soft sediment, intertidal marine ecosystems creates a complex web of dynamic conditions with easily changed short-term modifications (Reise 2002). A constant reworking or activity

by any community member is needed for any effect to persist, and if an organism is an ecosystem engineer, whose activities affect other species by either providing a habitat or transforming the environment, it can determine the proportion of energy flow to either producers, consumers or microbes (Widdows and Brinsley 2002; Reise 2002). In soft sediments, an ecosystem engineer can influence particle composition, distribution and re-suspension, sediment stability, microbial activity and nutrient flux rates, as well as modify the depth of oxic/anoxic chemocline, impacting other community members and their habitat (Reise 2002). As conditions in soft-sediment intertidal ecosystems are dynamic, a change that affects the interplay of community members can have repercussions on the entire ecosystem. If an ecosystem engineer is either removed or added to the community, it has the potential to create drastic changes in the habitat.

In Fall 2012, *Marenzelleria* was noted for the first time in Indian Pond, Newfoundland by Fiona Cuthbert of Memorial University during the collection of specimens for a Biology of Invertebrates course. *Marenzelleria* is a genus of spionid polychaete with five described species that are difficult to distinguish based on morphology alone, three of which are sibling species that have become successfully established in European waters (Sikorski and Bick 2004; Blank et al. 2006; Urban-Malinga 2013; Norkko et al. 2012). Members of the genus *Marenzelleria* occur on both sides of the Atlantic and the Arctic, as well as the Eastern Pacific (Sikorski and Bick 2004). Species known in the Western North Atlantic are: *M. neglecta* Sikorski and Bick, 2004; *M. viridis* Verrill, 1873*;* and *M. bastropi* Bick, 2005 (Sikorski and Bick 2004; Bick 2005; Blank et al. 2006). Arctic species include *M. neglecta* in the Canadian arctic, as

well as *M. wireni* Augner, 1913, and *M. arctia* Chamberlin, 1920 that are reportedly circumpolar although *M. arctia* have been noted only in Russian Seas (Sikorski and Bick 2004; Blank et al. 2006). All species typically occupy fine-grained sand to clay-grade sediments, and occur from the lower intertidal to depths of approximately 30 m (Hines and Comtois 1985; Sikorski and Bick 2004; Bick 2005; Blank et al. 2006). Despite being nearly morphologically identical, species can differ in their burrow structure and in the magnitude and quality of their irrigation activity, as demonstrated by the three sibling species *M. viridis, M. neglecta,* and *M. arctia* (Renz and Forster 2014). Most ecological studies have focused on these three species due to recent invasions in European seas, with less work focusing on *M. wireni* and *M. bastropi*.

The least studied of the three sibling species, *Marenzelleria arctia*, is found up to 30 m depth (where it is most abundant), in silty, sandy or mixed bottoms in regions with extreme changes in salinity (0 to 31.5) and temperature (0-12˚C) (Sikorski and Bick 2004; Bastrop and Blank 2008). This species usually constructs continuously ventilated, J, Y or U shaped burrows up to 8 cm deep, with about 0.4 m^2 of extended sediment-water interface below each square meter of surface sediment (Renz and Forster 2014). The constant, unidirectional flow created by *M. arctia* creates more stable conditions than the deeper burrowing *M. neglecta* and *M. viridis*.

Marenzelleria neglecta (up to 115 mm long and 2.0 mm wide) and *M. viridis* (up to 93 mm long and 1.1 mm wide) both occur along Atlantic coastlines of North America, with *M. neglecta* being distributed from Chesapeake Bay to Georgia in salinities up to 10, and *M. viridis* spanning Chesapeake Bay to Nova Scotia and the Gulf of St

Lawrence (easternmost location: eastern Bradelle Valley) in areas of higher than 10 or more fluctuating salinity (Brunel et al. 1998; Sikorski and Bick 2004; Blank et al. 2006). Both species construct 25-35 cm deep, I or J shaped burrows and induce a two-way directional flow through those burrows, creating oscillating, dynamic conditions that stimulate anaerobic bacterial metabolism (Quintana et al. 2007; Blank and Bastrop 2008; Renz and Forster 2014). The sediment-water interface extended by these species is similar, on average 2.8 m^2 for *M. viridis* and 2.1 m^2 for *M. neglecta,* however, *M. viridis* is capable of transporting almost twice as much water as *M. neglecta* – on average, 12 mL day^{-1} per individual, compared to 6.6 mL day^{-1} per individual for *M. neglecta* (Renz and Forster 2014). Since the magnitude of burrow ventilation is quite different between those two species, there might be differences in the degree to which they stimulate both microbial activity and nutrient exchange rates, and therefore different propensities to cause ecosystem change.

Since the introduction of *Marenzelleria* spp. into European waters in the 1980s, *M. viridis,* has become a prevalent invasive species. By 1993, *M. viridis* became the dominant macrobenthic species in the Baltic Sea, comprising up to 80% of species assemblages in some areas (Zettler et al. 1996). Occupying an empty niche and lacking predators, this species quickly propagated and reached high densities, from 100-200 individuals to 8000 individuals per square metre (Schneider 1996; Delefosse et al. 2012). Reports of the introduction of *M. viridis* correlate with a decrease in other macrobenthic species (Delefosse et al. 2012), and raise concerns that the increased production of hydrogen sulfide, changes in organic matter degradation and a re-suspension of deep

buried contaminants may further harm the already stressed ecosystem of invaded regions (Kristensen et al. 2011; Norkko et al. 2012; Quintana et al. 2013).

The discovery of *Marenzelleria* sp. in Indian Pond, NL, outside the reported ranges of all species within this genus, prompted an investigation to determine the species' identity and thereby confirm the first record of *Marenzelleria* in coastal Newfoundland. Species identity is also important in assessing the potential influence this species may have on community structure and ecosystem functioning. In a first attempt to examine ecosystem correlates, the abundance of *Marenzelleria* sp. and the characteristics of inhabited regions were investigated in four opportunistically selected intertidal locations in Newfoundland, including Indian Pond. Sediment core samples and macrofauna were collected at these locations to investigate associations between *Marenzelleria* sp., sediment characteristics and macrofaunal community structure.

2.2 Materials and Methods

2.2.1 Sampling Locations

This study considered two locations, Conception Bay and Bonne Bay, both in Newfoundland. The location in Conception Bay, Indian Pond (Figure 2.1), was chosen as this was the location of the first observed *Marenzelleria* spp. Indian Pond is a brackish lagoon with a narrow opening connecting it to Conception Bay; it serves as an industrial cooling water intake source for the nearby Holyrood Thermal Generating Station. Located in the center of the community of Seal Cove, Indian Pond receives anthropogenic impacts from boating activities and domestic runoff from nearby dwellings, in addition to the

influence of the power plant. Although protected from wave action, the narrow opening of the lagoon creates strong tidal currents with rapid water level changes (personal observation). Surface salinity varied from 10 to 22, and temperature varied between 4 °C and 26 °C through the sampling period (April to December 2013). In mid-August 2014, the time of this study, the water temperature was 19.5°C, and the salinity 21.

Figure 2.1: Map of sampling location in Conception Bay, Indian Pond. Section in red represents sampling area of 2013 and 2014. (Adapted from the National Topographic System Maps, Natural Resources Canada).

Bonne Bay is a sub-arctic fjord with two basins – South Arm and East Arm – with East Arm separated from South Arm and the Gulf of St. Lawrence by a shallow glacial sill (approximately 13 m depth). Bonne Bay sites were chosen due to similar communites

and potential habitat for *Marenzelleria* spp. For this study, two locations within East Arm, Neddy Harbour and Deer Brook Lagoon, and one on other side of the sill, Sandy Head, were selected (Figure 2.2).

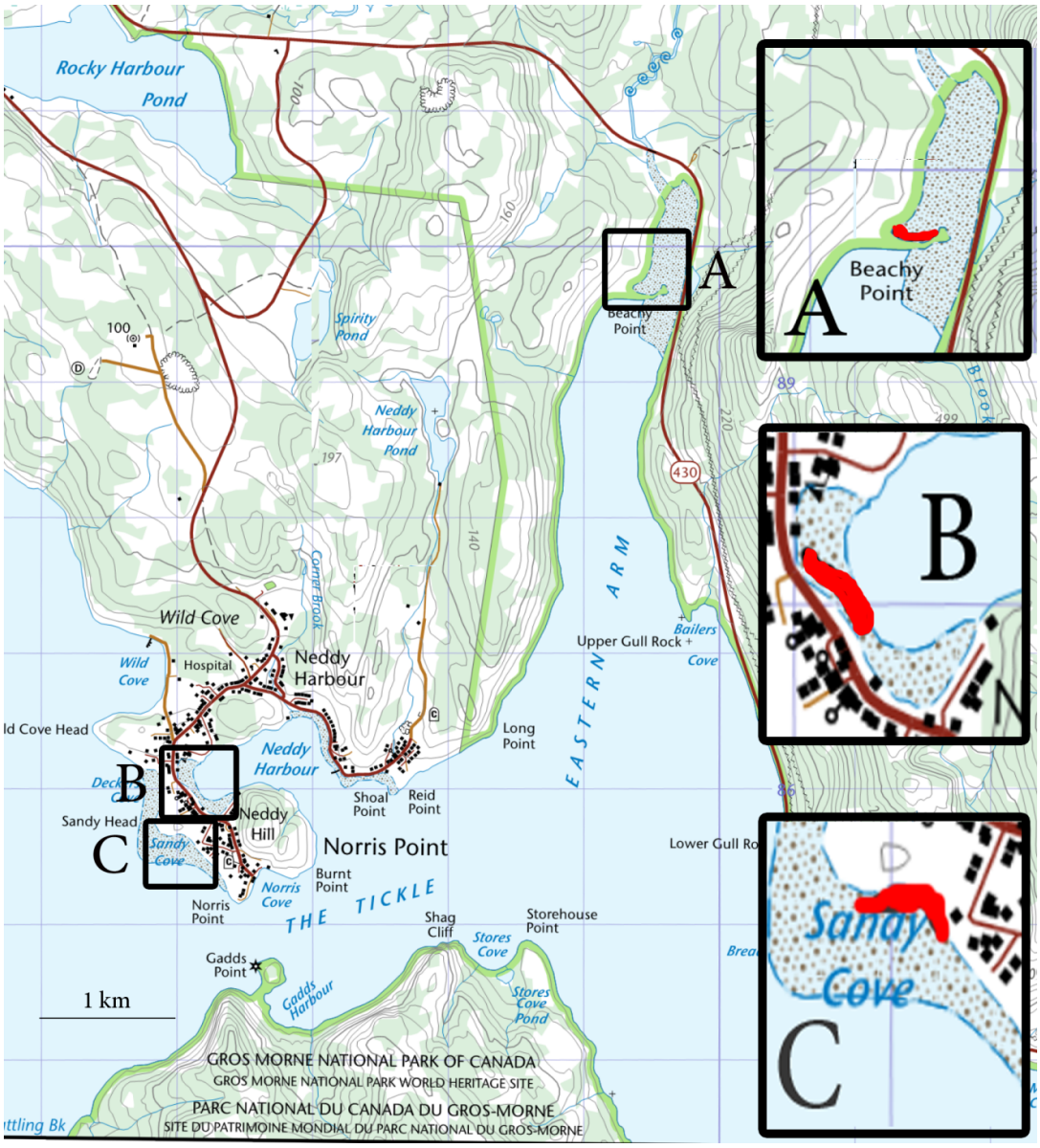


Figure 2.2: Map of sampling locations in Bonne Bay, Deer Brook Lagoon (A), Neddy Harbour (B) and Sandy Head (C). Sections in red represent sampling areas within each location for 2013 and 2014. (Adapted from the National Topographic System Maps, Natural Resources Canada).

Deer Brook Lagoon (Figure 2.2, inset A), located at the mouth of Deer Brook, has the least anthropogenic influences of the three Bonne Bay locations. This area receives abundant input of terrestrial organic matter and detritus from the Brook. The lagoon is sheltered from wave action and mobile sea ice by long sand bars, and substrate is fine sand to mud. During the lowest spring low tides, this location is almost completely exposed. Salinitiy is typically low but varies with tide and season. The salinity in Deer Brook measured 11.4 in early September 2013 and the temperature 22°C; in late August 2014 the temperature and salinity is unknown, although previous sampling in the area measured salinity as usually no higher than 15.

Neddy Harbour (Figure 2.2, inset B) is a sheltered cove that has with the greatest anthropogenic influences of the three Bonne Bay locations, primarily through the effects of dredging. It receives minor freshwater runoff from Neddy Pond Brook. In late August 2013, the water temperature at this site was 16.1°C and the salinity unknown; in late August 2014 the salinity was 23.1 and temperature 18.3°C.

Sandy Head (Figure 2.2, inset C) has the greatest exposure to the Gulf of St. Lawrence, with a line of boulders providing moderate protection from wave and sea ice action. The main feature of this site is a headland that is being rapidly eroded and experiences slumping, contributing to the sediments in this region. Similar to Deer Brook Lagoon, salinity and temperature varies with tides and precipitation. In late August 2013, the surface salinity was 18.8 and the temperature 18.5°C, and in late August 2014 the salinity was 28.9 and temperature 17.1°C.

All sampling was done haphazardly within the four study locations, with sites (N = 3 to 5) generally separated by a distance of 10 to 15 m and between 2 to 4 m from the water's edge at low tide. Sample sites were in locations only exposed during spring tides, when sampling took place. Fecal rods deposited at the surface of the sediment were taken to indicate the presence of *Marenzelleria* sp. within sampling locations, and sample plots were selected haphazardly in the general area where fecal rods were observed (about 25 m^2; see Fig. 3.1 for image of fecal rods). In 2013, sample collection took place on the low tides of August 27th for Neddy Harbour (5 sites) and Sandy Head (5 sites), and on September 2nd for Deer Brook Lagoon (3 sites). In 2014, sample collection took place in Indian Pond (4 sites) on August 13th, in Neddy Harbour (5 sites) on August 19th, in Sandy Head (5 sites) on August 25th and in Deer Brook (5 sites) on August 29th. Sample collection took place in the same general area where fecal rods were observed for each location in 2013 and 2014. Samples were collected at these times due to time constraints and available assistance.

2.2.2 Macrofaunal Community Sampling

Sediment samples for the characterization of macrofaunal assemblages were collected by measuring a 30 cm x 20 cm wide and 10 cm deep plot, and transferring the sediments into a bucket for transportation back to the Bonne Bay Marine Station or to Memorial University for processing. Sediment was run through 1.5 mm and then 1 mm mesh sieves, and all organisms collected. Specimens were visually identified to species based on morphological characteristics using a stereomicroscope, and enumerated.

To quantify the macrofaunal community diversity in sampled plots, the Shannon-Wiener diversity index (*H*) was calculated:

$$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

where:

p_i = proportion of individuals of the *i*th species

S = number of species encountered

$\sum$ = sum from species 1 to species S

2.2.3 Identification of *Marenzelleria* species.

As there is a *Marenzelleria* cryptic species complex consisting of three species (*M.arctia, neglecta* and *viridis*), the mitochondrial COI gene segment was amplified and sequenced to determine the identity of Newfoundland specimens, as in Bastrop and Blank (2006). Two individuals, one each from Bonne Bay and Indian Pond, were fixed in 100% ethanol. Total genomic DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit according to manufacturer's instructions and the concentration and purity of each extraction was quantified using a ND-1000 Spectrophotometer. The gene fragment COI (632 bp, Table 2.1) was amplified with polymerase chain reactions (PCR) performed with a BIO- RAD C1000 Thermal Cycler in 25 µL total volume, with a concentration of 25 ng/mL of DNA. The amplification profile consisted of denaturation for 60 seconds at 94°C, followed by 38 cycles of extension-elongations with 30 seconds at 94°C, 30 seconds at 50°C and 60 seconds at 72°C, and a final elongation at 72°C for 5 minutes.

PCR products were purified and sequenced at the Genomics and Proteomics lab, Memorial University. Sequences from the samples were edited using Sequencher (Version 5.0) and matched to closest known sequences in GenBank using the Basic Local Alignment Search Tool (BLAST).

Table 2.1: Primers used for amplification and sequencing to identify collected *Marenzelleria* sp.

Primer	Sequence	Reference
LCOI 1490	5'-GGTCAACAAATCATAAAGATATTGT-3'	Blank and Bastrop 2006
HCOI 2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Blank and Bastrop 2006

2.2.4 Sediment characteristics

The concentration of photosynthetic pigments, percentage of organic matter, median grain size and sorting were investigated for each sampled plot. One core sample for each plot was taken immediately adjacent to the plot sampled for macrofauna with a 5 cm diameter x 50 cm long plastic corer. Core samples were subsequently wrapped in aluminum foil to reduce light exposure, and brought back to the Bonne Bay Marine Station or Memorial University for further processing. Cores were sectioned into 0-2 cm, 2-4 cm, 4-6 cm, and 6-10 cm depth intervals in a darkened room. Sections were homogenized, wrapped in aluminum foil and frozen at -20°C. Each depth interval was subsampled for one measurement of each sediment characteristic listed above.

2.2.4.1 Photosynthetic pigment analysis

Photosynthetic pigment levels were measured from each depth section to characterize sampling site as well as investigate possible correlates with *Marenzelleria* sp., as *Marenzelleria* has been described as feeding on surface diatoms. To determine the

concentration of photosynthetic pigments, 2.0 g of thawed sediment was placed in a pre-weighed glass centrifuge tube with 8 mL of 90% acetone and left at 4°C for 18 hours for pigment extraction. Subsequently, the samples were transferred to plastic 15 mL centrifuge tubes and centrifuged at 3000 rpm for 10 minutes, with the supernatant immediately placed back into a glass centrifuge tube. One mL of supernatant was pipetted into a cuvette, transferred to a spectrophotometer, and absorbance measurements at wavelengths of 665 and 750 nm were recorded with 90% acetone as a blank. Then, 200 µL of 0.1M hydrochloric acid was added to the cuvette, and after two minutes a second reading at wavelengths of 665 and 750 nm was recorded. To determine the dry weight of sediment samples, 0.5 g of the same thawed sediment sample was dried overnight in an embedding oven at 60°C and the resulting weight difference used to calculate the equivalent weight loss of the sample from which pigments were extracted. To calculate the amount of chlorophyll a, the following formula was used (Danovaro 2010):

$$\text{Chl a } (\mu g/g) = 26.7(A_{665O}-A_{665aO}) * (v +(PP_u-PP_s)) / (CO*P_s)$$

where:

$A_{665O} = (A_{665}- A_{750})$

$A_{665aO} = (A_{665a-} A_{750a})$

A_{665} =absorbance of sample at 665nm before acidification

A_{750} = absorbance of the sample at 750nm before acidification

A_{665a}= absorbance of sample at 665nm after acidification

A_{750a} = absorbance of the sample at 750nm after acidification

V= volume of acetone

CO = optical length (in this case, 1 cm)

PP_u = weight of the tube containing wet sediment

PP_s = weight of the tube containing dry sediment

P_s = weight of dry sediment

2.2.4.2 Organic Matter

Organic matter percentage in each depth fraction to characterize the sampling sites, as well as correlate with *Marenzelleria* sp. as *Marenzelleria* has been described as a deposit feeder. To determine the percentage of organic matter, approximately 1.00 g of thawed sediment was transferred to a pre-weighed crucible, and left overnight in the drying oven at 60°C. The crucible and dried sample was reweighed the following day once the crucible had cooled. A bench-top furnace (Thermo Scientific, Thermolyne) set to 550°C was used to burn off organic matter present in the sample, and the weight of the crucible and resulting ash recorded. The following equation was used to quantify the percentage of organic matter:

% organic matter = ((dry weight of sediment-ash weight of sediment)/ (dry weight of sediment))*100

2.2.4.3 Median Grain Size and Sorting Coefficient

Median grain size and sorting coefficient were measured from each depth fraction to characterize the sampling site as well as determine correlates with *Marenzelleria* sp., as other marcrofauna have shown preference to certain sediment sizes. To determine median grain size and the sorting coefficient, grain size analysis by dry sieving, adapted from

Folk (1974), was used. Thawed sediment samples (55 g) were dried overnight at 60°C in a drying oven and reweighed the following day. Samples were transferred to pre-labeled 50 mL centrifuge tubes for analysis at the CREAIT TERRA facility in the Earth Sciences Department of Memorial University. Sieves (8 in diameter) of multiple screen sizes were selected for this analysis based on differentiation between different grain size classes of the Wentworth scale: gravel and very coarse sand (-1 Ø, 2.00 mm), very coarse to coarse sand (0 Ø, 1.00 mm), coarse to medium sand (1 Ø, 0.5 mm), medium to fine sand (2 Ø, 0.25 mm), fine to very fine sand (3 Ø, 0.125 mm), and very fine sand to coarse silt (4 Ø, 0.0625 mm). Screens were nested in order, with the coarsest screen on top and catch pan on the bottom, and placed in a Ro-Tap machine with the dry sample for 10 minutes. Each size fraction was carefully removed from the screen, and weighed to a precision of 0.01g.

Once the sample size fractions were weighed a cumulative weight graph was plotted, where the 50th percentile (Q_{50}) corresponded to the median grain size of the sample. To calculate the sorting coefficient, the following formula (Gray and Elliot 2009) was used:

$$(Q_{84} - Q_{16}/4) + (Q_{95} - Q_5/ 6.6)$$

where:

Q_{84} = 84th percentile on cumulative weight graph

Q_{16} = 16th percentile on cumulative weight graph

Q_{95} = 95th percentile on cumulative weight graph

Q_5 = 5th percentile on cumulative weight graph

2.2.5 Multivariate Analysis

Sediment characteristics were compared among locations (Indian Pond, Neddy Harbour, Sandy Head or Deer Brook) and years (2013, 2014) after data normalization; the dataset consisted of surface (0-2 cm) chlorophyll a, and organic matter, along with average chlorophyll a, organic matter, median grain size, and sorting coefficient. An analysis of similarity (ANOSIM) procedure was then run on the Euclidian distance matrix of sediment data, using 9999 permutations.

To explore macrofaunal communities classified by location-year, macrofaunal data were transformed to the fourth root to work on the same scale and nonmetric Multidimensional Scaling (nMDS) using the Bray Curtis similarity was performed. Species found in just one sampling plot were discarded from the analysis.

Then, in order to examine if structuring of assemblages might be driven by sediment characteristics, Pearson correlations of sediment data were plotted together with the macrofaunal nMDS.

To determine if there were significant differences in macrofaunal communities between locations and years, an ANOSIM was run on the previously obtained Bray Curtis similarity matrix, using 9999 permutations. In the ANOSIM, location and years were analyzed as cross-factors. To relate environmental variables with assemblages, a Biota and/or Environmental matching test (BEST) was conducted, based on Spearman rank correlations.

Finally, to determine whether *Marenzelleria* sp. presence or abundance may be related to community structure, another nMDS was run without *Marenzelleria* data (run

with dummyvaraibles to reduce the effect of zeros). All analyses were conducted using the software PRIMER 6.0 (Clarke & Warwick 2001).

2.3 Results

2.3.1 Presence of *Marenzelleria viridis* in Indian Pond and Bonne Bay

Analysis of the COI mitochondrial gene fragment amplified from total DNA extracted from the two specimens confirmed the presence of *Marenzelleria viridis* in both Indian Pond and Bonne Bay. The top 15 matches for the gene fragment (510 bp) from the specimen in Deer Brook were *Marenzelleria viridis* (top 3 matches: haplotype Mv 4, Mv 2 and Mv 1) all with an E-value of 0.0, query cover 100% and identity of 99%. The top 15 matches for the gene fragment (590 bp) from the Indian Pond specimen were also *Marenzelleria viridis* (haplotypes Mv 2, Mv 1 and Mv 4 in the top 4 matches) all with an E value of 0.0, query cover from 98 - 100%, and identity 99 - 100%. The species was found in all four locations sampled in both years, with average densities ranging from 3.7 ± 3.8 individuals m^{-2} in Neddy Harbour in 2013 to 22 ± 8.4 individuals m^{-2} in Deer Brook in 2014 (Table 2.2). In 2013, *M. viridis* accounted for 8% of the total macrofaunal abundance in Neddy Harbour, 28% in Sandy Head, and 48% in Deer Brook. In 2014, *M. viridis* accounted for 14% of the total macrofaunal abundance in Indian Pond, 31% in Neddy Harbour, 18% in Sandy Head, and 69% in Deer Brook.

Table 2.2: Densities (mean ± 1 SD individuals m^{-2}) of the five most common species found at all four sampling locations from 2013 and 2014. The Shannon-Wiener diversity (*H*) is also indicated. (See Appendix 1 for full list of species collected)

	Indian Pond		Neddy Harbour		Sandy Head		Deer Brook Lagoon	
	2013	2014	2013	2014	2013	2014	2013	2014
Diversity (*H*)	N/A	0.7 ± 0.5	1.1 ± 0.6	1.0 ± 0.3	0.9 ± 0.5	1.0 ± 0.4	1.0 ± 0.3	0.7 ± 0.4
Marenzelleria viridis	N/A	6.3 ± 6.3	3.7 ± 3.8	11 ± 8.6	9.7 ± 14	10 ± 5.9	8.9 ± 8.4	22 ± 8.4
Nereis virens	N/A	28 ± 14	4.0 ± 3.0	14 ± 9.9	3.3 ± 4.7	9.0 ± 8.8	3.3 ± 1.7	2.5 ± 3.1
Heteromastus filiformis	N/A	0	4.3 ± 7.0	15 ± 13	13 ± 16	30 ± 9.4	0.6 ± 1.0	6.0 ± 8.4
Mya arenaria	N/A	5.6 ± 6.6	6.3 ± 8.7	0.5 ± 1.1	1.7 ± 1.7	0.5 ± 1.1	0	0
Macoma balthica	N/A	0	24 ± 18	0	5 ± 1.5	1.5 ± 2.2	4.4 ± 4.2	2.5 ± 2.5

2.3.2 Characteristics of Locations with *Marenzelleria viridis*

2.3.2.1 Indian Pond

For 2014, the average Shannon-Wiener diversity (*H*) of macrofauna at Indian Pond was 0.7 ± 0.5. The most abundant species was *Nereis virens* with 28 ± 14 individuals m^{-2} (Table 2.2). Surface sediments in Indian Pond had an average chlorophyll a content of 9.43 ± 3.88 μg g^{-1} of dry sediment, an average organic matter content of 1.17 ± 0.13%, an average median grain size of 0.62 ± 0.24 mm and a sorting coefficient of 1.04 ± 0.13. Chlorophyll a and organic matter content decreased with depth, except for an increase in organic matter in the 6-10 cm depth fraction to 1.20 ± 0.29%. Both average median grain size and sorting coefficients remained similar with depth (Table 2.3).

Table 2.3: Average sediment characteristics (mean ± SD) at depth intervals for each sample location in 2013 and 2014.

	Chl a (ug g^{-1} dry sediment)		Organic Matter (%)		Median Grain Size (mm)		Sorting Coefficient	
	2013	2014	2013	2014	2013	2014	2013	2014
Indian Pond								
0-2 cm	N/A	9.4 ± 3.8	N/A	1.2 ± 0.1	N/A	0.6 ± 0.2	N/A	1.0 ± 0.1
2-4 cm	N/A	3.6 ± 1.4	N/A	0.8 ± 0.1	N/A	0.6 ± 0.1	N/A	1.0 ± 0.1
4-6 cm	N/A	1.8 ± 1.2	N/A	0.8 ± 0.2	N/A	0.6 ± 0.1	N/A	0.9 ± 0.1
6-10 cm	N/A	1.5 ± 1.0	N/A	1.2 ± 0.3	N/A	0.6 ± 0.1	N/A	1.1 ± 0.1
Neddy Harbour								
0-2 cm	3.4 ± 1.0	3.4 ± 2.5	0.7 ± 0.5	1.5 ± 0.5	0.3 ± 0.1	0.2 ± 0.1	1.5 ± 0.3	1.4 ± 0.1
2-4 cm	1.5 ± 0.6	1.7 ± 0.5	0.9 ± 0.6	1.3 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	1.4 ± 0.2	1.4 ± 0.2
4-6 cm	0.9 ± 0.5	1.0 ± 0.5	1.0 ± 1.0	1.2 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	1.5 ± 0.2	1.4 ± 0.3
6-10 cm	0.6 ±0.3	0.6 ± 0.3	0.8 ± 0.8	1.2 ± 0.3	0.3 ± 0.1	0.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.2
Sandy Head								
0-2 cm	2.0 ± 1.4	4.3 ± 2.1	0.6 ± 0.4	1.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.4	0.7 ± 0.9
2-4 cm	0.5 ± 0.2	0.9 ± 0.3	0.8 ± 1.5	1.3 ± 0.6	0.2 ± 0.0	0.2 ± 0.0	1.0 ± 0.6	0.9 ± 0.6
4-6 cm	0.4 ± 0.2	1.0 ± 0.8	0.5 ± 0.0	1.2 ± 0.4	0.2 ± 0.0	0.2 ± 0.1	0.6 ± 0.1	0.9 ± 0.6
6-10 cm	0.3 ± 0.2	1.3 ± 0.8	0.5 ± 0.0	1.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.7 ± 0.2	1.2 ± 0.5
Deer Brook Lagoon								
0-2 cm	17 ± 7.7	8.4 ± 2.5	1.7 ± 0.3	3.0 ± 0.8	0.2 ± 0.0	0.2 ± 0.0	1.0 ± 0.0	0.9 ± 0.6
2-4 cm	6.1 ± 1.0	5.5 ± 2.3	1.8 ± 0.3	3.4 ± 0.8	0.2 ± 0.0	0.2 ± 0.0	1.5 ± 0.0	1.0 ± 0.5
4-6 cm	2.1 ± 0.6	2.6 ± 1.7	5.9 ± 1.2	3.3 ± 1.0	0.2 ± 0.0	0.6 ± 0.9	1.3 ± 0.2	1.0 ± 0.6
6-10 cm	0.8 ± 0.1	1.7 ± 2.4	1.5 ± 0.6	4.1 ± 2.4	0.2 ± 0.0	0.1 ± 0.0	1.7 ± 0.2	0.8 ± 0.4

2.3.2.2 Neddy Harbour

The macrofaunal Shannon-Wiener diversity at Neddy Harbour averaged 1.1 ± 0.6 in 2013 and 1.0 ± 0.3 in 2014. The most abundant species in 2013 was *Macoma balthica* with 24 ± 18 individuals per m^{-2}. In 2014, this drastically changed as the abundance of *Macoma balthica* sampled went to 0, and *Heteromastus filiformis,* followed closely by *Nereis virens,* were the most abundant species, with 15 individuals m^{-2} and 14 individuals m^{-2}, respectively (Table 2.2).

In 2013, surface sediment samples (0-2 cm depth) had an average chlorophyll a content of 3.44 ± 1.00 μg g^{-1} dry sediment and 0.69 ± 0.55% organic matter. The average median grain size was 0.30 ± 0.08 mm, and the average sorting coefficient was 1.55 ± 0.33. In deeper fractions, chlorophyll a content decreased to 1.52 ± 0.98 μg g^{-1} dry sediment at 6-10 cm, with the average percentage of organic matter being higher in deeper sediment fractions, up to 0.96 ± 1.02%. Median grain size and sorting coefficient were relatively similar with depth (Table 2.3).

In 2014, surface sediment samples were similar to those collected in 2013 in their chlorophyll a content, with an average value of 3.37 ± 2.45 μg g^{-1} dry sediment, but the percentage of organic matter was slightly higher than in the previous year, with an average of 1.55 ± 0.49%. The average sorting coefficient and median grain size were also both similar to 2013 values, with a sorting coefficient average of 1.43 ± 0.14 and a slightly smaller median grain size of 0.20 ± 0.05 mm. Chlorophyll a content decreased with depth to 0.625 ± 0.265 μg g^{-1} dry sediment at 6-10 cm depth, with the percentage of

organic matter, median grain size and sorting coefficient remaining at similar values with depth (Table 2.3).

2.3.2.3 Sandy Head

The Shannon-Wiener macrofaunal diversity of Sandy Head averaged 0.9 ± 0.5 in 2013 and 1.0 ± 0.4 in 2014. For both 2013 and 2014, *Heteromastus filiformis* was the most abundant species, with 13 ± 16 and 30 ± 9.4 individuals m^{-2} in 2013 and 2014 (Table 2.2). In 2013, surface samples (0-2 cm depth) had an average chlorophyll a content of 1.95 ± 1.38 μg g^{-1} dry sediment, an average organic matter content of 0.62 ± 0.40%, an average median grain size of 0.20 ± 0.03 mm and a sorting coefficient of 0.62 ± 0.4. Chlorophyll a content and organic matter percentage decreased with depth down to 0.32 ± 0.22 μg g^{-1} and 0.48 ± 0.05% respectively. Median grain size and sorting coefficient were relatively consistent with depth, with the sorting coefficient slightly higher in the 2-4 cm depth fraction (0.98 ± 0.64).

In 2014, the average chlorophyll a content and organic matter were higher than in the previous year, with values of 4.25 ± 2.14 μg g^{-1} dry sediment, and 1.33 ± 0.14% respectively. The average median grain size was 0.19 ±0.01 mm and the sorting coefficient was 0.68 ± 0.85, both very similar to 2013 values. The average chlorophyll a content and organic matter percentage decreased with depth, to 1.33 ± 0.88 μg g^{-1} and 1.17 ± 0.13% at 6-10 cm depth. The median grain size remained the same with depth, although the sorting coefficient increased to 1.22 ± 0.49 in the 6-10 cm depth fraction (Table 2.3).

2.3.2.4 Deer Brook Lagoon

The Shannon-Wiener macrofaunal diversity in Deer Brook Lagoon averaged 1.0 ± 0.3 in 2013 and 0.7 ± 0.4 in 2014. *Marenzelleria viridis* was the most abundant macrofaunal species in both sampling years, with 8.9 ± 8.4 individuals m^{-2} in 2013 and 22 ± 8.4 individuals m^{-2} in 2014 (Table 2.2). In 2013, surface sediment had a chlorophyll a content averaging 16.95 ± 7.7 μg g^{-1} dry sediment, an average organic matter percentage of 1.69 ± 0.33%, an average median grain size of 0.17 ± 0.00 mm, and a sorting coefficient of 1.03 ± 0.00. As expected, chlorophyll a content decreased with depth to 0.78 ± 0.11μg g^{-1} dry sediment although the organic matter percentage remained relatively constant with depth, with an increase at the 4-6 cm depth fraction to 5.86 ± 1.15%. Median grain size remained similar with depth, and sorting coefficient increased to 1.55 ± 0.00 in the 2-4 cm depth fraction but remained similar for other depths.

In 2014, the surface chlorophyll a content was 8.41 ± 2.50 μg g^{-1} dry sediment, almost half the concentration measured in 2013; however, the percentage of organic matter was higher, at 2.95 ± 0.85%. The median grain size was similar to the previous year with an average of 0.17 ± 0.05 mm. Similar to the chlorophyll a content, the average sorting coefficient (0.86 ± 0.57) was lower than in 2013. Chlorophyll a content decreased with depth to 1.67 ± 2.43 μg g^{-1} dry sediment, the percentage of organic matter increased with depth to 4.14 ± 2.43, while median grain size and sorting coefficients remained similar with depth (Table 2.3).

2.3.3 Multivariate Analysis

The ANOSIM on sediment characteristics shows highly significant differences between all locations ($R = 0.722$, $p = 0.00001$) and, to a lesser degree, between years ($R = 0.258$, $p = 0.0024$). The nMDS showed some differences in macrofaunal assemblages between location-year groupings (Figure 2.3). The overlap between macrofaunal nMDS and Pearson correlation results from the sediment characteristics dataset revealed no evident driver of assemblage composition (Figure 2.3). The ANOSIM on macrofaunal community structure revealed that the effect of both location and year was significant ($R = 0.324$, $p < 0.0001$; $R = 0.207$, $p = 0.045$, respectively). Furthermore, pairwise comparisons indicate that Indian Pond assemblages were highly different from the other sites (R values between 0.4 and 0.9, $p < 0.005$).

Biota and or Environmental matching (BEST) revealed no significant relationships (p-value = 0.17 and Rho = 0.223) between environment characteristics and macrofauna. Removing *Marenzelleria viridis* from the dataset resulted in a similar pattern of sample assemblage composition (Figure 2.4A). When *M. viridis* abundance data were

then plotted for each of these samples, no particular structure was evident (Figure 2.4B).

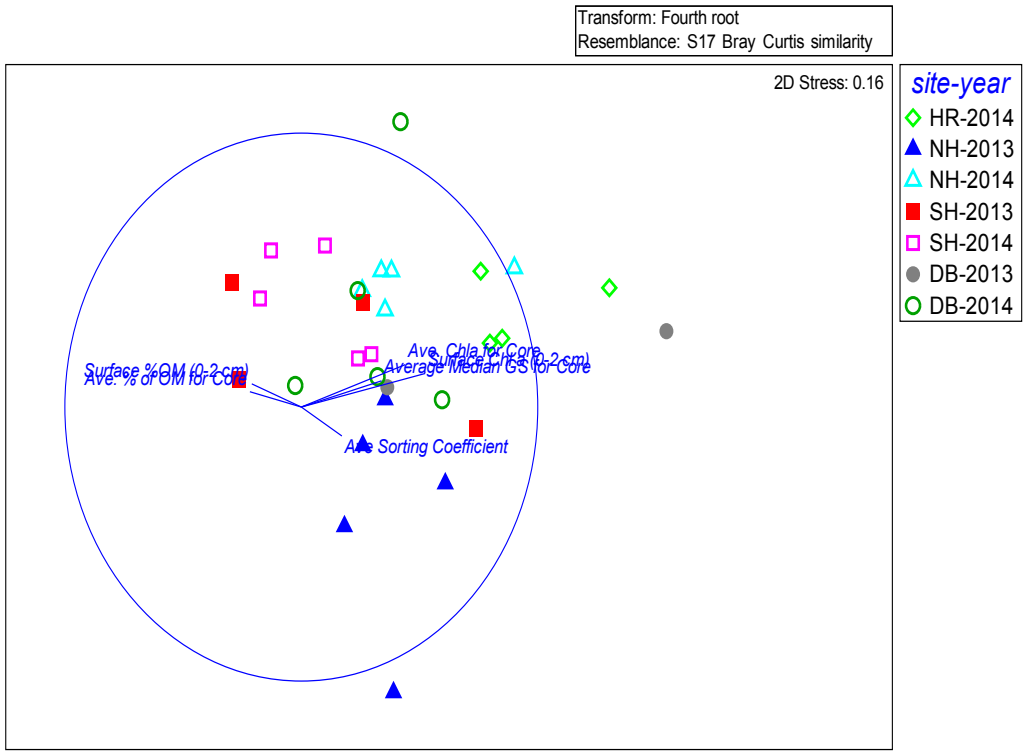
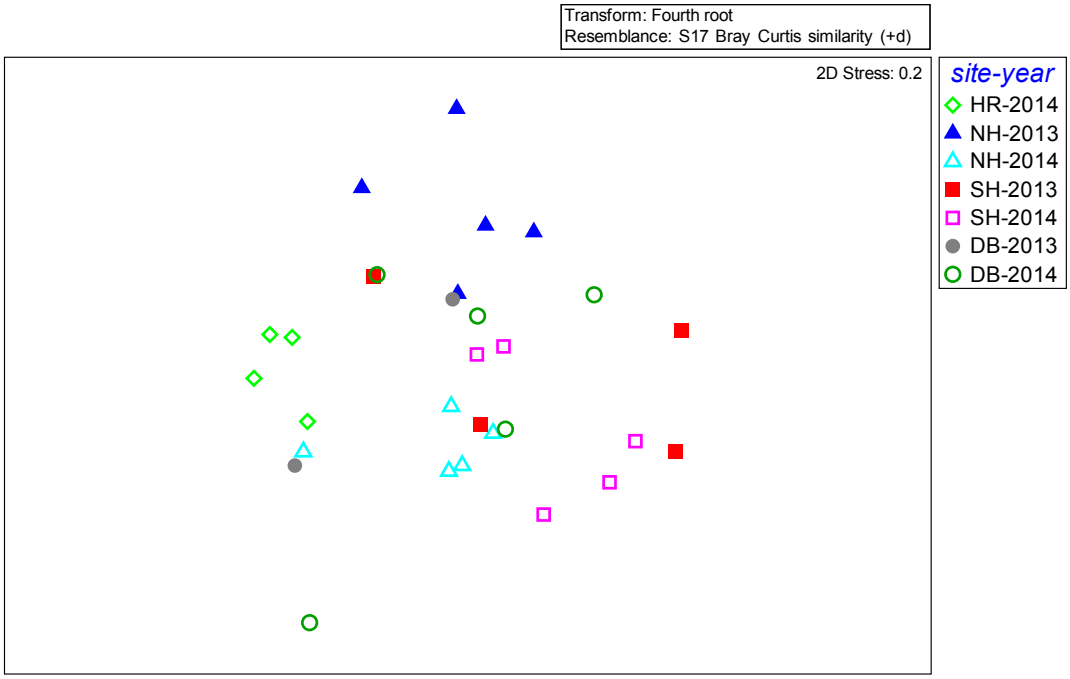
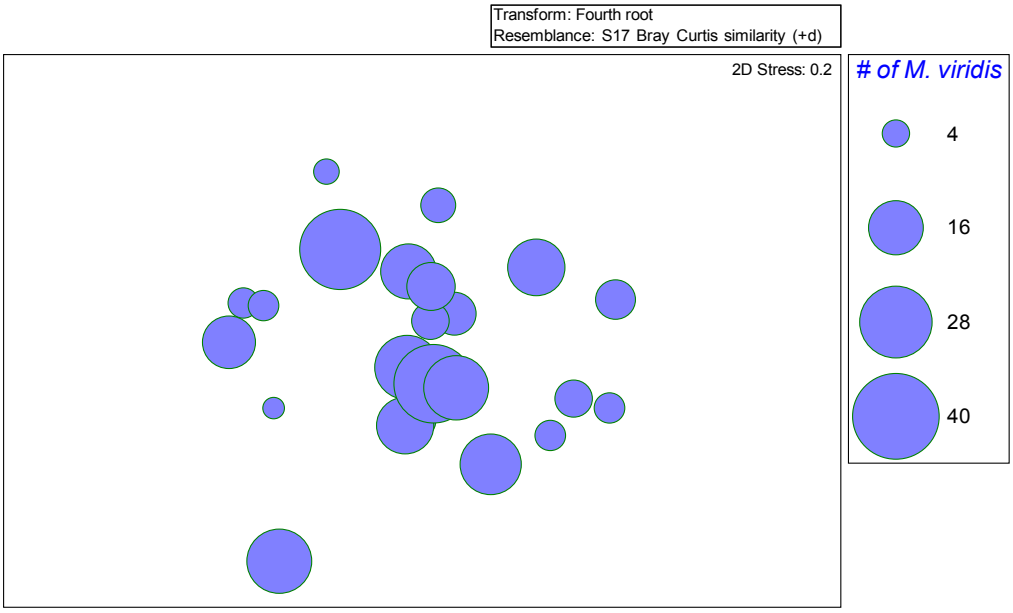


Figure 2.3: Superimposed Pearson correlation of sediment characteristics on Bray Curtis similarity of macrofaunal densities of sampling location and year (HR = Holyrood (Indian Pond), NH = Neddy Harbour, SH = Sandy Head, DB = Deer Brook Lagoon).



A)



B)

Figure 2.4: A) nMDS analysis of macrofaunal data, with the exclusion of *Marenzelleria viridis*. B) Densities of *M. viridis* (individuals m-2) superimposed on a). Numbers and circle diameter correspond to individuals m-2 in sampled plots. All locations and both years are considered.

2.4 Discussion

2.4.1 Confirmed Presence of *Marenzelleria viridis* in Newfoundland

This is the first confirmed report of the *Marenzelleria* genus in Newfoundland. As only two individuals were sequenced for this study, it is possible other *Marenzelleria* sp. besides *M. viridis* are present, but unfortunately due to time constraints more individuals could not be sequenced. Future studies could sequence more *Marenzelleria* individuals to confirm whether or not other species of the genus are distributed in Newfoundland.

Nonetheless, this is the first confirmed report of *Marenzelleria viridis* in Newfoundland, although other macrofaunal surveys have included this region (Maciolek 1984; Pocklington 1989; Blank et al. 2006). *Marenzelleria viridis* was found in the four investigated sites in both sampling years, and while it appears to be common, abundances are much lower than reported in both the native range and introduced regions. The highest density in this study (33 individuals m^{-2} in a plot from Deer Brook Lagoon, 2013) was low compared to the reported maximum densities of 1000 individuals m^{-2} in Nova Scotia (George 1966), and no fewer than 100-200 individuals m^{-2} in Europe. The lower density reported herein may be due to the modest human impacts at the sampling sites. *Marenzelleria viridis* is a highly opportunistic species and one of the first to colonize a region experiencing hypoxia (a common occurrence in polluted coastal regions); the species can reach extremely high abundances in these conditions (Norkko et al. 2012). While sites in Bonne Bay and Indian Pond were located near communities and likely experience some low-level pollution from the communities, both regions would naturally be eutrophic at times from organic inputs, such as leaves and wood particles in runoff

from Deer Brook into Deer Brook Lagoon. Numbers reported here could reflect relatively low anthropogenic influences in these regions compared to the stressed environments examined by other researchers, or could indicate recent introduction of this species, as discussed below.

The four sampling sites varied in average chlorophyll a content, percentage of organic matter, sorting coefficients, salinity, temperature, wave exposure, and to a lesser extent, median grain size. These factors, however, did not appear to structure macrofaunal communities to a great extent, likely due to the dynamic nature of the soft sediment and intertidal habitat of sampling sites. Strong conclusions on structuring factors cannot be made, however, since the low number of samples considered herein reduces the statistical power of multivariate analyses. Nevertheless, and despite observed differences in environmental conditions, *Marenzelleria viridis* reached similar abundances at all locations, and along with *Nereis virens*, was the only species to be found ubiquitously and in both sampling years. As a successful, established invader in Europe, typically inhabiting highly dynamic intertidal, low salinity regions of muddy sand (George 1966; Blank et al. 2006; Delefosse et al. 2012), it is not particularly surprising that *M. viridis* is can successfully inhabit the range of environmental conditions characterized by the sampling sites examined here.

2.4.2 A New Arrival?

Even though this is the first report of *Marenzelleria viridis* in Newfoundland, it is unclear whether it is a new arrival. While abundances are relatively low (<33 individuals

m^{-2}) compared to other regions, *M. viridis* is common, at least within the locations examined. It is possible the species has been present in very low abundance and went unnoticed; unless sampled carefully, *M. viridis* individuals often fragmented into small, unidentifiable pieces (personal observation). The presence of *M. viridis* in Newfoundland could represent an expansion of the species within its native range, and low abundances may indicate a maximum northern limit in the Western North Atlantic. On the one hand, colder temperatures, poor quality of organic matter, limited growth period of phototrophs, and even shore ice and pack ice could limit the population growth of *M. viridis*. On the other hand, the relatively low abundances may indicate a recent arrival to coastal Newfoundland ecosystems. In invaded regions of Europe, abundances of *M. viridis* are initially low before a sharp increase within a few years (Essink and Kleef 1988; Zettler et al. 1996). It is not known how long the species may have been present or its historical abundance, so no conclusion can be made at this time concerning how long *M. viridis* may have been present (i.e. a few years, decades or centuries).

If *Marenzelleria viridis* is a new arrival, it has the capacity to change ecosystem dynamics in Newfoundland coastal sediments. In regions where it has been introduced, *M. viridis* has been implicated with declines in, and even the displacement of local macrofauna through suspected increases in competition for food in nutrient poor environments, the stimulation of sulfate reduction or the re-suspension of buried contaminants (Kotta et al. 2006; Kristensen et al. 2011; Quintana et al. 2011; Delefosse et al. 2012; Urban-Malinga 2013). However, there is yet to be a report of a direct negative impact of *M. viridis* on other species (Norkko et al. 2012) and in this study, the

assemblage structure and abundance of other species did not seem affected by *M. viridis* presence. Additionally, the species may help increase oxygen levels in sediments, as *M. viridis* tolerates low oxygen levels, and is the first to settle in an area after a hypoxic event, which would lead to colonization by other species in otherwise inhabitable areas (Reise 2002; Norkko et al. 2012).

The potential for *Marenzelleria viridis* to be an ecosystem engineer and cause a change in communities depends on the population density of the species in a given area (Norkko et al. 2012). At present, densities of *M. viridis* in Newfoundland – or at the very least, the sampling locations – are quite low, and therefore *M. viridis* may be a "passenger" i.e., it does not significantly alter the ecosystem (Norkko et al. 2012). On the one hand if densities increase in coming years, the species may not necessarily have a negative impact on Newfoundland intertidal ecosystems –the arrival of *M. viridis* could fill an empty niche, provide an essential service, and create a more diverse community and a more resilient ecosystem. On the other hand, if abundances were to increase past some threshold, or if community structure changed, then *M. viridis* may drive ecosystem change, further altering community structure and possibly leading to the successful introduction of new, potentially harmful species (Reise 2002; Norkko et al. 2012). In this study, the abundances of *M. viridis* were, on average, higher in 2014 than in 2013. This increase coincided with increased abundance of *Heteromastus filiformis*, which has a similar lifestyle to *M. viridis,* and a decline in the abundance of the bivalve species *Macoma balthica* and *Mya arenaria*. One limitation in conclusions for this study is the lack of sampling during different times of year to investigate seasonal trends; future

studies should sample macrofauna during different times of the year, monitor the abundance of *M. viridis* in Bonne Bay and Indian Pond, and note any changes in species abundance. In addition, it would be beneficial to investigate other sites at different times of the year to determine the complete distribution of *M. viridis* in Newfoundland.

Chapter Three: Exploration of Prokaryotic and Bacterial Numbers in Sediments with *Marenzelleria viridis*

3.1 Introduction

Marine sediments, in the absence of bioturbation, become divided into stratified zones driven by the input of organic carbon (generally from above) and resulting from various redox processes, where electron acceptors of decreasing redox potentials are preferentially used at increasing depths (Nealson 1997; Matsui et al. 2004). Without fauna present, solute transport between sediments and overlying water occurs exclusively by passive diffusion or advection, limiting the aerobic respiration of organic matter to the depth to which oxygen can diffuse (Nealson 1997; Aller 2001). Beyond the depth of oxygen diffusion, a variety of anaerobic processes degrade organic matter, including nitrate reduction, manganese and iron oxidation, sulfur oxidation, metal reduction and finally, sulfate reduction in the deepest sediments (Nealson 1997; Matsui et al. 2004).

The most abundant inhabitants of marine sediments are the metabolically diverse prokaryotes. An enormous range of metabolic capabilities allows prokaryotes to exploit a variety of electron donors and electron acceptors (Nealson 1997). Generally, the availability of energy sources and metabolites, usually only available via diffusion, stratify prokaryote distributions although many prokaryotes are facultative in their metabolism and versatile in their requirements, with some capable of limited motility within the sediment (Nealson 1997; Aller 2001). The type of prokaryotes and their rate of

growth, in marine sediments depend upon the quality and the quantity of energy sources (Nealson 1997). Sulfate reducers and sulfur oxidizers are the two most common prokaryote groups that affect the sulfur cycle of marine sediments.

The sulfate reducers are a well-studied group of bacteria that dissimilate – or reduce - sulfate for energy gain (Jorgensen and Postgate 1982). Although considered obligate anaerobes, they can survive under aerobic conditions (Nealson 1997). In its highest oxidized state, sulfur exists as sulfate, a highly stable ion unless reduced biologically, e.g. by sulfate reducing bacteria (Gibson 1990; Nealson 1997). Sulfate is used as an electron acceptor for the oxidation of organic carbon (and thus the breakdown of organic matter), realeasing reduced sulfate as sulfide (Gibson 1990). About 50% of organic matter in coastal sediments is degraded by sulfate reducing bacteria, with the intensity of sulfate reduction dependent on the amount of organic matter present (Jorgensen and Postgate 1982; Gibson 1990). The sulfide produced by sulfate reducers contains a significant amount of energy, and as it diffuses down into the sediment it is converted to hydrogen sulfide (Jorgensen and Postgate 1982; Gibson 1990; Nealson 1997). Hydrogen sulfide is a strong reducing agent that is poisonous to most aerobic organisms because it binds to cytochrome-c oxidase, leading to metabolic breakdown (Gibson 1990; Hahlbeck et al. 2000). Hydrogen sulfide is, however, an important electron donor for another group of aerobic organisms – the sulfur oxidizing bacteria (Gibson, 1990).

Sulfur oxidizing bacteria are a highly diverse group of chemolithoautotrophs, chemolithoheterotrophs and mixotrophs, depending on their source of carbon (CO_2,

organic carbon or both; Jorgensen and Nelson 2004). One common feature of this group is the ability to oxidize reduced, inorganic sulfur compounds, such as hydrogen sulfide, elemental sulfur or thiosulfate, with oxygen or nitrate as the electron acceptor, and in doing so derive energy (Jorgensen and Postgate 1982; Nealson 1997). These bacteria are commonly found as endosymbionts or free-living in sediments, either forming filamentous mats on the sediment surface, living as individual filaments buried in the sediment, or as non-mat forming unicellular individuals, although the latter group has rarely been investigated (Jorgensen and Nelson 2004; Lenk et al. 2011). Sulfur oxidizers are highly abundant in intertidal sediments, and are important in both sulfur oxidation as well as inorganic carbon fixation, creating biomass and contributing to primary productivity (Lenk et al. 2011). Typically, these bacteria use oxygen as the electron acceptor to oxidize sulfur compounds, and require access to both sulfides and oxygen for their metabolism.Thus, most sulfur oxidizers ocuur at oxic-anoxic interfaces where oxygen and sulfides co-exist, where diffusing sulfides are rapidly chemically oxidized by metal oxides present in sediments or by oxygen (Nealson 1997; Jorgensen and Nelson 2004). One group of sulfur oxidizers can oxidize sulfur under anaerobic conditions using nitrate, which is incorporated into vacuoles under oxic conditions, as an electron acceptor instead of oxygen (Nealson 1997; Jorgensen and Nelson 2004). Nitrate is reduced primarily through dissimilatory nitrate reduction, producing ammonium, with sulfide suggested to be an important substrate for this pathway (Jorgensen and Nelson 2004).

While sulfate reducers and sulfur oxidizers are capable of motility (Jorgensen and Nelson 2004), in a system where passive diffusion is the main method of solute transport,

microbial growth and activity are primarily limited by the availability and diffusion rate of these energy sources. The diffusion driven stratification of the otherwise stratified biogeochemical system of sediments is broken down through processes of particle reworking and burrow ventilation, or bioturbation (Aller 2001; Kristensen et al. 2012). These activities alter the undisturbed sedimentary fabric as macrofauna move particles to build structures and irrigate them with oxygen for their own, oxic, metabolism. Particle transport can take place through sediment ingestion and/or egestion or via burrow construction, maintenance and subsequent collapse. Burrows vary from complex and semi-permanent to simple and transient (Aller 2001; Kristensen et al. 2012). Most notably, burrows create a "radial chemocline" with a vertical oxic-anoxic interface penetrating through the sediment layers (Steward et al. 1996; Matsui et al 2004). The exchange of solutes is enhanced by non-local and local transport across this interface as the burrow is ventilated and inhibitory metabolites are flushed out (Aller 2001; Kristensen et al. 2012, Renz and Forster 2014). Burrows created by a wide variety of infaunal invertebrate species stimulate metabolic activity and bacterial growth and increase the efficiency of organic matter breakdown (Alongi 1985; McIlroy and Logan 1999; Steward et al. 1996; Matsui et al. 2004; Papaspyrou et al. 2006; Ashforth et al. 2011; Bonaglia et al. 2013). The effects of macrofaunal activity on microbial communities depend on the depth and morphology of the burrow along with sediment characteristics, since selective grain sizes or a mucous lining can limit the exchange of certain solutes selectively (Kristensen et al. 2012; Renz and Forster 2014).

The polychaete *Marenzelleria viridis* constructs deep, blind ended I- or J-shaped burrows, creating an estimated 2.8 m^2 of burrow wall for every square metre of surface sediment (Renz and Forster, 2013). The 40 cm depth of this simple burrow construction, combined with a ventilation behavior described as a combination of ciliary and muscular pumping which drives water in and out of the burrows, stimulates anaerobic processes (particularly sulfate reduction) in sediments (Quintana et al. 2011, Bonaglia et al. 2013, Renz and Forster, 2014). Sulfate reduction was enhanced almost two fold in experimental cores with *M. viridis* (Kristensen et al. 2011), and other experiments have reported decreased denitrification rates (Bonaglia et al. 2013), increased production of ammonium (Renz and Forster 2014) and the growth of the sulfur oxidizing bacteria *Beggiatoa* spp. at the sediment surface attributed to the effusion of hydrogen sulfide (Quintana et al. 2013). Additionally, the ventilation behavior of *M. viridis* creates oscillations from oxic to anoxic conditions within the burrow and near the sediment-water interface as the cilia-driven irrigation draws oxygen from overlying surface waters deeper into the sediment, increasing the surface oxic zone for a short period (Jovanovic et al. 2014). The dynamic oxic-anoxic interfaces generated by *M. viridis* would likely create a favorable environment for microbes around the burrow and near the sediment-water interface surface, in particular sulfate reducers and sulfur oxidizers able to withstand fluctuations from complete anoxia to oxygenated conditions.

Previous work has demonstrated the enhancement of sulfate reduction and the growth of sulfur oxidizing bacteria in experimental cores with *Marenzelleria viridis* (Kristensen et al. 2011; Quintana et al. 2013), however, no study has quantified the

influence of *M. viridis* on the abundance of either sulfate reducers or sulfur oxidizers in sediments. Furthermore, there is a lack of experimental field studies looking at *M. viridis* and microbial communities under natural, uncontrolled conditions where seasonal effects, such as input of organic matter, may play a role. The goals of this chapter were to quantify sulfate reducing and sulfur oxidizing bacterial abundance using a fluorescence in situ hybridization (FISH) approach (cf. Manz et al. 1998; Ravenschlag et al. 2001; Matsui et al. 2004; Mermillod-Blondin et al. 2005), in order to: 1) determine the relative abundance of sulfate reducing and free-living sulfur oxidizing bacteria in *M. viridis* burrows and surrounding sediments; and 2) observe seasonal effects on the microbial communities therein.

3.2 Materials and Methods

3.2.1 Sample Site

Sediment samples were collected from Indian Pond, Newfoundland. Indian Pond is a brackish lagoon with a narrow opening into Conception Bay, with sediment composed mostly of sand (See Chapter 2 for more detail). During the course of this study, salinity varied from 10 to 20 and water temperature from 4°C in December to 26°C in July.

3.2.2 Sediment Collection and Preservation

To determine temporal trends of total prokaryotes, sulfur oxidizers and sulfate reducers, 3 g sediment samples (catergorized according to physical appearance and

hereafter refered to as sediment "type") were collected from *Marenzelleria viridis* rich sediments in Indian Pond (N 47° 27'21, W 53°05'42) in April, July, September and December of 2013 during the lowest low tide of each month. Triplicate surface sediment samples (to approximately 2 mm deep), and *M. viridis* fecal rods, identified by their characteristic "string-like" appearance as described by Renz and Forster 2013(Fig. 3.1), were collected using a stainless steel spatula rinsed with ethanol and transferred to 15 mL centrifuge tubes. Surface sediment was mainly composed of sand, light brown in colour, and appeared oxidized. Fecal rod samples were mostly similar to the surface sediment in appearance, with the exception of some fecal rods composed of finer, occasionally dark sediment. Following collection of surface and fecal rod samples, a shovel was used to carefully extract sediments. During this process, fracturing occurred and exposed *M. viridis* burrows (Fig. 3.2). Triplicate sediment samples were collected by scraping burrow linings occupied by *M. viridis*. Sediment collected from the burrow of *M. viridis* was composed mostly of sand, and brownish in colour, with grains bounded together, presumably by colorless mucus. In addition to burrow sediment samples of deeper (approximately 15-20 cm from the surface), reduced sediments, differentiated by color and termed "black reduced" and "grey reduced", were collected. Reduced sediment samples differed in colour but were similar in sand grain size and shape to each other and to surface samples.Surface and reduced sediments were sampled as far away as possible from the burrows to avoid potential influence of *M. viridis* ventilation activity.

Figure 3.1: Photograph of *Marenzelleria viridis* string-like fecal rods deposited on the sediment surface. Rods measure approxiamately 10mm in length (Photo Credit: Eli Schatz).

Figure 3.2: Photograph of *Marenzelleria viridis* occupied burrow, exposed by fracturing with a shovel. Samples from burrow sediment were collected by careful scraping; only sediment from occupied burrows were collected (Photo Credit: Eli Schatz).

Sediment samples were immediately fixed for cell counting with 4.5 mL of 2% formalin in 0.22 µm-filtered seawater buffered with 2% sodium tetraborate. Samples were left for 24 hours at 4°C, centrifuged at 16000x g for 5 minutes, and the supernatant removed. Samples were then rinsed with 4.5 mL of phosphate buffered saline (PBS) and preserved in a mixture of 1:1 PBS/ethanol 95% at -20°C until further processing (Danavaro, 2010).

3.2.3 Extraction and Filtering

The procedure to separate bacteria from sediment grains was modified from the extraction procedure outlined in Epstein and Rossel, 1995. Six mL of 0.1% sodium pyrophosphate, a detergent commonly used in extraction procedures to break apart mucus, was added to one gram of sediment sample. Samples were incubated at room temperature for 15 minutes, with 4 mL of filtered (0.22 µm), autoclaved seawater added afterwards. Samples were then sonicated for 30 minutes in a Bransonic 5510® ultrasonic bath to agitate the sediment grains and detach bacterial cells. Ice was added periodically to the ultrasonic bath to prevent samples from overheating. After sonication, samples were rinsed with 5 mL of filtered, autoclaved seawater, hand shaken, and particles left to settle for 1 minute. The resulting supernatant was then carefully decanted into a 50 mL centrifuge tube, and this washing process repeated 7 times. Samples were lightly centrifuged at 21000 rpm (500 g) for 5 minutes, and 1.5 mL of resulting supernatant carefully pipetted into a 2 mL Eppendorf tube. The extract was frozen at -20˚C until further processing.

Sample extracts were thawed, diluted by a factor of 83 (120 µL extract: 880 µL MilliQ water), pipetted onto GE black polycarbonate filters (0.22 µm, 25 mm diameter) and filtered using a Nalgene MittyVac hand vacuum pump with a pressure of no more than 5 mm Hg. Each filter was then transferred onto a glass slide, cut into quarters and air-dried. Individual quarter filters were transferred to clean, labeled glass slides for the hybridization procedure, using two of the quarters for each probe.

3.2.4 Hybridization

3.2.4.1 Selection of oligonucleotide probes and prokaryotic stain

Two oligonucleotide probes (GAM 660 and DSS 658) were selected to hybridize with sulfur oxidizers and sulfate reducers and determine their respective proportions within prokaryotic communities. The probe GAM 660 (Ravenschlag et al. 2001) targets sulfur oxidizing gammaproteobacteria and has previously been successfully used in marine sediment studies (Ravenschlag et al. 2001; Lenk et al. 2011). This particular group of bacteria was targeting as some studies have suggested *Marenzelleria viridis* ventilation activity may encourage the growth of sulfur oxidizing bacteria. The probe DSS 658 (Manz et al. 1998) targets one of the most common groups of sulfate reducing bacteria, the Desulfobacteraceae. This family is considered strictly anaerobic and includes *Desulfofrigus*, *Desulfococcus* and *Desulfosarcina* (Gibson 1990). This particular group of bacteria was targeting for three reasons: 1) the ventilation activity of *M. viridis* has been suggested to enhance the activity of sulfate reducers in sediment 2) this family is one of the most common groups of sulfate reducing bacteria and finally 3) this probe had similar

hybridization conditions as the GAM660 probe, and therefore hybridizations could be performed concurrently and a better comparison between the two groups for the same sample could be made. Both probes were labeled with fluorescein (excitation 450 nm, emission 518 nm) at the 5' end. Working solutions of probes were created by dilution with autoclaved TE (1 mM Tris buffer stock and 0.1 mM EDTA) to a final concentration of 25 ng/µL. Prokaryotes were counterstained using 4',6-diamidino-2-phenylindole (DAPI, excitation 365 nm, emission 463). Working solutions of DAPI were created by dilution of stock DAPI solution (5 mg/mL) with 0.22 µm filtered, autoclaved seawater for a final concentration of 5 ng/µL.

3.2.4.2 Hybridization Procedure

Filter samples were hybridized following the procedure outlined by Pernthaler et al. (2001), with all steps performed in the dark to avoid photosensitive flurochromes exposure to light and thus affecting counting of cells.. Hybridization solutions were prepared for each probe (DSS 658 and GAM 660) by combining 400 µL of 5x hybridization buffer (4.5 M NaCl, 0.05% SDS and 0.1M Tris HCl), MilliQ water and formamide, to obtain a final formamide concentration for each probe as in Table 3.1. Once prepared, 20 µL of this hybridization solution (2 µl of probe working solution + 18 µL hybridization solution) was pipetted directly onto each filter section, previously placed on a glass slides. The slides were then transferred into pre-warmed hybridization chambers (50 mL centrifuge tubes containing hybridization solution) and incubated in a 46°C water bath for 1.5 hours.

Following hybridization, filter sections were transferred individually into 2 mL Eppendorf tubes containing 1.5 mL of pre-warmed (48°C) 10x wash buffer (1.0M NaCl, 0.2M Tris HCl, 0.001% SDS, 50 mM EDTA) for 15 minutes in a 48°C water bath to remove non-specific staining. Filter sections were removed from the wash buffer, rinsed with distilled water and placed on clean, glass slides. Each filter section was then counter-stained with 50 µL of DAPI [5.0 ng/µL] for 3 minutes, rinsed with distilled water, and then rinsed with 80% ethanol for several seconds to remove non-specific DAPI staining and reduce background fluorescence. Filter sections were air dried on clean, labeled glass slides and mounted in PermaFluor (ThermoScientific). Slides were left in the dark overnight at 4°C before counting.

Table 3.1: Oligonucleotide Probes used in this study

Probe Name	Sequence	Formamide Concentration	Hybridization T^{o} & Duration	Reference
GAM 600	TCCACTTCCCTCTAC	35-40%	46°C for 90 mins	Ravenschlag,et al. 2001
DSS 658	TCCACTTCCCTCTCCCAT	60%	46°C for 90 mins	Manz et al., 1998

3.2.5 Counting

Slide preparations were examined in the dark at 1000x magnification with a light microscope fitted for epifluorescence with a Carl Zeiss Microscopy GmbH, Germany filter set 01 (for DAPI stained cells) and filter set 09 (for cells hybridized with fluorescein-labeled DSS 658 and GAM 660). All DAPI- and fluorescein-labeled cells within a single field of view were counted, for a minimum of 10 fields of view and 200-400 DAPI stained cells for each sample. These numbers were then used to calculate the proportions of prokaryotic cells that were sulfate reducers (DSS:DAPI) or sulfur oxidizers

(GAM660:DAPI) in each sample. All counts were made within a 24 hour period (following Kepner and Pratt 1994).

To calculate the total number of prokaryotes per gram of sediment, the following formula was used (Danovaro, 2010):

(Average cell number for each optical field) x [Optical field coefficient x extraction coefficient x dilution factor of the sediment/ [sediment wet weight]

Where:

Optical field coefficient =(filtration area/counting area), = 226.98 mm / 0.026 mm = 8730

Correction for the extraction co-efficiency = 1.44

To calculate the total number of sulfate reducers or sulfur oxidizers, the following formula was used (Mermillod-Blondin et al. 2005):

(Total number of prokaryotes) x (proportion of cells detected by probe to DAPI stained cells)

3.2.6 Statistical analyses

Once the number of prokaryotes and bacterial groups were calculated, the effects of the five types of sediment and four collection months were compared using a two –way ANOVA with type of sediment and collection months as main effects (Minitab® 17, Minitab® Statistical Software) for total prokaryotes, number of sulfate reducers and

sulfur oxidizers, and ratio of sulfate reducers and sulfur oxidisers. Tukey post-hoc tests determined which type of sediment or collection month differed significantly.

3.3 Results

3.3.1 Total Prokaryotic Abundance

The total number of cells stained with DAPI per gram of wet sediment was similar for all types of sediment sampled in all collection months, with the highest mean cell count of $9.76 \pm 1.68 \times 10^7$ cells g^{-1} for burrow samples collected in December (n =3), and the lowest mean count of $4.92 \pm 0.14 \times 10^7$ cells g^{-1} for fecal rod samples collected in July (n= 3) (Figure 3.3). Type of sediment did not significantly affect the number of cells stained by DAPI (F-value = 1.51, p-value = 0.218, df= 4), nor did the month in which samples were collected (F-value = 2.31, p-value = 0.091, df= 3). There were also no significant interactions between month and sediment sampled (F-value = 1.77, p-value = 0.088, df =12).

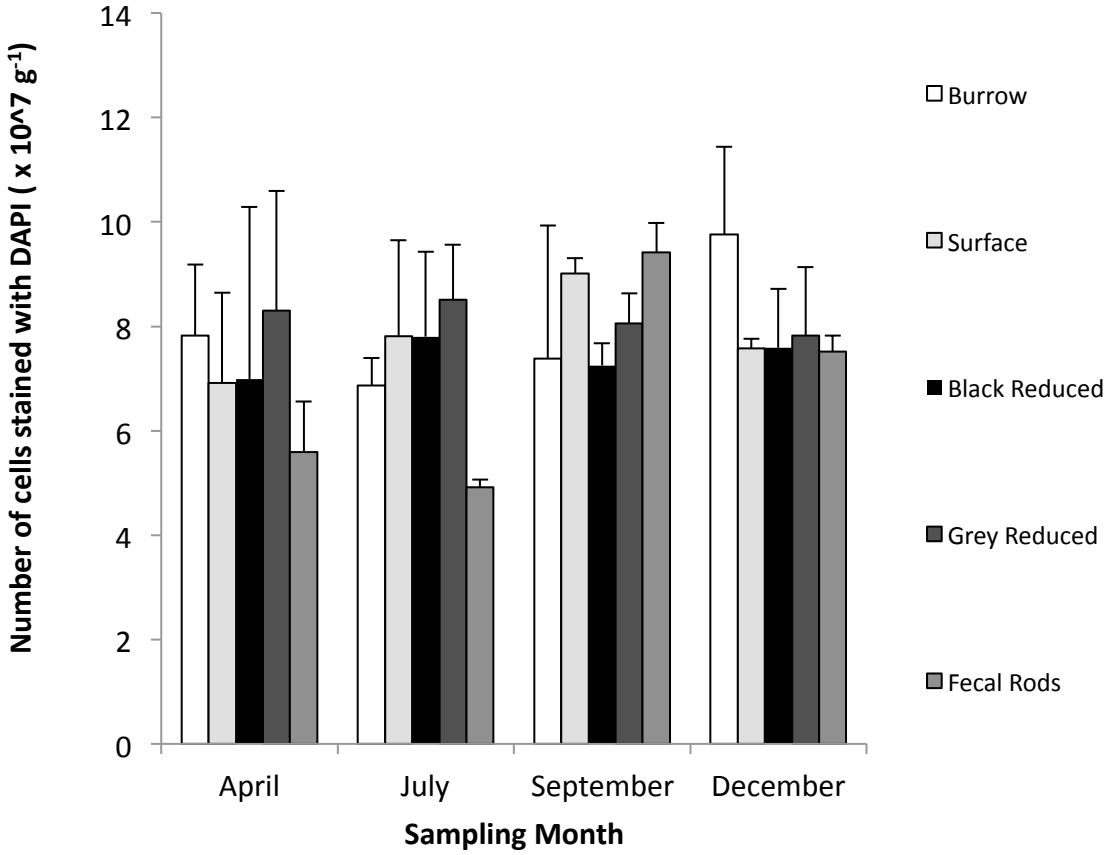


Figure 3.3: Abundance of prokaryotic cells per gram of wet sediment detected by DAPI in burrow sediment and fecal rods of *Marenzelleria viridis,* and in surface, black and grey reduced sediment samples (mean ± SD, n=3)

3.3.2 Sulfate Reducing Bacteria (DSS 658)

The mean number of cells detected by the DSS 658 probe per gram of wet sediment varied between types of sediment samples and collection month, with fecal rod samples collected in September having the highest mean of 4.20 ± 1.08 x 10^7cells g^{-1}, and grey reduced sediment samples collected in July having the lowest mean of 0.20 ± 0.23 x 10^7 cells g^{-1} (Figure 3.4; See Appendix 2 for full list of counts of DSS 658 cells). Type of sediment did not significantly affect the number of cells detected by the DSS 658 probe

(F-value = 0.68, p-value = 0.608, df= 4), nor was there a significant interaction between type of sediment collected and month sampled (F-value = 1.21, p-value 0.31, df= 12). Sampling month did significantly affect (F-value = 6.93, p-value = 0.00, df= 3) the number of cells detected by the DSS 658 probe for grey reduced sediment samples (F-value = 5.83, p-value = 0.02, df= 3), with significantly lower number of cells in samples collected in July than samples collected in September. Sampling month did not have a significant effect on number of cells in other types of sediment.

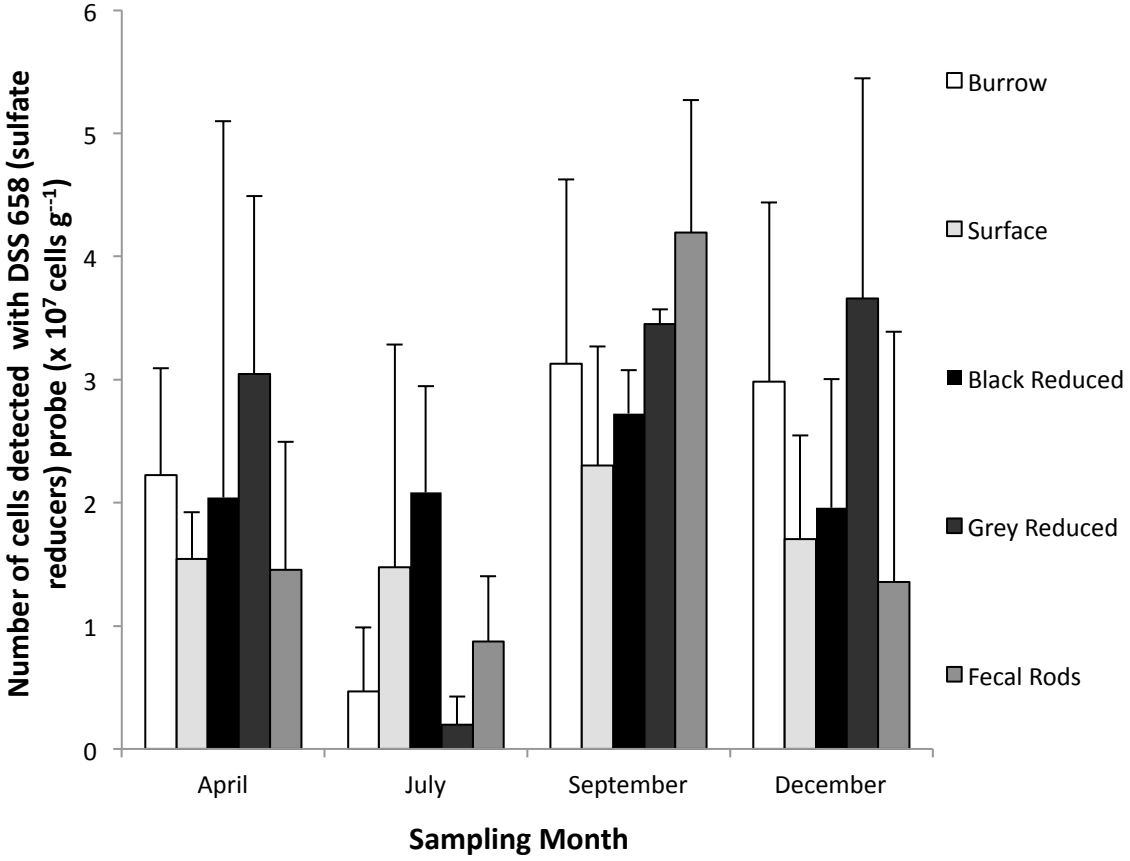


Figure 3.4: Abundance of sulfate reducers detected by the DSS 658 probe in burrow sediment and fecal rods of *Marenzelleria viridis*, and in surface, black and grey reduced sediment samples (mean ± SD, n=3)

3.3.3 Sulfur Oxidizing Bacteria (GAM 660)

As found with cells labeled with the DSS658 probe, the mean number of cells detected with the GAM 660 probe also varied among sediment samples and collection month. Fecal rod samples collected in September contained the greatest number of labeled cells, with $4.19 \times 10^7 \pm 0.27$ cells g^{-1}; black reduced sediment samples collected in July had the least: $0.78 \pm 0.70 \times 10^7$ cells g^{-1} (Figure 3.5; see Appendix 3 for full list of GAM 660 cells). Type of sediment did not significantly affect the number of cells labeled by the GAM 660 probe (F-value = 0.50, p-value = 0.739, df= 4), and there was no significant interaction between type of sediment and collection month (F-value = 1.51, p-value = 0.160, df= 12). Sampling month had a significant effect (F-value = 8.70, p-value = 0.00, df= 3) on the number of cells labeled by the GAM 660 probe for both black (F-value = 4.22, p-value = 0.05, df= 3) and grey (F-value = 5.79, p-value = 0.02, df =3) reduced sediments. The number of cells detected in both reduced sediments was significantly lower for samples collected in July compared to those samples collected in December, with a significantly lower number of cells in grey reduced sediment collected in July than in samples collected in September. Sampling month did not significantly affect the number of cells detected in other sediment samples.

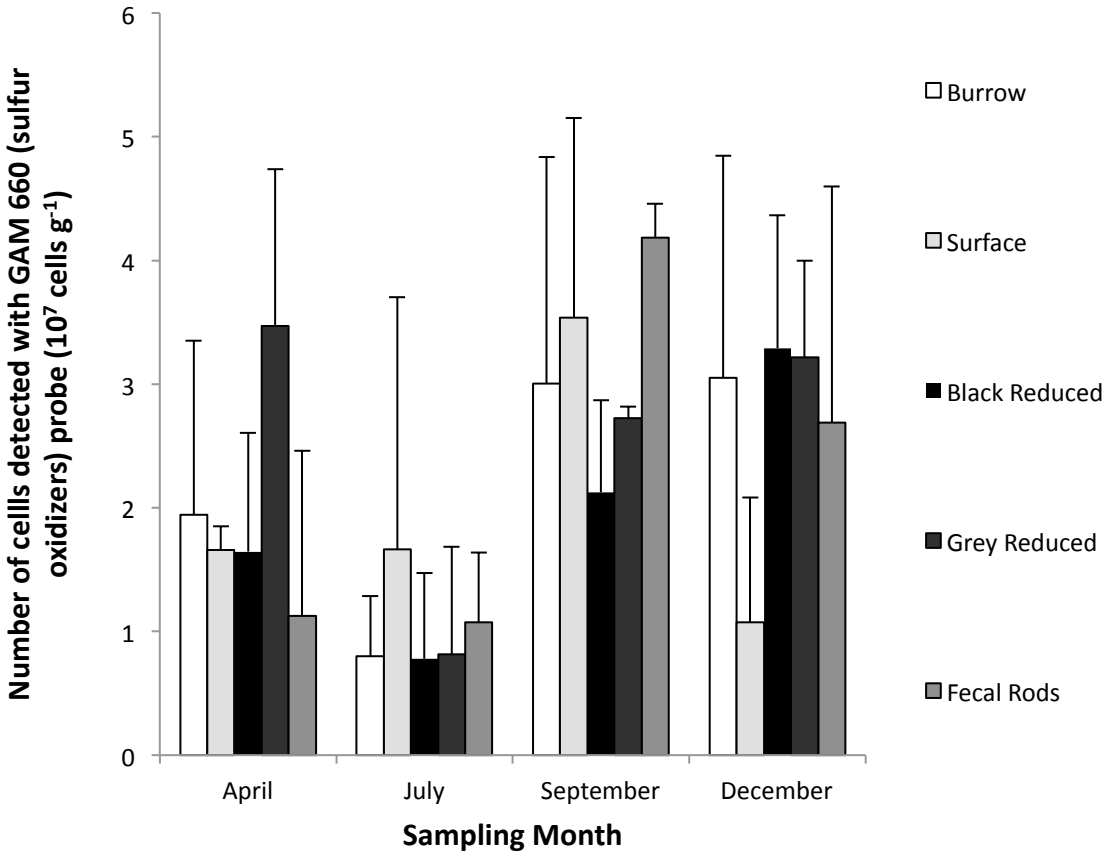


Figure 3.5: Abundance of sulfur oxidizers detected by the GAM 660 probe in burrow sediment and fecal rods of *Marenzelleria viridis*, and in surface, black and grey reduced sediment samples (mean ± SD, n=3)

3.3.4 Percentage of cells labeled with DSS 658 and GAM 660

The percentage of prokaryotic cells labeled with the DSS 658 and GAM 660 probes ranged widely, with mean percentages ranging from 3-43% for cells labeled by DSS 658, and a range of 8-45% for cells detected by GAM 660 (Table 3.2). Type of sediment did not significantly affect the percentage of cells detected by either the DSS 658 or GAM 660 probe (F-value = 0.74, p-value = 0.57; F-value = 0.70, p-value = 0.60, df= 4), nor was there a significant interaction between type of sediment and sampling

month on percentage of cells detected for either probe (F-value = 1.33, p-value = 0.243; F-value= 1.42, p-value = 0.197, df = 12). Sampling month did significantly affect the percentage of cells detected by both DSS 658 and GAM 660 (F-value = 7.33, p-value = 0.00; F-value = 10.23, p-value = 0.00, df = 3, respectively).

The percentage of cells detected by the DSS 658 probe was significantly lower (F-value = 6.74, p-value = 0.01, df = 3) in burrow samples collected in July compared to September, and the percentage of these cells were also significantly lower in grey reduced sediment samples collected in July compared to all other sampling months (F-value = 10.60, p-value = 0.00, df=3).

The percentage of cells detected by the GAM 660 probe was significantly lower in both black (F-value = 5.99, p-value =0.02, df = 3) and grey (F-value = 13.94, p-value = 0.00, df = 3) reduced sediment samples. The percentage of these cells detected in black reduced sediment was significantly lower for samples collected in July compared to those samples collected in December, and for grey reduced sediment the percentage of cells detected was significantly lower in July compared to all other sampling months.

The ratio of cells labeled with the two probes (number of cells detected by DSS 658 : number of cells detected by GAM 660 per sample) was similar among types of sediment and sampling months, with at least twice as many cells labeled with DSS 658 than with GAM 660 in burrow and fecal rod samples collected in April and black reduced sediment collected in July (Figure 3.6). Type of sediment or sampling month did not significantly affect the ratio of cells labeled with the probes (F-value = 0.57, p-value = 0.689, df=4; and F-value = 0.51, p-value = 0.679, df= 3, respectively). There was also no significant

interaction between type of sediment sampled and collection month (F-value = 1.91, p-value = 0.063, df = 12).

Table 3.2: Percentages of prokaryotic cells detected by oligonucleotide probes (mean ± SD, n=3) for each sediment type, at each sampling month. The total number of cells labeled by DAPI (# DAPI) per gram of wet sediment, the percentage of prokaryotic cells labeled by DSS 658 and GAM 660 (%DSS 658, %GAM 660) and the ratio of cells labeled by each of the probes (DSS 658:GAM660) are presented.

	April	July	September	December
Burrow				
# DAPI ($x10^7 \cdot$ g dry sediment^{-1})	7.8 ± 1.4	6.9 ± 0.5	7.4 ± 2.5	9.8 ± 1.7
% DSS 658	29.2 ± 6.9	6.4 ± 7.3	38.8 ± 6.2	28.9 ± 14.0
% GAM 660	23.2 ± 16.7	12.1 ± 5.9	39.6 ± 15.6	31.7 ± 12.8
DSS 658:GAM 660	2.2 ± 2.2	0.5 ± 0.7	1.2 ± 0.3	1.2 ± 0.9
Surface				
# DAPI ($x10^7 \cdot$ g dry sediment^{-1})	6.9 ± 1.7	7.8 ± 1.8	9.0 ± 0.3	7.6 ± 0.2
% DSS 658	22.5 ± 2.9	17.6 ± 19.0	26.2 ± 13.8	23.3 ± 10.8
% GAM 660	24.6 ± 4.7	18.1 ±18.6	38.6 ± 14.0	14.2 ± 14.3
DSS 658:GAM 660	0.9 ± 0.2	1.0 ± 0.7	0.8 ± 0.6	2.2 ± 1.0
Black reduced				
# DAPI ($x10^7 \cdot$ g dry sediment^{-1})	7.0 ± 3.3	7.8 ± 1.6	7.3 ± 0.4	7.6 ± 1.1
% DSS 658	20.3 ± 27.4	28.6 ± 8.5	35.1 ± 3.5	27.9 ± 8.2
% GAM 660	24.6 ± 13.5	8.4 ± 5.5	31.2 ± 8.7	38.5 ±6.7
DSS 658:GAM 660	0.8 ± 1.2	3.7 ± 1.9	1.3 ± 0.3	0.6 ± 0.3
Grey reduced				
# DAPI ($x10^7 \cdot$ g dry sediment^{-1})	8.3 ± 2.3	8.5 ± 1.1	8.1 ± 0.6	7.8 ± 1.3
% DSS 658	37.7 ± 9.2	2.1 ± 2.2	43.7 ± 2.0	43.2 ± 18.8
% GAM 660	39.0 ± 4.6	9.3 ± 10.4	33.4 ± 2.3	43.7 ± 8.2
DSS 658:GAM 660	0.9 ± 0.3	0.2 ± 0.1	1.3 ± 0.0	1.1 ± 0.4
Fecal Rods				
# DAPI ($x10^7 \cdot$ g dry sediment^{-1})	8.3 ± 2.3	8.5 ± 1.1	8.1 ± 0.6	7.8 ± 1.3
% DSS 658	37.7 ± 9.2	2.1 ± 2.2	43.7 ± 2.0	43.2 ± 18.8
% GAM 660	39.0 ± 4.6	9.3 ± 10.4	33.4 ± 2.3	43.7 ± 8.2
DSS 658:GAM 660	3.4 ± 4.6	0.9 ± 0.3	1.0 ± 0.2	0.6 ± 0.7

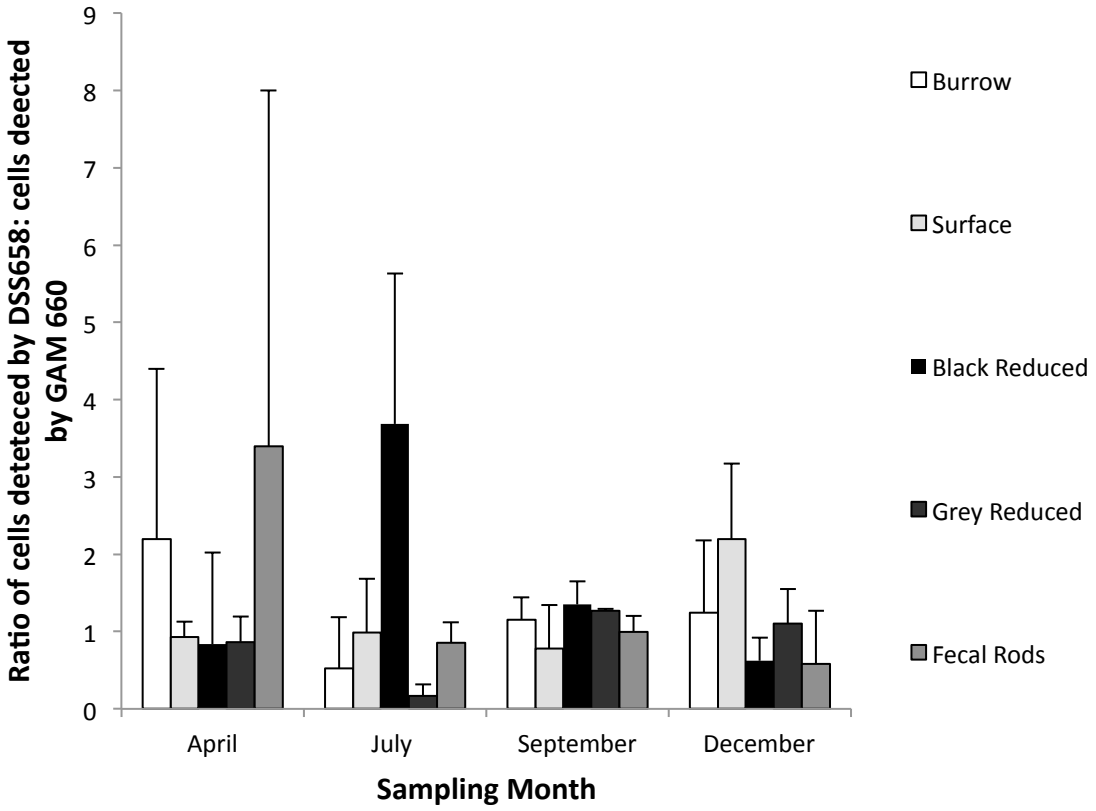


Figure 3.6: Prokaryotic cells detected by either DSS 658 or GAM 660 probe (expressed as a ratio of number of cells detected by DSS 658: number of cells detected by GAM 660 within a single sample) in burrow sediment and fecal rods of *Marenzelleria viridis*, and in surface, black and grey reduced sediment samples (mean ± SD, n= 3)

3.4 Discussion

3.4.1 Total Prokaryotes

The total abundance of prokaryotes in all sediments sampled, ranging from 4.48 x 10^7 cells g^{-1} and 1.08 x 10^8 cells g^{-1} is almost one to two orders of magnitude lower than other studies from similar environments. In Arctic marine sediments, abundances were found to range between 2.0 x 10^8 to 4.0 x 10^9 cells mL^{-1} in September/October, 2.1 to 4.7

x 10^9 cells mL^{-1} in July, and between 1.8 to 4.1 x 10^9 cells mL^{-1} when experimentally exposed to temperatures ranging from 0 to 20˚C (Sahm and Berniger 1998, Ravenschlag et al. 2001, Robador et al. 2009). Temperate, estuarine sediments had total prokaryotic numbers between 11.9 to 15.5 x 10^9 mL in July and 3.8 to 6.1 x 10^9 when exposed to temperatures ranging from 0 to 20˚C (Wellsbury et al. 1996; Robador et al. 2009). The numbers of prokaryotes reported here are slightly lower than in another study, also from Conception Bay, Newfoundland, which reported a range of 3.5 x 10^8 to 1.3 x 10^9 prokaryotic cells ml^{-1} at the sediment-water interface (Goudie 1997). A study of planktonic bacteria in Conception Bay reported values ranging from 1.52 to 4.12 x 10^5 cells ml^{-1} (Pomeroy 1991), which is slightly lower than worldwide abundances. Similarly to planktonic bacteria, the abundance of prokaryotes in intertidal sediments in Newfoundland may fall on the lower end of a worldwide abundance spectrum; confirmation requires more studies in other intertidal regions in Newfoundland.

Prokaryotic numbers obtained from four collection months were similar, despite differences in temperature during these time periods. Temperature has been positively linked to prokaryotic abundance (Garcia-Martinez et al. 2009). These findings suggest that the total prokaryotic abundances in Indian Pond are independent of temperature, and may be influenced by other physical or chemical factors, as suggested in other studies showing no or weak temporal trends in abundances (Boer et al. 2009; Garcia- Martinez et al. 2009). It is possible that the prokaryotic community itself varies, with certain taxa dominating under different temperature and substrate conditions; however, this study

focussed on two major functional groups, the sulfate reducers and sulfur oxidizers, rather than the diversity of the prokaryotic community as a whole.

Typically, surface sediments support higher abundances of prokaryotes than in deeper, reduced sediments (Ravenschlag et al. 2001; Llobet-Brossa et al. 2002; Matsui et al. 2004; Papaspyrou et al. 2006; Boer et al. 2009), and significantly higher abundances within or in the vicinity of an organism's burrow than surrounding sediments (Aller and Aller 1986; Papaspyrou et al. 2005, 2006). This study showed little difference in total prokaryotic abundance between the oxic surface, reduced deeper sediments, the burrow material and fecal rods of *Marenzelleria viridis*. A lack of vertical differentiation in the abundance of prokaryotes has been attributed to high energy input from either waves or tides, creating a high sediment turnover rate and therefore a mixing of prokaryotic numbers (Garcia-Martinez et al. 2009), or to higher porewater content within sediments (Ravenschlag et al. 2001). Indian Pond is a small, sheltered, brackish lagoon with relatively low energy input. While Indian Pond serves as a cooling water intake source for the Holyrood Thermal Generating Station and experiences strong tidal currents (personal observation), these processes do not generate the level of energy required to disrupted vertical sediment layers. It is possible that physical and chemical processes in Indian Pond promote equal numbers of prokaryotes at the surface and in deeper sediments, independent of mechanical disruption. The prokaryotic communities within each type of sediment sampled are likely to be distinct, and so the relative abundance of certain taxa may differ, however, the prokaryotic community as a whole was not investigated.

3.4.2 Sulfate Reducers

The total abundance of sulfate reducers targeted by the DSS 658 probe ranged from 8.29×10^5 cells g^{-1} to 5.56×10^7 cells g^{-1}, with no detected sulfate reducers in some samples. Unfortunately, no studies have investigated abundances of sulfate reducers in Newfoundland sediments using this probe, so it is not known if these values are within normal ranges for this region.

The abundance of sulfate reducers from samples was similar in the four collection months, with slightly (non-significant) lower abundances in July, with the exception of abundances in grey reduced sediment samples which were significantly lower than samples collected in September. Abundances of sulfate reducers were relatively constant temporally, although the abundance of specific groups of sulfate reducers declined with increasing temperature; some groups of sulfate reducers may be better able to use specific available substrates, such as diatom-derived carbohydrates, during a given time period (Haynes et al. 2007; Garcia-Martinez et al. 2009; Robador et al. 2009). The lower abundance of sulfate reducers in July samples may relate to increased temperatures and difference in available substrates. These variables may inhibit the growth of the targeted sulfate reducing bacteria and give other groups of prokaryotes, not identified in this study, a competitive advantage.

Sulfate reducers were detected in all types of sediments; this result was expected because the targeted *Desulfobacteraceae* is the most abundant group of sulfate-reducing bacteria (Garcia-Martinez et al. 2009). Although considered to be strict anaerobes, members of this group can temporarily survive under aerobic conditions and occur in a

wide variety of marine sediments, including oxic-anoxic interfaces and oxic surface sediments, with some reports of higher sulfate reducer abundance at the surface than at depth (Nealson 1997; Llobet-Brossa et al. 2002; Matsui et al. 2004; Buhring et al. 2005). Aggregates of these bacteria could exist within anoxic microhabitats at the surface and within *Marenzelleria viridis* burrows; furthermore, aggregations with oxygen consuming bacteria such as sulfur oxidizers could provide sulfate reducers with further protection from oxygen (Matsui et al. 2004).

The detection of sulfate reducers in all types of sediments is not unusual; however, the abundance of detected cells and the percentage of total prokaryotes are unexpected. As mentioned above, abundances of sulfate reducers in Newfoundland sediments are unknown so it is unclear whether these values are typical for this region. Studies elsewhere reported lower proportions of DSS 658 targeted sulfate reducers, between 16% of total prokaryotes in Arctic subtidal sediments, and 1.0 - 5.3% in mud flats in up to 20 cm depth (Ravenschlag et al. 2000; Llobet-Brossa et al. 2002), although Buhring et al. (2005) found this group accounted for 43% of total bacterial abundance in deep sediment (excluding Archaea). The *Desulfobacteriaceae* are metabolically diverse and therefore better competitors than most other sulfate reducing groups (Matsui et al. 2004). The high abundance and percentage of these bacteria in the sediment samples of the current study may be linked to the ventilation behavior and irrigation effects of *Marenzelleria viridis*. Oscillations between oxic and anoxic conditions presumably supply the *Desulfobacteriaceae* with a variety of fresh metabolites for sulfate reduction while simultaneously excluding competitors unable to cope with oxygen exposure and dynamic

conditions. Because the burrow is assumed to be nutrient rich from *M. viridis*-derived organic matter and influxes of reduced porewater, a higher number of sulfate reducers was expected in burrows compared to surrounding and surface sediments, especially given enhanced sulfate reduction in experimental sediments containing *M. viridis (*Matsui et al. 2004, and references therein; Kristensen et al. 2011). The lack of significant differences in the abundance and proportion of sulfate reducers between types of sediment is therefore unexpected; however, homogeneity between burrow, surface and reduced sediments are consistent with observations of oscillating oxic to anoxic conditions both within the burrow and at the sediment surface (Jovanovic et al. 2014). Altough *Desulfobacteriaceae* are highly abundant in all sediments, other sulfate reducers not targeted by the DSS 658 probe may have been present in the burrow and surrounding sediments, and gone undetected in this study. Future investigations using a similar methodology but with a variety of probes targeting other sulfate reducing groups could shed further light on the sulfate reducing communities associated with *M. viridis* burrows.

3.4.3 Sulfur Oxidizers

The mean total abundance of sulfur oxidizers targeted by the GAM 660 probe ranged from 1.06×10^6 cells g^{-1} to 5.02×10^7 cells g^{-1}. As for the DSS 658 probe, the lack of studies investigating sulfur oxidizers in Newfoundland using the GAM660 probe, precludes any conclusion on whether these values are within a typical range.

As for sulfate reducers, the abundance of sulfur oxidizers within samples from the four collection months did not differ significantly among most sediment types, with the

exception of significantly lower abundances in July within both reduced sediment samples. Studies of temporal trends in sulfur oxidizer abundance are few; however, sulfur oxidizers are also likely temperature sensitive, as demonstrated for sulfate reducers (Haynes et al. 2007; Robador et al. 2009). The decreased abundance of sulfur oxidizers in reduced samples from July may relate to higher temperature or different substrate availability.

Sulfur oxidizers were detected in all types of sediment sampled, which was expected since gammaproteobacteria are common and highly abundant in intertidal sediments (Jorgensen and Nelson 2004; Lenk et al. 2011). While unicellular, free living, non-mat forming sulfur oxidizers are rarely investigated, they are common at oxic-anoxic interfaces, can migrate up to 15 cm and can occur in large numbers in sub-oxic sediments a few centimeters beneath the sediment-water interface (Jorgensen and Nelson 2004; Lenk 2006).

The abundance of sulfur oxidizers detected by the GAM 660 probe did not differ significantly among types of sediment sampled. The abundance of the sulfur oxidizers appears consistent with values from Ravenschlag et al. (2001) who reported values up to 9.4×10^{7} cells mL^{-1}. The lack of a difference between surface and reduced sediments is consistent with thier study, which reported no vertical zonation. Some sulfur oxidizers can oxidize sulfur in anaerobic conditions, so the presence of abundant sulfur oxidizers in reduced sediment may not be uncommon. An abundance of sulfur oxidizers in burrow sediments is likely to be common, because burrows commonly concentrate at the oxic-anoxic transition zone.

Although the abundance of sulfur oxidizers appears within ranges reported in comparable studies, the proportions of sulfur oxidizers are much higher – up to 45% in this study, compared to 2.1% reported by Ravenschlag et al. (2001). The high percentage of sulfur oxidizing bacteria in all types of sediment may be a product of the dynamic redox conditions in the sediment created by *Marenzelleria viridis*. Additionally, the stimulation of sulfate reduction results in production of more sulfide, to the benefit of sulfur oxidizers. The highly dynamic conditions would also exclude competitors and allow sulfur oxidizers to flourish.

Admittedly, as was the case with sulfate reducers, the sulfur oxidizers targeted by the GAM 660 probe do not target all sulfur oxidizers that may have been present, given that they are a highly diverse group. Additionally, different sulfur oxidizers may have been present in different sediment samples, given that the probe targets a wide diversity of sulfur oxidizing bacteria, with some better able to cope with higher sulfide concentrations than others (Thomas et al. 2014). Future work should use a wider variety of probes to target more specific groups, or use a metagenomic approach to shed further light on the sulfur oxidizing communities associated with *Marenzelleria viridis* burrows.

3.4.4 Ratio of Sulfate Reducers to Sulfur Oxidizers

The similar and approximately 1:1 ratio between sulfate reducers and sulfur oxidizers for all sediment types and at the four collection months indicates similar abundances of both groups despite the varying sediment conditions. Because sedimentary conditions were expected to differ among samples, and because sulfate reducers are

considered strict anaerobes and sulfur oxidizers require aerobic conditions to undergo metabolism (Nealson 1997; Jorgensen and Nelson 2004), a predominance of one or the other group was expected, especially in sediments with different oxygen conditions. Ventilation by *Marenzelleria viridis* may obscure patterns by creating dynamic fluctuating oxygen conditions within the burrow, surrounding sediments and also at the sediment surface (Jovanovic et al. 2014). Given that Indian Pond sediments are highly porous and chemical conditions vary over short temporal scales, sulfate reducers and sulfur oxidizers tolerant of these changes may coexist in relatively similar abundances given that conditions fluctuate too much to have one group dominate over the other. Although this study investigated specific groups of sulfate reducers and sulfur oxidizers as opposed to sulfate reducing and sulfur oxidizing communities as a whole; future studies using a wider variety of probes could determine whether broader microbial communities associated with *M. viridis* occur in similar abundances. Addtioanally, examining oxygen conditions in the sediment in realtion to the prokaryotes could aid in the interpretation on the sulfur oxidizing and sulfate reducing bacterial communities.

3.4.5 Conclusions

The lack of significant differences in the abundance and relative proportions of total prokaryotes, sulfate reducers and sulfur oxidizers between different sediment types was unexpected; however low replication and lack of oxygen and sulfur characterization of the sediments limits interpretation and could be improved upon in future studies.The (presumably) nutrient rich environment of the burrow wall, enhanced by the dual

ventilation of *Marenzelleria viridis*, was expected to support a higher abundance of sulfate reducers and sulfur oxidizers than surrounding sediments with more stable redox conditions. Burrows with lower or similar total prokaryotic abundances compared to surrounding sediments still have as high, or sometimes higher, microbial activity than non-burrowed sediment (Alongi 1985; Papaspyrou et al. 2005, 2006). The enhanced activity of microbes along burrow linings has been attributed to grazing by meiofauna, protozoa or macrofauna, which maintains prokaryotes in a constant growth phase (Alongi 1985; Grossmann and Reichardt 1991; Mayer et al. 1995; Traunspurger et al. 1997; Papaspyrou et al. 2005, 2006). Deposit feeding by macrofauna is thought to selectively impact certain biogeochemical processes, with species-dependent enhancement of given processes (Grossmann and Reichardt 1991; Mayer et al. 1995). *Marenzelleira viridis* stimulates the microbial activity of specific groups of bacteria, in particular sulfate reducers (Kristensen et al. 2011; Bonaglia et al. 2013). While the activity of prokaryotes and abundance of meiofauna were not measured in this study, observations of *M. viridis* deposit feeding in its burrow (Essink and Kleef 1988) suggest that these polychaetes stimulate microbial activity through consumption of microbes from the burrow lining, but without increasing the total abundance of microbes in comparison to surrounding sediments. The unique ventilation habits of *M. viridis* may therefore create an environment favorable for specific types of bacteria, such as the *Desulfobacteriaceae* and sulfur oxidizers able to switch carbon and energy sources, which could subsequently be consumed by *M. viridis*.

Chapter Four: Investigation of the Diet of *Marenzelleria viridis*

4.1 Introduction

Polychaetes of the family Spionidae are common inhabitants of organic rich sediments, where they either surface deposit feed or suspension feed in overlying water using characteristic feeding palps (Dauer et al. 1981). The paired feeding palps of spionids contain a median, ciliated food groove, and when in contact with the sediment surface, particles are transferred to this food groove and transported via cilia to the pharynx for ingestion (Dauer et al. 1981; Dauer 1997). Under certain conditions, feeding palps are used to suspension feed and collect food particles in the water column (Dauer 1997).

The spionid *Marenzelleria viridis* has received recent attention as a result of its widespread invasion of European waters, with diverse consequences to benthic resident communities and habitats (Kristensen et al. 2011; Delefosse et al. 2012; Norkko et al. 2012; Quintana et al. 2013). Morphological specialization on the distinctive feeding palps of *M. viridis* may provide clues to better understand the ecological success of this species. Compared to other spionids, the feeding palps of *M. viridis* are relatively short and can produce only a small feeding radius at the sediment-water surface; consequently, these polychaetes must leave the safety of their burrow to surface deposit feed unless food particles are rapidly replenished (Dauer 1997). Additionally, the cilia of the palp food groove are distinct in *M. viridis*. On most spionids feeding palps, frontal cilia line the

median food groove to capture food particles, lateral and latero-frontal cilia produce currents and direct food particles to the frontal surface of the palps, and sensory cirri and cilia may be used to reject non-food particles from the pharynx (Dauer 1997). The number of ciliary types varies among species, and *M. viridis* only has frontal cilia and scattered cirri on the lateral and abfrontal surface of their palps (Dauer 1997). The feeding palps of all other spionids have a symmetrical arrangement of frontal cilia lining a median groove that directs food towards the pharynx. In *M. viridis*, the frontal cilia on one side of the palp extend away from the food groove on a flat lateral extension when the palp is in contact with the sediment surface (Dauer 1997). The functional significance of this ciliary arrangement has not been explored or determined by direct observation, but is hypothesized to aid in dislodging mucus-bound particles of bacteria (Dauer 1997).

Spionids are generally considered to either feed on freshly deposited material or diatoms at the surface, suspension feed, or use both feeding mechanisms facultatively (Dauer et al. 1981). *Marenzelleria viridis* exhibits different feeding strategies under different sediment conditions, with suspension feeding observed in silt-clay sediments, and deposit feeding in silt-sand sediments despite an abundance of suspended material (Dauer 1997). *Marenzelleria viridis* has been documented feeding on benthic diatoms at the surface, surface deposit feeding, suspension feeding and potentially feeding on reduced burrow sediments (Sanders et al. 1962; Dauer et al. 1981; Essink and Kleef 1988; Miller et al. 1992; Zettler et al. 1996; Urban–Malinga et al. 2013). It is considered herein that the feeding palps of *M. viridis* are likely inefficient in collecting food particles at the sediment-water interface because of their small size and ciliary arrangement; the potential

for suspension feeding is similarly weak in this species because no palp movement was observed at higher current speeds (Dauer 1997; Miller et al. 1992). The inferred inefficiency of both surface deposit feeding and suspension feeding strategies suggests an alternate method of food collection of *M. viridis*. Deep tier deposit feeding has been suggested based on reports of both light and dark fecal rods inferred to result from feeding on oxic (light) and reduced (dark) sediments respectively (Dauer 1997; Essink and Kleef 1988).

The unique ventilation behavior of *Marenzelleria viridis* enhances the flux of metabolites within sediments, stimulates anaerobic processes and creates oscillations of oxic and anoxic conditions (Hahlbeck 2000; Kristensen et al. 2011; Quintana et al. 2013; Jovanovic et al. 2014), and may play a role in meeting the nutritional requirements of *M. viridis* by enhancing a food source of chemosynthetic origin. The purpose of the alternating ventilation behavior is not clear, and the extreme tolerance of *M. viridis* to sulfide suggests that this compound may be important for this species. Enhancement of metabolites and competitive exclusion would presumably encourage chemoautotrophs, and sulfate reducers and sulfur oxidizers in particular, to grow along the burrow. Indeed, the abundance of these microbes was elevated around the burrow wall and in surrounding sediments (See Chapter 3). The enhancement of certain bacterial groups, or microbial gardening/farming, has been suggested as a feeding strategy for other deposit feeding polychaetes inhabiting sulfidic sediments, such as lugworms, *Heteromastus filiformis* and capitellids (Clough and Lopez 1993; Kikuchi and Wada 1996; Tsutsumi et al. 2001;

Ashforth et al. 2011). Results from Chapter 3 suggest grazing on the prokaryotic population in the burrow, possibly by *M. viridis*.

As a constructor of deep burrows that extend into the reduced sediment layer, *Marenzelleria viridis* is exposed to higher levels of ambient hydrogen sulfide that may be utilized for nutritional benefit. Many animals in reducing habitats, such as hydrothermal vents and organic rich sediments, harbor sulfur-oxidizing symbionts both to cope with toxic sulfide exposure, and to provide an energy source (Bagarinao 1992; Cavanaugh 1994; Childress 1995; Giere 1996). Chemosymbiosis occurs widely between invertebrates and chemoautotrophs, spanning at least 100 species across 5 invertebrate phyla (Cavanaugh 1994; Distel 1998). To meet the biogeochemical requirements of thiotrophic endosymbionts, which require oxygen or nitrate as well as either sulfide or thiosulfate, many symbiotic macroorganisms occupy the redox boundary (Giere 1996; Stewart and Cavanaugh 2006). The ventilation behavior of *Marenzelleria viridis* may provide metabolites to chemoautotrophs living in a chemosymbiotic relationship with *M. viridis* that may supply extra nutrients to the host. However, no previous ultra-structural studies of *M. viridis* have investigated the possible presence of symbionts.

No studies to date have investigated which of several reported feeding strategies is the primary feeding mode of *Marenzelleria viridis,* and whether the species depends on a chemosynthetic food source. This chapter explores potential chemosynthetic nutritional sources of *Marenzelleria viridis,* either in the form of 1) chemoautotrophic symbionts or 2) gardening or cultivation of chemoautotrophic microbes in sediments. Potential nutritional sources were investigated by sampling a variety of potential food sources from

the *M. viridis* environment, as well as the polychaetes themselves, to explore trophic strategies using stable isotope analysis. If *M. viridis* is dependent on a chemoautotrophic source of nutrition, stable isotope analysis will reveal isotopic signatures comparable to other organisms dependent on chemosynthesis. Additionally, transmission electron microscopy (TEM) was used to explore whether *M. viridis* may harbor sulfur-oxidizing endo- or ectosymbionts. In other symbiont bearing annelids (with the exception of specialized *Riftia* species), symbiotic bacteria typically occur in the cuticle, the epidermis or the cuticle to epidermis boundary (Giere 1996); therefore, TEM investigations concentrated in these areas of *M. viridis* tissue.

4.2 Materials and Methods

4.2.1 Stable Isotope Analysis of ^{13}C, ^{15}N and ^{34}S

To explore dietary sources of *Marenzelleria viridis*, the stable isotope ratios of organic C, N and S were determined for annelids and their potential food sources. Three *M. viridis* individuals were collected from Deer Brook in November 2013 (see Chapter 2 for descriptions of sampling sites) and kept in filtered seawater (0.22 μm) at 4°C for gut purging. Once purged, specimens were rinsed with distilled water and transferred to acid washed scintillation vials and frozen at -20°C.

Potential food sources of *Marenzelleria viridis* were sampled at Deer Brook. These included two types of organic matter deposited at the surface (distinguished by color, “brown organic matter” and “black organic matter”), surface sediment, black reduced sediment from about 10 cm depth, and *M. viridis* burrow sediment. Seawater

from Deer Brook was collected at high tide using an acid rinsed plastic jug dipped approximately 10 cm below the water surface for analysis of suspended particulate organic matter (SPOM). Seawater (2 L) was filtered on GF/F Whatman® filters (47 mm diameter, 0.7 μm porosity) to collect suspended particulate matter.

Sediment and tissue samples were freeze-dried and ground into powder with a mortar and pestle. Ground tissue samples were then transferred to acid-washed scintillation vials. One set of sediment subsamples were weighed in tin caps and treated with HCl to remove carbonates for analysis of ^{13}C and ^{15}N. For analysis of suspended particulate organic matter, one filter was fumigated with HCl to remove carbonates for analysis of ^{13}C and ^{15}N. Untreated sediment samples, acid treated sediment sub-samples in tin caps, tissue and filter samples in scintillation vials were sent to the G.G. Hatch Stable Isotope Laboratory, University of Ottawa for analysis (Table 4.1). For ^{13}C and ^{15}N, samples were analyzed with a Delta Advantage (Thermo, Germany) isotope ratio mass spectrometer, and for ^{34}S samples were analyzed with a Delta XP isotope ratio mass spectrometer.

Table 4.1: Total number of samples and isotopes analyzed for *Marenzelleria viridis* tissue and potential food sources from the sediment and the water column.

Samples Submitted for Analysis	Isotopes Analyzed	Number of Samples
Tissue		
Marenzelleria viridis	^{13}C, ^{15}N and ^{34}S	3 individuals
Sediment Samples (Food Sources)		
Surface	^{13}C, ^{15}N and ^{34}S	2 (1 acid treated, 1 untreated)
Black Reduced (10 cm depth)	^{13}C, ^{15}N and ^{34}S	2 (1 acid treated, 1 untreated)
Marenzelleria viridis burrow	^{13}C, ^{15}N and ^{34}S	2 (1 acid treated, 1 untreated)
Brown Organic Matter, surface film	^{13}C, ^{15}N and ^{34}S	2 (1 acid treated, 1 untreated)
Black Organic Matter, surface film	^{13}C, ^{15}N and ^{34}S	2 (1 acid treated, 1 untreated)
Suspended Particulate Organic Matter Samples (Food Source)		
Filter with high tide seawater sample	^{13}C and ^{15}N	2 (1 acid treated, 1 untreated)

4.2.2 Transmission Electron Microscopy

To search for potential chemosynthetic symbionts associated with *Marenzelleria viridis* tissues, one specimen collected from Indian Pond in December 2012 was selected for TEM analysis (see Chapter 2 for a description of sampling location in Indian Pond). The individual was left in a beaker of filtered seawater (0.22 µm) at 4°C until it had purged gut contents (approximately 24 hours). Two gills were removed from the anterior region, and to ensure that body tissue samples were small enough to be embedded, the specimen was cut into anterior, middle and posterior regions, and further cut transversely. Body tissue and gill samples were placed in separate scintillation vials with 2.5% gluteraldehyde in 0.1M sodium cacodylate- 1% HCl buffer, with enough volume to ensure complete immersion of the tissue. After 24 hours, the fixative was replaced with 0.1 M sodium cacodylate- 1% HCl buffer to cover the tissues.

Samples were post-fixed with 1% osmium tetroxide in 0.1M sodium cacodylate buffer for 15 minutes. Tissues were subsequently dehydrated in a series of increasing concentrations of ethanol (two changes of 50%, 70%, 90% and 100%) for 10 minutes in each change. Each tissue sample was placed in a drop of EPON resin (16.0 g TAAB 812, 8.0 g DDSA, 9.2 g NMA, and 0.4 g DMP) on a strip of Parafilm for 15 minutes. This process was repeated twice more. Tissue samples were transferred to molds filled with EPON resin, properly orientated to enable transverse sections to view epidermal and cuticle ultrastructure, and left in an embedding oven at 80°C overnight for curing.

Tissue samples were sectioned with a LKG Bromma 8800 ultramicrotome using glass knives to a thickness of approximately 3 μm. These semi-thin sections were placed on microscope slides, dried, and then stained using 1% toluidine blue in 1% sodium borate on a heated surface to allow the stain to permeate the tissue. Semi-thin sections were then viewed with a light microscope to help determine if the sections were appropriate for viewing with TEM (i.e. apporpriate orientation to view ultrastucture) and selected for further processing.

Ultrathin sections (approximately 60 nm) were cut with a diamond knife using the same ultramicrotome on the automated heating advancement setting. Sections were placed on 3.05 mm copper grids (Type G200) and brought to the Electron Microscopy and Flow Cytometry Unit of the Health Sciences Centre at Memorial University for post staining and examination. Grids were post stained with uranyl acetate for 15 minutes, rinsed three times with 50% methanol, and then stained with lead citrate for seven

minutes, followed by a rinse with distilled water. Once dry, grids and respective tissue samples were examined with a Phillips 300 transmission electron microscope.

4.3 Results

4.3.1 Stable Isotope Analysis

4.3.1.1 Carbon

Organic carbon isotopic values for sediment samples from the burrow and from black reduced sediment were similar, varying between -24.36 and -24.82 ‰ (Table 4.2). Surface sediment had a heavier $\delta^{13}C$ value of -23.84 ‰, and brown organic matter was lighter, at -25.12 ‰. Suspended particulate organic matter $\delta^{13}C$ was slightly lighter than the sediment samples at -27.66 ‰. The $\delta^{13}C$ of *Marenzelleria viridis* was heavier than the potential food sources tested (-17.8 to -17.45 ‰).

Table 4.2: Stable isotopic signatures of *Marenzelleria viridis* individuals collected in Deer Brook. Isotope signatures of potential food sources (sediment, surface films and suspended particulate matter) are presented. $\delta^{13}C$ signatures from investigated food sources are from fumigated samples; $\delta^{15}N$ and $\delta^{34}S$ signatures are from non-fumigated samples.

Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Marenzelleria viridis 1	-17.8	5.48	-9.45
Marenzelleria viridis 2	-17.45	5.43	-5.54
Marenzelleria viridis 3	-17.49	6.73	2.24
Surface sediment	-23.84	5.18	-18.28
Black reduced sediment	-24.82	4.32	-16.61
Burrow sediment	-24.36	4.4	-20.36
Brown organic matter	-25.12	0.92	0.22
Black organic matter	-24.69	1.15	-10.18
Suspended particulate organic matter	-27.66	3.54	-13.16

4.3.1.2 Nitrogen

Brown and black organic matter sediment samples were lightest in organic $\delta^{15}N$, with values of 0.92 and 1.15 ‰ respectively. Black reduced and burrow sediments had similar nitrogen isotopic values of 4.32 ‰ and 4.4 ‰. The surface sediment sample was the heaviest, with a value of 5.18 ‰. The SPOM had a nitrogen isotopic value between those of sediment samples, 3.54 ‰. The $\delta^{15}N$ of *Marenzelleria viridis* ranged from 5.48 to 6.73 ‰ (Table 4.2).

4.3.1.3 Sulfur

The organic $\delta^{34}S$ values of sediment and SPOM varied the most of the three elements examined. Burrow sediment samples were lightest in S, with a value of -20.36 ‰. The next lightest signatures were from surface sediment (-18.28 ‰), black reduced sediment (-16.61 ‰), and black organic matter (-10.18 ‰), and the heaviest $\delta^{34}S$ value was in brown organic matter (0.22 ‰). The sample of SPOM was intermediate at -13.16 ‰. The $\delta^{34}S$ of *Marenzelleria viridis* was relatively heavy and varied widely between individuals (-9.45 to 2.24 ‰) (Table 4.2).

4.3.1.4 Dual isotope plots

A graphical representation of $\delta^{13}C$ and $\delta^{15}N$ values along two axes separated the sample types into distinct groups (Fig. 4.1). The *Marenzelleria viridis* samples were grouped together and were characterized by the heaviest $\delta^{13}C$ and $\delta^{15}N$ signatures; the C isotopic signature was notably high compared to other samples. All sediment samples grouped together, and were relatively heavy in $\delta^{15}N$ compared to SPOM and surface

films. The surface films grouped together and had the lightest $\delta^{15}N$ values, while the SPOM had the lowest $\delta^{13}C$.

When graphing the $\delta^{13}C$ and $\delta^{34}S$ signatures together, *Marenzelleria viridis* samples grouped together and were distinguished by heavier $\delta^{13}C$, and relatively variable$\delta^{34}S$ values (Fig. 4.2). All other samples were lighter in $\delta^{13}C$. The surface films had the most similar $\delta^{34}S$ values to *M. viridis*, while the sediment samples were grouped together and had comparably light $\delta^{34}S$ values.

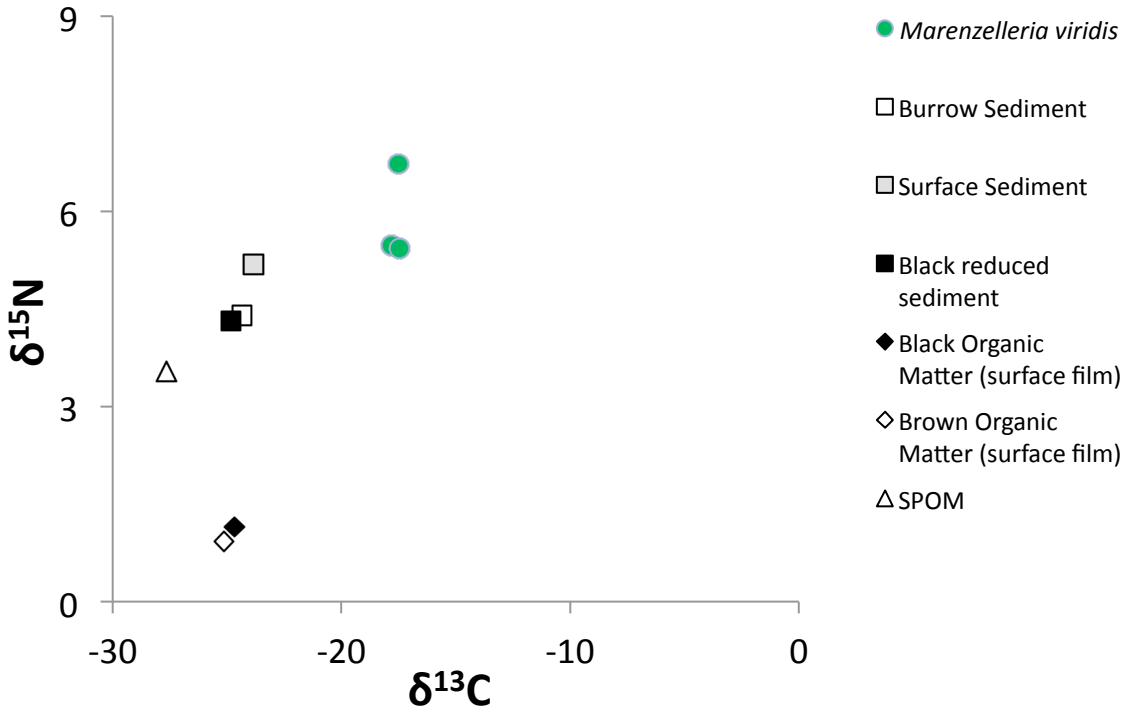


Figure 4.1: Stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of *Marenzelleria viridis* tissues and potential food sources.

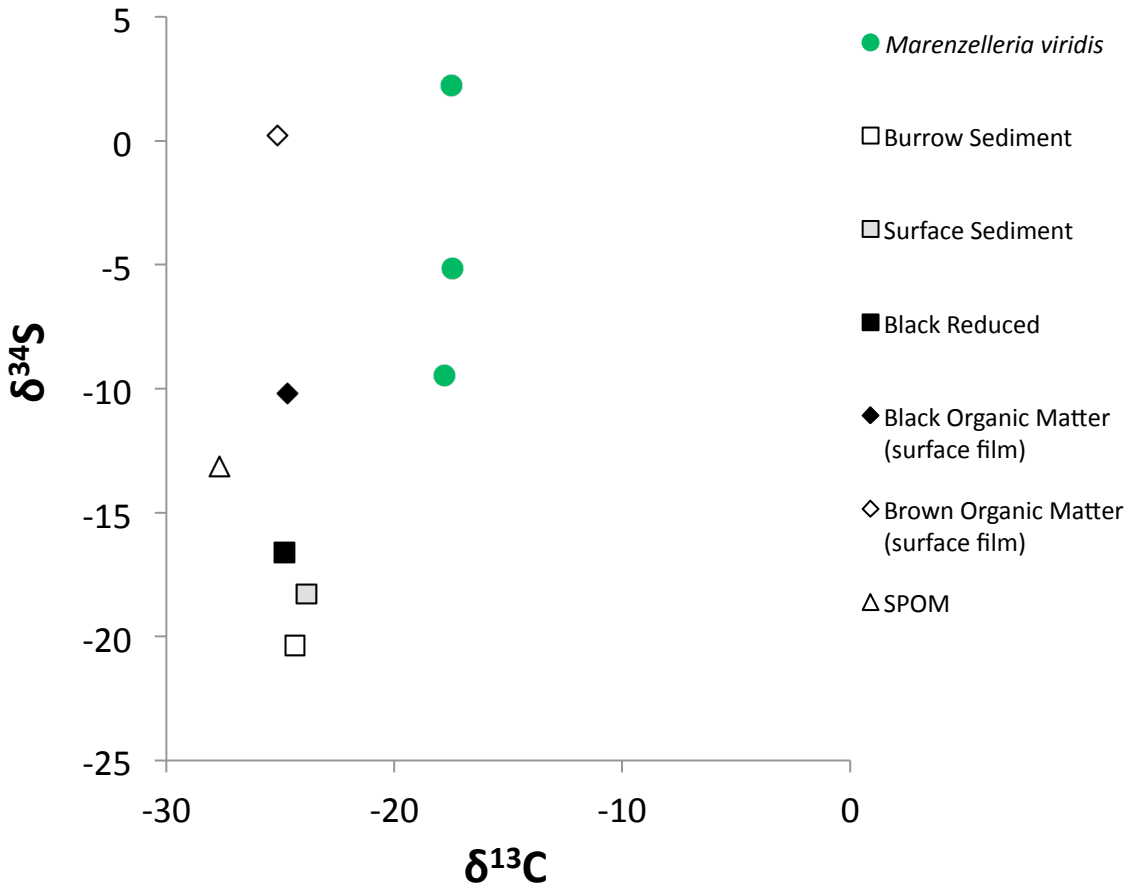


Figure 4.2: Stable isotopic signatures ($\delta^{13}C$ and $\delta^{34}S$) of *Marenzelleria viridis* tissues and potential food sources

4.3.2 Transmission Electron Microscopy

4.3.2.1 Transverse sections of Gill Tissue

The gill epidermis was covered by a cuticle about 0.75 µm thick, and a large blood vessel dominated the center of the gill filament (Figure 4.3A). The distance from the blood vessel and the outer surface of the cuticle was about 4 µm. Numerous mitochondria in the epidermal cytoplasm were electron dense and contained few cristae. Epidermal extensions (microvilli) crossed the cuticle and ended in somewhat inflated tips

laying flat on the cuticle surface. Surrounding the microvilli were round, electron dense epicuticle projections. Cuticle "pockets", approximately 0.42 µm across and 0.25 µm deep bordered the epidermal-cuticle interface (Figure 4.3B). These pockets contained an abundance of electron dense granules 0.08 µm in length. The electron dense granules were not observed elsewhere in other cells, cell layers or on the surface of the gill filament. No recognizable bacteria were found in either intracellular or extracellular spaces.

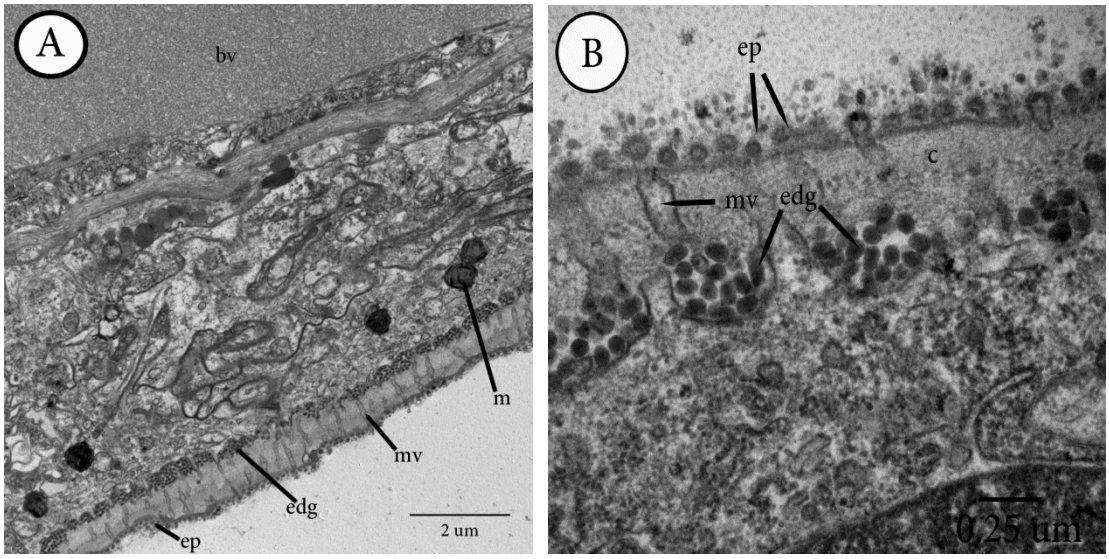


Figure 4.3: Transmission electron micrographs of *Marenzelleria viridis* gill tissue. (A) Electron micrograph depicting gill epithelium with large blood vessel and electron dense "pockets" bordering epidermal-cuticle interface. No obvious bacteria were observed. (B) "Pockets" of electron dense granules bordering epidermal-cuticle boundary. Granules were not observed elsewhere in gill epithelia. bv: blood vessel, c: cuticle, edg: electron dense granules, ep: epicuticle projection, m: mitochondria, mv: microvilli

4.3.2.2 Transverse Sections of Body Segments

4.3.2.2.1 ANTERIOR REGION

In the anterior region of *Marenzelleria viridis*, TEM revealed abundant, electron dense mitochondria throughout the cytoplasm of epidermal cells. Some mitochondria appeared swollen with few cristae and electron dense inclusions (Figure 4.4A). Round, electron dense bodies (approximately 0.5 μm in diameter) and vacuoles were also abundant in the epidermal cytoplasm (Figure 4.4B). Crescent and dumbbell shaped electron dense particles aggregated in the epidermal cytoplasm next to the epidermal-cuticle boundary, with some particles contained within inclusions (Figure 4.4C). These particles were approximately 0.5 μm in length and 0.1 μm wide, and not observed elsewhere in the epidermis or the cuticle. Microvilli crossed to cuticle and tips were somewhat inflated and flat on the surface. Similar to the gill filament, round, electron dense epicuticle projections surrounded the microvilli. Both microvilli and projections were coated with numerous long, thin filaments (approximately 2 μm), likely a type of glycocalyx. No identifiable bacteria were detected in either intracellular or extracellular spaces.

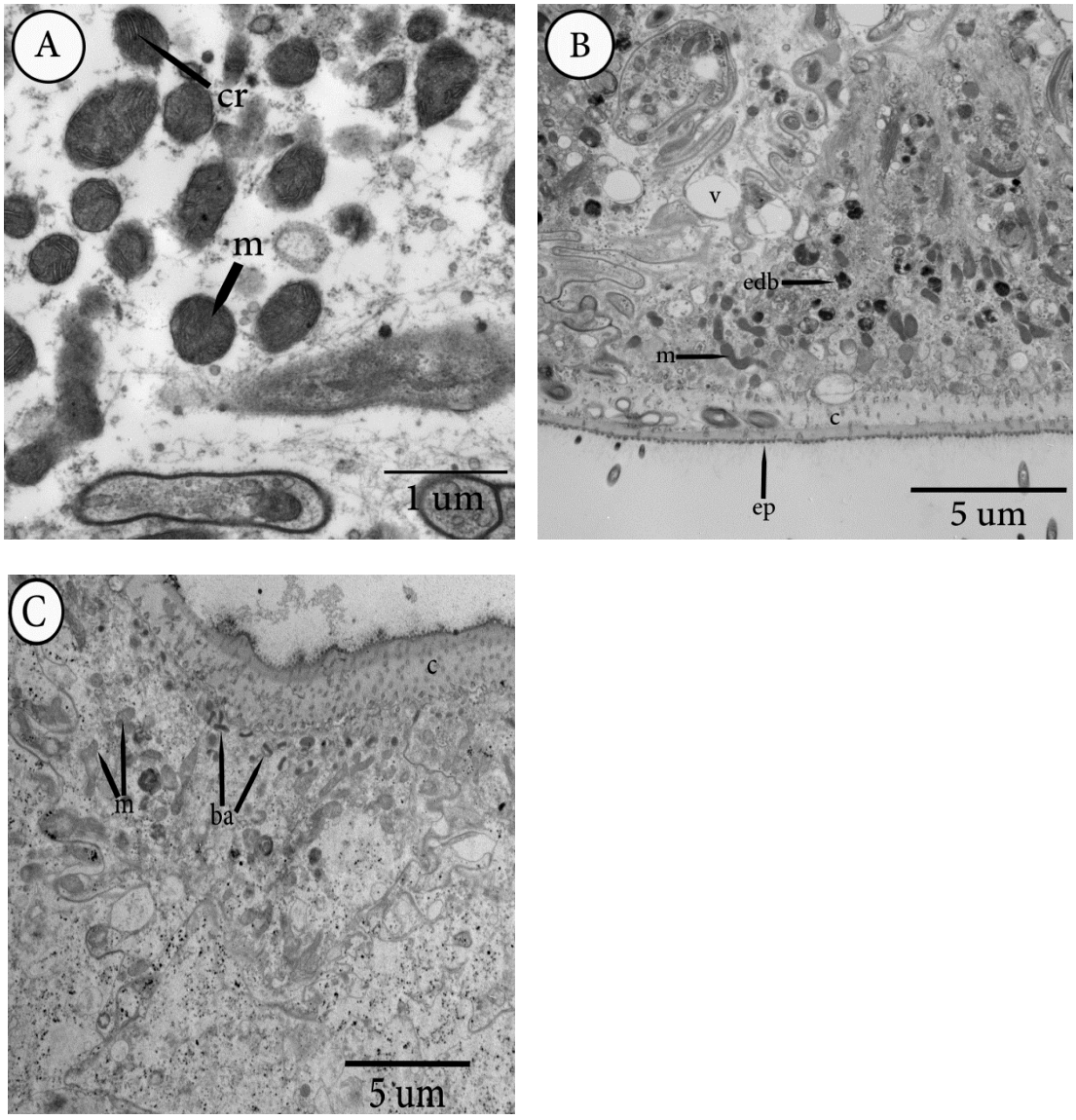


Figure 4.4: Electron micrographs of *Marenzelleria viridis* anterior body segment (A) Swollen, electron dense mitochondria with few cristae present in anterior epithelia. (B) Electron micrograph depicting abundant electron dense bodies and empty vacuoles in epithelia. No obvious bacteria were observed. (C) Putative baculoviruses bordering the epidermal-cuticle boundary. These particles were not observed elsewhere in epithelia. ba: baculovirus, c: cuticle, cr: cristae, edb: electron dense body, ep: epicuticle projections, m: mitochondria, v: vacuole.

4.3.2.2.2 MID BODY REGION

In the mid-body region of *Marenzelleria viridis*, TEM revealed a similar ultrastucture as observed in the anterior region. Electron dense bodies and mitochondria were present throughout the epidermal cytoplasm, with a higher abundance of mitochondria with electron dense inclusions (Figure 4.5A). The crescent and dumbbell shaped particles were more abundant compared to the anterior region, yet were still aggregated in the epidermal cytoplasm near the epidermal-cuticle boundary (Figure 4.5A, 4.5B). The epicuticle in this region, with microvilli, epicuticle projections and glycocalyx, was very similar to the anterior region. No bacteria were observed in either the intracellular or extracellular spaces.

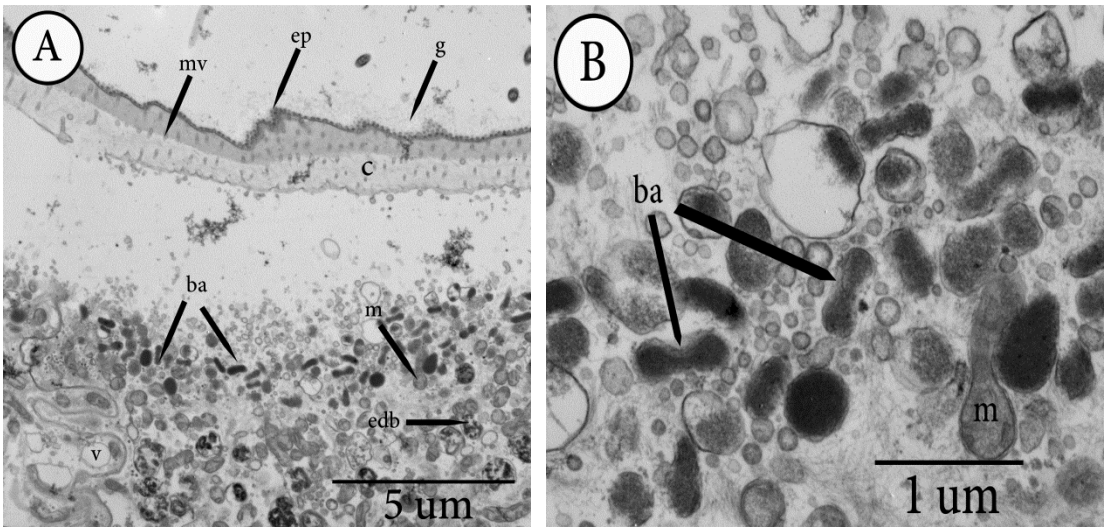


Figure 4.5: Electron micrographs of *Marenzelleria viridis* mid body section. (A) Electron micrograph depicting placement of putative baculoviruses bordering the epiderimal – cuticle interface. No obvious bacteria were observed. (B) Putative baculaviruses. Some appeared to be degrading in vacuoles. ba: baculoviruses, c: cuticle, edb: electron dense bodies, ep: epicuticle projections, g: glycocalyx, m: mitochondria, mv: microvilli, v: vacuole

4.3.2.2.3 POSTERIOR BODY REGION

In the posterior region of *Marenzelleria viridis*, TEM revealed a similar ultrastructure as the mid and anterior body region, with some minor differences. Mitochondria were abundant, electron dense, and vacuoles containing electron dense material and what appeared to be degrading mitochondria were observed throughout the epidermal cytoplasm (Figure 4.6 A, B). Electron dense bodies were numerous in the cytoplasm (Figure 4.6 B). Crescent shaped and dumbbell-shaped particles were also observed in this body region similarly to the anterior and median regions, albeit in lower abundance (Figure 4.6 C). Compared to the other two regions, more glycogen was observed in the cytoplasm of epidermal cells. The epicuticle, with microvilli, epicuticle projections and glycocalx, was similar to that in the other body regions. No recognizable bacteria were observed in either the intracellular or extracellular spaces.

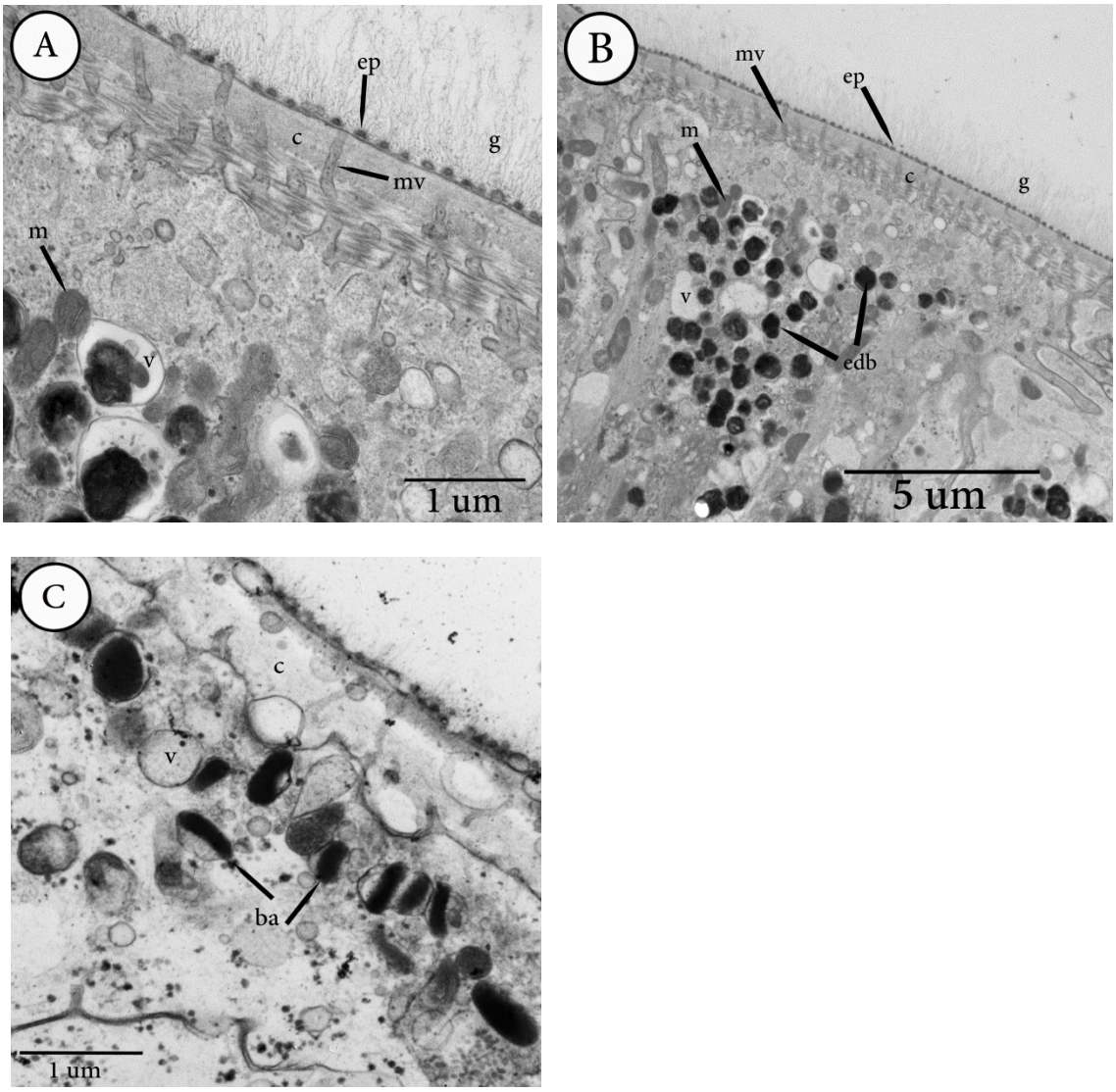


Figure 4.6: Electron micrograph of *Marenzelleria viridis* posterior body section. (A) Electron micrograph depicting epithelia and cuticle. No obvious bacteria were observed. (B) Electron dense bodies were numerous in cytoplasm of epithelial cells. (C) Putative baculoviruses were observed bordering the epidermal – cuticle boundary, and not elsewhere in cells. ba: baculoviruses, c: cuticle, edb: electron dense bodies, ep: epidermal projections, g: glycocalyx, m: mitochondria, mv: microvilli, v: vacuole

4.4 Discussion

4.4.1 Potential food sources of *Marenzelleria viridis*

4.4.1.1 Suspended Particulate Organic Matter (SPOM)

Suspended particulate organic matter (SPOM) is often predominantly planktonic in origin, and its isotopic signature depends on the sources of assimilated carbon, sulfur or nitrogen in its multiple components. In estuaries, these components can have terrestrial, marine or autochthonous sources, with each source having distinct isotopic signatures (Martineau et al. 2004). While the $\delta^{15}N$ values of primary producers in estuaries are highly variable, estuarine plankton can have a $\delta^{13}C$ range of -29‰ to -22‰, and a $\delta^{34}S$ of 7‰ to 20‰, whereas more brackish/freshwater plankton have signatures of -28‰ to -25‰ for $\delta^{13}C$ and -3.6‰ to 4.3‰ for $\delta^{34}S$ (Chanton and Lewis 1999; McKinney et al. 2001; Chanton and Lewis 2002). The sampling site was located close to Deer Brook, which drains the watershed of the peatland environment in the Long Range Mountains; this runoff can provide carbon, nitrogen and sulfur sources for plankton, and additionally contributes to the total SPOM of the sample site.

The SPOM of Deer Brook, collected at high tide, had a $\delta^{15}N$ value of 3.54‰, within the range of $\delta^{15}N$ values for marine phytoplankton (between 0-10‰, Fry et al. 1991; Peterson 1999). However the $\delta^{13}C$ value of -27‰ falls within the -28‰ to -25‰ range of fresh or brackish water plankton, and outside of typical marine phytoplankton values (-19‰ to -24‰), with temperate plankton between -20.7‰ and -20.9‰ (Peterson 1999; Kharlamenko et al. 2001; Chanton and Lewis 2002; Fry 2006). Furthermore, the very light $\delta^{34}S$ value of -13.54‰ is also unlike typical values for marine plankton (~ 17‰

to 21‰), and terrestrial plants (1.8‰), and is more depleted than chemosynthetic primary producers at hydrothermal vents, which have a $\delta^{34}S$ range of -2.8‰ to 10‰ (Peterson 1999; Fry 2006; Reid et al. 2012). It is possible that runoff from Deer Brook contributes the majority of carbon, nitrogen and sulfur for plankton at the sampling site. Runoff from Deer Brook would also carry POM from peatlands and other terrestrial sources as the water flows downstream. In estuaries, plankton $\delta^{13}C$ values can vary due to differences in the carbon sources of dissolved organic carbon, and can result in phytoplankton signatures more similar to organic matter from terrestrial plants (~-28‰) (Fry et al. 1991; Peterson 1999). In this study, $\delta^{13}C$ values for the SPOM, which is isotopically light, are similar to typical terrestrial plants or *Sphagnum* moss (about -28‰ and -26‰, respectively; Kracht and Gleixner 2000; Fry et al. 1991), and the $\delta^{34}S$ signature of SPOM is closest to saltmarsh plants (*Spartina alterniflora*), which can be as light as -7.7 ‰ (Peterson et al. 1986), suggesting an allochthonous source for C and S. Although care was taken during sampling to not re-suspend sediment, it is possible that contamination occurred; anoxic marsh and benthic sediments tend to be more depleted in $\delta^{34}S$ due to sulfate reduction, which could also explain the light $\delta^{34}S$ signature of the SPOM (Peterson 1999).

4.4.1.2 Surface, Black Reduced and Burrow Sediments

Bulk sedimentary organic matter in Deer Brook Lagoon is likely composed of a mixture of detritus from terrestrial (Deer Brook) and marine (tidal transport) sources, in addition to autochthonous organic matter from planktonic and benthic primary production as seen in other estuaries (Thornton and McManus 1994; Chanton and Lewis 2002).

Detritus from marine sources is usually isotopically heavier in nitrogen and carbon ($\delta^{13}C$: -18‰ to -23‰ and $\delta^{15}N$: 6‰ to 11‰) than terrestrial detritus ($\delta^{13}C$: -29‰ to -26‰ and $\delta^{15}N$ -5‰ to 3‰) (Tsutsumi et al. 2001; Clough and Lopez 1993; Thornton and McManus 1994; Karlson et al. 2014). Estuarine sediments, containing organic matter from a variety of sources, have reported ranges of -26.6 to -21‰ for organic $\delta^{13}C$, and 8 to 10‰ for organic $\delta^{15}N$ (Thornton and McManus 1994; Chanton and Lewis 2002). Estuarine sediments are depleted in organic $\delta^{34}S$ with values between -24‰ to 6.3‰, due to microbial sulfate reduction, which discriminates against ^{34}S and favors ^{32}S, producing isotopically light sulfides (Peterson 1999; Stribling and Cornwell 1997; Detmers et al. 2001; Chanton and Lewis 2002).

Surface, black reduced and burrow sediment samples were similar in organic $\delta^{13}C$ and $\delta^{15}N$, with surface sediments slightly more enriched in $\delta^{15}N$ (Table 4.2); these fall within reported ranges for estuarine sediments, in particular lagoons (around -25.6 ‰ for $\delta^{13}C$ and 4.3 to 6.7‰ for $\delta^{15}N$; Tsutsumi et al. 2001). Although organic sulfur isotopes were light, chemosynthetic carbon, which is typically ranges from -27‰ to -35‰ for chemoautotrophs with RuBisCo type I (Robinson and Cavanaugh 1995), was not obvious in these sediment samples; rather, bulk organic carbon was relatively enriched in ^{13}C, likely due to the presence of various types of organic matter in the sediment, in particular marine algae. Chemosynthetic carbon, however, is difficult to detect in sediments unless abundant within the bulk organic matter (Tsutsumi et al. 2001). The slightly more enriched $\delta^{15}N$ value of the surface sediment compared to the black reduced and burrow sediments likely results from either organic matter decomposition at the sediment

surface, which generally leads to ^{15}N enrichment, or nitrification in subsurface sediments, which depletes ^{15}N, resulting in a lighter signature (Peterson 1999, Tsutsumi et al. 2001, Roach et al. 2011). Nitrification in black reduced sediment, burrow sediment, and potentially in surface sediments, may result from increased production of ammonium from *Marenzelleria viridis* ventilation, which can then be used by nitrifying bacteria (Bonaglia et al. 2013).

The $\delta^{34}S$ values of the surface, black reduced and burrow sediments were more or less similar and distinct from the surface films and SPOM. Organic matter-degrading bacteria or fungi may either use sediment sulfide or seawater sulfate as a source of sulfur, resulting in a variety of $\delta^{34}S$ signatures in sediments (Peterson 1999). The light $\delta^{34}S$ value of burrow sediment likely results from microbial incorporation of porewater sulfides, which have $\delta^{34}S$ signatures of -19‰ and -22‰ in reducing sediments, such as those induced by *M. viridis* ventilation (Peterson et al. 1986; Stribling and Cornwell 1997; Kristensen et al. 2011; Bonaglia et al. 2013). Additionally, $\delta^{34}S$ depletion can occur after several cycles of sulfide oxidation to intermediate sulfur compounds through disproportionation, which likely occurs in the burrow of *M. viridis* (Canfield 2001). While high sulfate reduction rates are not expected to occur in oxic surface sediments, the ventilation activity of *M. viridis* may promote sulfate reduction near the sediment surface and influence the $\delta^{34}S$ value. The isotopic sulfur signature of benthic microalgae within surface sediments similarly varies depending on how reducing sediments are, with a lighter signature in algae growing over sulfidic muds than in oxic sediments (Chanton and Lewis 2002). The combination of elevated sulfate reduction rates and isotopically light

microalgae may have contributed to the depleted $\delta^{34}S$ signature of bulk surface sediment organic matter.

4.4.1.3 Black and Brown Surface Films

Black and brown surface films collected in Deer Brook lagoon were likely composed of different species of benthic microalgae (diatoms and other algae) and other microbes as well as terrestrial detritus and settling plankton. Isotopic signatures of the two films, therefore, result from a combination of these sources. Although $\delta^{15}N$ signatures of estuarine primary producers vary depending on N availability and species-dependent fractionation, marine photosynthetic organisms have a typical $\delta^{13}C$ signature around -21‰ and a $\delta^{34}S$ signature ranging from 17‰ to 22‰, with benthic microalgae ranging from 3.9‰ to 5.4‰ for $\delta^{34}S$ and around -15‰ for $\delta^{13}C$ (McKinney et al. 2001; Kharlomenko et al. 2001; Chanton and Lewis 2002; Fry 2006).

Signatures for both surface films were more depleted in $\delta^{13}C$ in comparison to the typical isotopic signature of benthic microalgae (around -15‰), and more similar to terrestrial values or estuarine plankton, which range from -29‰ to -22‰, reflecting the mixture of organic matter sources in both films (Fry 1986; Chanton and Lewis 2002; Fry 2006). While the $\delta^{13}C$ and $\delta^{15}N$ signatures of both films were similar, with the differences in $\delta^{15}N$ likely caused by different primary producer species in the two films, the $\delta^{34}S$ signatures were both isotopically light compared to benthic microalgae (i.e. 3.9‰ to 5.4‰), and variable. As suggested for the surface sediment, the reducing conditions of the sediment and the recycling of S in the sediment results in more depleted $\delta^{34}S$ values, thus driving the light $\delta^{34}S$ in both films (Canfield 2001; Kharlomenko et al. 2001;

Chanton and Lewis 2002; Fry 2006). As was the case for the $\delta^{15}N$ signatures, different microbial composition of the two films likely explains the large differences in $\delta^{34}S$ with species composing the black surface film possibly using more sulfides to degrade organic matter than species in the brown surface film, which may use seawater sulfate, resulting in a heavier $\delta^{34}S$ signature for the brown surface film (Peterson 1999).

4.4.2 Lack of Bacterial Symbionts and Chemosynthetically Derived Sulfur: a Case of Selective Deposit Feeding?

Stable isotopes are useful tools in studying food web dynamics because of differences in C, N and S isotopes from different food sources and the fractionation of isotopes during metabolism, although isotope shifts can vary depending on tissue and species (Peterson et al. 1985; Cifuentes et al. 1988; Kikuchi and Wada 1996; Post 2002; McCutchan et al. 2003). Typically, sulfur and carbon isotopes fractionate by a small amount, between + 2.0‰ to -0.5‰ for $\delta^{34}S$ and +0.5‰ for $\delta^{13}C$, and so the $\delta^{34}S$ and $\delta^{13}C$ signatures of the consumer generally reflect the $\delta^{34}S$ and $\delta^{13}C$ of the food source (Peterson et al. 1985; Kikuchi and Wada 1996; Bosheker and Middelburg 2002; McCutchan et al. 2003). Nitrogen isotopes become more enriched from food source to consumer, with fractionation depending on food source (+ 1.4 ± 0.21 ‰ if consuming invertebrates or + 2.2 ± 0.65 if consuming plants/algae): most consumers become 3-4 ‰ heavier with each trophic level (McKinney et al. 2001; Post 2002; McCutchan et al. 2003).

It is important to note this study as primarily an exploratory one; due to time and cost restraints, these is low replication of samples which limits interpretation of the data. Future work could increase the number of samples of food sources and *Marenzelleria viridis* individuals, as well as collect samples from different times of the year in case isotope values of individuals were a reflection of the polychaetes not feeding at time of sampling (November).

Nonetheless, considering fractionation and based on $\delta^{15}N$ values of the samples collected, either food source could form a component of the diet of *Marenzelleria viridis*. However, the $\delta^{13}C$ of *M. viridis* tissue is more enriched than expected considering the typical carbon fractionation through consumption and assimilation and the food sources considered. An enrichment of $\delta^{13}C$ in comparison to food sources has also been documented in other deposit feeding polychaetes (*Notomastus* sp.*, Heteromastus filiformis* and *Capitella* sp.), in filter-feeding zooplankton, in black fly larvae and in *Marenzelleria arctia,* with selective feeding on a isotopically distinct fraction of total organic matter suggested to be the cause of this discrepancy, as SPOM and sediment are composed of a wide variety of organic matter material with distinctive $\delta^{13}C$ signatures (Clough and Lopez 1993; Kikuchi and Wada 1996; Tsutsumi et al. 2001; Martineau et al. 2004; Karlson et al. 2014). Therefore, *M. viridis* selectively feed on an isotopically distinct fraction, not isolated in this study, in the water column, surface films or sediment.

The $\delta^{34}S$ signatures of *Marenzelleria viridis* are much lighter than the standard range for typical deposit feeders of 12‰ to 14.9‰ (Chanton and Lewis 2002). Other marine or estuarine animals with $\delta^{34}S$ signatures similar to *M. viridis*, such as the bivalves

Macoma incongrua ($\delta^{34}S$ = -3.9‰ to -3.5‰) and *Polymesoda erose* ($\delta^{34}S$ = -4.3‰) are considered to be among the most depleted in $\delta^{34}S$ (Kharlamenko et al. 2001). Depleted $\delta^{34}S$ values typify chemosymbiotic species such as the bivalve *Pillucina pisidium* (Kharlamenko et al. 2001), although ultrastructural analysis revealed a lack of both extra- and endo-cellular symbionts in *M. viridis*. Consumers of chemosynthetic primary production have $\delta^{34}S$ values of -9‰ to 10‰, in contrast to $\delta^{34}S$ values of 16‰ to 19‰ in consumers of photosynthetic primary production (Reid et al. 2012). Assimilation of considerable amounts of microbial biomass in detritivores may contribute to low $\delta^{34}S$ values (Peterson 1999). In this study, neither SPOM nor deeper sediments had the $\delta^{34}S$ signature of the expected main food source of *M. viridis*, and this species may consume an isotopically distinct fraction of organic matter from the bulk sediment or water column, consistent with the interpretation of $\delta^{13}C$ values. SPOM in the water column is generally composed of plankton using a consistent source of sulfur, mostly seawater sulfate; therefore, similar $\delta^{34}S$ values would be expected in individuals of a species depending mainly on SPOM as a main food source, which was not the case in this study. In comparison, $\delta^{34}S$ in sediments vary depending on the use of either seawater sulfate or porewater sulfide by producers (Peterson 1999). The variability of $\delta^{34}S$ among *M. viridis* specimens suggests consumption of this distinct organic matter source, from the burrow or black reduced sediments rather than the water column. The $\delta^{34}S$ signatures observed in different individuals of *M. viridis* suggest that they fed mainly on organic matter at different stages of decay, and on species of bacteria that assimilated sulfur from different sources, such as sulfur oxidizing bacteria whose $\delta^{34}S$ signature depends on the availability

of sulfate or thiosulfate (Peterson 1999; Detmers et al. 2001). Variable $\delta^{34}S$ signatures have also been reported in other detritivores, and in dorvilleid polychaetes that consume fish pellets at varying stages of decay, resulting in a range of $\delta^{34}S$ signatures (Kharlamenko et al. 2001; Salvo et al. 2015). *M. viridis* may also consume materials from surface mats, given the similarity in $\delta^{34}S$ signatures; however, the discrepancy in $\delta^{13}C$ signatures between surface mats and *M. viridis* tissues suggests that surface mats are not its sole food source and supports the hypothesis of selective microbial feeding.

The $\delta^{34}S$ signatures of *Marenzelleria viridis* suggest a food source of chemosynthetic origin, and although the $\delta^{13}C$ values of *M. viridis* are more enriched than would be expected from chemosynthesis using RuBisCo type I, they are close to the -9‰ to -15‰ range for chemoautotrophs that use RuBisCo type II (Robinson and Cavanaugh 1995). Like animal consumers, $\delta^{13}C$ signatures in bacteria reflect their food sources or substrates used for growth, unless they fix CO_2 (Robinson and Cavanaugh 1995; Boscheker and Middelburg 2002). Some bacteria use carbon sources with very specific $\delta^{13}C$ signatures or produce lipids with distinct isotopes ratios, a characteristic used to identify certain bacterial populations (Bosheker and Middelburg 2002). Although chemosynthetic products are usually $\delta^{13}C$ depleted, some bacteria, including sulfate reducers, utilize a reverse citric acid cycle (TCA) for carbon fixation, resulting in lipids enriched in $\delta^{13}C$ (Boscheker and Middelburg 2002, Buhring et al. 2005). The Desulfobacteraceae, a group of sulfate reducers, have distinct C_{17} fatty acids, $i C_{17:1w7}$ and $ai C_{17:0}$, and differ from most chemoautotrophic bacteria in $\delta^{13}C$ enrichment in fatty acids, with a signature of -17.1 and -17.6‰ (Buhring et al. 2005). The $\delta^{13}C$ values of -17.8 ‰ to -17.45 ‰ in *M. viridis*

suggest an exclusive feeding and/or assimilation of Desulfobacteraceae bacteria, or feeding on a carbon source from other chemoautotrophs that use RuBisCo type II.

4.4.3 Ultrastructure of Epithelia and Cuticle

While TEM did not reveal the presence of any obvious bacterial symbionts that may aid in sulfide detoxification or help meet nutritional requirements, the ultrastructure of epidermal and cuticle tissues from gill and body segments is consistent with that of other annelids and invertebrates that live in sulfidic and hypoxic environments. The branchial hemocoel of *Marenzelleria viridis* is much larger than in other polychaetes, indicating high oxygen uptake efficiency (Storch and Alberti 1978). The small distance between the hemocoel and the external environment (4 μm) may also lead to a high uptake of sulfides. The epithelium and cuticle of *M. viridis* are typical of polychaetes, with distally flattened microvilli crossing the cuticle, epicuticle projections, and a glycocalyx layer covering microvilli in the body sections (Hausen 2005). Microvilli and epicuticle projections may play roles in mucus production, and the abundance of these in *M. viridis* suggests the importance of mucus for the species (Giere et al. 1988; Hausen 2005). When collected in the field, sediment grains attached via mucus to the body surface of *M. viridis* were difficult to remove. Although some species, such as the polychaete *Tubificoides benedii*, produce a thick, bacteria-housing mucus layer that may help to prevent sulfide from entering body tissues (Giere et al. 1988; Menon et al. 2003), there was no evidence of a thick mucus layer or associated bacteria in the specimen of *M. viridis* examined (although TEM processing may have removed surficial mucus and any associated bacteria). The relatively long glycocalyx filaments may form a protective layer

against sulfides; while the function of the glycocalyx in the polychaete integument is not certain, in other cells it is involved in substrate uptake or protection. Therefore, the glycocalyx, combined with mucus, may limit sulfide diffusion into body tissues, with mucus also likely involved in burrow construction and maintenance.

The electron dense bodies (EDBs) and mitochondria observed in *Marenzelleria viridis* epithelia are consistent with life in sulfidic, low oxygen content sediments. Invertebrates in environments with either periodic or constant sulfide exposure typically have abundant mitochondria that are misshapen, swollen or have electron dense matrices with few cristae, possibly resulting from stressful low oxygen conditions, sulfide exposure or a poor development due to anaerobic metabolism (Giere et al. 1988; Dubilier et al. 1997; Menon et al. 2003). Additionally, the epithelial cells of animals exposed to sulfides typically have membrane bound, electron dense bodies, referred to as electron dense organelles, sulfur oxidizing bodies, precipitates, inclusions or granules (Giere et al. 1988; Menon et al. 2003; Wohlgemuth et al. 2007). Such EDBs have been reported in every sulfidic environment-inhabiting annelid examined, but the origin and function of these structures remain unknown (Menon et al. 2003; Wohlgemuth et al. 2007). In other annelids, these structures contain iron, copper and zinc and their presence and abundance depend on sulfide exposure, suggesting an adaptive function in sulfide oxidation, and/or the autophagic degradation of damaged organelles (Menon et al. 2003; Wohlgemuth et al. 2007). The EDBs in the body and gill epithelia of *M. viridis* may have the same function(s); in both epithelia, the EDBs were located at the periphery of cells, with the

smaller, more granular EDBs in “pockets” of the gill cuticle. The EDBs present in the gill may help limit the amount of sulfide entering the gill cells and hemolymph.

4.4.4 Possible Presence of Baculoviruses in *Marenzelleria viridis*

The electron dense, crescent and dumbbell shaped particles aggregated near the epidermal-cuticle boundary are similar in size and shape to baculoviruses. This family of large viruses causes disease in specific arthropods, and is commonly used as a biopesticide because these viruses liquefy their host (Clem 2001; Slavicek 2012). Baculoviruses can manipulate apoptosis and interfere with the immune response of the host cell (Clem 2001). Baculoviruses are not known to cause an infection in polychaetes, although they have been detected in polychaetes and in another annelid (Giere et al. 1998; Desrina et al. 2013). This is the first report of putative baculoviruses in *Marenzelleria viridis,* but, because only one specimen was observed with TEM, the prevalence of baculoviruses in *M. viridis* is uncertain. .

4.4.5 Conclusions: Microbial Gardening in *Marenzelleria viridis*?

Gardening of chemoautotrophic bacteria, whereby ventilation activities enhance the growth of some species of bacteria that are subsequently digested by macrofauna, have been suggested for some deposit feeders in reduced sediments because the bacteria would help with sulfide exposure and serve as a food source (Clough and Lopez 1993; Ashforth et al. 2011). Microbial gardening has been hypothesized for other deposit feeding polychaetes, including lugworms, *Heteromastus filiformis*, and capitellid species

(Clough and Lopez 1993; Kikuchi and Wada 1996; Tsutsumi et al. 2001; Ashforth et al. 2011). While bacteria in sediments alone may not provide an adequate food supply, given their lack of essential fatty acids and cell numbers too low to meet caloric requirements, polychaetes that feed on chemoautotrophic bacteria can synthesize missing fatty acids *de novo,* or may facultatively feed on other food sources to acquire missing fatty acids (Kharlamenko et al. 2001; Thurber et al. 2012; Salvo et al. 2015). Additionally, exopolymers produced by bacterial cells in biofilms can provide 10 times more food than cells alone, increasing the caloric content of a bacteria-based food source (Hall and Meyer 1998).

Marenzelleria viridis is known to enhance the activity, and likely growth, of sulfate reducing and sulfur oxidizing bacteria (Kristensen et al. 2011; Bonaglia et al. 2013). The surprisingly low abundance of Desulfobaceraceae in burrow sediment suggests grazing of the population (See Chapter 3), possibly by *M. viridis*. The $\delta^{34}S$ signature of *M. viridis* is of chemosynthetic origin, and the $\delta^{13}C$ signature is similar to the $\delta^{13}C$ signature of Desulfobacteraceae, suggesting a selective feeding, and potentially an active cultivation through ventilation behavior, of sulfur bacteria. Sub-surface deposit feeding, and likely consumption of farmed bacteria, has been observed in *M. viridis* (Essink and Kleef 1988). In this species, the short feeding palps, with their peculiar arrangement of frontal cilia, suggest an adaptation for dislodging particles (e.g. bacteria or diatoms) held together by mucus within a narrow space (i.e., the burrow wall) (Dauer 1997). Longer feeding palps would be unnecessary if feeding on a readily available source within the burrow. The behavior of *M. viridis* might support the active cultivation

of populations of bacteria in the burrow, through a constant supply of metabolites, providing *M. viridis* with a readily available food source. *M. arctia* has an isotopic niche distinct from other deposit feeders from the same habitat and is thought to consume old organic matter in the sediment more than freshly deposited phytoplankton bloom material (Karlson et al. 2011, 2014). It is possible that the old detrital organic matter in the sediment provides a food source for microbes. Periodically, *M. viridis* likely grazes on microbes and the microbial productivity on the burrow wall stimulated by bioirrigation behavior. The ultrastructural features, observed with TEM and adaptations of *M. viridis* to high sulfide environments (Schneider 1996; Bochert et al. 1997; Schiedek 1997; Hahlbeck et al. 2000) suggest potential importance of sulfur bacterial cultivation for this species.

Chapter Five: Thesis Summary and Conclusions

5.1 Summary of Results

This study focused on questions related to the unique, dynamic ventilation behavior of *Marenzelleria*; in particular, how this behavior may affect other organisms in surrounding sediments, and whether the unusual ventilation behavior of *Marenzelleria* is linked to its mode of feeding. This study is a first exploration to these questions with unfortunately low replication; as such, caution is needed in interpretations of results.

In Chapter 2, I determined the species identity of *Marenzelleria* sampled in coastal Newfoundland and examined it in context of the associated macrofauna and sediment characteristics. I confirmed the identity of the species as *M. viridis*, reported here for the first time in Newfoundland, although at lower maximum abundances than reported for other regions of the world. The abundances of *M. viridis* in Bonne Bay and Conception Bay sampling locations were relatively similar, despite differences in salinity, temperature, wave regime, sedimentary chlorophyll a, organic matter content, sorting coefficient, and median grain sizes at the sampling sites. The ubiquity of *M. viridis* in locations spanning a range of abiotic characters is not particularly surprising given that the species has successfully invaded several European seas, and its typical habitat encompasses dynamic, low salinity intertidal mud to sand (George 1966; Blank et al. 2006; Delefosse et al. 2012).

In Chapter 3, I determined the relative abundances of total prokaryotes, sulfate reducing bacteria and free-living sulfur oxidizing bacteria along the burrow lining of

Marenzelleria viridis and in surrounding sediments, as well as seasonal influences on these abundances. Because the highly dynamic, unique ventilation behavior of *M. viridis* creates oscillating redox conditions within the burrow that should favor microbial growth, I expected the burrow to contain the highest numbers of prokaryotes and sulfur bacteria among the five sediment types studied (*M. viridis* burrow sediment and fecal rods, surface sediment, and both black and grey reduced sediments). For each group of prokaryotes examined, abundances were not significantly different between collection months, a finding consistent with similar studies that have found weak to no temporal trends elsewhere (Boer et al. 2009; Garcia-Martinez et al. 2009). Relatively similar abundances of total prokaryotes, sulfate reducers, and sulfur oxidizers were found in all five sediment types considered. This homogeneity of sedimentary microflora is unexpected given reports of stimulated anaerobic activity and the growth of *Beggiotoa* mats in experimental cores with *M. viridis,* and reports of high abundances of prokaryotes in burrows relative to host sediments (Aller and Aller 1986; Papaspyrou et al. 2005, 2006). The relatively high abundances and apparently homogeneous distribution of the prokaryotic groups examined suggest far-field (cm to dm distance) microbial enhancement effects of bioirrigation by *M. viridis* in permeable sediments, combined with the grazing behaviour of *M. viridis*, which I inferred cropped prokaryote populations in near-burrow sediments.

In Chapter 4, I consider the potential nutritional sources of *Marenzelleria viridis*, including investigation of the possibility of chemoautotrophic symbionts and the culturing of free-living chemosynthetic bacteria in the burrow wall or surrounding sediments. The lifestyle of *M. viridis* includes the construction of deep burrows extending into reduced

sediment layers and a unique ventilation behavior that increases exposure of the polychaete to hydrogen sulfide. Such behaviors may indicate adaptations for utilizing chemosynthetic nutrition. Despite a ventilation behavior that would increase access of metabolites for symbiotic chemoautotrophic bacteria, *M. viridis* does not form a symbiotic association with such bacteria. Although *M. viridis* could feed on some material from surface mats, C, N and S stable isotopic signatures of *M. viridis* indicate that it also feeds on an isotopically distinct fraction of organic matter. The $\delta^{34}S$ signature provides evidence for a chemosynthetic origin for this food source, and the $\delta^{13}C$ signature, while more enriched then typical chemosynthetic values, is consistent with the biogeochemical activity of the bacteria family Desulfobacteraceae, suggesting selective feeding or assimilation of this group by *M. viridis*.

5.2 Conclusions

Members of the Spionidae family commonly inhabit organic rich sediments that either suspension or surface deposit feed using characteristically short feeding palps (Dauer et al. 1981). The genus *Marenzelleria* is abundant in intertidal, organically enriched, and often polluted, sediments, and while the five known species within this genus are nearly morphologically indistinguishable, they can differ in burrow construction and in the magnitude of ventilation activity (Sikorski and Bick 2004; Blank et al. 2006; Renz and Forster 2014). Three of the more commonly investigated (and cryptic) species, *M. arctia, M. neglecta* and *M. viridis*, are distinctive in both burrow structure and burrow ventilation (Renz and Forster 2014). *M. bastropi* and *M. wireni*

could also show different burrow construction and ventilation behaviors; however, these species have not yet been investigated. The most studied member of the genus, *Marenzelleria viridis*, constructs I or J shaped burrows that extend deep into reduced sediments. Unique dual ventilation habits cause fluctuation between oxic to anoxic conditions and stimulate sulfate reduction (Kristensen et al. 2011; Quintana et al. 2011; Jovanovic et al. 2014) that increase the amount of metabolites available for chemoautotrophic bacteria. An individual *M. viridis* affects on average 2.8 m^2 of the sediment-water interface, compared to 2.1 m^2 in *M. neglecta*. *M. viridis* can pump almost twice as much water during its burrow irrigation activities as *M. neglecta*; on average, 12 mL day^{-1} per individual versus 6.6 mL day^{-1} per individual for *M. neglecta* (Renz and Forster 2014).

The purpose of bi-directional burrow ventilation by *Marenzelleria viridis* has yet to be explained, however the ultrastructure of the epidermal tissue (Chapter 3) suggests adaptation for dealing with sulfide exposure. Based on the results of Chapters 2 and 3, I suggest that ventilation links to the cultivation of chemoautotrophic bacteria on the burrow wall, and in the near-burrow sediment, as a source of nutrition. Field studies demonstrated higher numbers of sulfate reducers and sulfur oxidizers than in similar sediment types, and may result from an enhancement effect of oscillating redox conditions created by the ventilation behavior of *M. viridis* (Jovanovic et al. 2014). The isotopic signatures of *M. viridis* tissue suggest that *M. viridis* diet consistentely includes chemosynthetic bacteria, such as members of the Desulfobacteraceae family. Cultivation of a chemosynthetic food source provides the benefit of a readily available food supply

without the need to move across the seafloor to feed. The feeding palps of *M. viridis*, which are shorter in comparison to other spionids with a different arrangement of frontal cilia (Dauer 1997), may be an adaptation for exploiting biomass cultivated by on the burrow wall by bioirrigation.

Because there are differences in burrow construction and ventilation between sibling species (Renz and Forster 2014), and given that previous isotopic work demonstrates *Marenzelleria arctia* feeding on old organic matter in the sediment (Karlson et al. 2011), food acquisition could be a significant driver for evolution and speciation in this genus. Phylogenetic work has revealed that the most basal species in the genus *Marenzelleria* are the Arctic species *M. arctia* and *M. wireni*, which suggests an Arctic origin for the genus (Blank and Bastrop 2008). The remaining three species, *M. viridis*, *M. neglecta* and *M. bastropi* are cryptic boreal species, with *M. bastropi* considered endemic to Currituck Sound (Blank and Bastrop 2008). Noting that *M. neglecta* and *M. bastropi* were only recognized as a distinct from *M. viridis* in recent years, it is possible that previous observations of suspension, surface deposit and sub-surface deposit feeding in *M. viridis* misidentified other species as *M. viridis*. However, more work comparing the feeding strategy of the sibling species, including stable isotopic studies in different locations, is needed before definitive conclusions can be made.

Potentially, as the genus *Marenzelleria* radiated outwards from the Arctic into warmer habitats, differences in feeding strategies developed along with differences in burrow morphology, burrow construction and ventilation habits. The sister species *M. neglecta* and *M. viridis* overlap in ranges and form hybrids, however, *M. viridis* forms

deeper burrows, reworks sediment more intensely and exchanges more water than *M. neglecta* (Renz and Forster 2013). Given that the feeding mode of *M. viridis* varies with substrate type (Dauer et al. 1981), it is possible that all members of the genus *Marenzelleria* have adaptable modes of feeding, with some species depending more on certain food sources than others. Differences in burrow construction and bioirrigation between *M. neglecta* and *M. viridis* may indicate greater dependence on farmed chemoautotrophic bacteria on the burrow wall, whereas *M. neglecta* relies more on either surface deposit feeding on diatoms or suspension feeding. Because *M. viridis* occurs further north than *M. neglecta*, chemoautotrophic bacteria may be a more reliable food source than photosynthetic food sources such as diatoms that are restricted by the short growing period in the sub-arctic.

5.3 Future Directions

Given that this is the first formal report of a member of the genus *Marenzelleria* in coastal regions of Newfoundland, investigations of other areas around Newfoundland could provide beneficial further information on the distribution of this species. Monitoring changes in the abundance of this species in both Bonne Bay and Indian Pond would be useful if *Marenzelleria viridis* is indeed a new arrival, and—in which case—study of such possible changes could help determine the potential impacts *M. viridis* on ecosystem processes in the region.

This thesis has also provided a first report of the prokaryotic abundance and relative numbers of sulfate reducers and sulfur oxidizers in field-collected sediments

inhabited by *Marenzelleria viridis*. Future work could either apply a wider variety of probes specific to other groups of bacteria or use metagenomics to investigate Archeal and bacterial communities in sediments colonized by *M. viridis*. Additionally, laboratory studies comparing prokaryotic communities in sediments containing *M. viridis* to control sediments would confirm the influence of the ventilation behavior on microbial populations in sediments.

Investigations of Archeal and bacterial communities in burrow sediments of other species of *Marenzelleria*, combined with stable isotope analyses, could indicate whether the ventilation behavior and burrow of *M. viridis* occur widely throughout the genus or represent unique adaptations for cultivating sulfate reducing bacteria as a food source. Furthermore, feeding studies of the different species could reveal whether species-specific burrow morphologies and irrigation behaviors link to dietary differences and speciation within the genus.

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Appendix 1

Macrofaunal Abundance in Indian Pond and Bonne Bay Sites in 2013 and 2014

Polychaetes

Site	Year	*Marenzelleria viridis*	*Nereis virens*	*Heteromastus filiformis*	*Nephtys ciliate*	*Arenicola marina*	*Lagis sp.*	*Phyllodocidae*
IP1	2014	5	42.5	0	0	0	0	0
IP2	2014	5	10	0	0	0	0	0
IP3	2014	15	35	0	0	0	0	0
IP4	2014	0	25	0	0	0	0	0
NH1	2013	0	5	16.7	0	0	0	0
NH2	2013	3.3	3.3	0	0	0	0	0
NH3	2013	8.3	8.3	1.7	0	0	0	0
NH4	2013	0	0	0	0	0	0	0
NH5	2013	6.7	3.3	3.3	0	0	1.7	0
NH6	2014	5	7.5	35	0	0	0	0
NH7	2014	7.5	20	20	0	0	2.5	0
NH8	2014	22.5	12.5	7.5	0	0	0	0
NH9	2014	2.5	2.5	0	0	0	0	0
NH10	2014	17.5	27.5	12.5	0	0	2.5	0
SH1	2013	1.7	6.7	0	0	0	0	0
SH2	2013	8.3	0	35	0	0	0	21.7
SH3	2013	5	0	6.7	1.7	0	0	0
SH4	2013	33.3	10	25	0	0	0	0
SH5	2014	7.5	0	35	0	0	0	0
SH6	2014	20	12.5	35	2.5	0	0	5
SH7	2014	5	0	25	0	0	0	0
SH8	2014	10	12.5	37.5	0	0	0	0
SH9	2014	7.5	20	15	0	2.5	0	0
DB1	2013	16.7	3.3	1.7	0	0	0	0
DB2	2013	0	1.7	0	0	0	0	0
DB3	2014	22.5	2.5	20	0	0	0	0
DB4	2014	17.5	0	7.5	0	0	0	0
DB5	2014	35	7.5	0	0	0	0	0
DB6	2014	22.5	0	0	0	0	0	0
DB7	2014	12.5	2.5	2.5	0	0	0	0

Molluscs

Site	Year	*Mya arenaria*	*Macoma balthica*	*Littorina saxatilis*
IP1	2014	12.5	0	0
IP2	2014	10	0	0
IP3	2014	0	0	5
IP4	2014	0	0	0
NH1	2013	20	53.3	8.3
NH2	2013	10	13.3	3.3
NH3	2013	1.7	8.3	0
NH4	2013	0	25	0
NH5	2013	0	18.3	1.7
NH6	2014	0	0	0
NH7	2014	0	0	0
NH8	2014	2.5	0	0
NH9	2014	0	0	0
NH10	2014	0	0	0
SH1	2013	0	3.3	0
SH2	2013	3.3	1.7	0
SH3	2013	1.7	0	0
SH4	2013	0	0	1.7
SH5	2014	2.5	0	0
SH6	2014	0	0	0
SH7	2014	0	0	0
SH8	2014	0	5	0
SH9	2014	0	2.5	0
DB1	2013	0	5	0
DB2	2013	0	0	0
DB3	2014	0	0	0
DB4	2014	0	5	0
DB5	2014	0	5	0
DB6	2014	0	0	0
DB7	2014	0	2.5	0

Crustaceans

Site	Year	*Carcinus maenas*	*Crangon septemspinosa*
IP1	2014	0	0
IP2	2014	0	0
IP3	2014	0	0
IP4	2014	0	0
NH1	2013	0	0
NH2	2013	0	0
NH3	2013	0	0
NH4	2013	0	0
NH5	2013	0	0
NH6	2014	0	0
NH7	2014	0	0
NH8	2014	0	0
NH9	2014	0	0
NH10	2014	2.5	0
SH1	2013	0	0
SH2	2013	0	0
SH3	2013	0	0
SH4	2013	0	0
SH5	2014	0	0
SH6	2014	0	0
SH7	2014	0	0
SH8	2014	0	0
SH9	2014	0	0
DB1	2013	0	0
DB2	2013	0	1.7
DB3	2014	0	0
DB4	2014	0	0
DB5	2014	0	0
DB6	2014	0	0
DB7	2014	0	0

IP= Indian Pond NH = Neddy Harbour

SH = Sandy Head DB= Deer Brook

Appendix 2

Direct Counts of Cells Labeled with the probe GAM 660

Month	Sediment	Wet Weight	Counted DAPI	FOV	Average Number FOV	(X/wet weight)	Total number of cells	GAM Ratio	# of GAM in Sample
Apr	S	1.014	202	26	7.769	7438580	57792044	0.2723	15736773
Apr	S	0.995	161	22	7.318	7580623	55476378	0.2733	15161694
Apr	S	1.003	208	16	13	7520160	97762074	0.1923	18799647
Apr	BR	0.999	200	14	14.285	7550270	1.08E+08	0.235	25347336
Apr	BR	0.997	204	29	7.034	7565416	53218790	0.1176	6258530
Apr	BR	1.003	202	33	6.121	7520160	46032492	0.3861	17773145
Apr	GR	1.003	200	23	8.695	7520160	65392691	0.375	24522259
Apr	GR	0.996	206	18	11.444	7573012	86668916	0.3544	30715464
Apr	GR	1.002	206	14	14.714	7527665	1.11E+08	0.4417	48924551
Apr	B	0.991	206	26	7.923	7611221	60304289	0.0534	3220249
Apr	B	0.991	211	22	9.59	7611221	72998529	0.3828	27943837
Apr	B	1.009	209	15	13.933	7475441	1.04E+08	0.2607	27153942
Apr	F	1.017	203	26	7.807	7416637	57906821	0.0887	5136335
Apr	F	1	204	23	8.869	7542720	66900647	0.3971	26566247
Apr	F	0.993	200	38	5.263	7595891	39978375	0.05	1998919
Jul	S	1.0048	201	25	8.04	7506688	60353771	0.0647	3904889
Jul	S	1.009	206	22	9.363	7475441	69997311	0.0825	5774778
Jul	S	1.0013	202	15	13.466	7532927	1.01E+08	0.396	40171594
Jul	BR	1.0285	150	15	10	7333709	73337093	0.0666	4884250
Jul	BR	0.9932	200	14	14.285	7594362	1.08E+08	0.145	15731178
Jul	BR	1.0184	204	22	9.272	7406441	68677912	0.0392	2692174
Jul	GR	1.0056	206	15	13.7333	7500716	1.03E+08	0.0534	5500725
Jul	GR	1.008	204	18	11.333	7482857	84805714	0.2108	17877045
Jul	GR	1.0068	200	21	9.523	7491776	71350247	0.0149	1063119
Jul	B	1.0163	200	23	8.695	7421746	64536918	0.125	8067115
Jul	B	1.0036	201	29	6.931	7515664	52091324	0.0597	3109852
Jul	B	1.0064	203	21	9.666	7494754	72449285	0.1773	12845258
Jul	F	1.0061	67	12	5.583	7496988	41858185	0.1044	4369995
Jul	F	1.0051	201	25	8.04	7504447	60335756	0.2139	12905818
Jul	F	1.0083	42	7	6	7480631	44883785	0.3333	14959765
Sep	S	1.0035	195	19	10.263	7516413	77142129	0.2359	18197828

Sep	S	0.9975	209	17	12.294	7561624	92963496	0.4067	37808254
Sep	S	0.9963	103	8	12.875	7570732	97473171	0.5146	50159694
Sep	BR	1.0169	205	21	9.761	7417367	72407625	0.2683	19426966
Sep	BR	0.99	206	22	9.363	7618909	71340694	0.4126	29435170
Sep	BR	1.0125	203	26	7.807	7449600	58164185	0.2562	14901664
Sep	GR	1.0175	208	17	12.235	7412993	90700145	0.3077	27908435
Sep	GR	1.0042	205	19	10.789	7511173	81041604	0.3415	27675708
Sep	GR	1.0097	139	14	9.928	7470258	74168995	0.3525	26144571
Sep	B	1.014	152	25	6.08	7438580	45226566	0.2171	9818687
Sep	B	1.0078	200	20	10	7484342	74843421	0.465	34802191
Sep	B	1.0043	204	17	12	7510425	90125102	0.5049	45504164
Sep	F	0.9955	202	17	11.882	7576816	90030398	0.4851	43673746
Sep	F	1.0131	205	15	13.666	7445188	1.02E+08	0.4244	43183083
Sep	F	1.0007	173	15	11.533	7537444	86931852	0.4451	38693367
Dec	S	0.996	205	20	10.25	7573012	77623373	0.0341	2646957
Dec	S	1	207	18	11.5	7542720	86741280	0.087	7546491
Dec	S	1.0089	204	21	9.714	7476182	72625768	0.3039	22070971
Dec	BR	0.9922	204	15	13.6	7602016	1.03E+08	0.348	35978820
Dec	BR	1.0089	203	25	8.12	7476182	60706598	0.3448	20931635
Dec	BR	1.019	208	17	12.235	7402080	90566632	0.4615	41796501
Dec	GR	1.009	201	22	9.136	7475441	68298348	0.3532	24122976
Dec	GR	0.9917	202	17	11.882	7605849	90375377	0.4406	39819391
Dec	GR	1.006	201	24	8.375	7497734	62793519	0.5174	32489367
Dec	B	1.011	200	14	14.285	7460653	1.07E+08	0.455	48494243
Dec	B	0.9911	206	15	13.733	7610453	1.05E+08	0.2913	30445770
Dec	B	1.0066	157	19	8.263	7493264	61918027	0.2038	12618894
Dec	F	1.0093	203	17	11.941	7473219	89239028	0.5123	45717154
Dec	F	1.011	200	15	13.333	7460653	99475371	0.075	7460653
Dec	F	1.009	205	21	9.761	7475441	72974543	0.376	27438428

Where:

Apr = April

Jul = July

Sep = September

Dec = December

S=Surface

BR = Black Reduced

GR = Grey Reduced

B = Burrow

F = Fecal Rods

FOV = Field of View

X = (Optical Coefficient) x (Extraction Coefficient) x (Dilution) = 7542720

Optical Coefficient = 8730

Extraction Coefficient = 1.44

Dilution Factor of Sediment = 600

Appendix 3

Direct Counts of Cells Labeled with DSS 658

Month	Sediment	Wet Weight	Counted DAPI	FOV	Average Number FOV	(X/wet weight)	Total number of cells	DSS Ratio	# of DSS in Sample
Apr	S	1.014	202	26	7.77	7438580	57797766	0.1931	11160749
Apr	S	0.995	200	23	8.7	7580623	65951421	0.25	16487855
Apr	S	1.003	203	19	10.68	7520160	80315304	0.2315	18592993
Apr	BR	0.999	200	14	14.29	7550270	1.08E+08	0.515	55565082
Apr	BR	0.997	200	19	5.68	7565416	42971564	0	0
Apr	BR	1.003	203	25	8.12	7520160	61063695	0.0936	5715562
Apr	GR	1.003	202	32	6.31	7520160	47452207	0.2921	13860790
Apr	GR	0.996	204	15	13.6	7573012	1.03E+08	0.3627	37355548
Apr	GR	1.002	202	18	11.22	7527665	84460398	0.4752	40135581
Apr	B	0.991	206	23	8.96	7611221	68196540	0.2233	15228287
Apr	B	0.991	202	30	14.43	7611221	1.1E+08	0.2913	31993455
Apr	B	1.009	202	28	7.21	7475441	53897930	0.3614	19478712
Apr	F	1.017	203	24	8.46	7416637	62744750	0.0493	3093316
Apr	F	1	201	26	7.73	7542720	58305226	0.398	23205480
Apr	F	0.993	202	31	6.52	7595891	49525211	0.3515	17408112
Jul	S	1.0048	203	26	7.807	7506688	58609909	0.115	6740140
Jul	S	1.009	209	18	11.611	7475441	86798176	0.0239	2074476
Jul	S	1.0013	206	17	12.117	7532927	91281353	0.3883	35444549
Jul	BR	1.0285	114	15	7.6	7333709	55736191	0.2895	16135627
Jul	BR	0.9932	209	19	11	7594362	83537978	0.3684	30775391
Jul	BR	1.0184	201	19	10.578	7406441	78352355	0.199	15592119
Jul	GR	1.0056	205	21	9.761	7500716	73221275	0.0195	1427815
Jul	GR	1.008	206	15	13.733	7482857	1.03E+08	0.0437	4490812
Jul	GR	1.0068	201	20	10.05	7491776	75292348	0	0
Jul	B	1.0163	202	21	9.619	7421746	71390124	0.1436	10251622
Jul	B	1.0036	201	20	10.05	7515664	75532419	0	0
Jul	B	1.0064	203	20	10.15	7494754	76071749	0.0488	3712301
Jul	F	1.0061	200	28	7.142	7496988	53549917	0.0842	4508903
Jul	F	1.0051	168	31	5.419	7504447	40669263	0.1726	7019515
Jul	F	1.0083	202	28	7.214	7480631	53967408	0.2723	14695325
Sep	S	1.0035	208	15	13.866	7516413	1.04E+08	0.2452	25556604
Sep	S	0.9975	196	16	12.25	7561624	92629895	0.1326	12282724

Sep	S	0.9963	202	20	10.1	7570732	76464390	0.4074	31151593
Sep	BR	1.0169	202	21	9.619	7417367	71348002	0.3614	25785168
Sep	BR	0.99	206	19	10.842	7618909	82605014	0.3786	31274258
Sep	BR	1.0125	202	19	10.631	7449600	79201011	0.3119	24702795
Sep	GR	1.0175	195	18	10.833	7412993	80307420	0.4462	35833171
Sep	GR	1.0042	198	18	11	7511173	82622904	0.4141	34214144
Sep	GR	1.0097	189	19	9.947	7470258	74309413	0.4497	33416943
Sep	B	1.014	154	25	6.16	7438580	45821652	0.3181	14575868
Sep	B	1.0078	198	17	11.647	7484342	87170573	0.4091	35661481
Sep	B	1.0043	199	15	13.266	7510425	99638307	0.4371	43551904
Sep	F	0.9955	200	15	13.3333	7576816	1.01E+08	0.53	53542831
Sep	F	1.0131	207	16	12.9375	7445188	96322120	0.4155	40021841
Sep	F	1.0007	165	14	11.785	7537444	88834159	0.3636	32300100
Dec	S	0.996	202	22	9.181	7573012	69534020	0.1089	7572255
Dec	S	1	206	23	8.956	7542720	67556536	0.2961	20003490
Dec	S	1.0089	204	19	10.736	7476182	80270585	0.2941	23607579
Dec	BR	0.9922	206	32	6.4375	7602016	48937976	0.2087	10213356
Dec	BR	1.0089	200	22	9.09	7476182	67965291	0.26	17670976
Dec	BR	1.019	203	18	11.277	7402080	83479019	0.3695	30845497
Dec	GR	1.009	202	22	9.181	7475441	68638140	0.2426	16651613
Dec	GR	0.9917	202	16	12.625	7605849	96023838	0.4356	41827984
Dec	GR	1.006	210	19	11.052	7497734	82869687	0.619	51296336
Dec	B	1.011	200	14	14.285	7460653	1.07E+08	0.42	44763917
Dec	B	0.9911	205	14	14.642	7610453	1.11E+08	0.1414	15757443
Dec	B	1.0066	202	16	12.625	7493264	94602464	0.3069	29033496
Dec	F	1.0093	161	18	8.9444	7473219	66843793	0.0124	828863
Dec	F	1.011	200	29	6.896	7460653	51452778	0.055	2829903
Dec	F	1.009	200	21	9.523	7475441	71194676	0.52	37021232

Where:

Apr = April

Jul = July

Sep = September

Dec = December

S=Surface

BR = Black Reduced

GR = Grey Reduced

B = Burrow

F = Fecal Rods

FOV = Field of View

X = (Optical Coefficient) x (Extraction Coefficient) x (Dilution) = 7542720

Optical Coefficient = 8730

Extraction Coefficient = 1.44

Dilution Factor of Sediment = 600