

MEDVEDEFF, CASSANDRA ANNE, M.S. Assimilation of Particulate and Dissolved Basal Carbon Resources by *Sphaerium nitidum* and *Grensia praeterita* in an Arctic Lake. (2008)

Directed by Dr. Anne E. Hershey. 40 pp.

During the summer 2006, I tested the importance of dissolved and particulate basal carbon sources to the caddisfly, *Grensia praeterita*, and the fingernail clam, *Sphaerium nitidum*, using intact sediment cores in a 1-month incubation experiment. Carbon sources included a  $^{13}\text{C}$  labeled seston treatment, an algal-derived  $\text{DO}^{13}\text{C}$  treatment, a  $^{13}\text{C}$ -methyl labeled acetate treatment, and a  $^{13}\text{C}$ -carboxyl labeled acetate treatment. *Grensia* and *Sphaerium* were both found to rely on both particulate and dissolved carbon sources. While enrichment from the  $^{13}\text{C}$  labeled seston treatment (particulate source) was the largest source for both organisms, *Grensia* and *Sphaerium* both showed reliance on microbial loop processing as seen by assimilation of the algal-derived  $\text{DO}^{13}\text{C}$  treatment. *Sphaerium* also assimilated carbon derived from methanogenic and non-methanogenic pathways of the microbial loop, based on differential assimilation of the two  $^{13}\text{C}$  labeled acetate treatments.

ASSIMILATION OF PARTICULATE AND DISSOLVED BASAL  
CARBON RESOURCES BY *SPHAERIUM NITIDIUM* AND  
*GRENSIA PRAETERITA* IN AN ARCTIC LAKE

by  
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A Thesis Submitted to  
the Faculty of The Graduate School at  
The University of North Carolina at Greensboro  
in Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

Greensboro  
2008

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## **CHAPTER I**

### **INTRODUCTION**

Lake organisms rely on autochthonous (phytoplankton and periphyton) and allochthonous (terrestrial) carbon sources to fuel growth and metabolism. More than 90% of dissolved organic carbon comes from allochthonous sources in oligotrophic lakes (Schlesinger 1997). Organic compounds are mostly derived from plant detritus during decomposition stages providing surrounding biota with a dissolved organic carbon source (Wetzel 1992). In arctic systems, including the Toolik Field Station vicinity, there is high organic input to lakes from allochthonous sources (O'Brien et al. 1997). Although the arctic habitat is above the tree line, the landscape is almost completely covered by types of tundra (Miller et al. 1986) providing a source of carbon to local lake systems (Oechel 1989). Tundra soils may contain more than 98% of the total carbon, 94-99% of the total nitrogen, and 95-99.5% of the total phosphorus in arctic terrestrial ecosystems (Bunnell and Scoullar 1981) while arctic soils contain 14%-15% of the global organic carbon budget (McGuire et al. 2000, Whalen and Reeburgh 1990).

In lake systems, both particulate and dissolved forms of organic matter are important contributors of carbon to various organisms. Particulate organic matter (POM)

is comprised of reduced carbon compounds within a water body in particle form (Cummins 1974). POM is further divided into two size categories; coarse and fine (CPOM and FPOM respectively). CPOM ranges in size from 1-54mm and can be found as large twigs, leaf packs or small plant fragments inhabited by many bacteria. CPOM is consumed by detritus shredders, collectors, macro-gatherers, and macro-filterers (Cummins 1974). The consumed organic matter which is not converted to biomass is released as feces to the microbial loop, added to the FPOM pool, or metabolized to CO<sub>2</sub>. FPOM consists of particles ranging from 0.0005-1mm. It can be found as plant and animal fragments, feces from invertebrates, and small detrital fragments. Algae are a main constituent of FPOM in lake systems. This type of POM is consumed by collectors such as macro-filterers, micro-filterers and micro-gatherers (Cummins 1974). Seston is a matrix of suspended matter consisting of dead and living particulate organic matter (Wetzel 1983). Once the suspended matter settles on the sediment it is considered fine benthic organic matter (FBOM), but can be re-suspended readily.

Dissolved organic carbon (DOC) is comprised of organic material which is operationally defined as anything passing through a 0.5µm filter (Cummins 1974). It consists mainly of dissolved materials such as algal exudates and the excretions of bacteria and producers (Cummins 1974). DOC can be catabolized into CO<sub>2</sub> or assimilated into tissues (Findlay 1986). DOC is a known carbon source for bacteria, and bacteria are important in invertebrate diets (Hall 1995).

The microbial loop is an aquatic energy pathway fueled by the uptake and recycling of nutrients in the water column by bacteria and the associated re-



mineralization of organic matter (Edwards et al. 1990). The microbial loop transfers about half of the dissolved organic matter from primary production to the particulate phase (Azam et al. 1994). This energy transfer between the dissolved and particulate phase is an important scavenger pathway which recovers energy which could be otherwise lost (See Figure 1). Bacteria play the most important role in the microbial loop since they take up the largest amount of DOM and can then be consumed by larger organisms, recycling the organic matter throughout the lake system. Zooplankton and other top level consumers rely on the microbial loop as an important component of their diets (Wetzel 1983).

### *Methanogenesis*

Some specialized bacteria, methanogens, reside in anaerobic lake systems and release methane as a byproduct of metabolic processes. In contrast, specialized bacteria which consume methane for metabolic processes in aerobic conditions are called methane oxidizing bacteria, or MOB. Methanogenesis is the production of methane as a byproduct of methanogen respiration under anoxic conditions (Whalen 2005).

Methanogenesis is important in carbon cycling of freshwater systems because methane that is not released as bubbles (Chanton 2005) can be consumed by methane oxidizing bacteria redirecting the methane into a particulate usable carbon source available to lake organisms via microbial loop processing (Kuivila et al. 1988, See Figure 1). Recent studies have provided strong evidence that methanogenic and methane oxidizing bacteria are important food sources for *Chironomus* and *Oligochaeta* (Grey et al. 2004, Grey and

Deines 2005, Eller et al. 2005, Hershey et al. 2006).

Important products of decomposition of organic matter by Eubacteria are acetate, H<sub>2</sub> and CO<sub>2</sub> which can then be converted to methane by Archaeobacteria such as methanogens (Schlesinger 1997). One pathway for methanogenesis is acetate splitting, or the acetoclastic pathway: CH<sub>3</sub>COOH → CO<sub>2</sub> + CH<sub>4</sub>. Another pathway for methanogenesis is the hydrogenotrophic pathway: CO<sub>2</sub> + 4H<sub>2</sub> → CH<sub>4</sub> + 2H<sub>2</sub>O.

The hydrogenotrophic pathway is more complex than the acetoclastic pathway (Schlesinger 1997) and the two pathways differentially fractionate carbon isotopes (Krzycki et al. 1987). Since the acetoclastic pathway releases methane and carbon dioxide as byproducts, the expired carbon dioxide may catalyze methanogenesis via the hydrogenotrophic pathway in anaerobic conditions (See Figure 1). The ratio of acetoclastic to hydrogenotrophic methanogenesis is generally 2:1 (Kotsyrbenko et al. 2004, Glissmann et al. 2004). Methanogens provide a large input of methane to the atmosphere and production is often regulated by temperature (Fenchel et al. 1998). Low temperatures (4°C), result in sediments dominated by acetoclastic methanogens where as higher temperatures (30°C) show dominance in the hydrogenotrophic methanogens (Glissmann et al. 2004) suggesting a shift from the acetoclastic to the hydrogenotrophic pathway as sediment temperatures increase (Zepp Falz et al. 1999). While the acetate precursor for methanogenesis yields most of the methane production, the hydrogenotrophic pathway may be more active in the upper 2 cm of sediments where nutrients are more abundant (Zepp Falz et al. 1999).

The atmospheric methane concentration has increased at an average rate of 1% /yr

over the last several decades (Schlesinger 1997). The amount of methane reaching the atmosphere from aquatic ecosystems is largely dependent on the prevalence of methane oxidizing bacteria at the sediment-water interface because they consume the majority of methane produced in sediments (Oremland and Culbertson 1992). The remaining methane is released to the atmosphere by bubbling when the dissolved methane exceeds the hydrostatic pressure (Schlesinger 1997, Happell and Chanton 1994, Dubey 2005), by diffusion, or via emergent plants (Yavitt and Knapp 1995).

Freshwater environments including wetlands contribute more than 70% of the naturally produced methane, while contributing more than 20% of the total global methane production (Michmerhuizen et al. 1996). Supersaturation of the water column with CO<sub>2</sub> and CH<sub>4</sub> is common in arctic lakes (Kling et al. 1992) due to the high solubility of gases in cold water. In addition, large quantities of methane are released at ice out because lakes store methane under ice cover (Michmerhuizen et al. 1996). Arctic lakes therefore represent an important greenhouse gas reservoir which can greatly augment the atmospheric methane concentration during ice melt. This is one of the reasons why the effects of global warming are more evident at higher latitudes (Hansen and Lebedeff 1987).

Methanogen populations have been shown to increase concurrent with temperature increases (Zeikus and Winfrey 1976) at a faster rate than methane oxidizing bacteria, suggesting that as the climate warms, there may be an accelerated production of methane released from these bacteria (King and Adamsen 1992) and proportionately less MOB to consume it.

The increased temperature associated with global warming leads to an increase in permafrost thawing, resulting in increased amounts of DOC entering water bodies from tundra soils (Petrone et al. 2006), resulting in further increases in the prevalence of methanogens in boreal systems (Petrone et al. 2006, Zimov 2006). A link between decreased DOC levels and decreased reliance on MOB was shown in an arctic population of *Chironomus* by exposing the study organisms to low levels of DOC from a mountain spring (Hershey et al. in press).

### *Stable Isotopes*

Stable isotope analysis provides a useful technique for examining bacterial carbon assimilation (Hall 1995) and can provide a reliable estimate of the source of carbon being assimilated into the tissues of an organism (Hesslein et al. 1993). Stable isotope compositions are measured using a mass spectrometer as a ratio of heavy to light elements, e.g.,  $^{13}\text{C}/^{12}\text{C}$ , expressed in ppt relative to a standard. The mathematical expression for the isotopic analysis of carbon is based on the PeeDee Belemite standard and is reported as the difference from this standard in ppt  $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$  (Peterson and Fry 1987). From stable isotope analysis one can not only determine the source of the carbon consumed by an organism, but also what carbon source is preferentially assimilated by the organism and therefore consumed by higher trophic level organisms.

The stable isotope carbon signatures of some macroinvertebrates in arctic lakes (especially offshore) have been shown to be depleted in  $^{13}\text{C}$  when compared to other

sources, suggesting that MOB may be increasingly important in arctic food webs (Hershey et al. 2006). Higher methane turnover rates yield a more depleted  $^{13}\text{C}$  value (Eller et al. 2007). When biogenic methane is consumed by MOB during methane oxidation the  $^{13}\text{C}$  value of methane becomes more depleted, which can be traced through bacterial metabolism and assimilation by higher trophic level organisms. Because acetoclastic methanogens utilize only methyl-labeled acetate, whereas aerobic bacteria utilize both methyl- and carboxyl-labeled acetate, we can experimentally detect utilization of methanogenic bacteria by assessing percent enrichment of methyl- versus carboxyl-  $^{13}\text{C}$  labeled acetate to organism biomass.

### *Trophic Fractionation*

Fractionation is the measure of preferential assimilation of the lighter isotopic form of an element over the heavier form of the element, i.e.  $^{12}\text{C}$  vs.  $^{13}\text{C}$  and  $^{14}\text{N}$  vs.  $^{15}\text{N}$ . Trophic fractionation of carbon is usually small,  $0.5 \pm 1.3\text{‰}$  (McCutchan et al. 2003), such that  $\delta^{13}\text{C}$  is typically not used to determine trophic position, unlike  $^{15}\text{N}$  which has a distinctive 3-4‰ shift indicative of changing trophic position (Post 2002). Carbon fractionation of methane is often higher than that of particulate matter, with reported values reported between -13 and -90‰ (Fuex 1977). Biogenic methane typically has a  $\delta^{13}\text{C}$  value between -50 and -80‰ (Whiticar 1996). The range of  $\delta^{13}\text{C}$  values of biogenic methane varies greatly based on the pathway of methanogenesis and the  $\delta^{13}\text{C}$  of the carbon source. Methanogens which utilize the acetoclastic pathway have been reported to fractionate C at a value close to -21‰ in comparison to methanogens utilizing the

hydrogenotrophic pathway fractionating around -45‰ (Krzycki et al. 1987), although these estimates depend on experimental conditions.

Mixing models have recently been used to assess source material for aquatic food webs (e. g., Vander Zanden and Rasmussen 2001, Hershey et al. 2006). However, none of these models considered the potentially large fractionation value due to the assimilation of methane-derived carbon in consumer diets. Therefore, when carbon fractionation is unknown and potentially quite variable due to experimental conditions and/or multiple pathways of organic matter processing, mixing models may not accurately decipher the assimilation of carbon sources for consumers. However, by using labeled substrates for aerobic and methanogenic microbes, we can detect the various pathways for C assimilation, and make inferences about their importance based on experimental conditions and previously published studies of feeding ecology.

In this study I chose to look at the assimilation of dissolved and particulate carbon sources by *Sphaerium nitidum* and *Grensia praeterita* in an arctic lake. More specifically I focused on the importance of particulate organic matter (POM), microbial loop processing, and two methanogenic and non-methanogenic pathways by *Sphaerium nitidum* and *Grensia praeterita* in an arctic lake. In the first objective, I looked at the assimilation of POM via direct utilization of pelagic seston. I hypothesized that POM would be utilized by both organisms since functionally *Sphaerium* is a filter feeder and *Grensia* is a collector-gatherer. In my second objective, I looked at the reliance of the microbial loop by using <sup>13</sup>C labeled algal DOC. I hypothesized that both organisms would rely on microbial loop processing. My last objective looked at the importance of

methanogenic and non-methanogenic pathways of the microbial loop by utilization of  $^{13}\text{C}$  labeled acetate treatments. I hypothesized that *Sphaerium* would have a higher dependence on the methanogenic pathways as they have been known to rely on pedal feeding.

## CHAPTER II

### STUDY SITES

The research was conducted at Toolik Field Station (68°38'N, 149°38'W) on the North Slope of the Brooks Range in arctic Alaska during summer 2006. The field station is surrounded by many oligotrophic kettle basin lakes which are ice covered for 9 months of the year (O'Brien et al. 1997). The organisms for my study, *Sphaerium* and *Grensia*, were both collected from the littoral zone of Lake N3 located 2 km north of the field station. This lake was chosen due to its close proximity to the field station and high availability of study organisms. Sediment cores were taken from Lake GTH 112 (68°40'N, 149°14'W) for use as mesocosms to measure assimilation of organic matter sources by *Sphaerium* and *Grensia*. Lake GTH 112 was selected as a sediment source for this experiment because previous studies have indicated high levels of methane production and oxidation in Lake GTH 112 sediments (Hershey et al. 2006, and unpublished data).

*Study organism: Grensia praeterita*

*Grensia praeterita* is a species of larval caddisfly found in lentic habitats.



They construct protective cases made of plant particles or rock fragments which cover their bodies (Merritt and Cummins 1996). They feed surficially by extending their legs and head outside the case pulling particles toward their mouth parts (Merritt and Cummins 1996).

*Study organism: Sphaerium nitidum*

*Sphaerium nitidum* is a fingernail clam which can obtain food by suspension and pedal feeding (McMahon 1991). During pedal feeding, cilia on the foot will capture bacteria and sediment while transporting the deposition to the mantle cavity (Thorp and Covich 2001). This type of feeding was thought to be minimal (Leff and Leff 2000) although some studies showed evidence of greater importance (Vaughn and Hakenkamp 2001). Hornbach et al. (1984) found a population of *Sphaerium striatinum* that assimilated 35% of its organic carbon via filter feeding suggesting 65% assimilation from sediment sources, i.e. possibly pedal feeding. A  $^{15}\text{N}$  tracer experiment found *Sphaerium striatinum* biomass to consist of 20% suspended and 80% deposited material (Raikow and Hamilton 2001).

## CHAPTER III

### METHODS

A D-net was used to collect organisms from macrophyte beds within the littoral zone of Lake N3. 108 *Grensia* and 540 *Sphaerium* were collected over a three day period from Lake N3 and were brought back to Toolik Field Station to be used in the experimental mesocosms. Sixty sediment cores were taken from Lake GTH 112 with a KB corer from a depth of about 4m.

After returning to the field station, sediment cores were immediately extruded into 20 cm depth experimental cores and sealed at the bottom. The cores were secured in metal racks in a vertical position and the racks were anchored to a pool containing water re-circulated from Toolik Lake in order to keep temperature constant for all sediment cores. The experiment consisted of sixty total cores; thirty containing *Sphaerium* and thirty containing *Grensia*. Each *Grensia* core contained three individuals while *Sphaerium* cores contained fifteen individuals. These densities were selected to provide sufficient mass for isotopic analysis while also minimizing oxygen stress and resource depletion within the cores. The top of each core was covered with a piece of window screen secured by a zip-tie so organisms could not escape. Light exposure was limited by a 60% shade cover mesh draped over the pool, which was secured to the pool edges by

clothes pins. This decrease in light exposure was to better simulate the organisms natural environment although the cores received more light than deep areas of Lake GTH 112.

### *Experimental Treatments*

Five treatments with six replicates were applied to the thirty cores for each species. Initial  $\delta^{13}\text{C}$  values were measured on organisms taken from Lake N3. The five treatments chosen were: algal  $\text{DO}^{13}\text{C}$ ,  $^{13}\text{C}$ - labeled seston,  $^{13}\text{C}$ -carboxyl labeled sodium acetate ( $\text{CH}_3^*\text{COONa}$ ),  $^{13}\text{C}$ -methyl labeled sodium acetate ( $^*\text{CH}_3\text{COONa}$ ), and a control. For the seston and  $\text{DO}^{13}\text{C}$  treatment, 15L of water were collected from the metalimnion of Lake NE9B with a Van Dorn bottle and placed in clear 4L cubitainers. This lake was chosen because it was known to have a high metalimnetic chlorophyll *a* maximum (Matt Keyes biology department UNCG, personal communication). This water was taken from 4.5 m. Each cubitainer was inoculated with an average of 0.125g of  $\text{NaH}^{13}\text{CO}_3$ . After inoculation, cubitainers were incubated in Toolik Lake for 10 days. The water was then filtered with a 0.2  $\mu\text{m}$  membrane filter. The resultant  $^{13}\text{C}$ - enriched seston on the filter was added to 1L of Lake GTH 112 hypolimnetic water. Filtrate remaining was used as the  $\text{DO}^{13}\text{C}$  treatment containing no bacteria. Preparations were frozen for later use. During freezing and thawing, algal leaching and excretions may have entered the seston treatment as DOC.

A 6mM concentration of acetate was utilized for labeled acetate treatments. Acetate is assimilated by most phototrophic bacteria (Goltschalk 1986). The 6 mM concentration was selected because sulfur reducing bacteria have been shown to out-

compete methanogens at low acetate concentrations (Lovely and Klug 1983, Yoda et al. 1987), although sulfate concentrations are very low in area lakes (Chalfant 2004). The natural concentrations of acetate in freshwater has been estimated to be between 0-6  $\mu\text{g liter}^{-1}$  (Allen 1968).  $^{13}\text{C}$  labeled acetate has been previously used to trace microbial carbon through a stream food web (Hall 1995).

A stock solution was prepared for both  $^{13}\text{C}$ -labeled acetate treatments,  $\text{CH}_3^*\text{COONa}$  and  $^*\text{CH}_3\text{COONa}$ . In a 1L volumetric flask 0.5004g  $^*\text{CH}_3\text{COONa}$  and 6.8805g of unlabeled sodium acetate was added to 1L of DI water. From the stock solution, 66.7ml of solution was added to 933.3 ml of glass filtered Lake GTH 112 water. 0.501g of  $\text{CH}_3^*\text{COONa}$  and 6.8814g of unlabeled  $\text{CH}_3\text{COONa}$  was added to a 1L volumetric flask and was filled to 1L with DI water. From the stock solution, 66.7ml of stock solution was added to 933.3 ml of glass filtered Lake GTH 112 water. The control contained unfiltered water from Lake GTH 112.

After a four week period, *Sphaerium* and *Grensia* were harvested from the cores. *Sphaerium* were removed from their shells and *Grensia* were removed from their cases. Animal tissues from each core were pooled and placed into microfuge tubes. Organisms were dried and a mass of 0.8-1.2mg was placed in tin capsules and sent to the UC Davis Isotope Facility. Liquid samples of treatment water were also sent to UC Davis for stable isotope analyses.  $\delta^{13}\text{C}$  of animal tissue from each core was expressed as a % of the  $\delta^{13}\text{C}$  of the respective treatment water. Percent (%) enrichment was compared among treatments. % enrichment was used as a response variable, in lieu of a mixing model approach because fractionation of carbon isotopes during methanogenesis was unknown

and unlikely to be similar to carbon isotope fractionation by aerobic processes. Unlike fractionation of whole organisms, which is small on average, carbon fractionation of the methanogenic process ranges from -21 to less than -55‰ (Krzycki et al. 1987). Percent enrichment analysis can detect assimilation of labeled substrates, but without a mixing model it cannot quantify importance of source materials.

#### *Data Analysis*

To evaluate the effect of the core experiment itself on utilization of ambient organic matter sources, two-way ANOVA was conducted using  $\delta^{13}\text{C}$  values of species, treatment, and their interaction, where control and initial values were considered the treatments. To detect differences between  $^{13}\text{C}$ -labeled treatments for each species, separate one-way ANOVA were performed on the arcsin transformed percent enrichment of *Sphaerium* and *Grensia* independently. All statistics were run on SAS v.9.1.3 and JMP v.6.0.3 (SAS Institute, Inc. Cary, NC).

## CHAPTER IV

### RESULTS

#### *Experimental Animal Survivorship*

Visual examination of the experimental cores over the 4 week duration showed *Sphaerium* to be active across all treatment types. *Sphaerium* were embedded in the surficial sediments of the experimental cores or were occasionally observed adhering to the core walls (Table 1). The overall survivorship of *Sphaerium* in experimental cores was 50% at the end of the four week experimental period.

Live *Grensia* were active on top of the surficial sediments in experimental cores (Table 1), except in the CH<sub>3</sub>\*COONa treatment where there was no *Grensia* survivorship. *Grensia* survivorship in control, seston, and algal DO<sup>13</sup>C treatments was approximately 67%, and approximately 11% in the \*CH<sub>3</sub>COONa treatment.

#### *Experimental Core Observations*

When the experiment was harvested, experimental treatment cores varied in sediment color and degree of turbidity in overlying water. In both acetate treatments, overlying headwater in cores was extremely turbid compared to the control cores. A

reddish/brown precipitate formed throughout the water column and stained core walls (Table 1). The  $\text{CH}_3^*\text{COONa}$  cores with no surviving organisms smelled anaerobic, and sediments were darker when compared to the cores with surviving experimental animals (Table 1). In the algal  $\text{DO}^{13}\text{C}$  and seston treatment cores, headwater was less turbid than when compared to acetate treatments (Table 1). In algal  $\text{DO}^{13}\text{C}$  and seston treatment cores with no surviving organisms, sediments were a darker brown color compared to the lighter brown sediments found in cores with surviving organisms (Table 1). In the control treatment cores, overlying water was clear and sediments were light brown in color across all cores (Table 1).

#### *Comparison of Initial and Control Experimental Organisms*

Examination of initial and control  $\delta^{13}\text{C}$  values of *Sphaerium* and *Grensia* showed a significant difference between species (Figure 2, p-value <0.001, Table 2), but not between treatment types, although there was a significant species x treatment interaction (p-value = 0.02, Table 2, Figure 3).  $\delta^{13}\text{C}$  *Sphaerium* was initially lower than  $\delta^{13}\text{C}$  *Grensia* (p-value <0.05, Figure 2). The *Sphaerium* control  $\delta^{13}\text{C}$  value also was significantly lower than *Grensia* control  $\delta^{13}\text{C}$  values (p-value <0.05 Figure 2). However, the initial and control  $\delta^{13}\text{C}$  values of the organisms changed differently over time (p-value = 0.03, Table 2, Figure 3) with *Sphaerium*  $\delta^{13}\text{C}$  increasing and *Grensia* staying relatively constant during the experiment (Figure 3).

The differences between initial and control  $\delta^{13}\text{C}$  values for both experimental *Grensia* and *Sphaerium* were small compared to treatment effects on  $\delta^{13}\text{C}$  (Table 3).

*Sphaerium* in the CH<sub>3</sub>\*COONa treatments were enriched in <sup>13</sup>C compared to all other treatment values. *Grensia* was highly enriched in the algal DO<sup>13</sup>C samples when compared to the control and initial treatments (Table 3).

*Sphaerium* δ<sup>15</sup>N of initial samples was slightly lower than all experimental *Sphaerium* values, but δ<sup>15</sup>N values of all experimental treatments were generally similar (Table 3). δ<sup>15</sup>N signatures for *Sphaerium* ranged from 4.38 - 5.01‰ (Table 3). δ<sup>15</sup>N values of experimental *Grensia* also showed small variation among treatments. δ<sup>15</sup>N means ranged from 2.85 to 3.51‰ (Table 3).

Percent enrichment from each respective treatment to *Sphaerium* biomass is as follows: 26% seston, 18% algal DO<sup>13</sup>C consuming bacteria, 10% CH<sub>3</sub>\*COONa, and 5% \*CH<sub>3</sub>COONa (Figure 4a). Percent enrichment of treatment label from *Sphaerium*'s diet showed that all four carbon sources were utilized (Figure 4a). Percent enrichment of <sup>13</sup>C label from CH<sub>3</sub>\*COONa was significantly greater than \*CH<sub>3</sub>COONa in *Sphaerium* (Figure 4a).

Stable isotope analysis indicated that percent enrichment from each respective treatment to *Grensia* biomass is as follows: 60% seston, 34% algal DO<sup>13</sup>C consuming bacteria, and 3% of \*CH<sub>3</sub>COONa (Figure 4b). However, one-way ANOVA of the arcsin transformation of percent enrichment of label in *Grensia*'s diet showed no statistically significant difference between treatments (P<0.05, Figure 4b).



## CHAPTER V

### DISCUSSION

Results from this work indicate that both *Sphaerium* and *Grensia* consumed carbon derived from both particulate and dissolved sources (Fig. 4), but carbon enrichment of the various sources varied between the two consumers. More labeled material was incorporated from seston than any other source for both *Sphaerium* (26%) and *Grensia* (60%), although *Grensia* may have been more dependent on algal detritus than was *Sphaerium*. Algal DOC, which would have been processed through the microbial loop, also was shown to be an important carbon basal resource for both study organisms, with 34% incorporation by *Grensia* and 18% incorporation by *Sphaerium* (Figure 4a, 4b). *Sphaerium* showed differential enrichment from  $\text{CH}_3^*\text{COONa}$  and  $^*\text{CH}_3\text{COONa}$ . Below we discuss that it is very unlikely that this result could have occurred through aerobic processing alone, and suggest that methanogenic microbial pathways were likely important to *Sphaerium* nutrition (see *Role of methanogenic processes*). Because there was no survivorship of *Grensia* in the  $\text{CH}_3^*\text{COONa}$ , we cannot make inferences about the role of methanogenic pathways for *Grensia* nutrition.

Utilization of both algal seston and algal DOC illustrate that carbon sources derived from direct detrital as well as microbial loop processes were important to both organisms. The importance of the microbial loop for entraining DOC into the particulate

pool has not been previously studied for either *Grensia* or *Sphaerium*. *Grensia* are known functionally as collectors (Merritt and Cummins 1996), whereas *Sphaerium* are predominately known as filter feeders (Thorp and Covich 2001), although pedal feeding has been reported (Vaughn and Hakenkamp 2001). Our data indicate that feeding niches of both organisms are broader than those classifications suggest, and provide a further insight into the origins of the particulate and dissolved carbon sources.

The survival rate for *Grensia* was low in both the acetate treatments (Table 1). The low survival rate in the acetate treatments was likely due to the high concentration of labeled (mM) acetate added to the experimental cores, utilized to circumvent possible competition from sulfate reducing bacteria. Experimental acetate cores were stained reddish/brown (Table 1) suggesting precipitation of iron from the water columns which may lead to increased darker un-decomposed organic matter and humic substances (Schlesinger 1997). Oxygen depletion from water may have been a result of increased decomposition and aerobic bacterial respiration in the high acetate sediments. Pedal feeding bivalves mix sediments increasing oxygen levels (Levinton 1995). Also, *Sphaerium* may be more tolerant of low oxygen conditions than *Grensia*, explaining the difference in overall survival rate between the two benthic consumers.

*Grensia* feeds surficially, extending its head and legs outside of its cases (Merritt and Cummins 1996), thus POM was expected to be the primary contributor to *Grensia* biomass. Furthermore, because filter feeding is not known for *Grensia*, nor likely given *Grensia*'s morphology, this result indicates that much of its diet was derived from deposited seston. In near-shore areas of arctic Lake E5, located in the Toolik Field

Station vicinity, *Grensia*'s diet was estimated to be derived from approximately 57% seston, based on utilization of a  $^{15}\text{N}$  tracer (Hershey et al. 2006), which is very consistent with the 60% biomass enrichment estimated in our study (Figure 4b).

Kasprzak (1986) found Sphaeriidae to consume close to 96% seston in a eutrophic lake. Seston enrichment (26%) was much lower in the present study. Clearly, *Sphaerium* do not rely exclusively on filter feeding; and may utilize pedal feeding (Vaughn and Hakenkamp 2001). Pedal deposit feeding, has been shown to supplement filter feeding via assimilation of bacteria from sediments by cilia on the foot (McMahon 1991, Vaughn and Hakenkamp 2001). In this study, biomass enrichment from  $^{13}\text{C}$  labeled algal DOC and  $^{13}\text{C}$  labeled acetate indicates that assimilation of foods derived through the microbial loop was quite important. Pedal feeding on sediment bacteria which would have utilized labeled DOC is one mechanism for *Sphaerium* to have accessed DOC basal resources, although filtering of water column bacteria that utilized the labeled DOC also likely occurred.

#### *Role of Methanogenic Processes*

Methane production via methanogenesis was highest at sediment depths in the range of 0-2 cm (Zeikus and Winfrey 1976), directly on or slightly below the sediment-water interface. These high rates of methanogenesis at the sediment water interface may be due to higher nutrient availability compared to deeper sediments (Zeikus and Winfrey 1976, Zepp Falz et al. 1999).

In a stream population of *Sphaerium striatinum*, Hornbach et al. (1984) found that

44% of assimilated carbon was incorporated into biomass and 56% was respired as CO<sub>2</sub>. The methyl group in acetate is lighter than the carboxyl group and is favored during respiration (Winfrey and Zeikus 1979). Under aerobic conditions, oxidation of the methyl C on acetate to CO<sub>2</sub> was virtually complete (Winfrey and Zeikus 1979). Thus, if *Sphaerium* respired 56% of assimilated carbon, similar to *S. striatinum* (Hornbach et al. 1984), then essentially all of the methyl-labeled C and 6% of the carboxyl-labeled C would have been respired to CO<sub>2</sub> under aerobic conditions. Only the remaining unrespired carboxyl C would accumulate as biomass. Therefore, if aerobic uptake of acetate was the primary means of acetate incorporation into the microbial loop, all of the <sup>13</sup>C label from \*CH<sub>3</sub>COONa should have been metabolized to CO<sub>2</sub> and none (or trace amounts) would accumulate in *Sphaerium* biomass. However, we observed 5% enrichment of \*CH<sub>3</sub>COONa into *Sphaerium*, with relatively low variability (Fig. 4a), which was clearly distinct from the trace amounts of \*CH<sub>3</sub>COONa enrichment that we observed for *Grensia* (Fig. 4b). During acetoclastic methanogenesis, the methyl group in \*CH<sub>3</sub>COONa would be used directly by methanogens (Zeikus 1977, See Figure 1). Therefore, the most likely interpretation of these data is that the 5% incorporation of \*CH<sub>3</sub>COONa observed for *Sphaerium* reflects utilization of methane-derived C. We cannot use these data to infer the quantitative importance of methane-derived carbon for *Sphaerium* due to the previously discussed problem that carbon isotopic fractionation by methanogens under the experimental conditions is unknown.

Enrichment of \*CH<sub>3</sub>COONa into *Sphaerium* biomass could also occur indirectly via hydrogenotrophic methanogenesis by either of two mechanisms, which are not

mutually exclusive. First,  $^{13}\text{C}$ - $\text{CO}_2$  produced by aerobic metabolism of  $^*\text{CH}_3\text{COONa}$  could be utilized by hydrogenotrophic methanogens to produce methane, which would then be assimilated by MOB and subsequently ingested by *Sphaerium*. Secondly,  $^{13}\text{C}$ - $\text{CO}_2$  also would be produced during acetoclastic methanogenesis of  $\text{CH}_3^*\text{COONa}$  and that  $^{13}\text{C}$ - $\text{CO}_2$  could then enter the hydrogenotrophic pathway, with the same net outcome as in the first mechanism. Thus, there are multiple pathways for assimilation of methane-derived carbon by *Sphaerium*, and the 5% enrichment from  $^*\text{CH}_3\text{COONa}$  to *Sphaerium* biomass indicates that at least one of these pathways was utilized. Further research would be needed to distinguish and quantify these pathways. Because fractionation by methanogens and MOB is unknown, it is not possible to use % enrichment of  $^*\text{CH}_3\text{COONa}$  versus  $\text{CH}_3^*\text{COONa}$  to quantitatively estimate the role of aerobic versus methanogenic processes to *Sphaerium* nutrition.

In a survey of lakes in the Toolik Lake vicinity,  $\delta^{13}\text{C}$  of offshore fingernail clams was -34.7‰, which was lower than  $\delta^{13}\text{C}$  of seston or DOC; Hershey et al. (2006) suggested this was due to feeding partially on methane-derived carbon. The  $\delta^{13}\text{C}$  value of pelagic seston from arctic Lake E6 was -29.5‰ and arctic Lake E5 was -31.7‰ (Hershey et al. 2006). Epilimnetic and hypolimnetic  $\text{DO}^{13}\text{C}$  values in area lakes ranges from about -25‰ - 27‰ (Hershey unpublished data 2007). Naturally occurring methane presents variable  $\delta^{13}\text{C}$  values, but is virtually always  $^{13}\text{C}$  depleted compared to reported values for seston and DOC (e.g., Krzycki et al. 1987). In Lake NE-9B methane  $\delta^{13}\text{C}$  in the epilimnion ranged from -43.53 to -48.83‰ (Hershey unpublished data 2007). These data also suggest that methane-derived carbon is very likely a basal carbon source for

*Sphaerium* in area lakes, consistent with the experimental results.

## CHAPTER VI

### CONCLUSIONS

This study provides insight to the importance of particulate and dissolved C sources to two benthic invertebrates, *Grensia* and *Sphaerium*. *Grensia* incorporated labeled C from both seston and algal derived DOC, as did *Sphaerium*, but *Sphaerium* also utilized methane-derived C. However, pathways for incorporating methane-derived C into consumer food webs are complex, and further research is needed to quantify the mechanisms that are most important to consumers.

In arctic lake food webs, both *Grensia* and *Sphaerium* are important in the diets of fishes (see Hershey et al. 1999), thus, to the extent that DOC and methane-derived carbon are important in supporting benthic consumers, they are also important basal resources for fishes. Furthermore, methane production occurs in lake sediments universally, thus its role in lake food webs is not restricted to the arctic. The DOC pool in lakes is chemically complex, and in many oligotrophic lakes, it is dominated by terrestrial inputs (Kritzberg et al. 2004). Further study of the relative importance of terrestrial and algal DOC in supporting higher trophic levels is needed for understanding C cycling in these ecosystems.

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Appendix A. Tables and Figures

Table 1. Observations of experimental cores and survivorship in experimental animals (n=6 for each treatment)

Core Treatments	No. Initial	No. Surviving per core (mean ±SE)	turbidity of overlying water	discolored sediments	location of animals
<i>Sphaerium</i> control	15	10±1.39	not turbid	cores with surviving organisms were lighter brown in color	buried/core wall
<i>Sphaerium</i> seston	15	4±2.25	low turbidity		buried/core wall
<i>Sphaerium</i> Algal DOC	15	12±0.73	low turbidity		buried/core wall
<i>Sphaerium</i> *CH <sub>3</sub> COONa	15	8±2.63	Turbid		buried/core wall
<i>Sphaerium</i> CH <sub>3</sub> *COONa	15	6±0.86	Turbid		buried/core wall
<i>Grensia</i> control	3	2±0.21	not turbid	cores with no surviving organisms were darker brown in color	top of sediments
<i>Grensia</i> seston	3	2±0.48	low turbidity		top of sediments
<i>Grensia</i> Algal DOC	3	2±0.41	low turbidity		top of sediments
<i>Grensia</i> *CH <sub>3</sub> COONa	3	1±0.21	Turbid		top of sediments
<i>Grensia</i> CH <sub>3</sub> *COONa	3	0±0	Turbid		top of sediments

Table 2. Two way ANOVA comparing control and initial  $\delta^{13}\text{C}$  values (‰) in *Sphaerium* and *Grensia*

	F-value	P-value
Overall ANOVA	9.37	<0.001
Species	20.39	<0.001
Treatment	1.8	0.2
Species*Treatment	5.91	0.03

Table 3. *Sphaerium* and *Grensia*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for each treatment in this experiment

trt type	trt $\delta^{13}\text{C}$	<i>Grensia</i> $\delta^{13}\text{C}$ (mean $\pm$ SE)	<i>Grensia</i> $\delta^{15}\text{N}$ (mean $\pm$ SE)	<i>Sphaerium</i> $\delta^{13}\text{C}$ (mean $\pm$ SE)	<i>Sphaerium</i> $\delta^{15}\text{N}$ (mean $\pm$ SE)
Initial	NA	-31.53 $\pm$ 0.42	2.97 $\pm$ 0.27	-33.48 $\pm$ 0.03	4.38 $\pm$ 0.18
Control	-28.86	-31.78 $\pm$ 0.44	3.24 $\pm$ 0.26	-32.36 $\pm$ 0.09	4.67 $\pm$ 0.10
Seston	199.09	118.07 $\pm$ 61.93	3.51 $\pm$ 0.21	51.47 $\pm$ 0.80	5.01 $\pm$ 0.14
Algal DOC	579.73	193.76 $\pm$ 70.26	2.97 $\pm$ 0.20	102.79 $\pm$ 18.43	4.86 $\pm$ 0.09
*CH <sub>3</sub> COONa	2816.35	77.28 $\pm$ 14.77	2.85 $\pm$ 0.85	123.76 $\pm$ 9.08	4.60 $\pm$ 0.13
CH <sub>3</sub> *COONa	3450.66	-	-	343.54 $\pm$ 27.04	4.94 $\pm$ 0.09



Figure 1. Summary of microbial loop processing including methanogenic pathways in aquatic systems. Sources of DOC include both allochthonous inputs from tundra runoff and autochthonous inputs such as organic matter re-entering the microbial loop from phytoplankton, protozoa, and zooplankton.

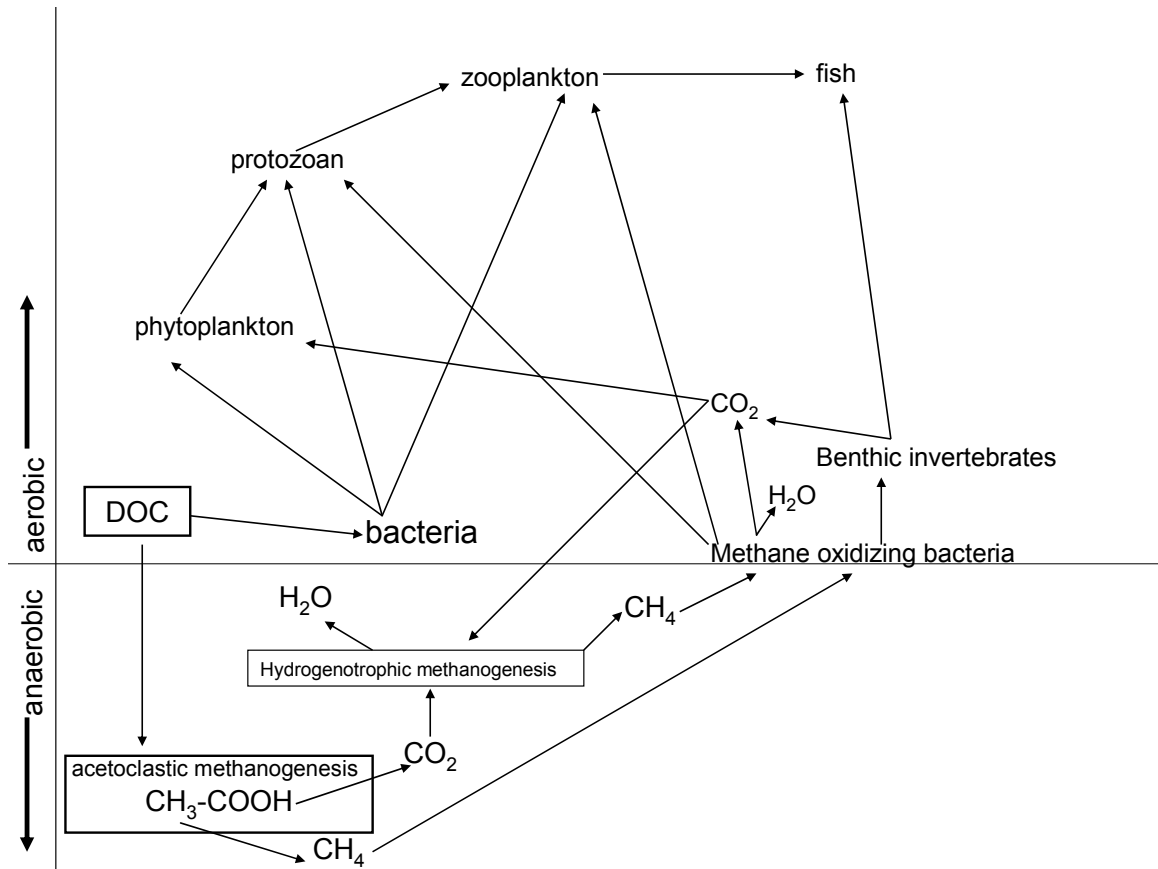


Figure 2. One way ANOVA of initial and control  $\delta^{13}\text{C}$  values to *Sphaerium* and *Grensia*  
\*=significant differences at  $P < 0.05$

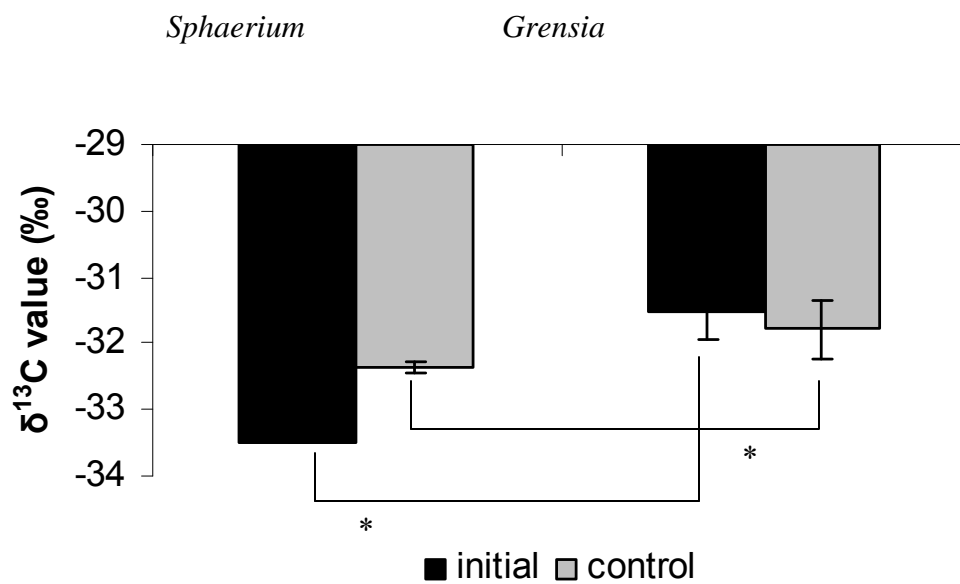


Figure 3. Initial and control  $\delta^{13}\text{C}$  values to *Sphaerium* and *Grensia*.

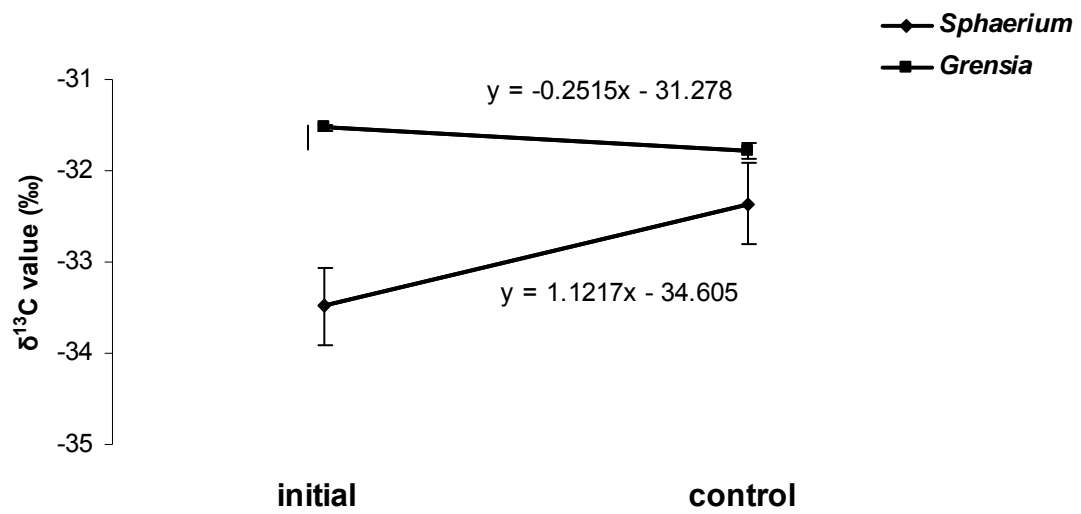


Figure 4a. Percent enrichment of *Sphaerium* biomass (Statistics run on Arcsin data)

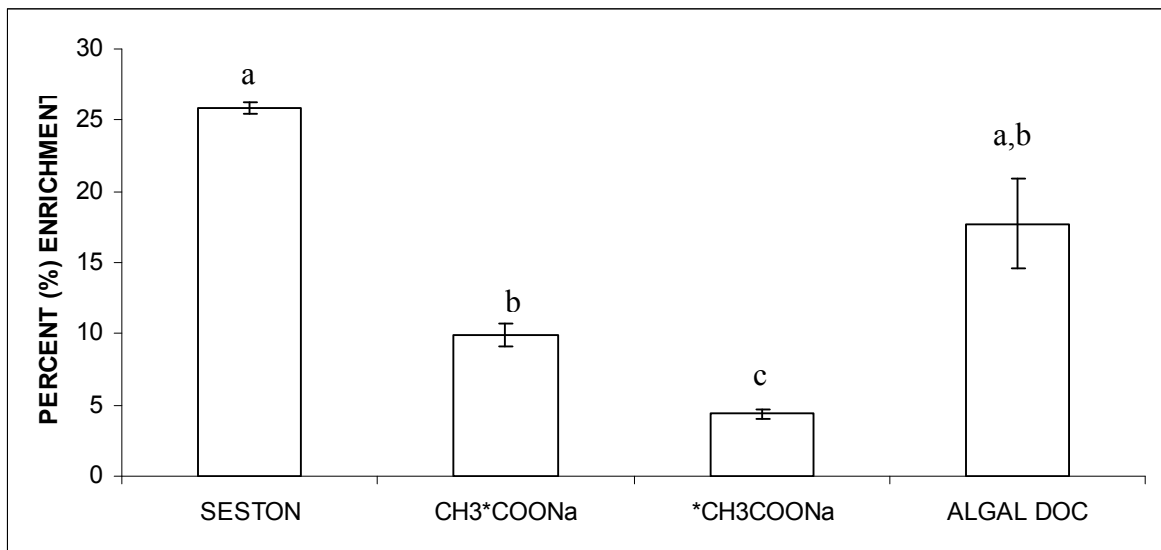
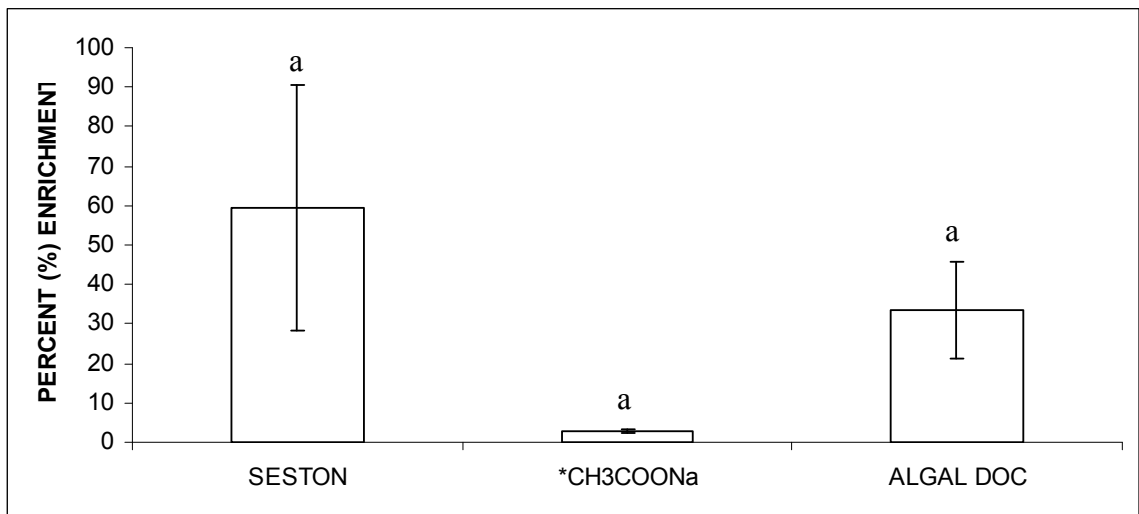


Figure 4b. Percent enrichment of *Grensia* biomass (Statistics run on Arcsin data)



Appendix B. Raw Data

*Sphaerium* stable isotope raw data of organism and treatment liquid

treatment type	Rep	treatment $\delta^{13}\text{C}$ value	organism $\delta^{13}\text{C}$ value
seston	1	199.1	52.3
seston	2	199.1	50.7
CH <sub>3</sub> *COONa	1	3450.7	414.6
CH <sub>3</sub> *COONa	2	3450.7	316.3
CH <sub>3</sub> *COONa	3	3450.7	401.6
CH <sub>3</sub> *COONa	4	3450.7	304.1
CH <sub>3</sub> *COONa	5	3450.7	281.1
*CH <sub>3</sub> COONa	1	2816.3	129.8
*CH <sub>3</sub> COONa	2	2816.3	136.8
*CH <sub>3</sub> COONa	3	2816.3	96.9
*CH <sub>3</sub> COONa	4	2816.3	131.6
Algal DOC	1	579.7	35.9
Algal DOC	2	579.7	104.0
Algal DOC	3	579.7	165.5
Algal DOC	4	579.7	133.1
Algal DOC	5	579.7	73.5
Algal DOC	6	579.7	104.6

*Grensia* stable isotope raw data of organism and treatment liquid

treatment type	Rep	treatment $\delta^{13}\text{C}$ value	organism $\delta^{13}\text{C}$ value
seston	1	199.1	273.8
seston	2	199.1	-5.2
seston	3	199.1	33.1
seston	4	199.1	24.7
seston	5	199.1	264
*CH <sub>3</sub> COONa	1	2816.3	62.5
*CH <sub>3</sub> COONa	2	2816.3	92.1
Algal DOC	1	579.7	122.0
Algal DOC	2	579.7	212.4
Algal DOC	3	579.7	58.6
Algal DOC	4	579.7	382.1