Role of Temperature and Organic Degradation on the Persistence of Stable Isotopes of Carbon and Nitrogen in Aquaculture Waste

by

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ABSTRACT

Role of Temperature and Organic Degradation on the Persistence of Stable Isotopes of Carbon and Nitrogen in Aquaculture Waste

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This study evaluated effects of temperature and organic degradation on the persistence of δ13C and δ15N in aquaculture waste. Organic degradation time showed a significant linear (p<0.0001) and quadratic (p<0.0096) effect of depletion on δ13C and a significant linear effect (p<0.0001) of enrichment on δ15N. Temperature showed a significant linear effect (p<0.0001) of depletion on δ13C and a significant linear effect (p<0.0001) of enrichment on δ15N. Sediment samples collected from active sites showed δ13C values ranging from -22.55 to -20.94‰ and δ15N values ranging from 3.27 to 5.87‰. Inactive site sediment samples showed δ13C values ranging from -26.34 to -18.96‰ and δ15N values ranging from 4.02 to 12.12‰. Aquaculture feed δ13C values ranged from -21.07 to -17.32‰ and δ15N values ranged from 4.89 to 9.66‰. Organic degradation and temperature had significant effects on the persistence of δ13C and δ15N in aquaculture waste therefore reducing the potential for tracking aquaculture waste.
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Declaration of Work performed

All work reported in this thesis was performed by me with the exception of the following:

Isotopic analysis of the samples was carried by William Mark and staff of the Environmental Isotopes Laboratory, University of Waterloo.

Statistical analysis of sediment samples and feeds used at farm sites was performed by Margaret Quinton and Bill Szkotnicki Animal and Poultry Science, University of Guelph.

Fourier analysis of temperature data and statistical analysis of incubation data performed by Bill Szkotnicki, University of Guelph.
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AWATS</td>
<td>Aquaculture Waste Transport Simulator</td>
</tr>
<tr>
<td>BCE</td>
<td>Below cage environment (BCE)</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CE</td>
<td>Cage environment (CE)</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>DF</td>
<td>Degrees of Freedom</td>
</tr>
<tr>
<td>DFO</td>
<td>Department of Fisheries and Oceans</td>
</tr>
<tr>
<td>DST</td>
<td>Decision Support Tool</td>
</tr>
<tr>
<td>GLM</td>
<td>General Linear Model</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>LSM</td>
<td>Least square means</td>
</tr>
<tr>
<td>LE</td>
<td>Linear effect</td>
</tr>
<tr>
<td>m</td>
<td>Meter</td>
</tr>
<tr>
<td>MOM</td>
<td>Modelling-On growing fish farms-Monitoring</td>
</tr>
<tr>
<td>n</td>
<td>Sample size</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>POM</td>
<td>Particulate organic matter</td>
</tr>
<tr>
<td>QE</td>
<td>Quadratic effect</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>δ</td>
<td>Stable isotope ratio</td>
</tr>
<tr>
<td>SOK</td>
<td>State-of-Knowledge Initiative</td>
</tr>
<tr>
<td>S</td>
<td>Sulfur</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
</tr>
<tr>
<td>WWC</td>
<td>Whole water column</td>
</tr>
<tr>
<td>δ\textsuperscript{13}C</td>
<td>Stable Isotope of Carbon</td>
</tr>
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<td>δ\textsuperscript{15}N</td>
<td>Stable Isotope of Nitrogen</td>
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Chapter 1. Literature Review

1.1 Aquaculture Introduction: A Global and Canadian Industry Perspective

Global aquaculture has reached a new milestone in recent years by producing half (50%) of the world’s annual total food fish supply of 148 million tonnes of fish in 2010 (FAO, 2012). Aquaculture is the fastest growing animal-food producing sector in the world, growing at an average annual growth rate of 8.7% since 1970, while capture fisheries production has not grown since the mid-1980’s (FAO, 2008). This trend is a result of over-exploited wild fisheries and a rapidly growing human population. Canadian aquaculture production in 2011 reached 163 036 tonnes, worth 845 million dollars, of which salmonids production made up 606 million (Statistics Canada, 2011). Although the industry has more than tripled over the past 20 years, from the year 2010 to 2011, the gross output by aquaculture producers decreased by 15.2% and the gross value was down 29.9% (Statistics Canada, 2011). These figures are a reflection of the structural changes taking place throughout the industry in the face of emerging new technologies and environmental concerns.

The province of Ontario, although by no means a major contributor to national production, is a prime example where the industry is making such changes. Ontario aquaculture is primarily focused on the production of rainbow trout (Oncorhynchus mykiss) with minor finfish species consisting of tilapia, Arctic char, brook trout, smallmouth and largemouth bass, as well as cyprinid baitfish (Moccia and Bevan, 2007). During the 1980’s and early 1990’s the primary production method of rainbow trout was land-based intensive culture, producing around 2000 tonnes annually (Moccia and Bevan, 2007). With the introduction of Norwegian cage-
aquaculture technology in the Great Lakes in the early 1980’s, the industry rapidly shifted from land-based to open water operations in the late 1990’s, which currently account for 78% of total provincial production at 4100 tonnes, worth 15.7 million dollars (Statistics Canada, 2007). In addition to this value, the aquaculture industry contributes significantly to community and regional economies, providing upwards of 60 million dollars in indirect economic contribution through a variety of industry sectors including: manufacturing, retail and wholesale trade, construction, transportation, and business services (NOAA, 2007).

Despite these benefits and the strategic position of Ontario with regard to availability of freshwater and a consumer market on both sides of the border, production of rainbow trout has fluctuated around 4000 tonnes with recent decline for over the last decade. A major contributing factor to the slow growth of open water cage culture in Ontario is the lack of functional government regulations and guidelines regarding expanding existing sites and accessing new ones. This stems from the fact that open-water cage aquaculture is a first generation industry, that has pioneered and researched many of its own structural changes, that have made it possible to successful move away from less efficient, land based systems. As is often the case with new industries, there is a lack of a sound scientific knowledge base on the environmental impacts of freshwater cage culture in the Great Lakes, leaving the numerous government regulatory agencies responsible for ensuring sustainability, lagging behind in creating a coherent framework of policies that could move the industry forward.

This work is aimed at addressing the environmental impacts of cage aquaculture waste deposited on the lake bottom, through the evaluation of new tools used to identify waste sediment, in
particular stable isotopes and how temperature and organic degradation play a potential role in these assessments.

1.2 Regulation and Environmental Management in Aquaculture

The main issues preventing the expansion of the industry in Ontario arise from the very nature of intensive open water cage aquaculture. Operations are located over a large region of public water that has different local environmental characteristics, making it difficult to predict environmental impacts and apply a universally functional regulatory framework. In addition to the input cage aquaculture wastes, open water sites are exposed to anthropogenic pollution that is often unidentified or quantified. Furthermore, by being within public recreational use zones, the industry has become the target of many special interest groups with powerful lobbying environmentalists that often provide misinformation, driven by conflicting interests, to the regulatory bodies. In Ontario, the agencies responsible for overseeing the environmental sustainability and licensing of operations on the Provincial level include: the Ministry of Natural Resources, Ministry of the Environment, the local Conservation Authorities, the Ontario Ministry of Municipal Affairs and the Housing and the local Municipalities. On the Federal level, these agencies include: Fisheries and Oceans Canada, Agriculture and Agri-Food Canada, Health Canada and Environment Canada. Each of these agencies administer their own respective legislations and permits, pertinent to cage aquaculture, and although there is considerable overlap in mandate between and within agencies, the operator must acquire all the separate permits necessary within a specific timeframe to proceed (Moccia and Bevan, 2000). This process is time consuming and complex because agencies have different definitions of sustainability and
each applies numerous Acts regarding the use, treatment, management and disposal of water used for culture (Moccia and Bevan, 2000).

Open water aquaculture has no specific point of water uptake or effluent release making it difficult to set limits and treatments on wastewater as is the case with land based operations. Cage aquaculture waste production estimates are made by the Ministry of Environment based on the maximum set limits of fish that can be farmed at particular sites which in turn is regulated by the Ministry of Natural Resources (Moccia and Bevan, 2000). This process is not necessarily effective in ensuring water quality considering that different sites, though similarly classified, can potentially respond differently to the same quantity of aquaculture waste. In addition, as previously mentioned, cage aquaculture occurs in recreational public waters and sites will have unknown background levels of anthropogenic and natural materials which can compromise further assessment of environmental impact across sites. Most importantly, it is the lack of knowledge on how aquaculture waste, composed primarily of fish metabolic waste and uneaten feed, is cycled by the freshwater environment that has caused public and regulatory concern, effectively stopping industry expansion. Furthermore, aquaculture operations around the world have shown their potential for causing environmental effects that include nutrient enrichment, habitat alteration and damage to wild fish populations (Carr et al., 1990; Gross, 1998; Jones et al., 2001; Mazzola and Sara 2001; Burford et al, 2002; Cole et al., 2002; Cromey et al., 2002; Bureau et al., 2003; Carroll et al., 2003; Fernandes and Reid, 2003; Brooks et al., 2004; Dempson and Power, 2004; Papatryphon, 2005; Thompson et al., 2005; DFO, 2006; Reid et al., 2006; Stewart et al., 2006; Kullman et al., 2007; Milne, 2012).
The environmental impact and management studies on cage aquaculture of salmonids have been predominantly concentrated in marine coastal areas, where in contrast, literature regarding Canadian aquaculture, especially in freshwater, has been limited in the past (Kullman et al., 2007; Blanchfield, 2009; Kullman et al., 2009; Johnston et al., 2010). In an effort to improve public confidence and global competitiveness in Canadian aquaculture a $75 million Program for Sustainable Aquaculture was launched by Fisheries and Oceans Canada in 2000 (DFO, 2003). The primary objective of the program was to ensure environmentally sustainable development of the aquaculture sector, achieved primarily through a scientific review of the potential environmental effects of aquaculture in marine and freshwater ecosystems. This review, referred to as the State-of-Knowledge (SOK) Initiative, described the current status of scientific knowledge and provided future research recommendations encompassing marine finfish and shellfish, and freshwater finfish in Canadian aquaculture under three main themes: impacts of waste (nutrient and organic matter), chemicals used by the industry (pesticides, drugs and antifoulants), and interactions between farmed fish and wild species (disease transfer, and genetic and ecological interactions) (DFO, 2006).

It is important to note that environmental effects of cage aquaculture are site-specific, depending on the environmental conditions and operational details at each site location (DFO, 2006). The SOK initiative does not address these issues but instead summarizes available scientific knowledge on recognized measurable affects that when observed at the ecosystem level, can generally be categorized into three main broad-scale changes:

1) Eutrophication (nutrient and organic matter enrichment leading to increased dissolved inorganic nutrient concentrations and decreased dissolved oxygen (DO)
2) Sedimentation (direct settling of feed pellets, faeces, increased organic matter flocculation leading to higher deposition rates of small particles, changes in turbidity)

3) Food web structure and function (changes in planktonic, fish and benthic community composition, enhanced/decreased productivity, behavioral avoidance, stimulation of harmful algal blooms, intertidal/macro algal community effects).

The effects outlined above are complex and directly related to the production and release of organic waste and the interactions between cultured and wild species. In order to reveal the potential environmental effects and fill knowledge gaps, a vast array of different studies have been conducted, from laboratory based computer modeling to direct infield sampling and observations. Some of the vital studies addressing environmental management will be summarized here.

**Key Studies**

Waste production or nutrient loading from a cage aquaculture facility is a function of fish size, species, water temperature, feed composition, ration, feeding methods and other factors. The nutritional components released as waste that are biologically limiting and responsible for the eutrophication in freshwater and marine water are phosphorous and nitrogen respectively (Tyrell, 1999; Hudson et al., 1999). Researchers have successfully used changes in levels of bacterial populations, chlorophyll, algal biomass and phytoplankton to demonstrate increased primary productivity as a result of aquaculture nutrient input in marine and fresh waters (Ruokolahti, 1988; Munro et al., 1985; Eloranta and Palomaki, 1986; Carr and Goulder, 1990; Kelly, 1993; Smith et al., 2001).
In order to quantify nutrient loading Cho and Bureau (1998) developed a mass balance bioenergetics based model to estimate waste output by using parameters of the nutritional energy of feed, the difference in nutrient ingestion and retention by the fish, and a thermal growth coefficient. The ability to estimate the amount of potentially released waste based on the above variables is a crucial step in predicting and managing the potential impact.

Regulatory agencies are primarily concerned with concentrations of suspended solids, total phosphorous and total nitrogen entering the receiving environment (Naylor et al., 1999). Waste produced by cultured fish is released in soluble (digested non retained feed materials) and solid (undigested material in faeces) forms. Components of environmental concern in the waste stream from cultured fish such as phosphorous (P), nitrogen (N) and biodegradable matter are closely associated with the settleable solid waste form (Naylor et al., 1999). The fecal solid waste component is estimated to range from 15-30% of applied feed and uneaten feed can range from 3-40% of that used (Weston et al., 1996; Bureau et al., 2003). It is clear that feed digestibility and nutrient content are the main biological factors at the source of determining waste output (Cho and Bureau, 2001).

Based on this knowledge and with the aim of reducing environmental impact at the source, new diet formulations with high digestibility and quality ingredients have increased nutrient retention and consequently reduced the amount of soluble and settleable waste components, in particular phosphorous (Thorpe and Cho, 1995). However significant amounts of remaining solid and soluble waste are released in open water cage culture and understanding the fate of these
effluents (especially the solid fraction) within the receiving environment are of main concern for environmental management agencies.

A major challenge to modeling waste loading and quantifying local and far-field effects at open water sites are the numerous, often highly site-specific, environmental variables involved. As described above, a main concern and recognized final ecosystem response to increased nutrient levels, both natural and anthropogenic, is the process of eutrophication, where the receiving environment shifts trophic status from low to high primary productivity (Nixon, 1995). A simple and common observation made within close proximity to a cage aquaculture site, is the increased level of suspended particulate matter in the water column, primarily from fish faeces as well as unconsumed feed.

The identification and direct attribution of environmental effects, in particular far-field, to a cage aquaculture operation of the solid and dissolved waste fraction is a fundamental environmental management goal and challenge for many researchers. Predicting or quantifying waste impacts at open water sites is difficult because potential effects depend on many variables starting with operating procedures, feed type and feeding technique and more importantly site characteristics. Physical, chemical and biological will also influence how the waste is dispersed and further cycled within the receiving environment (Beveridge, 1987; Cole, 2002; Findlay and Watling, 1997). Further complications can arise in attributing anthropogenic wastes to aquaculture sites when many other potential sources can contribute wastes very similar in biochemical composition and environmental effect within the geographical range of interest (Dell’Anno et al., 2002; Einen et al., 1995; Nixon, 1995; Strain and Yeats, 1999; Strain et al., 1995).
Many different modeling approaches have been used, however; they all share the same principle of creating a waste loading schematic based on relative site specific variables controlling waste input and a resulting, measurable environmental effect (DFO, 2003). Although the dissolved wastes of carbon (C), nitrogen (N) and phosphorus (P) released through gill and urinary excretions of cultured fish are of potential concern, their rapid dilution in open water sites with adequate water exchange has warranted less attention than solid wastes, with the assumption that effects will be highly localized and temporary (Brooks et al., 2002; DFO, 2003). Most of the solid waste settles on sediments directly below the cages where they undergo bacterial degradation (Enell and Lof, 1993; Findlay and Watling, 1997; Elberizon and Kelly, 1998; Johansson et al., 1998). However, potential for far-field dispersion of wastes due to hydrographic conditions such as currents, waves, tides and bottom topography make waste accumulation a spatially variable and highly site specific impact (Cromey et al., 2002).

Holmer (1991) collected sediment samples containing nutrients and elemental concentrations directly attributable to a Danish coastal aquaculture site 1.2 kilometers away. In another study off the central coast of British Columbia, Sutherland et al. (2001) successfully identified material collected in sediment traps placed 500 meters (m) from a salmonid cage aquaculture site using C and N concentration ratios. On the other side of Canada, in the Western Bay of Fundy, Smith et al. (2005) used the trace metals zinc (Zn) and copper (Cu) to identify aquaculture waste within sediment cores collected 200 m from a cage site that had been removed five years prior. In the Huon Estuary, Tasmania, Australia, following a 12-month fish cage fallowing period of fish McGhie et al. (2000) used concentrations of C and N along with their isotopes to directly
attribute sediments collected 30 m from the cage sites to the feed and faeces derived from the aquaculture operation. In another study with the purpose of identifying aquaculture waste impact Johnsen et al. (1993) measured fatty acids, elemental sulfur (S) and pristine variables to indicate enrichment of sediments 100 to 150 m from salmonid net-pens off the west coast of Norway. The aforementioned studies exemplify the spatial and temporal variability of aquaculture solid waste impacts and associated variables used in their identification.

Despite the long range of waste dispersal at many sites, the majority of significant long-term effects occur within close proximity (under 50 m) to the cages where organic waste sediment accumulation is greatest (Carroll et al., 2003; Brooks et al., 2004, Chou et al., 2002; McGhie et al., 2000; Johannessen et al., 1994; Morrisey et al., 2000). Sites in relatively shallow waters with weak or even moderate currents (low flushing rate) will accumulate significant amounts of organic waste beneath cages due to the fast sinking rate of faecal pellets (4 cm/s) and uneaten feed (10 cm/s) as well as the additional baffling of currents by the submerged cage structure (Findlay and Whatling, 1997; Sutherland et al., 2001; Moccia et al., 2007). Sites located in deep water with strong currents accumulate significantly less organic waste and the waste that does settle beneath the cages is further dispersed over a larger area by means of bottom currents overcoming the low critical erosion shear stress of the settled waste causing resuspension (Findlay and Watling, 1997; Cromey et al., 2002). Such sites are said to have a higher assimilative capacity for organic waste compared to locations where waste accumulates only below the cages.
Assimilative capacity is a site’s ability to receive wastes while maintaining environmental quality (Fernandes, 2003). The benthic effects of organic waste accumulation are a dynamic interaction of physical, chemical and biological reactions that vary over time and space depending on operating practices and site characteristics that govern assimilative capacity such as bottom topography, site hydrodynamics, depth and water quality (Walker et al., 2003). Settled organic waste initially provides nutrients for the macro-fauna and aerobic bacteria communities which increasing in abundance, assimilate and decompose the waste while consuming dissolved oxygen (DO) (Mann, 1982). As soon as the supply of DO through water exchange is exceeded by the demand of the benthos, the decomposition process shifts from aerobic to anaerobic, with a corresponding change in bacteria and macro-fauna community structure (Mann, 1982). As the sediments become increasingly anoxic, conditions become toxic to aerobic macro-fauna, accelerating the development of anoxia through the death and decomposition of sediment burrowing species. The aerobic bacteria are succeeded by anaerobic sulphate reducing and methanogenic bacterial communities which produce hydrogen sulfide (H₂S) and methane gas (CH₄) respectively from the decomposition of waste sediments (Mann, 1982). Such changes in benthic community structure have been a fundamental environmental monitoring variable. The relationship between macro-fauna species diversity, abundance and biomass has been the most widely used index of sediment organic enrichment not only from aquaculture waste but any source of sediment organic waste (Pearson and Rosenberg, 1978; Carroll et al., 2003; Wildish et al., 2004).

Although macro-faunal communities are a highly sensitive index of organic enrichment their accurate assessment requires labour intensive sampling, taxonomic expertise, and knowledge of
natural seasonal community dynamics as well as species sensitivities to specific physical and chemical conditions (Crawford et al., 2002; Crome et al., 2002; Hagrave et al., 2008, Borja et al., 2009). However, when used in conjunction with other indicators that may respond differentially to the same organic enrichment, benthic macro-faunal community indexes increase the scope and accuracy of an environmental assessment.

Sediment chemistry provides additional indicators of benthic organic enrichment of which the most successfully used have been sulphide concentration, redox potential, metals (copper (Cu), nickel (Ni), zinc (Zn), iron (F), manganese (Mn)) and pH. Alternative indicators used for measuring benthic impact include total organic carbon (C), nitrogen (N), phosphorous (P) and particle size. As mentioned above, sediment chemistry parameters can give mixed results, identifying some sampled sites as not impacted by waste when biological parameters such as macro-faunal community index indicate a significant impact (Carroll et al., 2003). Due to the inconsistencies in indicator responses to organic enrichment it has been suggested that multiple variables be included in monitoring the benthic impact of aquaculture waste (Crawford et al., 2002; Wildish et al., 2004). As a result a variety of approaches have been taken, using different combinations of environmental indicators to model potential impacts and monitor existing effects.

A fundamental step in predicting the potential benthic effects at an aquaculture site begins with an estimate of the amount and type of waste released. Cho and Bureau (1998) developed a fish nutrition and bioenergetics based computer model, Fish-PrFEQ, that uses data on feed composition, fish growth, water temperature and fish energy utilization to calculate final waste
output in the form of total solid wastes as well as dissolved and solid forms of nitrogen and phosphorous. Although developed for hatchery use, the model has been successfully applied to marine fish species and is recommended as an accurate method of estimating aquaculture waste production (Davies and Slaski, 2003; Papatryphon et al., 2005).

The dispersion of the solid waste component is of primary environmental concern and several mathematical sedimentation and transport models have been developed for the purpose of predicting benthic enrichment. Ocean circulation models such as the Princeton Ocean Model (Blumberg and Mellor, 1987) and the CANDIE ocean model (Sheng et al., 1998) predict coastal zone transport and mixing and have been successfully used in estimating the dispersion of organic matter from coastal aquaculture sites. Dudley et al. (2000) developed a mathematical model, Aquaculture Waste Transport Simulator (AWATS) that uses a two-dimensional flow model coupled with a particle-tracking waste transport model to generate a simulation of waste dispersion. The accuracy of the predicted waste dispersion can then be validated by measuring benthic indicators of enrichment within the predicted sedimentation zone. This approach is taken in the more recently developed computer particle tracking DEPOMOD model of benthic enrichment which combines a particle tracking model and correlations between solid waste disposition and resulting changes in benthic community structure (Cromey, 2002; Wildish et al., 2004). The DEPOMOD model also includes a resuspension model which redistributes the settled waste based on predicted erosion and deposition events determined by the magnitude of the shear stress at the sediment water interface. In Norway a management system called Modelling-On growing fish farms-Monitoring (MOM) is used to adjust the local environmental impact of marine fish farms to the holding capacity of the sites in use.
The MOM system uses sediment traps as a first step to monitor the organic waste load of a farm, then a detection of macro fauna presence is used to determine sediment acceptability, azoic being unacceptable. The main test of the MOM system is a measurement of sediment pH and redox potential with the results plotted on a scoring diagram predicting the level of site exploitation. In addition, a group of qualitative sediment variables such as thickness of accumulated organic material, smell, colour, consistency and gas bubbles are assessed. Long term environmental changes are determined by benthic macro faunal community studies based on fauna investigations of the sediment. The MOM system also includes a simulation model consisting of three major models as follows:

1) Fish- sub-model simulating emission of dissolved and particulate material from the farm.

2) Dispersion sub-model that simulates dispersion and sedimentation rates of excess feed and faecal pellets (based on current variability and sinking rates of feed pellets).

3) Sediment sub-model which simulates the maximum organic deposition on the sediment allowing for a viable benthic in fauna (determined using current velocity above the sediment and the difference between the oxygen concentration in the turbulent boundary layer at the sediment surface and above water column).

The remaining two sub models measure water quality in the net cage environment (oxygen (O₂) and ammonia (NH₃) levels) and the recipient waters surrounding the farm (Secchi depth and oxygen). By combining the dispersion and sediment sub-models it is possible to calculate the maximum fish production that a site can sustain without the benthic infauna disappearing due to
oxygen deficit. The MOM model has been implemented gradually since 1997 and represents a good example of environmental management in aquaculture preventing over-exploitation of sites and adjacent areas and ensuring quality rearing conditions for the fish.

1.3 Stable Isotopes as tools in Environmental Management

Stable isotopes can be defined as different “species” of atoms of the same element that differ in their number of neutrons and therefore have different atomic masses but similar chemical properties. Different stable isotopes of the same element have the same chemical properties however, because of the difference in atomic mass they have different reaction rates causing products and reactants to accumulate different ratios of heavy to light isotopes, a process called fractionation. All materials on the planet have been formed through physical and chemical processes that through fractionation have created unique distinguishable ratios of heavy to light stable isotopes between source and product materials. The stable isotope ratio of a source material is considered a distinct signature when it does not change or changes in a predictable way. The unique signature can therefore be used to attribute unknown materials to their source through comparison of their stable isotope ratios.

In ecosystem and environmental studies, the most extensively used stable isotopes are those of carbon (C), nitrogen (N), sulfur (S), hydrogen (H) and oxygen (O). Isotopic compositions (δ values) in ecosystem and environmental studies are expressed as parts per thousand differences from a designated international standard:
\[
\delta X = \left[\frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}}\right] \times 1000
\]

where \( \delta X = \delta^{13}\text{C} \) or \( \delta^{15}\text{N} \) and \( R = \frac{13\text{C}}{12\text{C}} \) or \( \frac{15\text{N}}{14\text{N}} \)

All international standards are set at 0‰ by convention. Carbonate rock from the Pee Dee Belemnite formation (Craig, 1957) and nitrogen gas in the atmosphere (Mariotti, 1983) are normally used as the C and N standards.

Many biological reactions, such as digestion, cause changes to the ratio of heavy to light isotopes for C and S isotopes. The reactant or source material sets an isotopic baseline ratio that can be altered in favour of the light or heavy isotope depending on the reaction (Peterson and Fry, 1987). A 10% difference in ratios of heavy to light isotopes between reactants and products involves only minute absolute changes of 0.04% and 0.11% for \( \delta^{15}\text{N} \) and \( \delta^{13}\text{C} \), heavy isotopes respectively (Peterson and Fry, 1987). An understanding of the potential for fractionation of a given sample is crucial in evaluating the use of unique source material isotope signatures for the identification of the sample source.

A well-studied example of fractionation is that of the \( \delta^{13}\text{C} \) in the process of photosynthesis. Troughton et al. (1974) showed that terrestrial C3 plants are consistently depleted in the heavy \( \delta^{13}\text{C} \) compared to their source of C. In another study of \( \delta^{13}\text{C} \) fractionation, terrestrial C4 plants retained more of the heavy \( \delta^{13}\text{C} \) during photosynthesis compared to C3 plants in the latter study because of different C fixing pathways of the two plant groups (Farquhar, 1983). A laboratory study on fractionation effects in animals consuming isotopically distinct diets showed that \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) isotope signatures of the whole bodies of animals reflected the \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) values of the diet consumed, with a general fractionation of the diet resulting in consistent enrichment of
the animal body in $\delta^{13}C$ and $\delta^{15}N$ heavy isotopes (Deniro and Epstein, 1978; Deniro and Epstein, 1981).

Minagawa and Wada (1984) conducted a food web study in marine and freshwater environments using $\delta^{15}N$ and results showed that all consumers; zooplankton, fish and birds exhibited an enrichment in $\delta^{15}N$ with increasing trophic level. Furthermore, it has been shown that starving animals, through catabolism of their own tissues, become enriched in $\delta^{15}N$ despite not consuming any external nutrients (Hobson et al., 1992). In general, trophic fractionation of $\delta^{13}C$ and $\delta^{15}N$ isotopes results in consistent enrichment of the consumer body as whole however Lorraine et al. (2002) found that individual organs and tissues of the consumer will have different degrees of enrichment and can vary seasonally with metabolism.

The degree of fractionation depends partly on the quality and type of source material (Post 2002). Guelinckx (2008) studied the effects of digestion on $\delta^{13}C$ and $\delta^{15}N$ of fish gut contents and found fractionation effects differ between the foregut and hindgut and suggested further research to identify other influencing factors such as temperature, diet quality, food ration, consumer’s physiological status, as well as the underlying physiological mechanisms behind diet and tissue fractionation.

Although fractionation can be an unpredictable and difficult to interpret process, with the use of controlled laboratory experiments to elucidate patterns in isotope discrimination for specific reaction pathways, stable isotope fractionation becomes an accurate and informative variable of many physical and chemical cycles. When fractionation patterns are well understood, stable isotopes provide two kinds of valuable information: process information (reaction conditions) and information on the origin of a material (source information) (Peterson and Fry, 1987).
Stable isotopes have originally been used extensively by physical chemists and geochemists to study global elemental cycles however their potential use in many other fields of study such as microbiology, soil science, forensic science, biomedical sciences and food science has been recognized over recent decades. Specifically, stable isotopes have become important tools in studying elemental cycles in the ecosystems (Fry and Parker, 1979; Lichtfouse, 2000; Peterson and Fry, 1987). Stable isotopes of δ^{13}C and δ^{15}N are commonly used in the study of trophic dynamics and animal migrations in aquatic and terrestrial ecosystems (Gannes et al., 1997; Hobson, 1998; Thompson et al., 2005). For example, the pelagic food web in Lake Baikal was shown to have an ideal, isotopically ordered structure using stable isotopes of δ^{13}C and δ^{15}N examined across five ecological groups: phytoplankton, mesozooplankton, macrozooplankton amphipod, fish and seal (Yoshii et al., 1999).

Yoshioka and Wada (1994) studied the seasonal food web dynamics of a eutrophic lake using stable isotopes of δ^{13}C and δ^{15}N ratios of phytoplankton, zooplankton, benthic invertebrates and pond smelt, revealing seasonal variation in isotope composition based on diet shifts. In another study aimed at revealing ontogenetic shifts in dietary habits of the omnivorous opossum shrimp (Mysis relicta), Branstrator et al. (2000) used δ^{15}N to show an increasing trend towards carnivory with increased maturity. The trophic status and food web interactions of five Pacific salmon species as well as their historical response to oceanic physical processes was determined using stable isotopes of δ^{13}C and δ^{15}N (Satterfield and Finney, 2002). In a Florida freshwater lake, Gu et al. (1997) studied the feeding diversity of a population of blue tilapia (Oreochromis aureus) using δ^{13}C and δ^{15}N in the dorsal musculature revealing a surprisingly broad range of
isotope values indicative of a large diet variation within the population. In a similar study under laboratory conditions, Salazar used δ¹³C and δ¹⁵N to show the isotopic signature of salmonid feed ingredients can be identified in different diets and traced post consumption to dorsal musculature and fish faeces (Salazar –Hermoso, 2007).

Trophic dynamics have been studied using isotope techniques in many other commercially important finfish such as carp (Cyprinus carpio) (Gaye-Siessegger et al., 2004), red drum (Sciaenops ocellatus) (Herzka and Holt, 2000), Nile tilapias (Oreochromis niloticus) (Gaye-Siessegger et al., 2003) and Atlantic cod (Gadus morhua) (Schwarcz et al., 1998). Salazar –Hermoso (2007) data revealed that diets with varying ingredients showed unique δ¹³C and δ¹⁵N signatures that could be traced to fish musculature and waste collected in a laboratory setting. In another study of trophic dynamics, Hansson et al. (1997) described the pelagic food-web structure of three Baltic Sea coastal areas through the analysis of δ¹⁵N in particulate organic matter (mainly phytoplankton), zooplankton mysids (Mysis mixta and M. relicta), sprat (Sprattus sprattus), smelt (Osmerus eperlanus), four size classes of herring (Clupea harengus), and pikeperch (Stizostedion lucioperca), uncovering details of trophic interactions and the level of migration of the finfish species and the effect on the isotope signatures by nearby sewage treatment plant.

In addition to providing information on local food web dynamics, changes in isotope composition of consumers can be indicative of shifts in dietary sources as a result of migration between isotopically distinct food webs (Hobson, 1998). Limburg (1998) used δ¹³C and δ¹⁵N to distinguish between sea migrating (anadromous) blueback herring (Alosa aestivalis, Alosa
*Alosa sapidissima, Alosa pseudoharengus* and non-anadromous (freshwater residents) specimens of different age groups in the Hudson River estuary, giving insight into migratory plasticity.

In a study of a population of northern fur seals (*Callorhinus ursinus*) from the Pribilof Islands, Alaska, Kurle and Worthy (2001) used $\delta^{13}C$ and $\delta^{15}N$ analysis of fur skin and potential prey to distinguish between the feeding ecology and foraging location of migrating and nursing fur seal adult females and migrating juvenile males. In another study on pinniped migration and foraging, Burton and Koch (1999) used $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions of bone collagen of northern fur seals (*Callorhinus ursinus*), harbor seals (*Phoca vitulina*), California sea lions (*Zalophus californianus*), and northern elephant seals (*Mirounga angustirostris*) to distinguish between near-shore and offshore foraging locations and long-distance migrations from high to middle latitudes. McCarthy and Waldron (2000) successfully used $\delta^{13}C$ and $\delta^{15}N$ isotopes in adipose fin tissue and eggs of brown trout (*Salmo trutta*) to accurately identify eggs as the progeny of either a migratory adult female or a freshwater resident.

In addition to applications in animal migrations, stable isotopes have been used to investigate the relationship between seasonally varying ocean surface water productivity and corresponding variations in type and distribution of suspended and sinking particles (Altabet, 1988; Altabet et al., 1991; Altabet et al., 1999; Holmes et al., 2002; Nakatsuka et al., 1992; Saino and Hattori, 1987; Wada and Hattori, 1976). Stable isotopes have also been applied in numerous environmental studies involving the tracking of foreign materials and pollutants, and assessments of the ecosystem response to changing environmental conditions (Kidd et al., 2001; Lajtha and Michener, 1994; McGhie et al., 2000; Yokoyama and Ishihi, 2007). For example, sources and historical levels of atmospheric lead pollution have been calculated using lead stable isotope analyses of lake
sediments, ice cores, peat bogs as well as corals, trees and herbariums (Weiss et al., 1999). The input of sewage into different ecosystems is routinely traced and the environmental response measured extensively using $\delta^{15}$N values (Carlier et al., 2007a; Costanzo et al., 2001; Piola et al., 2006; Rogers, 2003; Savage and Elmgren, 2004). The roles of allochthonous and autochthonous carbon in small aquatic ecosystems have been traced using stable isotope values of $\delta^{13}$C to label autochthonous primary production (Cole et al., 2002).

Various toxic pollutants such as petroleum products and industrial solvents, the source of hazardous volatile chemicals have been tracked between gaseous, aqueous and non-aqueous liquid phases using $\delta^{13}$C (Slater et al., 1999). In studies done on acid precipitation, sulphur isotopes have been used to trace sulphur in atmospheric fallout (Ohizumi et al., 1997) in addition to measuring seasonal variations in sulphate from precipitation and streams (Hesslein et al., 1988).

Yoshinari and Wahlen (1985) investigated the contribution of a wastewater treatment facility to levels of atmospheric nitrous oxide using measures of oxygen stable isotopes in nitrous oxide produced from nitrification of waste at the facility. In a large-scale study across 40 sites in the Mississippi, Colorado, Rio Grande, and Columbia River basins, $\delta^{13}$C and $\delta^{15}$N isotopes were used to differentiate between seasonal changes over four years in particulate organic matter source materials and the effects of local nutrient sources and in-stream biogeochemical processes (Kendall et al., 2001).
Sources of organic matter and their fate in different ecosystems such as rivers, estuaries, lagoons, salt-marshes, and lake sediments have also been determined using stable isotope techniques (Andrews et al., 1998; Boschker et al., 1999; Hodell and Schleske, 1998; Machas and Santos, 1999; Maren and Struck, 1997; McClelland et al., 1997; Peterson and Howarth, 1987; Thornton and McManus, 1994).

1.4 Stable isotope applications in Aquaculture

The aquaculture industry faces many challenges related to environmental sustainability, and over recent years, stable isotopes have become a common tool applied to such issues as the fate of waste effluent from open-water cage sites, identifying sources of pollution within the vicinity of aquaculture sites, measuring effects of organic wastes on the receiving environment, recovery of organic waste affected sediments, nutrient allocation of newly formulated aquaculture diets in cultured species as well as differentiating between farmed finfish escapees and their wild conspecifics.

A major issue in aquaculture has been differentiating between aquaculture waste and other foreign pollutants. Jones et al. (2001) used $\delta^{15}$N and biological indicators to compare treated sewage effluent with the waste effluent of a shrimp farm, successfully distinguishing the source and degree of impacts. In another study of shrimp farm effluent, Burford et al. (2002) traced the fate of N enriched feed through the food web in shallow outdoor tank systems using $\delta^{15}$N ratios and revealed rapid microbial community remineralization of the $\delta^{15}$N enriched waste and suggested a large fraction of the waste probably remains in soluble organic form. Schroeder (1983) used $\delta^{13}$C and $\delta^{15}$N ratios to compare nutrient allocation from natural food versus formulated feed between tilapia (Tilapia aurea) and paeneid shrimp, concluding that tilapia
consume large quantities of natural food in addition to the feed provided as opposed to the
shrimp that consumed mostly formulated feed.

In addition to differentiating aquaculture waste from foreign pollutants, farm raised fish have
been differentiated from their wild counterparts based on differences in isotopic composition of
natural versus formulated diets. Dempson and Power (2004) distinguish between farmed and
wild Atlantic salmon (*Salmo salar*) by analysing the δ^{13}C and δ^{15}N composition of tissue
musculature showing significant enrichment in nitrogen and depletion in carbon for wild versus
aquaculture specimens.

In addition to revealing current feeding habits of aquaculture species, stable isotopes have been
applied to sediment core samples around fish farms in Gokasho Bay, Japan, revealing historical
changes in δ^{13}C and δ^{15}N signatures indicative of different diets successively used at the farm
sites over three decades (Yamada et al., 2003). Similarly, Salazar – Hermoso (2007), used δ^{13}C
and δ^{15}N signatures to assess the traceability of rainbow trout feces at an open water aquaculture
site in Georgian Bay, Ontario, by measuring isotope signatures in formulated and commercial
diets, fish musculature and in field sediment samples, concluding that in field observations were
inconclusive despite correlations between formulated and commercial diets and sampled
musculature under controlled conditions. These conclusions are consistent with results obtained
by Dempson and Power (2004) which showed considerable overlap in δ^{13}C and δ^{15}N signatures
between farmed waste and wild biota. However, isotope signatures did not differ significantly
between cage and reference sites (Dempson and Power, 2004).

The fate of aquaculture waste effluent in the receiving environment is one of the most important
factors affecting aquaculture sustainability and stable isotopes have been applied successfully in
determining waste dispersion and allocation. Franco-Nava et al. (2004) used $\delta^{13}$C and $\delta^{15}$N isotopes to determine the main contributors of suspended particulate matter and found three main isotopically distinct sources to be: feed, faeces and biofilm within a sea bass recirculating aquaculture system.

Mazzola and Sara (2001) analyzed stable isotope signatures of $\delta^{13}$C and $\delta^{15}$N of mussels (*Mytilus galloprovincialis*) and clams (*Tapes* sp.) grown around an open water intensive fish farm to demonstrate that molluscs uptake a significant amount of fish farm derived nutrients thereby reducing the environmental impact through waste assimilation. In another study aimed at tracking organic waste effluent from an aquaculture site in the Huon Estuary, Tasmania, isotopes of $\delta^{13}$C and $\delta^{15}$N were analysed over a 12 month falling period in cultured fish faeces and their feed as well as sediment trap material collected up to 30m with results showing that aquaculture derived organic waste was persistent up to 20 m from the centre of cages throughout the 12 month falling period (McGhie et al., 2000).

In order to assess the use of stable isotopes of $\delta^{13}$C and $\delta^{15}$N in tracking open water cultured fish faeces, this study’s primary aim was to measure and compare in field isotope values of sediment and feed samples, as well as conduct a controlled experiment of fish faeces incubation for the purpose of determining the potential effect of organic degradation on the persistence of $\delta^{13}$C and $\delta^{15}$N stable isotopes in the waste fraction.

In addition, in consideration of the variability of environmental conditions at open water aquaculture sites, and previous results obtained by Johnston et al. (2010) which suggested that direct consumption of farm waste by wild biota is limited despite considerable overlap in $\delta^{13}$C and $\delta^{15}$N signatures, a secondary aim of this study was to measure whole water column
temperatures from the lake surface to benthos across different aquaculture sites over a complete
growth season in order to gain insight and a general baseline of site environmental variability
and ultimately to infer the potential of site characteristics affecting benthic waste biodegradation
and the persistence of $\delta^{13}$C and $\delta^{15}$N stable isotopes.

Chapter 2. Assessing the Use of Stable Isotopes as Tracers of Aquaculture Waste Effluent
in Sediments at Cage Aquaculture Sites

2. 1. Introduction

Commercial fish feeds used at open water salmonid aquaculture operations are formulated
primarily from marine sourced ingredients that are $\delta^{13}$C and $\delta^{15}$N enriched. Digested and
undigested feed settles on the sediment beneath culture sites creating an environmental footprint
not readily identifiable with the source feed. Of the primary feed ingredients, fishmeal is
exclusively derived from marine sources and contributes the most $\delta^{13}$C and in particular $\delta^{15}$N.
This is of significant interest in freshwater applications of fishmeal based feed where the
enriched $\delta^{13}$C and $\delta^{15}$N isotope signature has potential to be a natural tracer of aquaculture waste.
Although digestion fractionates the isotopic signature of the feed causing minor isotope
depletion, it is assumed the faeces isotope signature closely reflects the signature of the source
feed and retains this signature once settling on the lake bottom.

In order to test whether settled wastes at a freshwater aquaculture site can be identified with the
feed used, seven aquaculture sites around Georgian Bay were investigated. Sediment samples
were collected at active and decommissioned aquaculture sites and their $\delta^{13}$C and $\delta^{15}$N isotope
signatures measured. The results showed a high variability of $\delta^{13}\text{C}$ and in particular $\delta^{15}\text{N}$ values within and between sampled sites. Decommissioned sites showed the highest level of enrichment suggesting that the signature of the isotopes changes with ongoing biodegradation.

2. 2. Methodology

2.2.1 Inactive site sediment sampling

Sediment samples from two inactive sites, La Cloche and Grassy Bay, were collected using an Ekman grab sampler from February 20 to February 26, 2007. A total of 7 samples were collected at Grassy Bay site and 36 samples at La Cloche site. Refer to figures 1 and 2 below for sampling patterns for each site relative to the shoreline.

Figure 1. Grassy Bay (inactive) site sampling pattern relative to shoreline.
Figure 2. La Cloche (inactive) site sampling patterns relative to shoreline
2.2.2. Active site sediment sampling

Sediment samples from six active sites were collected using an Ekman grab sampler during July 22 to July 24, 2007. A total of 18 samples were collected, 3 samples per site, in a transect through the middle of the site in the direction of the prevailing current.

Table 1. Active site coordinates

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45°19'11</td>
<td>-80°6'39</td>
</tr>
<tr>
<td>2</td>
<td>45°45'20</td>
<td>-81°47'30</td>
</tr>
<tr>
<td>3</td>
<td>46°14'4</td>
<td>-81°57'31</td>
</tr>
<tr>
<td>4</td>
<td>46°0'30</td>
<td>-81°44'3</td>
</tr>
<tr>
<td>5</td>
<td>46°4'49</td>
<td>-81°57'38</td>
</tr>
<tr>
<td>6</td>
<td>45°59'27</td>
<td>-81°43'58</td>
</tr>
</tbody>
</table>
Figure 3. Locations of Ekman grab sediment sampling sites around the Lake Huron drainage basin, depth contour interval: 5m and 10m, maximum depth 230m.
2.2.3. Feed sample and preparation

Single samples of feed, approximately 1 kg in weight, were collected from each of the 6 active sites at the same time of the sediment sampling. All samples were stored on ice and taken to the University of Guelph for preparation for analysis of \( \delta^{13} \)C and \( \delta^{15} \)N. Samples were divided in half (500 grams each) and each half was acid washed in order to remove contributions of bicarbonate derived \( \delta^{13} \)C isotopes according to the method of Lajtha and Michener (1994). Samples were dried at 60°C for 24 hours and ground to a fine powder using mortar and pestle. Samples were delivered to the University of Waterloo for mass spectrometry analysis of \( \delta^{13} \)C and \( \delta^{15} \)N. Samples were analyzed for \( \delta^{13} \)C and \( \delta^{15} \)N ratios on an Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108). Stable isotope ratios are expressed as delta values (\( \delta \)) and are measures of the per thousand (‰) differences between the isotope ratio of a sample and that of a known international standard material for that same isotope:

\[
\delta X = \left[ \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 1000
\]

where \( \delta X = \delta^{13} \)C or \( \delta^{15} \)N and \( R = ^{13} \)C/\(^{12} \)C or \(^{15} \)N/\(^{14} \)N.

All international standards are set at 0‰ by convention. Carbonate rock from the Pee Dee Belemnite formation (Craig, 1957) and nitrogen gas in the atmosphere (Mariotti, 1983) were used, respectively, as the carbon and nitrogen standard.

2.2.4. Statistical Analysis

For the purpose of this study only results obtained from acid washed samples were analyzed in order to exclude skewed results due to contamination from inorganic C sources such as bicarbonates.
Four different statistical analyses were performed using Statistical Analysis Software (SAS) program version 9.1 (SAS Institute Inc., Cary, NC, USA) on samples collected from both active and inactive sites, as well as feeds used at the active sites.

An analysis of variance using a General Linear Model (GLM) was performed on samples collected from active sites with the site as a block effect.

The second analysis consisted of correlating means of isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at active sites versus $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the feed.

A comparison between sediment stable isotope data collected at active versus inactive sites was performed using the GLM procedure via mixed model analysis of variance, assuming samples within sites were collected randomly.

The fourth analysis compared the variances of isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at active and inactive sites using the Brown and Forsythe’s test for homogeneity of variance method, assuming samples within sites were collected randomly.

2.3. Results

The values of $\delta^{15}\text{N}$ for samples taken from active sites were significantly different between sample locations within a site (F value =17.60, Pr>F= 0.0015, DF = 5) as well as measurements taken between the sites (F value = 9.4, Pr>F= 0.001, DF = 5).

No significant differences were found between $\delta^{13}\text{C}$ values of sediment samples taken within a particular site (F value = 0.08, Pr>F =0.788, DF = 5) as well as among sediment samples from different active sites (F value 0.43, Pr>F= 0.820, DF = 5).
No significant correlation was found between $\delta^{13}$C of sediment and $\delta^{13}$C of any feed samples tested (Pearson Correlation Coefficient of 0.0086).

A weak correlation was found between $\delta^{15}$N of sediment and the feed (Pearson Correlation Coefficient of 0.1611).

The mean $\delta^{13}$C and $\delta^{15}$N signatures of the feed samples were more enriched than mean $\delta^{13}$C and $\delta^{15}$N signatures of sediment samples.

Isotope values of $\delta^{13}$C from active sites were significantly different from $\delta^{13}$C values of inactive sites $F$ value = 18.7, $Pr > F = 0.01$).

The variance of the $\delta^{13}$C was similar between active and inactive sites ($F$ value =0.39, $Pr > F=0.5359$).

The $\delta^{15}$N values were not significantly different between active and inactive sediment samples ($F$ value = 3.81, $Pr .F = 0.1137$ however, because the variance of $\delta^{15}$N values were significantly different between the site types ($F$ value = 10.14, $Pr > F=0.0021$) therefore the comparison of $\delta^{15}$N values between active and inactive sites is not valid.

Table 2. Simple statistics of $\delta^{13}$C and $\delta^{15}$N signatures of sediment samples from active sites, inactive sites and feed samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean $\delta^{13}$C±(SD)</th>
<th>Mean $\delta^{15}$N±(SD)</th>
<th>Range $\delta^{13}$C min,max</th>
<th>Range $\delta^{15}$N min,max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active site sediment</td>
<td>18</td>
<td>-22.28(0.36)</td>
<td>4.66(0.61)</td>
<td>-22.88,-20.99</td>
<td>3.27,5.87</td>
</tr>
<tr>
<td>Inactive site sediment</td>
<td>58</td>
<td>-24.23(1.25)</td>
<td>7.35(2.18)</td>
<td>-26.34,-18.96</td>
<td>4.02,12.12</td>
</tr>
<tr>
<td>Feed</td>
<td>6</td>
<td>-19.93(1.56)</td>
<td>6.17(1.94)</td>
<td>-21.07, -17.32</td>
<td>4.89,9.66</td>
</tr>
</tbody>
</table>

SD = standard deviation; n = sample size; values with common superscript in each vertical column are not significantly different ($P > 0.5$)
Table 3. Signatures of $\delta^{13}$C and $\delta^{15}$N isotopes from sediment samples and feed at active sites. Refer to figures 3 and 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}$C (‰)</th>
<th>$\delta^{15}$N (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1 – West</td>
<td>-21.28</td>
<td>5.87</td>
</tr>
<tr>
<td>Site 1 – Centre</td>
<td>-22.78</td>
<td>5.33</td>
</tr>
<tr>
<td>Site 1 – East</td>
<td>-22.82</td>
<td>5.17</td>
</tr>
<tr>
<td>Site 2 – North</td>
<td>-21.04</td>
<td>4.23</td>
</tr>
<tr>
<td>Site 2 – Centre</td>
<td>-22.44</td>
<td>4.99</td>
</tr>
<tr>
<td>Site 2 – South</td>
<td>-23.48</td>
<td>4.41</td>
</tr>
<tr>
<td>Site 3 – North</td>
<td>-22.36</td>
<td>4.05</td>
</tr>
<tr>
<td>Site 3 – Centre</td>
<td>-22.67</td>
<td>4.80</td>
</tr>
<tr>
<td>Site 3 – South</td>
<td>-22.33</td>
<td>4.16</td>
</tr>
<tr>
<td>Site 4 – West</td>
<td>-23.89</td>
<td>3.42</td>
</tr>
<tr>
<td>Site 4 – Centre</td>
<td>-20.99</td>
<td>5.55</td>
</tr>
<tr>
<td>Site 4 – East</td>
<td>-22.58</td>
<td>4.80</td>
</tr>
<tr>
<td>Site 5 – North</td>
<td>-22.88</td>
<td>3.27</td>
</tr>
<tr>
<td>Site 5 – Centre</td>
<td>-22.46</td>
<td>4.35</td>
</tr>
<tr>
<td>Site 5 – South</td>
<td>-22.31</td>
<td>3.72</td>
</tr>
<tr>
<td>Site 6 – North</td>
<td>-21.33</td>
<td>5.03</td>
</tr>
<tr>
<td>Site 6 – Centre</td>
<td>-22.35</td>
<td>5.74</td>
</tr>
<tr>
<td>Site 6 – South</td>
<td>-21.05</td>
<td>4.95</td>
</tr>
<tr>
<td>Feed – Site 2</td>
<td>-20.24</td>
<td>7.23</td>
</tr>
<tr>
<td>Feed – Site 1</td>
<td>-18.79</td>
<td>9.66</td>
</tr>
<tr>
<td>Feed – Site 6</td>
<td>-17.32</td>
<td>5.43</td>
</tr>
<tr>
<td>Feed – Sites 3,4,5</td>
<td>-21.07</td>
<td>4.89</td>
</tr>
</tbody>
</table>
Figure 4. Signatures of $\delta^{13}$C and $\delta^{15}$N sediment samples and feed from active sites.
Table 4. Sediment $\delta^{13}$C and $\delta^{15}$N signatures from inactive site LaCloche. Refer to figure 3 and 5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}$C (‰)</th>
<th>$\delta^{15}$N (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x11</td>
<td>-24.87</td>
<td>5.08</td>
</tr>
<tr>
<td>x8</td>
<td>-24.47</td>
<td>7.09</td>
</tr>
<tr>
<td>x7</td>
<td>-24.36</td>
<td>9.68</td>
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<tr>
<td>x5</td>
<td>-24.38</td>
<td>7.51</td>
</tr>
<tr>
<td>x4</td>
<td>-23.75</td>
<td>11.63</td>
</tr>
<tr>
<td>w3</td>
<td>-24.57</td>
<td>4.63</td>
</tr>
<tr>
<td>w5</td>
<td>-22.53</td>
<td>11.34</td>
</tr>
<tr>
<td>w6</td>
<td>-23.49</td>
<td>7.91</td>
</tr>
<tr>
<td>w7</td>
<td>-24.23</td>
<td>7.97</td>
</tr>
<tr>
<td>w8</td>
<td>-24.47</td>
<td>8.88</td>
</tr>
<tr>
<td>w11</td>
<td>-25.29</td>
<td>5.23</td>
</tr>
<tr>
<td>v11</td>
<td>-25.62</td>
<td>5.85</td>
</tr>
<tr>
<td>v10</td>
<td>-24.83</td>
<td>5.86</td>
</tr>
<tr>
<td>v9</td>
<td>-24.13</td>
<td>6.48</td>
</tr>
<tr>
<td>v8</td>
<td>-24.93</td>
<td>10.58</td>
</tr>
<tr>
<td>v5</td>
<td>-24.38</td>
<td>6.07</td>
</tr>
<tr>
<td>v4</td>
<td>-23.88</td>
<td>6.56</td>
</tr>
<tr>
<td>v3</td>
<td>-24.73</td>
<td>5.60</td>
</tr>
<tr>
<td>u3</td>
<td>-24.45</td>
<td>6.64</td>
</tr>
<tr>
<td>u4</td>
<td>-24.38</td>
<td>7.58</td>
</tr>
<tr>
<td>u5</td>
<td>-24.39</td>
<td>8.20</td>
</tr>
<tr>
<td>u7</td>
<td>-23.35</td>
<td>6.39</td>
</tr>
<tr>
<td>u8</td>
<td>-24.63</td>
<td>6.11</td>
</tr>
<tr>
<td>t11</td>
<td>-23.55</td>
<td>7.61</td>
</tr>
<tr>
<td>t10</td>
<td>-24.46</td>
<td>7.67</td>
</tr>
<tr>
<td>t9</td>
<td>-19.26</td>
<td>11.97</td>
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<tr>
<td>t8</td>
<td>-23.49</td>
<td>7.03</td>
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<td>x3</td>
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<td>6.99</td>
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<td>11.98</td>
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<td>u1</td>
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<td>y7</td>
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<td>8.14</td>
</tr>
<tr>
<td>z7</td>
<td>-24.91</td>
<td>4.33</td>
</tr>
<tr>
<td>u12</td>
<td>-22.43</td>
<td>9.74</td>
</tr>
</tbody>
</table>
Table 5. Sediment signatures $\delta^{13}$C and $\delta^{15}$N from inactive site Grassy Bay. Refer to figures 3 and 5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}$C (%)</th>
<th>$\delta^{15}$N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>h4</td>
<td>-25.89</td>
<td>4.74</td>
</tr>
<tr>
<td>1 control</td>
<td>-24.68</td>
<td>5.05</td>
</tr>
<tr>
<td>f5</td>
<td>-24.08</td>
<td>4.71</td>
</tr>
<tr>
<td>j9</td>
<td>-25.49</td>
<td>6.19</td>
</tr>
<tr>
<td>g5</td>
<td>-26.01</td>
<td>4.98</td>
</tr>
<tr>
<td>g6</td>
<td>-26.34</td>
<td>4.89</td>
</tr>
<tr>
<td>g7</td>
<td>-25.21</td>
<td>7.79</td>
</tr>
<tr>
<td>i8</td>
<td>-24.58</td>
<td>5.57</td>
</tr>
<tr>
<td>2 control</td>
<td>-25.23</td>
<td>5.20</td>
</tr>
</tbody>
</table>

Figure 5. The $\delta^{13}$C and $\delta^{15}$N signatures from inactive sites: Grassy Bay and LaCloche.
2.4. Discussion

2.4.1. Carbon Isotope ($\delta^{13}C$)

For the purpose of this study only results from acid washed samples were considered for discussion in order to exclude potentially skewed results due to contamination from inorganic C sources, such as bicarbonate. Sediment samples from active sites showed high variance of $\delta^{13}C$ and $\delta^{15}N$ signatures. The $\delta^{13}C$ values were less variable than $\delta^{15}N$ ranging from -22.88 to -20.99‰ (±SD 0.358) compared with $\delta^{15}N$ values of 3.27 up to 5.87‰ (SD± 0.61). The $\delta^{13}C$ isotope values obtained in this study were enriched relative to the typical particulate organic matter values recorded for the Great Lakes in previous studies ranging from -25.55 to -29.90‰ (SD±0.5 ‰) (Keough et al., 1996; Hodell and Schelske, 1998; Leggett et al., 1999; Sierszen et al., 2004). This is also consistent with previous studies demonstrating that increased productivity is correlated with $\delta^{13}C$ enrichment, suggesting that elevated $\delta^{13}C$ values in sediments below cage sites are a direct result of organic input from the farm (Gu et al., 1996; Kidd et al., 2001). It is important to note that $\delta^{13}C$ values at active sites were depleted relative to values from feed samples obtained at those sites, where mean $\delta^{13}C$ of the feed was -19.93‰ (SD ±1.56‰) in comparison to $\delta^{13}C$ values of -22.28‰ (SD ±0.36) of the sediment samples. It has been shown in study by McGoldrick et al. (2008) that during glucose and ammonia uptake, bacteria exhibit higher discrimination against the heavier $\delta^{13}C$ isotope and therefore the bacterial biomass grows and accumulates depleted $\delta^{13}C$.

Fractionation of the $\delta^{13}C$ during biodegradation of organic waste is currently not well understood. Although fish digestion of consumed feed results in an enrichment of the hindgut contents and consequently of the excreted wastes (Guelinckx, 2008), further bacterial degradation of the settled organic carbon waste fraction, resulting in methanogenesis, has in
some cases been shown to deplete the $\delta^{13}C$ isotope signature, where the lighter isotope $\delta^{12}C$ is consumed faster in the reaction releasing carbon depleted CO$_2$ and CH$_4$ gases (Guelinckx, 2008). However other studies show that depending on the precursor of methane (methanol and methylamine or acetate and carbon dioxide hydrogen) and the bacteria strain involved in turning over the substrate, fractionation effects vary significantly (Krzycki et al., 1987; Goevert and Conrad, 2009). In freshwater environments methane is produced primarily from acetate as well as carbon dioxide and hydrogen, where as these precursors in marine environments are consumed by sulphate reducing bacteria and methanogenesis occurs using methanol and methylamine as the main substrate (Krzycki et al., 1987; Goevert and Conrad, 2009). Whiticar et al. (1986) suggested that in environments low in sulphate such as freshwater lakes where sulphate reducing bacteria does not out-compete methanogens, fractionation rate of the carbon isotope is lower compared to methanogenesis from methanol. Furthermore, although studies show methanogenesis fractionates carbon leaving an enriched residual fraction, it has been shown that fractionation rates decrease with decreasing substrate concentration. This implies that although the heavy isotope is discriminated against during methanogenesis it is eventually turned over by bacteria when the light isotope reaches low levels (Goevert and Conrad, 2009). Therefore it is probable that the sediment fractionation effect enriched the waste fraction at the beginning of deposition. However, over time methanogenesis depleted the signature below levels found in the feed. This is supported by the results since all but one sediment sample showed depleted $\delta^{13}C$ compared with the feed used at the particular site.

Also of interest is the difference in standard deviation of the feeds compared with that of the sediments. Feed values, despite using same brand type at more than one farm, exhibited a large
range of $\delta^{13}$C ratios ranging from -17.32 to -21.07‰. Although commercial feeds guarantee specific levels of ingredients, in particular fishmeal levels (which affects the nitrogen isotope ratio), the actual $\delta^{13}$C signature can be expected to vary between feed samples since various producers can use different plant species resulting in different $\delta^{13}$C isotope values (Salazar-Hermoso, 2007). Also, same plant species used in different feeds, depending on their source of origin may have different carbon isotope signatures.

Although $\delta^{13}$C of sediment samples did not correlate directly with values from feed, they fell in between values typically found in particulate organic matter (POM) in freshwater temperate lakes and the feeds used at the sites. This suggests that the isotope signature of settled wastes is partially depleted either through biodegradation and/or diluted through re-suspension and addition of local depleted C sources. In addition, bacterial biomass likely contributed a depleted $\delta^{13}$C signature as a result of discrimination against the heavy $\delta^{13}$C isotope as has been found in previous studies of bacterial glucose and ammonia uptake (McGoldrick et al., 2008).

Inactive site samples were more depleted in $\delta^{13}$C then feed and active site sediment samples. The mean value for $\delta^{13}$C, from -24.23‰ (SD ±1.25‰), was closer to background levels observed in the Great Lakes and results showed a wider range compared to active site samples, ranging from -18.96 to -26.34‰, suggesting locally variable biodegradation and/or mixing with naturally occurring depleted organic matter. Interestingly despite being inactive for many years and showing an overall depletion consistent with biodegradation of organic polymers through methanogenesis, the $\delta^{13}$C levels at inactive sites registered higher maximum enrichment than at active sites.
In addition, no data is available on the $\delta^{13}$C values of the feeds used at inactive sites, preventing a comparison with sediment values of feeds used at active sites. It is likely that with advances in feed formulation and emerging new alternatives in ingredients, the feeds used at inactive sites contained different plant matter that was potentially more enriched with in $\delta^{13}$C. Furthermore the lack of freshly deposited fecal waste and prolonged period of biodegradation may have resulted in the establishment of different biodegradation zones within the same local site as result of local sediment type, bathymetry, hydrological conditions (in particular resuspension) and quantity and pattern of original waste deposition. Anaerobic bacteria populations establish distinct population dynamics within the sediment depending on the prevailing environmental conditions that are often highly variable within a site. This results in different population dynamics with different rates of digestion within the sediment of a single site and consequently over time can lead to significantly different isotope signatures of sediment samples within close proximity to each other.

2.4.2. Nitrogen Isotopes

The $\delta^{15}$N values at active sites were more variable within sites than across sites, which was unexpected as samples collected from single sites were expected to have similar $\delta^{15}$N signatures that would correlate with the particular feeds used. This suggests that nitrogen underwent more differential fractionation within a site than across sites, unlike the $\delta^{13}$C which was not significantly different between or across sites. Other possible explanations for the variability of the $\delta^{15}$N signature is a dilution of the sediment sample with foreign settled depleted nitrogen sources such as local fish faeces, invertebrates, terrestrial runoff containing nitrates and nitrites as well as the bacterial biomass degrading and consuming the deposited waste. Although some
local dilution of the nitrogen sample may have occurred, considering the rarity of naturally occurring $\delta^{15}N$ in freshwater and that majority of samples contained fish faeces and uneaten feed (odour and visual observation of pellets in samples), it is more probable that the observed values are the result of differential nitrogen cycling, in particular coupled nitrification and denitrification resulting in nitrogen isotope fraction (Jenkins and Kemp, 1984; Robinson, 2001).

It has been shown in studies of N cycling in forest, raw humus and catchments, marine and freshwater sediments, as well as trophic ecological studies (plant uptake of N from bird guano) that nitrification and de-nitrification play important roles in fractionating the rare nitrogen isotope. The fractionation processes and resulting differences in nitrogen isotope pools form the basis of natural $\delta^{15}N$ tracer studies (Robinson, 2001). However when source nitrogen pools mix or their signature is altered by fractionation then successful tracking of source nitrogen pools can become impossible due to differences between different sources being removed. Furthermore, it is not always feasible to predict when mixing and fractionation will occur as well as obtaining the immediate resulting change in isotope signature which makes interpreting data values difficult (Robinson, 2001).

Although the main condition for a nitrogen tracer study was met, aquaculture feed used at the rainbow trout farms provided a unique and distinct, naturally enriched source of $\delta^{15}N$ there are still several concerns raised by the results. Feed values for $\delta^{15}N$, mean 6.17, were well above typical values found in oligotrophic temperate lakes, ~3.00, however values obtained from active site sediment samples fell in between these values with a mean of 4.66 (some values approached background levels, min of 3.27). The literature suggests that biodegradation of
nitrogenous organic waste compounds through denitrification leads to an enrichment of substrate being decomposed (Blackmer and Bremner, 1977; Jenkins and Kemp, 1984). It is possible that due to fresh accumulation of wastes at active sites the δ¹⁵N signature of fresh sediment does not undergo extensive denitrification, which requires an established anaerobic environment. Instead the surface sediment is depleted during settling and immediate aerobic degradation, in particular nitrification which has been shown to deplete δ¹⁵N. It should be noted that feeds displayed a large range of values δ¹⁵N ranging from 4.89 to 9.66‰, similarly with δ¹³C suggesting different levels of fishmeal or perhaps different sources of the ingredient resulting in isotopically distinct feeds. In addition no comparison with feeds used in the past at decommissioned sites can be compared. However sediment samples from decommissioned sites interestingly show the largest range of δ¹⁵N values ranging from 4.02 to 12.12‰. This may be attributable to differences in feeds used at the decommissioned sites however it is likely that biodegradation is responsible for such highly enriched values. Without the addition of fresh waste and a documented hypoxia in the hypolimnion it can be assumed that nitrification and denitrification, hydrogen sulfide and methane production played a significant role in shaping the isotopic profile at these sites. In addition, long-term periodic resuspension at decommissioned sites may have contributed to the aeration and depletion of certain samples. The nitrogen δ¹⁵N values confirm the presence of enrichment while at the same time reveal the need to further investigate causes of fractionation and signature change.
2.5. General Conclusion

Overall δ\textsuperscript{13}C and δ\textsuperscript{15}N signatures measured in commercial salmonid feeds were reflected in both active and inactive sediment samples. From the observation that inactive sites had not received waste input in over 5 years since the sampling period and showed the highest enrichment of δ\textsuperscript{15}N, it can be inferred that the δ\textsuperscript{15}N signature is a potential long term tracer of salmonid aquaculture waste. In addition, the δ\textsuperscript{15}N signature is reflected at active sites as well, though to a lesser degree, this, coupled with findings from previous studies tracing commercial diets to dorsal musculature of reared rainbow trout (Salazar – Hermoso, 2007) suggests the fractionation pathway of δ\textsuperscript{15}N has the potential to be accurately assessed through all stages of freshwater salmonid cage aquaculture production including after production has ended in the long term.

Although the δ\textsuperscript{13}C signature of commercial salmonid feed was also reflected in sediment samples of active and decommissioned sites, the values at inactive sites were significantly more depleted than δ\textsuperscript{13}C of feeds sampled, suggesting than unlike the δ\textsuperscript{15}N signature δ\textsuperscript{13}C is fractionated and or degraded at a greater rate during biodegradation which can further be supported by the high proportion of protein content of salmonid feeds relative to rich in C ingredients. (Reid et al., 2009).

However, δ\textsuperscript{13}C signature of active site samples was consistent, had the lowest SD, and was more similar to the feed δ\textsuperscript{13}C signature than δ\textsuperscript{13}C of decommissioned site samples, suggesting that δ\textsuperscript{13}C can be an effective tracer when combined with δ\textsuperscript{15}N although the persistence of δ\textsuperscript{13}C in lake sediment may be shorter than δ\textsuperscript{15}N. The difference in the rate of biodegradation between carbon and nitrogen rich compounds, within settled aquaculture waste sediment, has a potential effect on the persistence of their respective isotope signatures and sediment temperature is one of
the most important factors affecting the establishment and dynamic composition of the microbial community and therefore rate of substrate turn over. Therefore the current study included empirical data collection of water column temperature profiles at different open water aquaculture sites in order to gain insight into the variability of the cage and benthos environment and to establish a baseline of temperature data for use in designing the biodegradation experiment.

**Chapter 3. Long Term Monitoring of Large–Lake, Vertical Temperature Profiles:**

**Implications to Aquaculture Management**

**3.1. Introduction**

The tremendous growth of aquaculture in Ontario over the past two decades can attributed to the development of open-water cage culture of trout (78%) and now contributes $55-60 million to the provincial economy. Of all freshwater fish species produced in Canada in 2006 (trout, tilapia, Arctic char, brook trout, smallmouth and largemouth bass, cyprinid baitfish) 40% (3,800 tonnes) was trout produced through cage culture in Lake Huron and Georgian Bay, worth $15.7 million (Statistics Canada, 2006). Recently, licensing of new open-water facilities has stopped as a result of potential environmental impacts and a lack of standardized procedures for measuring the degree of impact and mitigation. In order to allow the industry to continue expanding, a new ‘Decision Support Tool’ (DST), developed through federal and provincial agencies as well as the University of Guelph, will aid in streamlining the license approval process by considering criteria for environmental, social and economic sustainability; water quality, operations, ecosystem impacts, user conflict, physical site aspects, sediment impacts, among others.
This study looked at temperature as a physical site aspect, with the primary objective of recording at high frequency (15 minute interval between each data log point) water temperatures from surface to bottom depths at 8 rainbow trout cage culture sites around Manitoulin Island and Georgian Bay during the summer production period. Two previous studies on temperature done in the same region, the Fathom Five National Marine Park by M.G. Wells and S. Parker (2010) and Cape Croker by Rich Moccia (2002, unpublished data), showed large amplitude high-frequency temperature fluctuations throughout the water column. At Cape Croker the temperature profile was so variable, 4°C - 22 °C during the summer that a previously existing aquaculture site had lower than expected production, likely attributable to the high stress level of the fish at the site. In addition, studies done further away in Lake Michigan by Hawley and Muzzi (2003), and Lake Ontario by Rao and Murthy (2001) also reveal highly varying temperatures throughout the water column, showing that this hydrological feature is ubiquitous to the Great Lakes.

The temperature profiles at potential or existing aquaculture sites give an idea of the degree of physiological stress fish at these sites can be, or are exposed to, as well as the movement of water masses and their stratification indicative of Georgian Bay and North Channel limnology. Furthermore aqua-culturists with access to long term temperature monitoring data can make site specific management decisions to improve the health of their fish and mitigate potentially harmful changing environmental conditions. The main goal is to make the aquaculture industry as environmentally dynamic as possible by incorporating all relevant monitoring parameters into adaptive management strategies which may be useful in minimizing the impacts of farms on the
environment, and improving the health and quality of the fish produced. The large temperature range, 4-26 °C, and frequent fluctuations of up to 10°C within a few hours are consistent with other temperature monitoring data from the Great Lakes (mentioned above) and reveal the suitability of current sites for fish culture as well as more of the challenges that the open-water aquaculture industry in Ontario faces from an environmental monitoring and operational management perspective. In addition, this study should bring to attention the importance of temperature in relation to fish physiology, the significance of the differences in temperature profiles that exist between sites and the need for further water quality monitoring in large stratified freshwater lakes of Ontario in order to make use of their great aquaculture potential.

3.2. Methodology

3.2.1. Temperature Logging Equipment Set Up

This study was done in Georgian Bay and North Channel of Manitoulin Island waters; refer to Fig. 6 and Table 6 for map of site distributions and coordinates. Temperature logging equipment consisted of 41 Onset temperature loggers (0.2°C accuracy); 14 Tidbit (0.4°C accuracy) models and 27 TempProV2 (0.2°C accuracy) models. An additional 8 Tidbits were used by Environment Canada for logging at site 8. A total of eight aquaculture companies kindly gave access to their sites for water column temperature monitoring within the vicinity of their open-water cages. Environment Canada launched the temperature string at site 8 and provided this data set.

Depth measurements were taken at each site prior to equipment assembly using a metered polypropylene line with brick anchor. The depth of water being monitored varied with site and
as a rule each site’s water column was split up into two environmental regions of interest; cage environment (CE), depth from surface to bottom of cage net, and below cage environment (BCE), depth from bottom of cage net to lake-bottom. Loggers were attached to ¼ inch steel cable using horseshoe brackets and key rings such that three logger depth intervals representing the cage environment were always located at subsurface, mid-cage and bottom cage depths for each site (variations in cage depth varied depending on site, see Table 7 and figure 6 for site locations and logger distributions). The remaining depth intervals below the cage environment were distributed evenly based on logger quantity and site depth with a logger 0.5m above maximum depth at each site. Collectively this assemblage is referred to as a ‘temperature string’. Temperature strings were anchored to the lake bottom using cinder blocks and suspended with a buoy or attached directly to the edge of the cage. One temperature string was assigned to a site at each aquaculture company except for one company which had two strings assigned at two different sites for better temperature representation because it was exceptionally large and had a highly varying depth range of approximately 34 to 74 metres. Due to issues of privacy, names of sites have been issued numbers from 1 – 8, each representing one temperature string.
Figure 6. Great Lakes Region showing location of the eight sites monitored in the North Channel and Georgian Bay of Lake Huron.
Table 6. Site coordinates

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45°19'11</td>
<td>-80°6'39</td>
</tr>
<tr>
<td>2</td>
<td>45°19'11</td>
<td>-80°6'39</td>
</tr>
<tr>
<td>3</td>
<td>45°45'20</td>
<td>-81°47'30</td>
</tr>
<tr>
<td>4</td>
<td>46°14'44</td>
<td>-81°57'31</td>
</tr>
<tr>
<td>5</td>
<td>46°0'30</td>
<td>-81°44'3</td>
</tr>
<tr>
<td>6</td>
<td>46°4'49</td>
<td>-81°57'38</td>
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<td>7</td>
<td>45°59'27</td>
<td>-81°43'58</td>
</tr>
<tr>
<td>8</td>
<td>45°48'5</td>
<td>-82°32'50</td>
</tr>
</tbody>
</table>

Temperature strings at all sites, except site 8, were deployed May 22\textsuperscript{nd} - 25\textsuperscript{th}, 2007 and recovered December 4\textsuperscript{th} - 12\textsuperscript{th} 2007 at all the aquaculture companies. The site 8 temperature string was deployed by Environment Canada May 1\textsuperscript{st}, 2007 and recovered November 29\textsuperscript{th}, 2007. All loggers were set to record temperature every 15 minutes (some exceptions, see table 2). Offloading of data was done on site using a water proof shuttle for TempPro V2 loggers and optic base station with laptop for Tidbit loggers during the logging period to ensure proper function and location of temperature strings, refer to Table 8 for specific dates.
Table 7. Site location and logger deployment details.

<table>
<thead>
<tr>
<th>Temperature String Sites</th>
<th>Site Depth (m)</th>
<th># Loggers per String</th>
<th>Logger Depths (m)</th>
<th>Logging Interval (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>34</td>
<td>7</td>
<td>0.5,10,15,20,25,30,33.5</td>
<td>15</td>
</tr>
<tr>
<td>Site 2</td>
<td>74</td>
<td>11</td>
<td>0.5,10,20,26.5,33,39.5, 47, 53.5, 60, 66.5, 73.5</td>
<td>15</td>
</tr>
<tr>
<td>Site 3</td>
<td>16.5</td>
<td>4</td>
<td>0.5, 4.5,9,16</td>
<td>15</td>
</tr>
<tr>
<td>Site 4</td>
<td>27.5</td>
<td>5</td>
<td>0.5,7,14,21,27</td>
<td>15</td>
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<tr>
<td>Site 5</td>
<td>16.5</td>
<td>4</td>
<td>0.5,6,12,16</td>
<td>15</td>
</tr>
<tr>
<td>Site 6</td>
<td>18.5</td>
<td>5</td>
<td>0.5, 4.5,9,15,18</td>
<td>15</td>
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<tr>
<td>Site 7</td>
<td>22</td>
<td>5</td>
<td>0.5,6,12,17.5,21.5</td>
<td>15</td>
</tr>
<tr>
<td>Site 8</td>
<td>15.5</td>
<td>8</td>
<td>1.5,7,9,11,13,15, 3</td>
<td>12/10</td>
</tr>
</tbody>
</table>
Figure 7. Schematic of cages and depths of individual loggers at each monitoring site
Table 8. Initial data logging start, offloading, and final logging dates at 8 aquaculture sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Launch date</th>
<th>Offloading/relaunch dates (2007)</th>
<th>End of logging period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>May 24, 2007</td>
<td>June 15, August 2</td>
<td>December 4, 2007</td>
</tr>
<tr>
<td>2</td>
<td>May 24, 2007</td>
<td>TempProV2 all loggers*: July 24</td>
<td>December 4, 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Surface logger: June 15, July 24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tidbits**: July 24, relaunched</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>**No logging July 24 – August 2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>May 24, 2007</td>
<td>June 15, July 22, October 4</td>
<td>December 12, 2007</td>
</tr>
<tr>
<td>4</td>
<td>May 22, 2007</td>
<td>June 14, July 23, October 4</td>
<td>December 10, 2007</td>
</tr>
<tr>
<td>5</td>
<td>May 23, 2007</td>
<td>June 14, July 23, October 5</td>
<td>December 11, 2007</td>
</tr>
<tr>
<td>6</td>
<td>May 23, 2007</td>
<td>June 14, July 24, October 5</td>
<td>December 11, 2007</td>
</tr>
<tr>
<td>7</td>
<td>May 23, 2007</td>
<td>June 14, July 23, October 5</td>
<td>April 28, 2008</td>
</tr>
<tr>
<td>8</td>
<td>May 1, 2007</td>
<td>August 22</td>
<td>November 29, 2007</td>
</tr>
</tbody>
</table>
3.2.2 Temperature Data Statistical Analysis

Simple statistics for temperature data recorded during period of May 22\textsuperscript{nd} to Nov 28\textsuperscript{th}, 2007, were performed using Microsoft Excel 2010 software.

A Fourier analysis on all temperature series was done using the R program in order to determine if temperature changes occur in well-defined periodicities potentially related to internal waves.

Temperature values were averaged every 4 time interval points. The trend of seasonal rise and fall of water temperatures was removed. Periodogram and spectral density values were calculated and plotted for each temperature series using the functional analyses below.

Fourier series

\[ f(x) = a_0 + \sum_{n=1}^{\infty} \left( a_n \cos\frac{n\pi x}{L} + b_n \sin\frac{n\pi x}{L} \right) \]

\[ x_t = \mu + A \cos \omega t + B \sin \omega t + \epsilon_t \]

resulting in,

\[ x_t = \frac{a_0}{2} + \sum_{k=1}^{m} (a_k \cos(\omega_k t) + b_k \sin(\omega_k t)) \]

Where

- \( t \) is the same subscript \( t = 1, 2, \ldots n \)
- \( x_t \) are equally spaced time data series
- \( n \) is the number of observations
- \( m \) is the number of frequency of Fourier decomposition \( m = n/2 \) if \( n \) is even; \( m = n-1/2 \) if \( n \) is odd
- \( a_0 \) is the mean term: \( a_0 = 2 \mu \)
- \( a_k, b_k \) are cosine and sine coefficients, respectively

\( w_k \) represents the Fourier frequencies:

\[ \omega_k = \frac{2\pi k}{n} \]
Functions of the Fourier coefficients \( a_k \) and \( b_k \) can be plotted against frequency or against wave length to form \textit{periodograms}. The amplitude periodogram \( J_k \) is defined as follows:

\[
J_k = \frac{n}{2} (a_k^2 + b_k^2)
\]

3. 3. Results
The temperature loggers were successfully deployed and recovered and temperature profiles were determined for all eight sites from May through December as can be observed in Figures 7 through 12 below. During this period, the cage environment varied from 4.0 to 25.9 °C with an overall average temperature of 14.7°C. A similar range was observed for the whole water column (WWC) from 3.9°C to 25.9°C, reflecting complete mixing of water column towards the end of recording period. Refer to summary statistics of temperature data recorded from May 25 – November 28 at the 8 aquaculture sites, with 3 depth zones; whole water column (WWC), cage environment (CE), below cage environment (BCE) in Table 10
Figure 8. Site 1 temperature profile of cage environment (0.5 & 20m) and maximum depth (33.5m) from May through December 2007.

Figure 9. Site 2 temperature profile of cage environment (0.5 & 26.5m) and maximum depth (73.5m) from May through December 2007.
Figure 10. Site 3 temperature profile of cage environment (0.5 & 9m) and maximum depth (16m) from May through December 2007.

Figure 11. Site 4 temperature profile of cage environment (0.5 & 14m) and maximum depth (27m) from May through December 2007.
Figure 12. Site 5 temperature profile of cage environment (0.5 & 12m) and maximum depth (16m) from May through December 2007.

Figure 13. Site 6 temperature profile of cage environment (0.5 & 9m) and maximum depth (18m) from May through December 2007.
The total range of temperatures recorded during this period was 22.24 °C and their summary statistics are shown graphically in Fig. 7, Fig. 8 and Fig. 9 for whole water column (WWC), cage environment (CE) and below cage environment (BC) respectively.

Figure 14. Whisker box-plot of whole water column (WWC) temperature range at the 8 monitored sites from May 25 – November 28, 2007, yellow box represents 50 percent of the data points between the 1st and 3rd quartiles, whiskers mark the total range of temperatures.
Figure 15. Whisker box-plot of cage environment (CE) temperature range at the 8 monitored sites from May 25 – November 28, 2007, yellow box represents 50 percent of the data points between the 1st and 3rd quartiles, whiskers mark the total range of temperatures.
Figure 16. Whisker box-plot of below cage (BC) environment temperature range at the 8 monitored sites from May 25 – November 28, 2007, yellow box represents 50 percent of the data points between the 1st and 3rd quartiles; whiskers mark the total range of temperatures.
At the beginning of the logging period in May the water column was already stratifying and the temperatures though highly fluctuating were steadily increasing. By the end of August, beginning of September the temperatures began declining as the water column destratified. The lowest temperature recorded was 3.66 °C at site 2 and highest was at site 4, 25.9 °C. Whole water column mean temperatures ranged from 7.47 °C at site 2 to 15.8 °C at site 8. Cage environment mean temperatures ranged from 11.8 °C at site 2 and 16.39 °C at site 8. Below cage environments had the largest range of mean temperatures from 4.97 °C to 15.09 °C. Site 3, site 5 and site 7 had similar cage environment mean temperatures with 50% of their temperatures falling with the ranges 12.39-17.82 °C, 12.12-17.49 °C and 11.88-17.87 °C, respectively, refer to box plots above (Figure 13 and Figure 15) for first and third quartiles showing temperature distributions. Site 4, 6 and 8 had higher cage environment mean temperatures ranging between 12.97-19.63 °C, 12.97-19.7 °C and 12.36-20.33 °C respectively. Sites 1 and 2 had the lowest cage environment mean temperatures ranging from 8.33-17.08 °C and 7.09-17.23 °C, respectively, 50% of the time. The end of the logging period was marked by a cooling of the whole water column leading to destratification and mixing of bottom and surface waters, termed fall turnover as seen in Table 9.

Referring to Table 9 below the earliest and latest fall turnover, based on temperatures at all depths becoming equal or within ~0.1 °C prior to gradual decline, occurred at site 8 on August 19, 2007 and site 2 on November 28, 2007 respectively. No turnover was observed during the entire logging period at Site 6. During summer peak production, within cage environment temperature fluctuations every 15 minutes were predominantly between 0.1°C and 1°C, however
fluctuations between 2 °C and 3 °C were not uncommon and in rare instances a fluctuation of 10 °C within 15min was observed. Fluctuations of up to 10 °C within 5 hours were not uncommon.

Table 9. Estimated fall turnover dates at each aquaculture site

<table>
<thead>
<tr>
<th>Site</th>
<th>Fall turnover date (2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 20</td>
</tr>
<tr>
<td>2</td>
<td>November 28</td>
</tr>
<tr>
<td>3</td>
<td>October 9</td>
</tr>
<tr>
<td>4</td>
<td>October 15</td>
</tr>
<tr>
<td>5</td>
<td>September 17</td>
</tr>
<tr>
<td>6</td>
<td>No turnover</td>
</tr>
<tr>
<td>7</td>
<td>October 15</td>
</tr>
<tr>
<td>8</td>
<td>August 19</td>
</tr>
</tbody>
</table>

Refer to Appendix I for bi weekly temperature profiles at each of the eight sites during the month of August, 2007, when the surface water is generally above 20°C with strong temperature stratification existing at most sites.
Table 10. Summary statistics of temperature data recorded from May 25 – November 28 at the 8 aquaculture sites, with 3 depth zones; whole water column (WWC), cage environment (CE), below cage environment (BCE).

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth zone</th>
<th>Mean °C</th>
<th>Median °C</th>
<th>Mode °C</th>
<th>Range °C</th>
<th>Max. °C</th>
<th>Min. °C</th>
<th>1st Quartile °C</th>
<th>3rd Quartile °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WWC</td>
<td>9.87</td>
<td>7.88</td>
<td>5.80</td>
<td>21.56</td>
<td>25.58</td>
<td>4.02</td>
<td>6.17</td>
<td>12.37</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>12.55</td>
<td>11.30</td>
<td>8.81</td>
<td>21.11</td>
<td>25.58</td>
<td>4.47</td>
<td>8.33</td>
<td>17.08</td>
</tr>
<tr>
<td></td>
<td>BCE</td>
<td>6.32</td>
<td>6.11</td>
<td>5.8</td>
<td>9.25</td>
<td>13.27</td>
<td>4.02</td>
<td>5.49</td>
<td>6.91</td>
</tr>
<tr>
<td>2</td>
<td>WWC</td>
<td>7.47</td>
<td>5.41</td>
<td>4.57</td>
<td>22.18</td>
<td>25.84</td>
<td>3.66</td>
<td>4.59</td>
<td>7.65</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>11.80</td>
<td>9.44</td>
<td>8.10</td>
<td>21.67</td>
<td>25.84</td>
<td>4.17</td>
<td>7.09</td>
<td>17.23</td>
</tr>
<tr>
<td></td>
<td>BCE</td>
<td>4.97</td>
<td>4.78</td>
<td>4.57</td>
<td>7.7</td>
<td>11.36</td>
<td>3.66</td>
<td>4.43</td>
<td>5.35</td>
</tr>
<tr>
<td>3</td>
<td>WWC</td>
<td>14.25</td>
<td>14.19</td>
<td>15.92</td>
<td>18.92</td>
<td>23.35</td>
<td>4.43</td>
<td>11.69</td>
<td>16.70</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>14.87</td>
<td>15.37</td>
<td>15.92</td>
<td>18.59</td>
<td>23.35</td>
<td>4.77</td>
<td>12.39</td>
<td>17.82</td>
</tr>
<tr>
<td></td>
<td>BCE</td>
<td>12.39</td>
<td>12.17</td>
<td>11.69</td>
<td>15.84</td>
<td>20.27</td>
<td>4.43</td>
<td>10.49</td>
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<td>4</td>
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<td>14.75</td>
<td>14.24</td>
<td>16.63</td>
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<td>25.91</td>
<td>5.82</td>
<td>11.47</td>
<td>18.31</td>
</tr>
<tr>
<td></td>
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<td>12.56</td>
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<td>22.90</td>
<td>5.82</td>
<td>10.17</td>
<td>14.30</td>
</tr>
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<td>5.95</td>
<td>12.12</td>
<td>17.49</td>
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<tr>
<td></td>
<td>BCE</td>
<td>12.06</td>
<td>11.81</td>
<td>16.53</td>
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<td>18.79</td>
<td>5.90</td>
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<td>15.70</td>
<td>16.96</td>
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<td>16.82</td>
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<td>5.77</td>
<td>11.88</td>
<td>17.87</td>
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<tr>
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<td>10.66</td>
<td>13.76</td>
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<td>18.70</td>
<td>5.62</td>
<td>9.34</td>
<td>12.94</td>
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<td>15.80</td>
<td>17.54</td>
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<td>22.87</td>
<td>3.93</td>
<td>11.6</td>
<td>18.83</td>
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</tbody>
</table>
3.4. Discussion

The large amplitude and high frequency temperature fluctuations, several degrees Celsius within 15 minutes and up to 10°C within a few hours, during the summer production period recorded at the different aquaculture sites have important implications to the management of aquaculture in Georgian Bay and North Channel waters. Though these temperature fluctuations are counter-intuitively large in amplitude and high in frequency, they are consistent with the circulation and thermal structure of the North Channel and Georgian Bay. In order to understand the temperature profiles recorded at the 8 aquaculture sites one must also understand the driving forces responsible for establishing these temperature regimens.

The Georgian Bay and North Channel are 2 of 4 interconnected water bodies (Main Lake and Saginaw Bay the other two) that make up Lake Huron (Sheng and Rao, 2006). The main inflows of water enter Lake Huron from Lake Superior via St. Mary’s River into the North Channel and from Lake Michigan at the Straits of Mackinac (Sheng and Rao, 2006). Water leaves the system through the south end via the St. Clair River (Sheng and Rao, 2006). The North Channel and Georgian Bay, as a result of high inflow from St. Mary’s River, with corresponding outflow in the south of Lake Huron have flushing times that are very short, 2 and 8.5 years, respectively (Weiler, 1988). The North Channel can therefore be seen as a flow through system with a complete water exchange in just 2 years (Weiler, 1988). The Georgian Bay water body being much larger nevertheless also has a short flushing time with a major water exchange with the Main Lake occurring at the Main Channel. The exchange that occurs at the Main Channel is responsible for the oligotrophic status of Georgian Bay by
providing cooler surface waters to offset summer solar heating while allowing nutrient rich bottom waters of the bay to flow into the Main Lake (Bennett, 1988).

The main driving force of the temperature distributions observed is the climate of the region (Wetzel 1975), with four distinct seasons, a long cold winter and strong winds throughout the year with frequent storms along with a hot humid summer (Weiler, 1988). In addition the irregular shoreline and highly varying basin morphology contribute to changes in current flow and wave action (Sheng and Rao, 2006).

As seen from the temperature profiles at all the sites except Site 6, this weather pattern leads to a very important hydrodynamic feature of seasonal changes in the thermal structure in Georgian Bay and the North Channel, from completely vertically mixed in early spring and late fall to stratified during the summer. As surface waters cool during late fall, due to strong vertical convection from wind cooling, the summer stratification weakens (Sheng and Rao 2006). Warm summer surface waters cooling faster than deep waters approach the maximum water density near 4 °C and begins mixing with deeper waters, forming a vertically homogenous water column (Sheng and Rao, 2006). As winter cooling progresses, surface waters pass the 4 °C mark and become less dense at 0-3 °C, thus forming a weak inverse stratification near the surface during the winter with densest ~4 °C water below (Wetzel, 1975).

The process then occurs in reverse during late winter and early spring, as temperatures near the surface warm up to 4 °C, again forming a vertically homogenous water column before the onset of summer stratification due to continuous solar heating (Sheng and Rao, 2006). The
retention and distribution of the surface absorbed solar energy depends on the physical work of wind energy, currents and swells, morphometry of the basin and water losses (Wetzel, 1975). Depending on these factors at a particular site the resulting patterns of thermal stratification will drive the physical and chemical cycles of the water body, thus determining its production, utilization and decomposition (Wetzel, 1975).

During the summer stratification period, the water column can be divided into three main bodies of decreasing temperature from surface to bottom; the epilimnion, metalimnion and hypolimnion (Wetzel, 1975). The final temperatures of the spring turnover determine the initial summer stratification temperatures (Wetzel, 1975). The epilimnion is the most turbulent and heated layer, the bottom hypolimnion layer is the densest and coldest layer making it the most stable and isolated (Wetzel, 1975). The metalimnion is the layer of thermal discontinuity between these two layers, characterized by a steep thermal gradient, where largest decrease in temperature with regard to depth is referred to as the thermocline (Wetzel, 1975).

An important characteristic of these water layers with regard to aquaculture site suitability and consequently the assimilative capacity of a site is the accompanying dissolved oxygen profile. Specifically, dissolved oxygen concentration drops in the hypolimnion during summer stratification while surface layers remain aerated through wind and wave action turbulence (Wetzel, 1975). Oxygen is responsible for driving the chemical and biological degradation of deposited organic material on the lake bottom by affecting the biological diversity and abundance responsible for it (Wetzel, 1975). Water at the sediment-water interface, where
oxygen demand is highest due to bacterial breakdown of organic compounds will consequently lose oxygen quickest (Wetzel, 1975). Since the temperature differences between the hypolimnion and overlying waters prevent mixing, the oxygen in the hypolimnion can be completed used up by organic degradation (Wetzel, 1975). Complete anoxia in the hypolimnion leads to production of toxic sulphur gas, hydrogen sulphide and methane gas (Wetzel, 1975). Furthermore a change from hypoxic to anoxic conditions brings the redox potential to 0mv and creates a reducing environment in the sediment (Wetzel, 1975).

This condition along with the presence of deposited aquaculture waste with further reducing properties, can lead to metal enrichment by complexing and adsorption to the acid molecules within the waste (Wetzel, 1975). Since aquaculture feed contains trace metals copper, zinc, iron and manganese as dietary requirements for salmonids (Chou, 2002), their accumulation in long term deposited sediments may have significant effects under anoxic conditions. Chou et al. (2004) found high concentrations of copper and saturation levels of zinc in marine sediments related to the inputs of fecal and metabolic waste metals and organic carbon originating from salmon aquaculture feed. In such cases of hypolimnetic anoxia and organic waste loading, spring and fall turnovers become important mechanisms in restoring oxygen levels in the hypolimnion and bringing nutrients/waste to the surface to be utilized or assimilated by primary producers.

Observing the lower depth temperature profiles it is evident that a pronounced stable hypolimnion is found only at the deeper sites 1 and 2 and the more wind sheltered site 6 site
located between two islands. However site 6 is the only one of the three sites with a pronounced hypolimnion that does not show a full turnover in the fall suggesting an anoxic hypolimnion with high anaerobic hydrogen sulphide and methane gas by-products of the aquaculture waste degradation.

Sites 3, 4, 5, 7, and 8 show weak hypolimnetic stratification with temperatures in the hypolimnion often fluctuating in the metalimnetic and even epilimnetic temperature ranges suggesting adequate mixing with overlying water. This may be attributable to the higher wind exposure and relatively shallow depths of these sites where as a result, solar heating of the hypolimnion occurs and sufficient winds cause turbulent vertical convection mixing during the stratification period. This can be clearly seen at site 8’s profile with no evidence of a hypolimnion, weak stratification, no natural wind barriers and consequently the earliest fall turnover, September 17, 2007. Correspondingly the latest turnover date occurs at the deepest site, 2, over 2 months later on November 27, 2007. With regard to assimilative capacity of a site, summer stratification along with spring and fall turnovers are some of the fundamental determinants and complete seasonal temperature profiles are necessary for their identification.

Rainbow trout (Oncorhynchus mykiss) reared at the aquaculture sites are poikilothermic and consequently have their metabolic rate, growth, energy expenditure and requirements and feed intake highly influenced by water temperature (Azevedo et al., 1998). Knowing the average environmental rearing temperature, growth period (days) initial weight and thermal growth coefficient of the fish species an aquaculture manager can accurately predict final product weight (Azevedo et al., 1998). Such information is useful in determining stocking density
based on desired final product weight, initial fingerling weight, known temperature regime and length of production cycle. The maximum and minimum temperatures recorded during the production cycle in this study approach the lower and upper incipient lethal temperatures for rainbow trout. Rainbow trout prefer a temperature range between 10-15 °C (Lund and Tufts, 2003) however can acclimate to higher temperatures where the growth rate is optimal around 18-19 °C (Werner et al., 2005). The upper and lower incipient lethal temperatures for rainbow trout depending on acclimation temperature is approximately 26-28.9 °C and 0-1 °C, respectively (Moloney, 2001). The lowest maximum temperature that occurred within the cage environment during the summer production cycle was 23.35 °C at the site 3. Site 4 had the highest maximum temperature at 25.91 °C and sites 1 and 2 and site 6 all exceeded the 25 °C mark as well. In the winter months on the other hand the temperatures approach the freezing point of 0 °C, the lower lethal limit. This is highly significant considering rainbow trout exposed to temperatures within their tolerance zone, between the preferred range (above) and lethal limits, experience adverse effects, discussed later (Viant et al., 2003).

More surprising than the temperature extremes observed at the sites, are the high frequency large amplitude fluctuations in temperature on the order of 5 °C within an hour. This spatial heterogeneity of mixing is usually attributed to turbulent mixing of water layers however, recent studies suggest that oscillating baroclinic currents along the lake bottom causing shear-driven turbulence along with high-frequency internal waves breaking up on sloping boundaries at the depth of the metalimmion, are also responsible for interior mixing (Boegman et al., 2003). It has been shown that high-frequency internal waves exist in narrow discrete frequency bands and some common mechanisms for their formation have been proposed; shear instability, nonlinear steepening of basin-scale internal waves, internal hydraulic jumps,
excitation by intrusion and gravity currents, and flow interaction with boundaries (Boegman et al., 2003). Another type of internal wave that could likely cause the observed temperature fluctuations is the Poincare wave REF or inertia-gravity wave that is of longer duration and more likely identifiable by the 15 minute sampling interval resolution applied in this study. These waves, sometimes kilometers long, have periods long enough to be affected by the rotation of the earth and are common throughout large lakes (Gill, 1982; Antenucci et al., 2000; Bouffard et al., 2012).

Internal waves are of great significance to both aquaculture managers and monitoring agencies due to their role in sediment resuspension (Sakai et al., 2002). Sakai et al. (2002) found internal waves to increase total dissolved and particulate phosphorous, and cause the release of methane from the bottom sediment at the end of the stratification period. Furthermore winter winds cause frequent internal waves and complete vertical mixing due to a thermally homogenous water column suggesting this plays a role in previously observed surface maximums of methane (Sakai et al., 2002). A possible scenario of such an effect is seen in the popular aerial photograph of the LaCloche Channel decommissioned aquaculture site, displaying melted ice contours of cages due to methane accumulation at the surface. Hawley and Muzzi (2003) concluded that suspended particulate material present in the hypolimnion during stratification can mix with suspended particulate material at mid-depths do to upwelling and downwelling caused by internal waves, characterized by a changing thermocline depth. In addition the amount of suspended material between mid-depths and the bottom can vary up to 50% as a direct result of resuspension, suggesting that short-term mixing events caused by internal waves are responsible for significant exchange of materials between the epilimnion and hypolimnion (Hawley and Muzzi, 2003). With particular regard to phosphorous these
findings are significant due to the fact that regulatory agencies monitor total phosphorous loading at these aquaculture sites within the water column at spring and fall turnover as well as during the stratification period (Reid et al., 2006). It would be expected therefore that higher levels of total phosphorous would be found during the mixing periods, controlled by the temperature regime, and during increased temperatures which promote bacterial activity which in turn also promotes phosphorous leaching (Hua et al., 2008) to explain. Therefore monitoring practices should take these effects into consideration when estimation overall total phosphorous loading at these particular sites. A more accurate method of estimating total phosphorous at an aquaculture site could be using a bioenergetics model such as Fish-PrFEQ, currently being researched (Hua et al., 2008).

Since temperature as a physical parameter has such a profound effect on the physical, chemical and biological properties of lake water columns, as well as sediments and organisms, it is considered one of the most important environmental parameters influencing not only the suitability of an aquaculture site but the distribution and success of all wild populations of salmonids (Mathews and Berg, 1997). Rainbow trout respond to adverse temperature conditions with the ‘heat shock protein response’, an important mechanism used to repair denatured cellular proteins and prevent further damage from thermal cellular stress (Werner et al., 2005). Upon exposure to thermal stress there is a rapid induction and expression of heat shock proteins whose roles are to correct folding, repair, translocate intracellular proteins, suppress protein aggregation and reactivate denatured proteins (Werner et al., 2005). Heat shock protein expression has also been linked to cellular signalling molecules and receptors controlling development and growth (Werner et al., 2005). Werner
et al. (2005) found that *Onchorynchus mykiss* (steelhead) acclimated to 15 °C chronically exposed to 20°C was associated with a decrease in high-energy compounds in liver and muscle tissue. Correspondingly, Viant et al. (2003) found that based the on heat shock protein expression profile, fish can acclimate to a constant 5 °C elevation in temperature from 15 to 20 °C, within 3 weeks. Thermal stress is thus considered to cause energy diversion from growth to maintenance, minimizing thermal damage using heat shock proteins to reorganize plasma membranes, particularly in fish with chronic exposure at 20 °C (Viant et al., 2003).

Reid et al. (1995) also suggested of such energy repartitioning due to heat shock protein induction within tissues of juvenile rainbow trout when he observed a 20% decrease in growth, appetite, food conversion efficiency, protein turnover and accretion of the fish exposed to 24-26 °C for 30 days. In addition to causing a heat shock response, higher temperatures also reduce haemoglobin oxygen affinity thus further raising the basic metabolic cost for the fish (Lund and Tufts, 2003). Considering the effects of thermal stress and the cage environment temperature distributions at Site 4, Site 6 and Site 8 which have the highest medians, all above the upper optimal range of 15 °C at approximately 16 °C, frequently fluctuating and exceeding 20 °C, it can be inferred that the fish are experiencing considerable thermal stress. The 1st and 3rd quartile temperature marks for these sites are above the lower and upper optimal range values at approximately 12.5 and 20 °C respectively, meaning that 50 percent of the time the temperatures are found within this range and beyond it reach maximums of approximately 25°C. Site 3, Site 5 and Site 7 have a similar 1st quartile mark of around 12 °C however the 3rd quartile mark is 2 °C cooler and so are the maxima at just below
24 °C. This cooler upper temperature regime probably coincides with markedly reduced heat shock response thus drawing less energy away from growth.

The most optimal temperatures were observed at the sites with the deepest nets (~25m vs. ≤14m at remaining sites), Site 1 and Site 2, where the 1st quartile marks were below the optimal range of 10 °C at approximately 8 °C and 7 °C respectively and the 3rd quartiles both at ~17 °C. Despite maximum temperatures at these sites exceeding 25 °C, the lower range of temperatures, specifically in the lower area of the cages by comparison with the remaining sites provides the fish with more thermoregulatory opportunities. Fish given the opportunity to move between water bodies with temperatures ranging from optimal to near lethal limits, will seek refuge in thermally optimal waters (Mathews and Berg, 1997). This is of significant importance to aquaculture managers of sites providing optimal temperatures below their cages and near lethal temperatures within their cages such as can be seen at all 8 sites in this study.

Submersible cage systems as well as pumping deeper cold water up through the cages in such situations are options that can significantly improve production and well-being of the fish while staying within the operating budget. Furthermore optimal stocking density may need to be reconsidered if upper cage environment temperatures frequently cause fish to seek refuge in lower parts of the cage causing overcrowding. Winfree et al. (1998) found increased temperature, density and food deprivation to be interactive and additive with regard to fin erosion in juvenile steelhead, and suggested that a combination of treatments would improve fish condition. Temperature stress can also increase the risk of disease in fish exposed to bacterial pathogens. Schisler et. al (2000) found that elevated temperature from 12.5 °C to 17 °C contributed significantly to mortality of fingerling rainbow trout when exposed to
*Myxobolus cerebralis* (cause of fish whirling disease) and *Flavobacterium psychrophilum* (cause of bacterial coldwater disease and rainbow trout fry syndrome). The increase in mortality at 17 °C vs. 12.5 °C of fish exposed to *F. psychrophilum* was unexpected, as the bacterium is generally found to be more virulent at temperatures below 15 °C. Schisler et al. (2000) hypothesized that the increased metabolic rate of both the fish and the pathogen contributed to this effect at high temperatures. Considering Snieszko’s model from (1974) for fish disease which states that in order for disease to occur, a fish must not only be exposed to a pathogen but also a physiological stressor. This may not hold true for all pathogens but may explain why majority of fish populations exposed to *M. cerebralis* show no signs of disease (Schisler et al., 2000). Taking this into account it is clear that fish exposed to an environmental stressor, such as temperature, as is the case at all the aquaculture sites in this study, are more prone to disease if exposed to a pathogen.

Based on the effects of temperature on lake physical, chemical and biological characteristics as well as on fish physiology it is evident that not all the sites are equally suitable for the aquaculture operations they currently have in place. The two deepest sites, Site 1 and Site 2 provide deep enough cages to allow their fish to thermo regulate between the below optimal temperatures at the bottom cage and the hot surface throughout the production cycle. In addition these sites experience a complete fall turnover suggesting that an anoxic hypolimnion is unlike to form at these deep highly exposed sites. Site 3, Site 5, and Site 7 have cage environments that are several degrees warmer on average than the latter sites however they also have a narrower over all temperature range with lower maximums thus making them suitable sites regarding temperature. High temperature issues may play a role at Site 4, Site 6 and site 8 where majority of the temperature profile within the cages lies above the optimal range, is
narrow as in the latter sites and reaches maximums close to and above 25 °C. The reasons for these sites having the warmest profiles are most likely a smaller influx of cool waters due either to wind sheltering, low current flow or water exchange with warmer water masses than at the other sites. Of particular concern is the Site 6 site with no evidence of a fall turnover and a well-defined hypolimnion. The potential for negative environmental impacts at this site is therefore higher than at other sites and should be investigated.

3.5. Conclusion and Future Research Ideas

Future research involving other parameter measurements coupled with high frequency time series temperature logging such as dissolved oxygen, phosphorous levels, current meters, air temperature, resuspension monitoring, sediment composition and redox potential, wind speed as well as precise production parameters at the sites would reveal significantly more cause and effect relationships between the observed temperature profiles and site suitability. With the current rises in climate temperatures and changing environment, year to year monitoring of water quality with this regard can provide invaluable information on possible future trends at current sites and can be used to assess the future suitability of new sites. The more parameters included in the monitoring process the better the resolution on the physical, chemical and biological processes involved allowing for the best mitigation strategies. The sustainability of the Ontario freshwater open net cage aquaculture industry depends solely on the understanding of all environmental effects at a particular site both from the environment and the operation itself.
Chapter 4. Role of Temperature and Organic Degradation on the Persistence of Stable Isotopes of Carbon ($\delta^{13}\text{C}$) and Nitrogen ($\delta^{15}\text{N}$) in Aquaculture Waste

4.1 Introduction

The receiving environment at open-water cage culture sites can be divided into two main parts: the water column that receives the dissolved wastes and the sediment where the solid wastes settle. This study focuses on the persistence of isotopes in the solid waste fraction. The settled waste at aquaculture sites is immediately subject to bacterial degradation that causes the fractionation of the isotope ratios leading to a shift in the signature. The rate of organic degradation of the waste through bacterial aerobic and anaerobic processes is determined by the sediment environmental conditions, sediment chemistry, bacterial dynamics as well as overlying water column biochemistry and hydrodynamics. The scope of this study was to assess the use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios in identifying the sediment fraction by determining the effect of organic degradation and temperature on the isotope signature in a laboratory setting.

In previous field studies of sediment $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at Ontario cage aquaculture sites (Salazar-Hermoso, 2007), sediment samples showed a high variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures ranging from depleted to enriched isotope ratios. Salazar-Hermoso (2007) showed diets formulated with different ingredients have isotope signatures that reflect the proportion of ingredients used, in particular the amount of enriched fishmeal component. However, the isotope values of feeds used at open water aquaculture sites did not reflect the signature in sediment samples (Salazar - Hermoso, 2007). It is speculated that the breakdown of the organic waste by the surrounding environment, specifically the bacterial degradation, causes
differential shifts in the $\delta^{13}C$ and $\delta^{15}N$ signatures. McGoldrick et al. (2008) measured bacterial and substrate during glucose and ammonia uptake by three bacterial strains and results showed higher discrimination against heavier isotopes of $\delta^{13}C$ and $\delta^{15}N$. The depleted bacterial biomass that forms as a result of fractionation and enters the food web may help explain why field observations show depleted isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ (McGoldrick et al., 2008).

In order to measure the potential effect of temperature and organic degradation on the persistence of $\delta^{13}C$ and $\delta^{15}N$ isotopes in aquaculture waste, an incubation trial of fish faeces collected from a flow through facility was setup with different temperatures and incubation times. Fish faeces incubated at different times and temperatures had significantly different $\delta^{13}C$ and $\delta^{15}N$ ratios. Increased incubation time and temperature significantly depleted the $\delta^{13}C$ signature and significantly enriched the $\delta^{15}N$ signature of faeces samples. The results show the need for further controlled laboratory studies with varying parameters of organic degradation to determine further fractionation effects that may help explain the observed variation in the field.

4.2. Hypothesis and Objectives

The hypothesis of this chapter is defined as follows:

*Carbon and nitrogen isotope signatures of faeces samples will be affected when incubated under different temperature and time intervals, with the changes in isotopic signatures correlating with increased incubation time and temperature, respectively.*
The following methods were used to test this hypothesis:

1. Measure the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signature of faeces collected at the time of excretion (time = 0) from farm raised rainbow trout (*Oncorhyncus mykiss*).

2. Measure the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures of the same faeces after incubation at different temperatures and time intervals.

3. Determine the linear, quadratic and cubic effects of incubation temperature and time on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of incubated faeces using mixed model statistical analysis.

### 4.3 Methodology

#### 4.3.1. Set Up of Fish Faeces Collecting Tanks, Fish Distribution within Tanks and Feeding Schedule

All fish used for fresh faeces collection came from a single population of animas stocked in an outdoor circular concrete tank at the Alma Research Station, Alma, Ontario. Total of six 0.33 m$^3$ fiberglass tanks were set up indoors, side by side in a single row, and equipped with a removable overflow settling column attached to the drain pipe at the base of each tank. Tanks were supplied with the same single source 8.3°C water well with a flow rate of 12lpm, an oxygen concentration of approximately 8ppm and a 12hour light: 12h dark cycle. The six tanks were stocked with approximately 15kg of 200-250g of fish. Individual tanks received 150g daily rations of Martin Mills Profishent commercial feed using an automatic belt feeder from 9am to 5pm. Fish were acclimated and average daily dry weight faeces production from each tank was calculated over a period of 12 weeks from November 19, 2008 – February 21, 2009.
Three days prior to the collection day on February 22, the feed rations were increased to 200g in order to achieve maximum satiety and maximize fecal matter production. After feeding, on the last day, the tanks were cleaned out of any leftover feed and the collecting cylinder was attached to the overflow column 3 hours from the end of feeding, 8:00pm, Feb 21. Faeces settled in the collecting cylinder from 8:00pm, Feb 21, until 8:00pm, Feb 22, 2009.

4.3.2. Faeces Incubation Preparation

At 8:00pm, Feb 22, collecting cylinders with faeces from each of 6 tanks were assigned a random order of preparation and subsequently removed in that order from the tank settling columns for faeces preparation. All equipment used to handle the faeces was sterilized with ethyl alcohol and rinsed with deionised water. The following methodology is described for one block of faeces and the remaining blocks were prepared using the same method.

Each block of faeces had the supernatant removed using a siphon and remaining 240ml of settled wet faeces was transferred into a 500ml graduated beaker. The 240ml of settled wet samples was inoculated with 0.025ml of N3000 Bactapur commercial bacteria culture and homogenized using a magnetic stirrer for 5 minutes. After inoculation and homogenization, the block sample was split into 4 subsamples of 60ml each. The 4 subsamples were randomly assigned to each of 4 temperature treatments.
Each randomly assigned 60ml subsample was further split into six 10ml samples using 20ml test tubes. The samples were then topped off with 8ml of fresh well water, identical to one used the in the tanks, and sealed with para-film. A pinhole was made in the para-film of each test tube to allow minimum gas exchange. Each of the 6 samples was then randomly assigned to 6 incubation time intervals: 0hrs, 48hrs, 7days, 14days, 21days, and 35days at each temperature. The above preparation was repeated for the remaining 5 block samples of faeces in the randomly assigned order. A total of 144 samples of faeces were prepared. Refer to Figure 17 below for experimental design.

![Diagram](image)

**Figure 17.** Incubation experimental design setup, showing faecal sample separation and treatment allocation.
4.3.3. Temperature Incubation Set Up

Four treatment temperatures were chosen as follows: 4°C, 8.5°C, 16°C, 25°C. Temperatures were maintained using two incubators and two water baths set up, in a thermostat regulated recirculation room at the Alma Research Station. Temperature of 4°C was maintained using a chilling incubator. Temperature of 8°C was maintained in a water bath using a flow through of well water through a trough at a constant 8°C. 16°C was maintained in a standing water bath at room temperature set to 16°C. 25°C was maintained using an incubator set to 25°C.

Test tubes of samples were placed in four test tube racks, each designated for one of four temperatures and each rack was placed in a closed plastic bin with vents to prevent light entry. The four bins containing 36 test tubes each, 6 blocks and 6 incubation times were then assigned to their respective temperature treatment in random order at 10pm, February 22, 2009. Temperature for each treatment was monitored using HOBO ware temperature loggers placed within the incubation environment which recorded real time incubation temperature every 10 minutes.

4.3.4. Sample Collection and Preparation for Stable Isotope Analysis

Samples at time of excretion (0 hours) were taken on ice to the Aquaculture lab at the University of Guelph for stable isotope analysis preparation. The samples were dried at 60°C for 24 hours, grounded to a fine powder and stored for analysis of δ^{13}C and δ^{15}N at the University of Waterloo.
The remaining samples were collected and prepared using the same method at the end of their respective incubation intervals.

4.3.5. Feed samples preparation

Feed samples of 5 grams were collected from each automatic belt feeder at 9am February 21, dried at 60°C for 24hrs and ground to a fine powder.

4.3.6. Statistical Analysis

Analysis of carbon and nitrogen isotope values was done using the GLM and Mixed Model procedures Statistical Analysis Software (SAS) version 9.1 (SAS Institute Inc., Cary, NC, USA). A mixed model analysis using orthogonal contrasts was performed to determine linear, quadratic and cubic effects of incubation temperature and time.

4.4 Results

4.4.1. Carbon Isotope (δ¹³C) Results

Isotope values for δ¹³C varied from -20.68 to -22.80‰ as shown in figures 17, 18 and 19. There was no significant effect of the tanks (block effect) on the δ¹³C as seen in table 11 below. There was no interaction between incubation temperature and time as shown in table 11. Incubation time and temperature had a strongly significant linear effect (LE) of depletion on the δ¹³C isotope signature. Refer to table 11 and 12 of fixed effects and orthogonal contrasts of δ¹³C.
At 25°C, time has a quadratic effect (QE) of depletion on the $\delta^{13}$C signature shown in table 12. There were no significant effects of time or temperature at 4°C or at 48 hours of incubation time.

Overall the $\delta^{13}$C depleted slightly with time and temperature. Refer to table 13 of least square means (LSM) and figures 17, 18 and 19 showing wireframe plots of $\delta^{13}$C over time and temperature.
Figure 18. Wireframe plot of $\delta^{13}$C signature change over time (h) and incubation temperature ($^\circ$C).
Figure 19. Plot of the change in LSM of $\delta^{13}$C over temperatures ($^\circ$C).
Figure 20. Plot of change in the LSM of $\delta^{13}$C signature over time (hours).
Table 11. Summary of fixed effects on the $\delta^{13}$C isotope signatures

<table>
<thead>
<tr>
<th>Effect</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>10.67</td>
<td>0.0005 *</td>
</tr>
<tr>
<td>Tank</td>
<td>0.34</td>
<td>0.8806</td>
</tr>
<tr>
<td>Time</td>
<td>10.26</td>
<td>&lt;0.0001 *</td>
</tr>
<tr>
<td>Temperature and Time</td>
<td>0.84</td>
<td>0.6101</td>
</tr>
</tbody>
</table>

*significant at 95% confidence level (p < 0.05)
Table 12. Orthogonal contrasts of mean C $\delta^{13}$ isotope values between incubation temperatures and time intervals, showing significance of linear (L), quadratic (Q) and cubic (C) effects of time and temperature.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F-value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. time</td>
<td>32.69</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Q. time</td>
<td>6.98</td>
<td>0.0096*</td>
</tr>
<tr>
<td>C. time</td>
<td>0.74</td>
<td>0.3909</td>
</tr>
<tr>
<td>L. temperature</td>
<td>31.07</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Q. temperature</td>
<td>1.02</td>
<td>0.3286</td>
</tr>
<tr>
<td>C. temperature</td>
<td>0.00</td>
<td>0.9932</td>
</tr>
<tr>
<td>L. time at 4C</td>
<td>2.76</td>
<td>0.1003</td>
</tr>
<tr>
<td>L. time at 8C</td>
<td>0.97</td>
<td>0.3270</td>
</tr>
<tr>
<td>L. time at 16C</td>
<td>0.02</td>
<td>0.8965</td>
</tr>
<tr>
<td>L. time at 25C</td>
<td>1.17</td>
<td>0.2826</td>
</tr>
<tr>
<td>Q. time at 4C</td>
<td>1.05</td>
<td>0.3089</td>
</tr>
<tr>
<td>Q. time at 8C</td>
<td>0.21</td>
<td>0.6486</td>
</tr>
<tr>
<td>Q. time at 16C</td>
<td>1.77</td>
<td>0.1872</td>
</tr>
<tr>
<td>Q. time at 25C</td>
<td>6.22</td>
<td>0.0144*</td>
</tr>
<tr>
<td>C. time at 4C</td>
<td>2.76</td>
<td>0.1003</td>
</tr>
<tr>
<td>C. time at 8C</td>
<td>0.97</td>
<td>0.3270</td>
</tr>
<tr>
<td>C. time at 16C</td>
<td>0.02</td>
<td>0.8965</td>
</tr>
<tr>
<td>C. time at 25C</td>
<td>1.17</td>
<td>0.2826</td>
</tr>
<tr>
<td>L. temperature at 48 hours</td>
<td>1.03</td>
<td>0.3127</td>
</tr>
<tr>
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</tr>
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*significant at 95% confidence level (p <0.05)
Table 13. The estimate of LSM of $\delta^{13}C$ values corresponding to individual and combined effects of temperature and time

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Time (hrs)</th>
<th>LSM estimate</th>
</tr>
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<td>-21.6526</td>
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<td>-21.7736</td>
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<td>-21.4136</td>
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<td>840</td>
<td>-21.9544</td>
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</tbody>
</table>
4.4.2 Nitrogen Isotope Results

The $\delta^{15}$N values varied from 3.69 to 9.71‰. There was a significant effect of incubation time, temperature and tank (block) on the $\delta^{15}$N signature as shown in Table 14 of fixed effects and figures 20, 21, and 22 below. In addition there was a significant interaction between the effect of temperature and time on $\delta^{15}$N as shown in table 15 and 16 of orthogonal LSM means below.

The linear effect of temperature is significant at all times except 48 hours of incubation and the effect of time is strongly significant at 25°C. Refer to table 15 of orthogonal contrasts below. At 25°C, time has a cubic effect on the $\delta^{15}$N signature as shown in table 15.
Figure 21. Wireframe plot of the $\delta^{15}$N signature change over time (hours) and temperature ($^\circ$C).
Figure 22. Plot of change in LSM of $\delta^{15}$N signature over temperature ($^\circ$C).
Figure 23. Plot of change in LSM of $\delta^{15}$N signature over time (hours).
Table 14. Summary of fixed effects on the $\delta^{15}N$ isotope signature

<table>
<thead>
<tr>
<th>Effect</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>32.36</td>
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</tr>
<tr>
<td>Tank</td>
<td>6.89</td>
<td>0.0016 *</td>
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<tr>
<td>Time</td>
<td>15.63</td>
<td>&lt; 0.0001 *</td>
</tr>
<tr>
<td>Temperature $^x$ Time</td>
<td>4.94</td>
<td>&lt;0.0001 *</td>
</tr>
</tbody>
</table>

*significant at 95% confidence level (p <0.05)
Table 15. Orthogonal contrasts of mean $\delta^{15}$N isotope values between incubation temperatures and time intervals, showing significance of linear (L), quadratic (Q) and cubic (CE) effects of time and temperature on the $\delta^{15}$N signature

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
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<td>L. time</td>
<td>60.09</td>
<td>&lt; 0.0001 *</td>
</tr>
<tr>
<td>Q. time</td>
<td>0.03</td>
<td>0.8671</td>
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<tr>
<td>C. time</td>
<td>2.75</td>
<td>0.1008</td>
</tr>
<tr>
<td>L. temperature</td>
<td>96.63</td>
<td>&lt; 0.0001 *</td>
</tr>
<tr>
<td>Q. temperature</td>
<td>0.32</td>
<td>0.5818</td>
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<tr>
<td>C. temperature</td>
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</tr>
<tr>
<td>L. time at 4C</td>
<td>0.24</td>
<td>0.6220</td>
</tr>
<tr>
<td>L. time at 8C</td>
<td>0.24</td>
<td>0.6282</td>
</tr>
<tr>
<td>L. time at 16C</td>
<td>1.12</td>
<td>0.2935</td>
</tr>
<tr>
<td>L. time at 25C</td>
<td>19.91</td>
<td>&lt; 0.0001 *</td>
</tr>
<tr>
<td>Q. time at 4C</td>
<td>0.78</td>
<td>0.3802</td>
</tr>
<tr>
<td>Q. time at 8C</td>
<td>0.21</td>
<td>0.6501</td>
</tr>
<tr>
<td>Q. time at 16C</td>
<td>1.89</td>
<td>0.1728</td>
</tr>
<tr>
<td>Q. time at 25C</td>
<td>2.12</td>
<td>0.1484</td>
</tr>
<tr>
<td>C. time at 4C</td>
<td>0.24</td>
<td>0.6220</td>
</tr>
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<td>C. time at 8C</td>
<td>0.24</td>
<td>0.6282</td>
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<tr>
<td>C. time at 16C</td>
<td>1.12</td>
<td>0.2935</td>
</tr>
<tr>
<td>C. time at 25C</td>
<td>19.91</td>
<td>&lt; 0.0001 *</td>
</tr>
<tr>
<td>L. temperature at 48 hours</td>
<td>0.51</td>
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<tr>
<td>L. temperature at 168 hours</td>
<td>20.31</td>
<td>&lt; 0.0001 *</td>
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<tr>
<td>L. temperature at 336 hours</td>
<td>17.17</td>
<td>&lt; 0.0001 *</td>
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<tr>
<td>L. temperature at 504 hours</td>
<td>14.18</td>
<td>0.0003 *</td>
</tr>
<tr>
<td>L. temperature at 840 hours</td>
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<tr>
<td>C. temperature at 168 hours</td>
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<td>C. temperature at 336 hours</td>
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<td>C. temperature at 840 hours</td>
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*significant at 95% confidence level (p < 0.05)
Table 16. Estimate of LSM of $\delta^{15}$N isotope values corresponding to individual and combined effects of temperature and time

<table>
<thead>
<tr>
<th>Effect</th>
<th>Temperature (C)</th>
<th>Time (hrs)</th>
<th>LSM estimate</th>
</tr>
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4.5 Discussion

Results of the incubation trials revealed significant effects of temperature and degradation time on δ¹³C and δ¹⁵N signatures consistent with results in the literature (Kelly and Chynoweth, 1981; Cole et al., 2002; Ostrom et al., 1997; Benner et al., 1987). It should be noted that LSM of δ¹³C and δ¹⁵N at time zero (0h) were slightly depleted compared to values of feeds used at active sites and slightly more enriched than active site sediments. This can be explained by comparing the sample sources. Settled faeces for incubation were collected within 24 hours using a settling column (minimum depletion from fish digestion) whereas samples from active sites collected using an Ekman grab, were more exposed to post excretion, aerobic degradation, break up and leaching accelerated by water currents leading to a depleted signature (Benner et al., 1987; Mancinelli and White, 2000). The δ¹³C isotopes showed less variation than δ¹⁵N suggesting greater isotope discrimination of nitrogen during the degradation process or a higher rate of substrate turnover, as confirmed by previous studies using δ¹³C and δ¹⁵N as integrators of their respective cycles (Koike and Hattori, 1978; McGoldrick et al., 2008; Meyers and Ishiwatari, 1993; Ostrom et al., 1997; Seitzinger, 2009).

The δ¹³C signature of samples prior to incubation (time = 0h) varied from -20.78 to -22.50‰ and were mostly within the range of values found in sediment samples found at active sites from -22.55 to -20.94‰ as shown in Table 2 in Chapter 2. Compared to δ¹³C signatures of feeds used at active sites (range of -21.07 to -17.32‰), samples at time 0hrs were generally slightly depleted with some overlap in values around -21.00‰. However, after 840h of incubation, δ¹³C values became depleted relative to feed values, and were in the minimum value range of active sediment samples at around -22.00‰, with individual values being more depleted.
than the minimum for active sites. All δ^{13}C values prior to and after incubation, were within the range of inactive site sediment samples (-26.34 up to -18.96‰).

Salazar - Hermoso (2007) reported isotope values of δ^{13}C in 3 experimentally formulated diets made with varying proportions of plant and animal ingredients as well as a commercial diet. According to Salazar 2007, the experimental δ^{13}C diet signatures (-20.78‰, -19.55‰, -19.86‰) and commercial δ^{13}C signatures (21.08‰) were very similar and within the range of the δ^{13}C values measured in present incubation study (commercial feed values: -18.77‰, -22.19‰; feeds from active sites: -21.07‰, -17.32‰). In addition, Salazar – Hermoso (2007), collected faeces produced from each of the four diets above, and the values of δ^{13}C for the experimental diets ranged from -20.80 to -21.76‰ which is very similar to the range of values obtained in incubated samples and only slightly depleted compared to average feed values found in this study.

By contrast the commercial diet used by Salazar-Hermoso (2007), produced unexpectedly depleted values of the δ^{13}C for the faeces samples, with an average value of -23.22‰ being more depleted in comparison to the incubated feed samples used for this experiment. However, feed sampled from an active site by Salazar –Hermoso (2007) produced a δ^{13}C value of -20.12‰ which is more consistent with the feed tested in this study (-18.77‰, -22.19‰) and slightly depleted compared to the faeces prior to the incubation (-20.53‰, -22.5‰). Compared to δ^{13}C values of sediment samples collected by Salazar- Hermoso (2007) an active site, δ^{13}C values of faeces incubated in this study were enriched. Kullman et al. (2009), recorded similar feed and faeces δ^{13}C values with ranges of -21.25‰, -19.31‰ and a single value of 21.47‰, respectively.
The $\delta^{15}$N value of incubation samples showed a greater fractionation shift toward enrichment than carbon and at time zero (0h) had a range of 3.69 to 5.42‰ and mean of 4.92 ‰ which closely reflects the range and mean of $\delta^{15}$N values of feed used, 4.93‰ to 8.3‰ and 5.61‰ respectively. The mean $\delta^{15}$N value at time zero (0h) of 4.92‰, falls within the range of active (3.27 - 5.87‰) and inactive (4.02 - 12.12‰) aquaculture site sediment samples, described in Chapter 2, as well as the active sites commercial feed range of 4.89 up to 9.66‰.

In general, $\delta^{15}$N values prior to incubation were most similar and slightly enriched relative to active site sediment samples (mean 4.66‰) and depleted compared to inactive site sediment samples (mean 7.35‰) and the feed from active sites (mean 6.17‰). The $\delta^{15}$N values recorded by Salazar 2007 (unpublished data) for formulated diets (9.35‰, 7.09‰, 5.27‰) and a commercial diet (6.12‰) were more enriched than $\delta^{15}$N values prior to incubation.

In addition $\delta^{15}$N values of faeces generated from the formulated diet (9.28‰, 8.08‰, 6.37‰) and commercial diets (8.74‰) Salazar – Hermoso (2007) were more enriched than incubation samples at time zero (4.92‰) obtained in current study. Incubated samples were more similar and slightly enriched compared to $\delta^{15}$N values of sediment samples collected by Salazar – Hermoso 2007 at an active site, range of 3.63-4.26 ‰ and mean of 4.06‰.

However, after 840hrs of incubation the $\delta^{15}$N values had a mean of 6.71‰ and a range of 5.33 to 9.71‰. The $\delta^{15}$N values after incubation are even more enriched compared to mean active site sediment sample values of 4.66‰ and closer to inactive site sample $\delta^{15}$N values than before incubation, although still slightly depleted. Also, the mean $\delta^{15}$N value at 840h was more
enriched than mean of the feed δ¹⁵N values used in the incubation experiment (5.61‰) and at active sites (6.17‰) as discussed in chapter 2. Compared to δ¹⁵N values of formulated diets (9.28‰, 8.08‰, 6.37‰) and commercial feed (8.74‰) used by Salazar – Hermoso 2007 , with the exception of one diet formulated with low fishmeal content (15%), mean δ¹⁵N values of incubation samples were depleted, however samples at 840h and 25°C were very similar ranging from 7.31 to 9.71‰.

All incubation samples were enriched in δ¹⁵N compared to mean δ¹⁵N values of sediment samples collected at an active site (4.06‰) and control sites in Lake Wolsey (3.93‰). Compared to δ¹⁵N values of feed (6.34‰, 6.84‰, 7.35‰) and faeces (7.18‰) measured by Kullman et al. (2009), incubation samples at time zero (mean 4.92‰) were depleted and over the course of incubation became very similar (mean 6.71‰), with values at 840h and 25°C becoming more enriched (mean of 8.38‰).

The observed enrichment of the δ¹⁵N during biodegradation of fish faeces was likely primarily caused by the rate and fractionation effect of the nitrification reaction (as it requires oxygen, it is often the rate limiting step in converting ammonia to nitrogen gas) and to a lesser extent denitrification. Erler and Eyre (2010) quantified nitrogen process rates using δ¹⁵N in a constructed wetland treating domestic effluent and results showed an enrichment of all nutrient compartments which was postulated to be have been driven by high rates of nitrification enriching the residual pool of NH₄⁺ which is then mineralized and incorporated into the wetland sediment, plants and fauna. Similarly in an experimental finfish aquaculture farm at the Experimental Lakes Area in north-western Ontario, Kullman et al. (2009) found that introduced
aquaculture waste caused a shift in $\delta^{15}$N of littoral, pelagic, and profundal invertebrates and minnows towards the signature of fish feed, where minnows sampled had $\delta^{15}$N values closest to the feed.

Nitrification and denitrification reaction rates themselves depend on environmental conditions such as organic substrate type and fluxes and loading of new substrate, lighting, temperature, pH, redox potential, available oxygen, relative abundance of other microbes such as sulfur reducing bacteria, methanogens and other chemotrophs. Population dynamics of biodegrading microbial communities are not well understood however it is known for example that some microbes such as sulfur reducing bacteria can change the biodegradation environment through the release of toxic hydrogen sulfide which inhibits nitrifying bacteria (Joye and Hollibaugh, 1995). Joye and Hollibaugh (1995) found that prior hydrogen sulfide exposure of estuarine sediment bacteria reduced nitrification for minimum of 24 h and that nitrification rates resume slowly. In the absence of inhibiting factors, coupled nitrification and denitrification are major degradation pathways in which the microbes create a chain reaction where the bacteria *Nitrosomonas* oxidizes $\text{NH}_4^+$ to $\text{NO}_2^-$, the substrate for the next bacteria, *Nitrobacter*, thus providing energy for cell growth through carbon fixation, which is exactly what occurs at the sediment water interface of settled aquaculture waste (Lehmann et al., 2003; Reinhardt et al., 2006; Lehmann et al., 2004). *Nitrobacter* subsequently oxidizes $\text{NO}_2^-$ to the less toxic $\text{NO}_3^-$ which is in turn reduced to $\text{N}_2(g)$ by the microbe *Pseudomonas*.

Overlying water supplies the top sediment layer with oxygen and sediment oxygen penetrating depth varies depending on sediment porosity, type and grain, oxygen saturation, temperature and
water flow. It has been shown that nitrifying and to a lesser extent denitrifying bacteria
discriminate between the heavy $\delta^{15}\text{N}$ and light $\delta^{14}\text{N}$ nitrogen isotope (Lehmann et al., 2003;
Lehmann et al., 2004; Delwiche and Steyn, 1970; Franco-Nava et al., 2004; MacGregor et al.,
2001; Macko et al., 1984; Mariotti et al., 1981; Peters et al., 1978). Results from previous
studies showed that an enriched residual fraction is formed following nitrification and
denitrification reactions (Handley and Raven, 1992; Barford et al., 1999; Casciotti et al., 2003;
Lehmann et al., 2004). The results show that although the bacterial biomass becomes depleted as
a result of assimilation of the lighter isotope into bacterial biomass at a faster rate, the released
nitrogen gas and left over ammonia at the end of denitrification is also depleted (Peters et al.
1978), resulting in overall enrichment of the residual fraction. These dynamic relationships
between microbes that are affected by a multitude of environmental factors and heavy $\delta^{15}\text{N}$
isotope discrimination are most probably responsible for the large variation in nitrogen isotope
signatures observed in the field as opposed to the uniform results obtained from the controlled
degradation experiment.

Furthermore, comparing the more sheltered and hypolimnetically isolated decommissioned sites
$\delta^{15}\text{N}$ signature to those of active sites, it can be argued that the highly enriched $\delta^{15}\text{N}$ values at the
decommissioned sites could be the result of a well-established, prolonged, relatively undisturbed
biodegradation (via nitrification and denitrification) that resulted in highly localized enriched
residual sediment fractions. Depending on the bathymetry and benthic community, sediments
directly next to these highly enriched fractions may well have been disturbed through
resuspension or benthic fauna activity resulting in a disturbance of the established microbial
community and mixing of the sediment, preventing further enrichment and leading to leaching and possible depletion of the δ^{15}N signature.

On the contrary, at the more exposed active sites, fresh sediment layers of faeces are continuously added, resuspension is more frequent and the bottom is more likely to be disturbed during farm operations, resulting in higher rates of leaching, lower rates of denitrification and therefore a more depleted and less variable δ^{15}N signature compared to the decommissioned sites. It should also be considered that more eutrophic environments and environments with well-established nitrifying and denitrifying microbial communities (optimal temperatures closer to 25-30°C) will respond quicker and assimilate ammonia faster than oligotrophic environments (especially colder ones) therefore suggesting the argument that although well flushed sites will have lower sedimentation rates directly beneath their cages, the waste that does settle will degrade slower than at a eutrophic site with higher sedimentation rates. This may partially explain the reason why some sites that are eutrophic do not accumulate waste beneath their cages.

Temperature is a major factor in the establishment of biodegrading microbial communities and despite the presence of nitrifying and denitrifying bacteria, the rate of biodegradation and relative abundance of different microbial species will directly correlate with changing temperature and environmental conditions (Capone and Kiene, 1988). The effect of temperature, regarding the δ^{15}N was consistent with the literature, showing an overall significant linear effect of enrichment at 25°C, the closest to optimal temperature treatment (25-30°C produces highest substrate
turnover rate for nitrosomas) for nitrifying and denitrifying bacteria (Bhaskar and Charyulu, 2005).

In addition there were two relatively weakly significant quadratic effects of temperature for the 168hr and 504hr incubation times. The quadratic effect at 168hrs maintains an increasing $\delta^{15}N$ signature and may likely reflect the exponential bacterial population growth, however the quadratic effect at 504hrs does not follow the same trend and unexpectedly decreases $\delta^{15}N$ signature during the 25°C treatment after linearly increasing from treatments of 4°C up to 16°C. The unexpectedly low $\delta^{15}N$ signature during the 504h treatment at 25°C may have been caused by a disturbance of those samples causing a slowing of the degradation process (i.e. fractionation) or by an inaccurate isotope measurement stemming from error in sample preparation or mass spectrometry analysis.

It should be noted that the samples used for incubation were relatively small and subject to variation in initial bacteria substrate dynamics. The linear effect of temperature was significant at all times after 48 hrs, which is supported by previous findings that suggested nitrifying bacteria require several days to fully establish themselves. The importance of temperature is further shown by the results of the effects of biodegradation, which were linearly significant only at the 25°C treatment, further supporting previous observations of maximum nitrifying activity between 25°C-30°C. Although there was a cubic effect of biodegradation time at 25°C, it is possible this occurred due to experimental error such as accidental disturbance of the sample and thus a breakdown of the microbial community dynamic and slowing of fractionation, accidental
exposure of the sample to light inhibiting nitrification, or an error occurred during the sample preparation for mass spectrometry.

The $\delta^{13}$C signature was much less variable than $\delta^{15}$N and was depleted during biodegradation, suggesting that microbial enzyme discrimination of the heavier carbon isotope is less than that of the carbon isotope. Also, carbon is used by microbes for cellular growth more than nitrogen and is therefore more assimilated into bacterial biomass (Ostrom et al., 1997). This assimilation of carbon for cell growth causes a large depleted bacterial biomass to form (McGloirdrick et al., 2008) while the carbon containing by-products, carbon dioxide and methane gas are released containing more of the heavy isotope (Galand et al., 2010). In addition, carbon fixation of CO$_2$ present is used by ammonia oxidizing bacteria as a carbon source for bacterial growth and since the lighter carbon isotope is assimilated faster it creates a depleted nitrifying and denitrifying bacterial biomass.

In addition, the CO$_2$ that escapes as a gas depletes the residual fraction as well. Methanogens and methanotrophs have been shown to significantly fractionate the $\delta^{13}$C during methanogenesis as well as carbon assimilation for cell growth (Goevert and Conrad, 2009). Overall the $\delta^{13}$C results supported the results for nitrogen by reflecting the same pattern of fractionation although in the opposite direction, depletion. There was a significant linear effect of time and temperature (depleting effect) and the linear effects, similarly to the $\delta^{15}$N results, were significant only after the 48hr incubation time, which further supports the argument that microbial communities take several days to establish themselves and begin significant biodegradation of the substrate.
In addition the quadratic effect of time was significant only at the 25°C treatment supporting the argument that optimum microbial degradation rates occur at temperatures range from 25°C to 30°C and are exponential. Since no cubic effects were observed for carbon at the same treatment levels where cubic effects were significant for δ¹⁵N, it can be presumed that the likelihood of experimental error during sample incubation or sample preparation for isotope measurement is low and may suggest the cause to be the establishment of nitrifying and denitrifying bacteria differentially than at other treatment levels due to minute differences in sample composition which may have facilitated the accelerated growth of one microbe species over another.

The current incubation study was not set up as a complete anaerobic system however the large BOD of the substrate and minimal oxygen access (paraffin cap with pinhole) resulted in a dynamic degradation through aerobic and anaerobic processes similar to those that occur in sediments at the sampling sites, coupling nitrification at the sediment water interface with denitrification in buried sediment, also referred to as “benthic-pelagic” coupling (Capone, 1988).
Figure 24. Schematic of the benthic nitrogen cycle showing major degradation pathways associated with organic degradation.

4.6 General Conclusions

Overall the incubation study results prove that significant changes in $\delta^{13}$C and $\delta^{15}$N signatures of salmonid faeces exposed to biodegradation are consistent and can be accurately measured over different time intervals under laboratory conditions. In addition the change in the rate of fractionation over a broad temperature range of treatments is significant and was consistently reflected in $\delta^{13}$C and $\delta^{15}$N signatures suggesting strong potential for use as an environmental parameter in monitoring the fate of waste in open water aquaculture. For example $\delta^{13}$C and $\delta^{15}$N signatures could be used in conjunction with aquaculture waste dispersal model physical parameters such as the amount of waste, settling velocity, solid waste composition, faecal density and stability to demonstrate sustainable thresholds of nutrient assimilative capacity, where the
isotopes could potentially conclusively identify and trace deposition of organics to the benthos as well as pelagic ecosystem transfer (Reid et al., 2009).
Chapter 5. Overall Conclusion and Recommendations

Field sampling failed to significantly correlate feed signatures used at farms with sediment samples collected below cages. The higher variance and similarly enriched values of $\delta^{15}\text{N}$ signatures of samples at decommissioned sites relative to $\delta^{15}\text{N}$ enrichment during the incubation experiment, as well as the higher variance of $\delta^{13}\text{C}$ and similarly depleted values at decommissioned sites and in feeds (from active sites) relative to $\delta^{13}\text{C}$ signatures in active site sediments suggests $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have potential to identify aquaculture waste below active and inactive sites. However, the distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and patterns of enrichment and depletion both in the field and laboratory setting, suggest further research is required to quantify fractionation effects due to biodegradation in order for the isotopes to be used as tracers of aquaculture waste in the field.

In addition the quantification of fractionation effects due to biodegradation can lead to the development of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as tools in determining a site’s assimilative capacity and benthic pattern of waste degradation. In general, field results demonstrated the potential for naturally occurring $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in aquaculture feed to be significantly fractionated causing isotope signatures to be highly variable even at heavy point source loading of an enriched waste fraction. Despite signature shifts being detrimental for isotope use as a tracer of aquaculture waste, the presence of enriched $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ at aquaculture sites that have been inactive for several years suggests potential in identifying aged deposits of aquaculture waste undergoing biodegradation.

Furthermore, the isotope shifts reflect specific degradation pathways such as nitrification and denitrification as well as methanogenesis which can be used in continuous monitoring of active
and inactive sites assimilation of settled wastes by identifying rates of degradation and spatial
distribution of microbial activity in the footprint zone. This information could be used in
assessing a sites potential for expansion or down scaling due to differences in waste assimilation.

Also, sites being considered for aquaculture can be examined using stable $\delta^{13}$C and $\delta^{15}$N to
determine biochemical activity in the sediment prior to and after loading which would help
predict the fate of waste from a long term operation. Even more information could be obtained
from stable isotope samples of sediments by using different sampling methods to an Eckman
grab, such as a core sample. Eckman grabs often disturb the sediment water interface layer
where denitrification and nitrification are coupled within only a few millimetres or a maximum
of several centimeters where the anoxic sediment meets the oxygen supplied surface layer at the
interface. This disturbance removes the possibility of measuring the isotope signature in a
sediment sample subjected to aerobic and anaerobic degradation separately and instead mixes the
two layers. In addition, Eckman grab has a limited sampling depth such that the well-established
anaerobic denitrification layer, responsible for much of the fractionation effect, is ultimately not
sampled. Core samples would allow researchers to correlate isotope changes with changing
sediment depth which could reveal a more precise fractionation path of $\delta^{13}$C and $\delta^{15}$N as well as
a potential pattern of isotope signature distribution unique to deep organically rich sediments
created from aquaculture waste deposition. Core sampling would also allow other sediment
parameters such as temperature and redox potential to be correlated with changing sediment
depth and $\delta^{13}$C and $\delta^{15}$N isotopic signatures.
Although δ^{13}C and δ^{15}N measurements were not correlated with sediment parameters other than temperature and biodegradation, it would be of interest to include parameters such as redox potential, hydrogen sulfide and methane concentrations, as well as the use of metal compounds often associated with sediments with a high negative redox potential. Metals of particular interest would be manganese, zinc, copper and iron due to their natural occurrence in feed, low abundance in freshwater systems and high affinity for anions present in aquaculture waste. Their elevated concentrations would likely correlate with levels of waste, degree of biodegradation and consequently rate of fractionation and isotope signature shifts. The correlation of additional parameters would allow one to identify the presence or past presence of organic material and its state of degradation despite a lack of isotope signature. Although δ^{13}C and δ^{15}N measurements are required for positive identification of aquaculture waste, the presence of other parameters can give insight into the fate of the signature.

In order to more precisely determine the suitability of δ^{13}C and δ^{15}N as tracers of aquaculture waste future studies should also collect data before, during and post aquaculture activities. Collecting data on changes in site benthic composition, oxygen profile and sediment redox potential, hydrodynamics of surrounding water column, metal compound concentrations along with sediment temperatures and isotope signatures will give greater insight into how a site progresses biologically, chemically and physically during the establishment and cessation of an open water cage aquaculture site. This information can then be streamlined to include the most indicative parameters that along with δ^{13}C and δ^{15}N can be used in not just tracer studies but also in determining the waste assimilation dynamics of a site.
Open water sites show specific waste deposition patterns unique to each site where one site may accumulate large quantities of waste directly beneath a cage and another site does not accumulate any waste sediment due to higher flushing rates and resuspension. Future studies could also include tests on the effects of sediment disturbance or resuspension on the rate of biodegradation, comparing aerobic and anaerobic degradation, specifically the difference in rates of fractionation between nitrification and denitrification.

Using naturally occurring δ^{13}C and δ^{15}N in commercial feeds for the purpose of tracking faecal waste at open water sites could be improved by measuring variation in δ^{13}C and δ^{15}N signatures of feed batches over time (due to changes in ingredient sourcing as well as proportions), in addition to long term sampling of background levels of δ^{13}C and δ^{15}N near and distant from proposed cage sites of which natural variation can be used to more accurately measure and attribute differences in receiving environment δ^{13}C and δ^{15}N levels to site operation. Future studies on the contribution of δ^{13}C and δ^{15}N from isolated feed ingredients (in particular commercial brands) to the overall signature of formulated feed could make it possible to study biodegradation and its effect on δ^{13}C and δ^{15}N shifts of individual and or combinations of ingredients. Data from such studies could then be used in conjunction with studies on biophysical properties of salmonid faeces to more accurately design aquaculture waste dispersal models, where δ^{13}C and δ^{15}N signatures of specific feed ingredients could trace and identify specific faecal waste fractions throughout the receiving environment.

Another recommendation for future research in δ^{13}C and δ^{15}N organic waste tracking is identifying naturally occurring bacterial populations within the benthic receiving environment prior to and during aquaculture activities, and then incorporating these strains of bacteria in
further studies of their effect on the persistence of $\delta^{13}$C and $\delta^{15}$N in faecal waste during biodegradation. By identifying the major bacteria strains within the sediment below a cage site, laboratory experiments can be designed to reflect more precisely the natural biodegradation pathways of faecal waste at a given site and their fractionation effects which can be used to better predict and interpret $\delta^{13}$C and $\delta^{15}$N results from the field.

In addition, sediment thermistors can be applied at aquaculture sites to establish sediment temperature profiles used in controlled faecal incubation experiments to predict expected changes in $\delta^{13}$C and $\delta^{15}$N of organic waste at different temperatures. Additional research in freshwater sediments using deep cores, with larger sample sizes and more parameters covering a larger area than the footprint zone would give a better idea of the dynamics of C and N cycling around cage aquaculture sites.

This study has revealed further potential uses of $\delta^{13}$C and $\delta^{15}$N isotopes in the aquaculture industry in addition to traceability of waste effluent, although the potential for $\delta^{13}$C and $\delta^{15}$N isotope use solely as tracers is limited and warrants further research into fractionation effects due to biological degradation. Bacteria substrate dynamics are complex and require laboratory controlled incubation experiments to determine specific fractionation pathways in order to explain results obtained in the field. Furthermore, alternatives to tracking waste from aquaculture sites using $\delta^{13}$C and $\delta^{15}$N isotopes should be explored, such as research into incorporating genetic markers into commercial feed and subsequent identification in the field, the labelling of feed with specific isotope signatures and applying more advanced sediment sampling techniques.
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APPENDIX I

Wire frame charts from period of July 30 to August 27th for all of 8 sites tested

Site 1

Figure 25. Wireframe plot of temperature profile for Site 1 July 30 to August 13, 2007
Figure 26. Wireframe plot of temperature profile for Site 1 from August 13 to August 27, 2007
Figure 27. Wireframe plot of temperature profile for Site 1 August 27 to September 10, 2007
Site 2

Figure 28. Wire frame chart for Site 2 July 30 to August 13, 2007
Figure 29. Wire frame chart for Site 2 August 13 to August 27, 2007
Figure 30. Wire frame chart for Site 2 August 27 to September 10, 2007
Figure 31. Wire frame chart for site 3 July 30 to August 13, 2007
Figure 32. Wire frame chart for site 3 August 13 to August 27, 2007
Figure 33. Wire frame chart for site 3 August 27 to Sept 10, 2007
Site 4

Figure 34. Wire frame plot for site 4 July 30 to August 13, 2007
Figure 35. Wire frame plot for site 4 from August 13 to August 27, 2007
Figure 36. Wire frame plot for site 4 from August 27 to September 10, 2007
Figure 37. Wire frame chart for site 5 July 30 to August 13, 2007
Figure 38. Wire frame plot for site 5 from August 13 to August 27, 2007
Figure 39. Wire frame chart for site 5 from August 27 to September 10, 2010.

Site 6
Figure 40. Wire frame diagram for site 6 from July 30 to August 13, 2007
Figure 41. Wire frame diagram for site 6 from August 13 to August 27, 2007
Figure 42. Wire frame diagram for site 6 from August 27 to September 10, 2070
Site 7

Figure 43. Wire frame diagram for site 7 from July 30 to August 13, 2007
Figure 44. Wire frame diagram for site 7 from August 13 to August 27, 2007
Figure 45. Wire frame diagram for site 7 from August 27 to September 10, 2070
Figure 46. Wire frame chart diagram for site 8 from July 30 to August 13, 2007
Figure 47. Wire frame diagram for site 8 from August 13 to August 27, 2007
Figure 48. Wire frame diagram for site 8 from August 27 to September 10, 2007
APPENDIX II

Spectral density plots
Spectral Density Estimate by Period (0.72) for CWEI Depth 0.55

Spectral Density Estimate by Period (0.72) for CWEI Depth 4.5 SJ

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