Effect of waste loading from freshwater cage aquaculture on benthic invertebrates and sediment chemistry

By

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Abstract

This study combined sedimentation, sediment chemistry and benthic community data from three *Oncorhynchus mykiss* cage farms in Ontario, along with a laboratory bioassay to examine the effects of aquaculture waste loading. Waste loading rates, as well as sediment TC, TN, TP and metal (Cu, Zn) concentrations were highest beneath the cage and decreased exponentially with distance. During the 21-day bioassay, *Tubifex* (Oligochaeta, Naididae), *Chironomus* (Diptera, Chironomidae) and *Sphaerium* (Bivalvia, Pisidiidae) were subjected to a gradient of waste loading. Survival and growth of *Sphaerium simile* was highest at intermediate levels of waste loading. *Chironomus riparius* growth increased with increasing waste addition. *Tubifex tubifex* growth increased with exposure to fish waste, compared with the control. Until this study, there were no predictions for thresholds of effect at freshwater cage farms in the literature. The proposed threshold of effect on freshwater benthos is 2.0 - 3.0 g C m^{-2} day^{-1}.

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Dedication

This thesis is dedicated in loving memory of my mother, S. Jill Wetton (1952-2012). Her battle with breast cancer taught me to face a difficult challenge one day at a time, despite how overwhelming it might seem. I hope I made her proud.

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1.1. State of world fisheries

It was once thought that the aquatic environment contained a limitless supply of fish to feed the increasing human population (Tidwell and Allan 2002). The world population has surpassed 6.8 billion, and fishing has become far more industrialized (FAO 2010). Demands for food fish now far exceed the sustainable yield (FAO 2010). Increased demand has intensified the pressure on the harvesters, which has translated into increased overfishing of many commercial fisheries (FAO 2010). In 2008, 53% of stocks were fully exploited, (*i.e.*, producing catches at or close to their maximum sustainable limits), and 28% of stocks were overexploited or depleted (FAO 2010). The worldwide consumption of food fish is increasing, having risen from 40 million tonnes in 1970 to 117.8 million tonnes in 2009 (FAO 2010). The Food and Agriculture Organization (FAO) of the United Nations estimated that about 3.0 billion people worldwide rely on fish for 15% of their average intake of animal protein (FAO 2010). Given the projected population growth over the next two decades, it is estimated that at least an additional 40 million tonnes of fish will be required by 2030 to maintain the current rate of consumption (FAO 2006).

To meet the increasing demand for fish, aquaculture has expanded rapidly to become the world's fastest growing animal food-producing sector (FAO 2002). While capture fisheries production stopped growing in the mid-1980s, the aquaculture sector has continued to grow at an average of 8.7% per year worldwide (FAO 2008). Fish produced by marine and freshwater aquaculture account for over half of the world's supply for

human consumption (FAO 2010). World aquaculture has grown tremendously during the last 50 years from a production of less than a million tonnes in the early 1950s to 52.5 million tonnes by 2008 (FAO 2010). The Asia-Pacific region is the world leader in terms of aquaculture, contributing 88.8% to worldwide production (FAO 2010).

1.2. Aquaculture

Aquaculture, or the farming of aquatic organisms, is achieved through the manipulation of the life cycle of an organism and control of the environmental factors which influence it (Beveridge 1987). Aquaculture began over 2000 years ago as a means of subsistence farming in China, where farmers would culture common carp (*Cyprinus carpi* Linnaeus*)* in their rice fields (Beveridge and Stewart 1998; Moccia and Bevan 2007). Aquaculture involves three main factors: the control of reproduction, control of growth and the elimination of natural causes of mortality (Beveridge 1987). Capture fisheries and aquaculture have a similar aim: to maximize the yield of organisms from the aquatic environment (Beveridge 1987). However, two criteria differentiate aquaculture from capture fisheries: ownership of stock, and intervention in the production cycle (husbandry) (Naylor *et al.* 2000).

The majority of worldwide aquaculture production (59.9%) uses freshwater as compared to seawater (32.4%) or brackish water (7.7%) (FAO 2010). In 2008, freshwater fishes represented the largest proportion of world aquaculture production at 28.8 million tonnes, followed by molluscs (13.1 million tonnes), crustaceans (5 million tonnes), diadromous fishes (3.3 million tonnes), marine fishes (1.8 million tonnes), and other aquatic animals (0.6 million tonnes) (FAO 2010). Freshwater finfish farms can be on land

(ponds, raceways, tanks) or water-based (natural-based enclosures, pens with natural bottoms, or cages) (Beveridge 1987).

1.3. Freshwater net-pen aquaculture operations

Cage farming is a water-based system where fish are reared in lakes within a net pen or cage that permits the free flow of water (Beveridge 1987). A commercial farm may consist of many cages secured together, with each cage ranging in size from 100 to 300 m^2 in surface area (Beveridge and Stewart 1998). Nutrition for finfish aquaculture is usually supplied by formulated feeds (Naylor *et al.* 1999). Feed wastage at modern farms is typically less than 5% (Cromey *et al*. 2002). Unlike land-based systems, in net-pen aquaculture, faecal material is released directly into the aquatic environment (Beveridge 1987; Reid *et al*. 2009).

In Canada, eleven freshwater cage farms are responsible for the majority of food fish production in freshwater (Northern Ontario Aquaculture Association (NOAA), pers. comm., January 19, 2012). In Ontario, 78% of the total rainbow trout (*Oncorhynchus mykiss* Walbaum) production is from net-pen culture operations (approximately 2964 tonnes) (Moccia and Bevan 2007). In the North Channel of Lake Huron and in Georgian Bay there are eight cage culture operations (NOAA, pers. comm.). Public opposition to net-pen farming has been generated over environmental concerns such as nutrient pollution, impact on benthic organisms, escapees, and disease transmission to wild fish populations (FAO 2006). To understand the actual effects of the aquaculture industry, the impact of farm operations on the receiving environment must be understood.

1.4. State of the aquaculture industry

Capture fishery production has plateaued since peaking in the 1980s, thus the dependence on aquaculture to meet growing demands for protein is expected to continue to rise (Boyd 2003; FAO 2002). However, growth of the aquaculture industry has not been uniform across the world (FAO 2010). The Latin American and Caribbean regions show the highest average annual growth (21.1%), whereas production growth in North America has been only 1.2% per year since 2000 (FAO 2010). Decisions affecting the expansion of the freshwater industry in Canada are based upon a cautious approach, as there is a scarcity of data to aid regulators in making informed decisions when granting licenses to new farm locations, or when reviewing proposed expansion plans for existing operations (Podemski and Blanchfield 2006). As aquaculture production intensifies, feed inputs increase and deposition of waste material will increase, which directly impacts the receiving waters and the underlying sediment (Lin and Yi 2003). Increased aquaculture development will require a larger share of natural resources, and will have a greater environmental impact than ever before (Lin and Yi 2003). Because of the expected worldwide growth of the aquaculture industry, it is important to understand its implications.

1.5. Literature review objectives

In this review of the literature, I shall focus on the effects of cage aquaculture on the benthic environment, and how the responses of the benthos can be measured in the field and predicted using biological assays in the laboratory. I shall provide the rationale for my thesis research where I investigated the effects of sedimentation of aquaculture waste

on benthic invertebrate communities under two commercial farms in Lake Huron, at an experimental farm and during a multispecies microcosm experiment in the laboratory. This information will assist in developing a monitoring strategy for commercial farms in Lake Huron, where the majority of commercial freshwater cage farming occurs in Canada.

1.6. The effects of cage aquaculture

Fish farming involves intensive management techniques with addition of high concentrations of biomass and the release of animal waste (Carroll *et al.* 2003; Bureau and Cho 1999). The greatest impact is a result of the release of nutrient-rich, untreated solid waste directly into the aquatic environment (Beveridge 1984). Impacts associated with cage aquaculture include increased nutrient levels in the sediment and in the water column, increased turbidity, reduced concentrations of dissolved oxygen, lower pH in the sediment, and altered benthic communities beneath the farms (*e.g.*, Rooney and Podemski 2009, 2010; Cornel and Whoriskey 1993; Gowen and Bradbury 1987; Beveridge 1984). The scale of the impact of a farm depends on the capacity of the site to assimilate farm waste, in relation to the loading rate and the degree of waste dispersal (FAO 2006; Carroll *et al.* 2003). The focus of the remainder of this literature review will be on the effects of waste loading on the benthic environment and the implications for the invertebrate community living in the immediate vicinity of aquaculture operations.

1.7. Sedimentation of farm waste

Lake-based cage aquaculture facilities are open systems in which wastes (uneaten food, faeces, fish scales, mucus and soluble wastes) are released directly into the water body (Kutti *et al*. 2007*a*; Tartari and Biasci 1997; Loch *et al.* 1996; Cornel and Whoriskey 1993). Sedimentation rates of organic matter increase depending on the type and location of the culture system (extensive, semi-intensive and intensive aquaculture in marine, brackish, or inland areas), the species under cultivation, and individual management practices, such as stocking density, cage configuration and feeding rates (Arthington and Bluhdorn 1996). Sedimentation rates can be measured using sediment traps which give an estimate of the scale and amount of waste dispersal from the farm (Droppo *et al.* 2007; Håkanson 1984; Staresinic *et al.* 1978). However, problems related to resuspension, mineralization, grazing, periphyton, and turbulence must be considered when using them for measuring waste deposition (Tartari and Biasci 1997). Researchers who use sediment traps are generally convinced that traps are the best tool to measure downward settling fluxes, and the instrumental errors associated with the traps are within reasonable limits (Bloesch and Burns 1980). Kalantzi and Karakassis (2006) showed that sedimentation rates are directly related to the intensity of fish farming, and therefore are important data to gather.

Much of the released material settles rapidly at or near the farm, though there is potential for some horizontal transport and dispersion (Cromey *et al.* 2002). Water depth and current velocity are critical factors determining patterns of sedimentation around cage sites (Cromey *et al.* 2002). Materials derived from fish farms sink rapidly because of

their large particle size compared to that of the ambient particulate matter (Cromey *et al.* 2002). Chen *et al.* (1999) estimated settling velocity for salmonid faecal matter at 3.7 to 9.2 cm/s. Rapp *et al.* (2007) measured sedimentation around a fish farm in Puerto Rico, moored in 27.2 meters of seawater with a current velocity of up to 40 cm/s. Ninety per cent of the solid waste fell within 30 m of the cage mooring block (Rapp *et al.* 2007). Holmer *et al.* (2007) deployed sedimentation traps along transects from three Mediterranean Sea fish farms. These farms were in 16 to 28 m of seawater and current speeds ranged from 5 to 20 cm/s (Holmer *et al.* 2007). Sedimentation rates decreased rapidly with increasing distance from the farms, diminishing by up to 91% at 40 m away from the net-pens (Holmer *et al.* 2007). At farms with little to no current, such as those in sheltered freshwater lakes, the dispersal of waste material should be minimal.

When comparing sedimentation rates at different cage aquaculture sites, attention must be paid to the feed conversion ratio (FCR), if available. The FCR is calculated as the number of kilograms of feed provided to produce one kilogram of fish. To minimize costs for farm operation, the farmer will try and keep their FCR at ≤1.2 (Boucher and Vandenberg 2005). Whoriskey and Smith (1988) measured sedimentation rates at a freshwater cage farm in Lac du Passage, Quebec. The sedimentation rate at the farm site was 5.5 times greater than at sampling stations >200 m and >600 m away from the cages. However, the reported FCR of the farm was 3.7:1, *i.e.*, it took 52 125 kg of feed to produce 14 tonnes of rainbow trout. With this high FCR, it is difficult to compare the results to modern operations due to more efficient feeds and feeding techniques. Weston (1990) measured sedimentation rates immediately below and near to a fish farm in Puget

Sound, Washington. Beneath the farm, 36.4 g/m^2/day of particulate organic matter were collected, compared to next to the cage where the sedimentation rate was 9.86 g/m^2/day (Weston 1990). The author failed to mention the FCR of the farm; however, he noted the sediment surface below the farm was covered in undigested pellets, indicating a high percentage of waste feed. It is important to consider the improved palatability and digestibility of feeds, and therefore the reduction in solid waste, when comparing the results in older literature to modern commercial farms. Estimates of feed wastage of up to 20% by Gowen and Bradbury (1987) are no longer expected, as farmers have a strong financial interest in keeping waste feed to a minimum.

1.8. Modelling the deposition of farm waste

Regulators monitor hydrodynamics, water quality and benthic conditions to evaluate environmental effects of net-pen farms (Dudley *et al.* 2000). The efficiency of this work would be enhanced through the use of models that incorporate information regarding the physical conditions of the area (Dudley *et al.* 2000). Models that simulate the sedimentation of waste material from cage farms are considered valuable management tools for predicting environmental impacts (Chamberlain and Stucchi 2007). A number of models have been used to link fish farming production, depth and current regime to sedimentation of waste material with distance from marine fish farms (Kalantzi and Karakassis 2006; Chamberlain and Stucchi 2007).

Aquaculture Waste Transport Simulator (AWATS) is a model that provides estimates of the dispersion of marine finfish aquaculture waste for regulatory use (Dudley *et al.* 2000). AWATS provides not only a profile of waste distribution, including complex

hydrodynamics and associated resuspension processes, but also information regarding spatial and temporal variations in current velocity (Dudley *et al.* 2000). This information could be used along with oxygen demand data to determine the intensity of waste loading has the potential to exceed the assimilative capacity of the environment (Dudley *et al.* 2000).

Another example of a model developed for monitoring deposition at marine aquaculture sites is DEPOMOD. This computer particle-tracking model was developed to enable better predictive capability of the impact from large marine cage fish farms on the benthos (Cromey *et al.* 2002). The model is used to predict accumulation of solids on the sediment and associated changes in the benthic community (Cromey *et al.* 2002). The goal of the model is to improve the site selection process by being able to predict the outcome of a proposed farm location and intensity of production (Cromey *et al.* 2002). DEPOMOD has been validated using sediment trap studies at a marine commercial fish farm in Scotland (Cromey *et al.* 2002). Predictions of waste loading under the cages using the model generally agreed ±13% with field data for a depositional site (Cromey *et al.* 2002). There is a need for the development of such a model for freshwater cage farms to assist regulators and farmers in making informed decisions on site-selection, stocking densities and cage configuration.

1.9. Nutrient enrichment of receiving environment

Cage aquaculture affects the benthic environment through the addition of solid waste, consisting primarily of fish faecal material, with some uneaten feed, scales and mucus (Walker *et al.* 2003; Camargo 1992; Gowen and Bradbury 1987). Approximately

3 to 10 kg of phosphorus and 39 to 55 kg of nitrogen are released to the environment for every metric tonne of fish produced (Bureau *et al.* 2003; Cho *et al.* 1994). The majority of phosphorus in fish waste settles quickly (≥6 cm/s) to the sediment in solid form (Azevedo *et al.* 2011; Reid *et al.* 2009; Phillips *et al.* 1995). The dissolved portion of the excreted material is largely composed of nitrogenous waste, such as ammonia and urea (Podemski and Blanchfield 2006). The amount of waste loading varies according to the size of the farm, the intensity of feeding, the feed conversion ratio (FCR), and the physical parameters of the receiving lake basin (Shikano and Kurihara 1988).

Although there may be significant variation in waste loading among farms, enrichment of the sediment below and immediately surrounding aquaculture operations has been documented at farms all over the world (*e.g.*, Rooney and Podemski 2010; Edgar *et al*. 2005; Lin and Yi 2003; Wildish *et al*. 2003; Ackefores and Enell 1994; Johnsen *et al.* 1993; Penczak *et al.* 1982). Hargrave *et al.* (1997) described organic enrichment due to sedimentation of organic matter from a farm in the Bay of Fundy on the east coast of Canada. The average value for sediment organic carbon was 40% higher than the value for reference sites, resulting from the direct sedimentation of particulate organic matter (Hargrave *et al.* 1997). At four Norwegian fish farms, total organic carbon levels in sediments were significantly higher immediately adjacent to cages compared to reference sites (Carroll *et al.* 2003). At a farm in Hong Kong, the total carbon, nitrogen, and phosphorus levels at the sampling station closest to the fish cages were 82.8%, 128.5% and 1315.7% higher, respectively than those of reference stations (Gao *et al*. 2005). The phosphorus load can be quite significant. An estimated 720-780 kg of phosphorus was loaded into Lac du Passage, Quebec per year for an annual trout

production of 14 tonnes (Cornel and Whoriskey 1993). However, this farm reported an FCR of 3.7:1 during the study, so this degree of waste must be taken into account when comparing these results with those of more efficient cage farms.

1.10. Effects of aquaculture solid waste on benthic invertebrates

When exposed to increased sedimentation from aquaculture, feeding and spawning habitats for benthic organisms may be affected due to an increase in turbidity, and the density of prey items subjected to inhospitable conditions or burial (Triffault-Bouchet *et al*. 2005; Wood and Armitage 1997; Camargo 1992). Sedimentation of wastes from cage aquaculture may alter substrate composition and stability which may result in some benthos being favoured at the expense of others (Wood and Armitage 1997; Death 1996). Pearson and Rosenberg (1978) described the decline of suspension feeders and increase in deposit feeders along a gradient of organic input. Hargrave *et al*. (1997) also found a significantly greater biomass of deposit feeders in the sediment under marine aquaculture cages (22 g/m^2) compared to sediment sampled at nearby reference locations (13 g/m^2). Deposit feeders such as the oligochaete worm, *Tubifex tubifex* (Müeller), may be better adapted to feed on the nutrient-rich material, whereas filter-feeding organisms such as the seed shrimp, Ostracoda, may have difficulty coping with increased suspended sediment (Wood and Armitage 1997). Rooney and Podemski (2009) examined the development of changes in zoobenthos along a transect from an experimental rainbow trout farm. They observed development of two distinct invertebrate communities: one occurring under the cage to 5 m beyond the cage edge, and another 15 to 45 m away from the cage (Rooney and Podemski 2009). The community close to the cage was composed of 75%

Chironomini larvae and 25% nematodes, while the community further from the cage was numerically dominated by Ostracoda, with presence of Nematoda, Orthocladiini, Tanytarsini and Sphaeriidae. Ostracods require a stable substrate (Delorme 2001) so reduced abundance may be due to the accumulation of organic matter and transformation of the substrate into a flocculent surface (Holmer *et al*. 2007).

Increased organic matter can modify sediment characteristics and promote chemical processes with products that can be detrimental to the benthos (Droppo *et al.* 2007; McGhie *et al.* 2000). This includes the stimulation of bacterial activity, which may result in oxygen depletion causing the production of anaerobic organic breakdown products such as sulphide and ammonia (Holmer *et al.* 2007). Ammonia is a toxic compound generated in natural waters and sediments as a primary by-product of organic matter decomposition by heterotrophic bacteria (Berner 1980). The loading of N-rich organic matter into the aquatic environment from aquaculture can increase the concentration of ammonia in the sediment pore-water (Whiteman *et al.* 1996; Snodgrass and Klapwijk 1984). In a study in Ontario, Canada, sediment quality was assessed at an experimental fish farm using sediment chemistry, benthic community analysis and pore-water ammonia concentrations (Rooney 2006). Pore-water total ammonia was the first variable to respond after the initiation of production (Rooney 2006). In the sediment under the cage, pore-water ammonia concentrations reached over 480 times the level in the reference sediment after only four months of production (Rooney 2006). In the same time period, the density of invertebrates under the cage was significantly reduced to 442 individuals m^{-2}, compared to 11089 individuals m^{-2} at reference stations (Rooney 2006). Rooney and Podemski (2010) suggested that measurement of pore-water ammonia be

included in freshwater sediment monitoring programs because of its rapid rate of response, sensitivity and biological significance.

1.11. Effect of environmental stress on benthic invertebrates

Benthic invertebrates are important in the function of natural ecosystems through a range of diverse activities, from assisting in decomposition of organic matter to providing food for higher trophic levels (*e.g.,* Nickell *et al*. 2003, Mirto *et al.* 2002; Rosenberg *et al.* 1986; McCall and Tevesz 1982; Brinkhurst 1974). Through their burrowing, feeding, locomotive, respiratory and excretory activities, benthic invertebrates control the cycling of energy, nutrients, and organic matter (Burton *et al.* 1992; Matisoff *et al.* 1985; McCall and Tevesz 1982; Brinkhurst 1974). Benthos live in intimate contact with bottom sediments and interstitial water for extended periods of their life cycles, and this increases the likelihood for adverse effects occurring in the presence of an environmental stressor within the sediment (Chapman 2007; Turesson *et al*. 2007; Burton *et al*. 1992). The overall measured response of the fauna to the stressor depends on whether the community is dominated by species which live deep within the sediment (mesobenthic, or true interstitial forms) or epibenthic and endobenthic species, where species exhibit surface-dwelling and shallow-burrowing lifestyles (Sutherland *et al.* 2007a). Epi- or endobenthic organisms may temporarily escape adverse conditions within the interstitial environment, whereas mesobenthos must tolerate the conditions in order to survive (Raffaelli 1987).

Benthic invertebrates respond to environmental change at longer temporal scales, integrating changes that occurred before the sampling event into their spatial pattern

(Pearson and Rosenberg 1978). When sampling water or sediment chemistry alone, the researcher is able to study the intensity of effects of the stressor on the habitat only. By including benthos in their sampling regime, the researcher is better equipped to interpret the effects of a stressor on the ecosystem (Kalantzi and Karakassis 2006).

The structure of aquatic ecosystems can be divided into successive layers of biological organization: individual, population, and community level (Attrill and Depledge 1997). Organism-level studies can be used to describe individual growth rates and physiological responses, whereas community-level studies allow examination of food webs and the indirect effects of stressors (Culp *et al.* 2000). It is important to explore how the effects on different levels of biological organization are linked to understand how individual stress responses may be expressed and which may produce community-level changes in stressed systems (Attrill and Depledge 1997).

1.12. Community-based responses to stress

Sheehan (1984*a*) defined communities as "assemblages of populations structured by biotic interactions and the constraints of their physical and chemical environment". The community is the most popular level of investigation for environmental assessment, and has been suggested as the most important level for impact studies (*e.g.*, Attrill and Depledge 1997; Martin and Richardson 1995; Clements and Kiffnet 1994; Warwick 1993) because it provides a multispecies response, likely over a wide taxonomic range with a range of sensitivities to a stressor (Attrill and Depledge 1997). On a temporal scale, changes in community structure reflect conditions integrated over a long period of time, or immediate changes due to catastrophic events (Attrill and Depledge 1997).

Endpoints that could be examined include abundance, biomass, composition, and species richness; as these properties summarize effects in the whole community. Analysis of the community may reveal those species most sensitive to the stressor, and assist in the prediction of consequences at the ecosystem level (Attrill and Depledge 1997).

Rapport *et al.* (1985) and Schindler (1987) compiled a list of symptoms of stress on a community. This list includes increased frequency of disease, a reduction in species richness and biomass, and retrogression to opportunist species (Schindler 1987; Rapport *et al.*1985). In undisturbed communities, larger bodied species with relatively longer life spans and more limited tolerances are present. In disturbed communities, species richness will be low as smaller organisms with shorter life spans, higher reproductive rates, and shorter food chains normally dominate the community (Cairns and Pratt 1989). This shift allows communities to maintain function and structure in the presence of a stressor (Schindler 1987).

Although reduction in species richness and biomass may be evidence of stress in some ecosystems (Cairns and Pratt 1989; Gray 1989; Mills 1971), this is dependent on the community being studied. At a deepwater salmon farm in Norway, invertebrate biomass was 35 times greater, within 250 m from the farm than 3 km away (Kutti *et al*. 2007*b*). Richness was highest at an intermediate distance (550-900 m) from the farm and at peak farm production, species richness doubled from 20 to 40 species per 0.1 m^2 (Kutti *et al*. 2007*b*). These observations correspond well with the intermediate disturbance hypothesis (Connell 1978). This hypothesis predicts that richness will be greatest in

communities subjected to moderate levels of disturbance (Connell 1978). Species richness is promoted by the spatial and temporal heterogeneity of the habitat resulting from the disturbance (Connell 1978). Deeper benthic communities are often limited by nutrient availability (Kutti *et al*. 2007*b*). Therefore, an increased supply of food to the local ecosystem, through a moderate and continuous loading of organic matter, may cause increased benthic species abundance and biomass of opportunistic species. The term 'opportunist' is applied to species whose reproductive and growth characteristics allow them to take advantage of a sudden environmental change, providing them with a favourable, unexploited niche (MacArthur 1960). This may be at the expense of some less tolerant species, so species richness may decline as a result. Sites that have been affected by high levels of organic loading from fish farming are often characterized by the dominance of one or two pollution-tolerant species (*e.g.*, Rooney and Podemski 2009; Kutti *et al*. 2007*b*; Dobrowolski 1987; Moore 1981).

There are several limitations to using community analysis in assessing the effects of a stressor. Community level analysis is not sensitive enough to determine deterioration in an ecosystem as early as could be detected using analysis at the individual level (Attrill and Depledge 1997). Following exposure to a stressor, the first effects can usually be detected as behavioural and physiological changes in individuals (Sibly 1996). Schindler (1987) felt that monitoring community-level changes would be a poor approach to detecting early signs of stress in an ecosystem. Once an effect is detectable at the community level, the system may have already been severely impacted. Another limitation of community-based analysis is that there are often no properly randomised controls in a field situation, so it is difficult to explain the influence of natural variables

(Attrill and Depledge 1997). The process of community-based analysis is also extremely labour-intensive and time-consuming, due to the expertise and person-hours required to sort and identify organisms from benthic samples.

1.13. Effects of a stressor at the population level

The effects of a stressor on a population can be examined by studying two components of life-history analysis: survivorship and reproduction (Sibly 1996). A species should theoretically be well-adapted to its environment, and any interference of normal patterns due to an environmental stressor can cause severe impacts (Sheehan 1984*b*). For example, prolonging of normal life history through delays in development or metamorphosis may alter population fitness (Sheehan 1984*b*). Many organisms reproduce at specific times, coupled with short adult lifespans; *i.e.*, mass emergence of mayflies. The overall reproductive success of such a population would decline if exposure to a stressor resulted in a loss of synchrony. The impact on one population may not change the function of the whole ecosystem (Sheehan 1984*b*). However, this depends on the type of organisms affected, and the diversity of the remaining community. Unless stressors can be shown to have an impact at the population level, they are unlikely to have detectable effects in the ecosystem (Gray 1989; Sheehan 1984*b*). For this reason, an assessment of the effect of a stressor, such as organic loading, must be examined on several levels of biological organization.

Stressors may damage organisms with immediate lethal consequences, or may have sublethal effects. Rand and Petrocelli (1985) discussed lethal effects as those that

caused death or failure to produce viable offspring, whereas sublethal effects include deleterious behavioural or physiological changes. Since the death of an individual is easily recognized in most cases, LC_{50} investigations of lethal effects are preferred for reasons of simplicity of interpretation. According to Rosenthal and Alderdice (1976), sublethal effects may be defined as "those responses to environmental changes, (histological, morphological, physiological, or ethological) that may be induced in one stage of development but be expressed at a later stage in terms of reduced survival potential". For example, sublethal impacts can involve effects on the "machinery of resource acquisition and uptake" such as mouthparts or digestive enzymes (Sibly 1996). This may not cause immediate death, but an organism may not be able to grow as efficiently if digestion has become more challenging. If energy is being partitioned to deal with the stressor, it is unavailable for growth and reproduction (Sibly 1996). The ability of individuals to reallocate energy contingent on their physiological status enables an organism to survive under a number of environmental circumstances.

1.14. Measuring stress in individual organisms

Stress in individual organisms is measured by the organism's ability to compensate for or to acclimate to the effects of a stressor (Maltby *et al.* 1990). Individual organism response to stress may be influenced by several factors, including sex, age, development stage, surface area, reproductive condition and nutritional status (Sheehan 1984*b*). Altered performance of individuals based on behavioural and biochemical changes from a stressor links individual organisms to community structure and dynamics (Verslycke *et al.* 2004; Sheehan 1984*b*). Behavioural changes such as avoidance may occur before any

effect is detected at higher levels of biological organization. In a study by Wentsel *et al.* (1977), midge larvae avoided sediments containing more than 8990 ppm Zn (dry weight). Although avoidance behaviour may be beneficial to avoid a brief exposure to a stressor, long term behaviour modification may prevent an organism from feeding or reproducing.

Adams (1990) defined stress at the level of the individual as the sum of all physiological responses that occur when animals attempt to establish or maintain homeostasis. Selye (1973) described the "general adaptation syndrome" model of the physiological response of an organism to a stressor. A single application of the stressor may lead to a measurable alarm response within the organism followed by compensation to the stressor and then a return to pre-stressor activity (Selye 1973). Measurable responses in individuals include biochemical, physiological and histological responses, which are linked to metabolism and energetics (Sheehan 1984*b*). Physiological energetics provide information on key processes in the organism's energy acquisition and expenditure (Verslycke *et al.* 2004). Those indices which best reflect individual performance and are most easily related to population fitness are growth and fecundity (Sheehan 1984*b*). Growth and fecundity are the net result of many essential processes such as consumption, excretion and respiration and, therefore, are a useful measure of physiological status (Maltby and Naylor 1990; Sheehan 1984*b*).

The effect of a stressor (*e.g.,* organic loading, high concentration of metals) on an individual can be measured by its effect on 'scope-for-growth' (SfG) (Sibly 1996; Maltby *et al.* 1990; Bayne 1975). Scope-for-growth is the difference between energy intake and total metabolic losses such as excretion and respiration, and measures the energy status of

an organism (Sheehan 1984*b;* Bayne 1975). If SfG has increased, the animal has more energy for growth and reproduction, and if SfG has decreased, the animal has less energy for these processes (Sibly 1996). Maltby and Naylor (1990) measured SfG in the amphipod, *Gammarus pulex* (Linnaeus), exposed to zinc at 0.3 mg L^{-1}. At this concentration, SfG was reduced, and there was a decrease in the size of offspring produced from a subsequent brood. A series of estimates of SfG allows the estimation of relative growth rate correlated with the intensity of the stressor (Sheehan 1984*b*). The growth and fecundity of individuals directly affects the survival of the population; therefore, stress-induced changes in SfG are indicative of more long-term population-level effects (Maltby and Naylor 1990). With to this ability to estimate effects on higher levels of organization, SfG might also be an effective method to estimate changes in growth with varying concentrations of organic matter from an aquaculture facility.

1.15. Observing biological responses to environmental stress

The use of life-history endpoints (*e.g.*, growth, survival and fecundity) as a measure of stress is common in environmental assessment studies (Giesy and Graney 1989). However, detecting a significant effect on these endpoints is difficult to measure in the field due to environmental variability. Therefore, there was a need to develop a method of standardizing conditions to examine the isolated impact of the stressor. Bioassays are a method of assessing the actual or potential impacts of stressors on the natural environment using the reaction of test species in the laboratory or a contained field experiment. The goal is to monitor or predict the effects of single compounds, elements or mixtures on the long-term health of individual organisms, populations, communities,

and ecosystems (Giesy and Graney 1989). The results can be used for comparison, to create meaningful regulations, for prediction of environmental effects, or for monitoring known stressors in the natural system (Cairns and Cherry 1993).

Scientists use biological assays to predict and monitor potential environmental impacts by screening new chemicals, testing effluent from factories, and monitoring sediment quality (Cairns and Pratt 1989). These tests are designed to study biological endpoints, which are used to determine the effects of certain intensities of a stressor to the test organisms (Malins 1989; Cairns and Pratt 1989). The response by the test species should be consistent, directly related to the environmental hazard, and easy to interpret for the public and government regulators (Cairns and Pratt 1989). The tests themselves should be rapid and inexpensive, with wide applicability and high sensitivity to stress (Cairns and Pratt 1989).

There are several examples in the literature where environmental bioassays have been used to predict ecological effects of stressors. Rooney (2006) suggested that bioassays using sediment from different distances from a cage farm could be used to differentiate between changes due to sediment chemistry and the effect of increased sedimentation and invertebrate burial. Kullman *et al*. (2007) developed a bioassay to determine whether the survival, growth and reproduction of a fingernail clam, *Sphaerium simile* (Say), would be affected by exposure to sediment collected from varying distances from an aquaculture cage. One hundred per cent of the sphaeriid clams exposed to sediment from directly under the cage died; however, those clams exposed to sediment 1

m from the cage had the greatest increase in length, and most embryos after six weeks of exposure (Kullman *et al*. 2007). Using endpoints such as survival, growth and fecundity, the authors concluded that the environment beneath the cage was inhospitable to some benthic organisms; however, organisms living just beyond the cage boundary might have been able to feed on and benefit from settled aquaculture waste (Kullman *et al*. 2007). Without testing the effects of a range of waste concentrations, one might have wrongly concluded that aquaculture waste is completely toxic to benthic organisms. This ability to determine the level of risk to the organisms at varying levels of an environmental stressor is one benefit of ecotoxicological testing.

The literature describing biological assays for freshwater systems has been reviewed and classified into two types: studies concerned with prediction or screening of effects, and those involving the assessment and monitoring of impacted environments. For the predictive tests, a stressor is applied to the system and a response is described that should be interpreted relative to its implications in the field (Calow 1989; Maltby and Calow 1989). In monitoring mode, the impacts of a stressor are studied by assessment of the intensity of the disturbance that is being applied to it (Calow 1989). Direct correlation to field effects is important for monitoring assays which are designed to address specific questions for the affected environments (Giesy and Hoke 1989). Within these divisions, bioassays are also classified into single- and multi-species tests, which are then categorized by the scale of the enclosures. The choice of the test depends on the fundamental scientific questions and the needs of regulators (Calow 1989). Different levels of testing form a range of simplicity, repeatability, complexity and applicability

(Buikema and Voshell 1993). These range from highly artificial single-species tests (acute and chronic), to multispecies tests, to those done in the field within natural ecosystems (Buikema and Voshell 1993). The precision is high but accuracy low in single-species tests and the reverse is true in tests using natural communities.

1.16. Single-species biological assays

Aquatic toxicology developed from mammalian toxicology which was designed for the protection of one species: *Homo sapiens* (Linnaeus) (Cairns and Pratt 1989). This may have contributed to the belief that single-species bioassays were appropriate for environmental toxicology (Cairns and Pratt 1989). Single-species tests have historically been the source of biological data for hazard assessment as they are simple test systems, focusing on a few well understood species, and the tests developed can be run by moderately trained people (Sánchez and Tarazona 2002). The use of single-species tests for sediment toxicity testing began with freshwater benthos, *i.e.*, the mayfly larvae *Hexagenia limbata* (Serville) (Prater and Anderson 1977), and the midge larvae, *Chironomus tentans* (Fabricius), in 1977 (Wentsel *et al*. 1977). The authors indicated survival, growth and emergence were related to bulk sediment contaminant concentrations (Prater and Anderson 1977; Wentsel *et al*. 1977). The toxicity of contaminated freshwater sediments is now most often assessed using short-term (3-14 days) monospecific bioassays with benthic species such as the amphipod, *Hyalella azteca* (Saussure), and midge larvae, *Chironomus riparius* (Meigen), or *C. tentans* (Clément *et al*. 2004; Burton *et al.* 1992). Two types of single-species bioassays are listed in the literature based on the duration of the test: acute and chronic. Though experimental

procedures may be similar, acute and chronic bioassays vary greatly in their ability to describe ecologically relevant endpoints such as survival, growth and reproduction.

Acute bioassays are the most developed and standardized test methods in aquatic toxicology. They are relatively rapid, simple and easy to control in a laboratory (Maltby and Calow 1989). Acute bioassays can be easily replicated; therefore, variability can be statistically tested (Kimball and Levin 1985). The length of acute bioassays is limited (48-96 hrs) because organisms used are not fed during the test (Buikema and Voshell 1993). The acceptance of standardized measures (or endpoints) provides a basis to use the results in establishing regulations to limit the effect of the stressor (Kimball and Levin 1985). Regardless of whether or not the concentration causing mortality is ecologically relevant, regulators often use LC_{50} values, or the lethal concentration in half of the test subjects within a given time period, to communicate possible risk to aquatic communities (Kimball and Levin 1985).

Chronic single-species biological assays typically are conducted to examine growth, reproduction, as well as lethality. Endpoints such as growth or reproduction are more sensitive discriminatory measurements of biological effects than acute tests, as the test occurs over a longer portion of the life cycle (Reynoldson *et al*. 1991; Buikema and Voshell 1993). There are three types of chronic bioassays: 1) a life cycle test, where the duration is one generation and endpoints include survival, growth and reproduction, 2) a sub-life cycle test, carried out during an early life stage to test the response to a stressor in terms of mortality, growth, behaviour, and metamorphosis, and 3) life history, where the bioassay lasts from egg to death or cessation of reproduction and changes in mortality,

growth, behaviour, metamorphosis, and reproduction between treatments are used as test endpoints (Buikema and Voshell 1993). The choice of test depends on the purpose of the bioassay and the needs of the regulators.

1.17. Limitations of single-species tests

The application of single-species laboratory tests to accurately predict the effects of stressors on communities has been widely debated (*e.g.*, Maltby and Calow 1989; Calow 1989). These tests can be used to assay the direct effects of stressors on individuals; however, they cannot provide information on the impact of indirect effects such as interspecific interactions (Maltby and Calow 1989). The behaviour of large-scale, complex systems cannot be predicted from the responses of its subunits (Cairns 1983; Kimball and Levin 1985). The importance of information such as the interactions among individuals and between species cannot be ignored (Kimball and Levin 1985). The difficulties in extrapolating data from single-species bioassays are well recognized and adequately documented. There can be variation in response of test individuals within the same species that are the same age under control conditions (Cairns and Pratt 1989; Slooff 1985). The variation becomes greater when a population of organisms containing various ages, condition or genetic strains are tested (Slooff 1985). This makes extrapolation to the natural ecosystem difficult as it is unclear if the response to the stressor was due to the stressor itself or if it was due to some combination of organism health and laboratory conditions.

Most of the basic scientific questions about how to extrapolate from one level of biological organization to another are unanswered (Cairns and Pratt 1989). The confidence we can place on bioassays would be improved if more work was done to validate these predictions in the field (Cairns and Pratt 1989). Attempting to estimate population data from single-species experiments to describe effects on emergent properties of systems is a correlation that is difficult to justify (Cairns and Pratt 1989). Single-species tests do not provide information on possible higher order effects such as changes in ecosystem energy flow or nutrient cycling, both of which play a large part in ecosystem level effects (Pontasch 1995; Cairns and Pratt 1989; Slooff 1985). Therefore, single-species tests may be unsuitable for determining ecologically acceptable stressor concentrations with a high degree of reliability, and not acceptable for predicting community level effects of aquaculture waste sedimentation on benthic invertebrates.

1.18. Multispecies biological assays

There are plenty of alternative testing strategies to single-species toxicity tests, which include a wide range of experimental designs, from simple indoor multispecies assemblages to field experiments (Sánchez and Tarazona 2002). Giesy and Allred (1985) suggested that by testing many species simultaneously, multispecies tests may be more realistic than several single-species tests in estimating the range of responses of organisms to a stressor. Multispecies tests use artificial assemblages or components of natural communities and allow studies of community dynamics in relatively long term or periodic exposures (Buikema and Voshell 1993). These tests range from small-scale, two-component systems with life-history endpoints, to whole ecosystem manipulations

examining endpoints such as diversity, abundance and biomass (Cairns and Cherry 1993). Organisms can interact with each other, and therefore can influence other species' responses and partitioning of the stressor between abiotic and biotic compartments such as the sediment, pore water, overlying water and biota (Triffault-Bouchet *et al*. 2005). Since more endpoints are incorporated in multispecies tests due to increased complexity, the likelihood of sampling the both extremes of tolerance is improved (Cairns and Cherry 1993).

A variety of test species have been used in multispecies tests, consisting of artificial or natural assemblages or organisms (Livingston 1988). An artificial, or gnotobiotic, assemblage is typically created from organisms cultured in the laboratory (Buikema and Voshell 1993; Livingston 1988). The endpoints studied in this type of assemblage can include mortality, growth, competition, predator-prey interactions, biomass, density, diversity, taxa richness, and community metabolism (Buikema and Voshell 1993). A natural assemblage in a multispecies test is one created from organisms that were collected in the field (Livingston 1988). The endpoints studied in this type of assemblage can include taxa richness, community metabolism, secondary production, behaviour, mortality, growth, competition, predator-prey interactions, biomass, density, diversity, and functional feeding groups (Buikema and Voshell 1993). The examination of more endpoints is possible since species interactions represent those present in the natural community (Buikema and Voshell 1993). The duration of bioassays using either type of assemblage ranges from days to months, depending on the endpoint being studied. There are various advantages and disadvantages based on the need for sensitivity, simplicity,

cost-effectiveness, practicality and predictability (Livingston 1988). These will be further described based on the scale of the experimental enclosure.

1.19. Microcosms and mesocosms

Multispecies bioassays can occur in either microcosms or mesocosms, depending on scale and the level of complexity desired. The literature contains many examples of freshwater benthic macroinvertebrate communities being tested successfully in microcosms (*e.g.*, Turesson *et al*. 2007; Sánchez and Tarazona 2002; Balch and Evan 1999; Borgmann and Munawar 1989; Arthur 1980). Microcosms typically have a volume less than 10 m^3 so they can be situated either indoors or outdoors, though they are usually found in the laboratory (Buikema and Voshell 1993; Clément *et al.* 2004; Odum 1984). These systems are relatively small so they are subject to greater control by the experimenter, which translates into a higher level of precision between replicates (Buikema and Voshell 1993). The term mesocosm was proposed by Banse (1982) to describe isolated or partially enclosed experimental setups larger than benchtop containers but smaller than any subunit of the natural environment (Buikema and Voshell 1993; Odum 1984). Mesocosms typically have a volume greater than $10m^3$ and can represent lotic (open or partially closed) or lentic aquatic environments (enclosures and experimental ponds) (Buikema and Voshell 1993). Mesocosms often contain representative portions of complete natural assemblages (Liber *et al.* 1992). Multispecies tests in both types of enclosures are a method of identifying interactions and compensation of test organisms that would be missed by single-species tests in response to a stressor (Mount 1985). Therefore, endpoints for multispecies assemblages include

changes in taxa richness, species diversity and density, shifts in dominant species, and changes in predator-prey relationships (Buikema and Voshell 1993).

1.20. Limitations of multispecies tests

When multispecies tests are designed relative to the hypothesis being tested, they can be useful tools for predicting and monitoring the effect of environmental stressors (Livingston 1988; Dickson *et al*. 1985). Multispecies tests are more costly than single-species tests, but the cost is offset by improved quality and quantity of information (Cairns and Cherry 1993). The advantage of these systems is in the ability to isolate and study mechanisms while retaining a certain amount of complexity (Dickson *et al*. 1985). However, the more a test system resembles a specific natural community, the less other scientists are able to duplicate the results (Calow 1989; Dickson *et al*. 1985). Also, the more site-specific a test is, the less certainty there is in extrapolating the results to other communities (Dickson *et al*. 1985; Giesy and Allred 1985). Complex assemblages may produce unique responses to the stressor each time the experiment is run; therefore, what is gained in realism is lost in repeatability (Calow 1989; Giesy and Allred 1985). Surprisingly few studies have been conducted specifically to assess replicability in multispecies biological assays, considering the importance of reliable results that can be extrapolated to the natural ecosystem.

Two problems often encountered in mesocosm studies are pre-treatment variability among sets of enclosures assigned to different future treatments, and post-treatment variability among replicate enclosures (Liber *et al.* 1992). The number and magnitude of

factors, and the longer time frame over which they can be expressed increases the potential for variability of measured parameters (Giesy and Allred 1985). Multispecies tests will naturally have higher background variability due to increased complexity and realism (Cairns and Cherry 1993). Since effects will be more difficult to distinguish from background noise, the effects must be greater and more replicates will be required for statistically significant results (Cairns and Cherry 1993).

The knowledge of how to assemble species into artificial test systems deserves much more attention. Some researchers have simply grouped benthic species together based on ease of culture and sensitivity to other related stressors. When selecting an assemblage for a multispecies assay, the experimenter must be sure that the group of species is likely to occur together naturally. Testing unlikely combinations of organisms together on account of their tolerance to stress has no benefit towards extrapolation to the natural field community (Mount 1985). This would defeat the purpose of doing a multispecies assay, as the endpoints are meant to include interspecific interactions that are likely to occur in the natural environment. Even with a representative community, there is no guarantee that that results will apply across all habitats (Maltby and Calow 1989). In order to have a better understanding of a specific location, separate biological assays must be run using the habitat features of the region of interest.

Multispecies biological assays have rarely been used by regulators due to a combination of legislative constraints and uncertainty about how to use assays to study intricate problems associated with complex ecosystems (Maltby and Calow 1989; Ireland

and Ho 2005). There is no guarantee the effects of stressors on complex systems will be the same as in simplified test systems. Laboratory tests may overestimate or underestimate the response seen in the field by not testing the appropriate intensity of the stressor (Ireland and Ho 2005). For multispecies tests to be accepted for regulatory use there should be examples of cases in which a standardized single-species bioassay proved inadequate at predicting ecological effects compared to a multispecies test addressing the same issue (Mount 1985). Improved testing is only important if it cannot be correlated with impact (Calow 1989; Mount 1985); therefore, this should be a focus of future multispecies biological assays.

Multispecies tests have been used to identify problems, and now should be used to answer questions and provide solutions (Mount 1985). Although ideal, bioassays are not capable of accurately predicting ecosystem level effects. Each type of stressor, the environment on which it is acting, and the affected community will vary; therefore, bioassays can estimate potential effects only based on the response of test organisms. In order to extrapolate this information to benthic communities, field surveys must be conducted to have some idea of the intensity of the disturbance, the quality of the sediment, and the state of the resident community.

1.21. Field surveys and experiments

The use of well-replicated, controlled field surveys and experiments has resulted in increased understanding of the structure and function of freshwater ecosystems (Cooper and Barmuta 1993). Relationships among biotic and abiotic factors at chosen sites or times are examined in field surveys, using endpoints such as species composition,

biomass, density, and diversity to assess the alterations of a community in response to a stressor such as organic loading (Cooper and Barmuta 1993; Giesy *et al.* 1990). Environmental variables of interest are rarely manipulated directly during field surveys, so analyses of these methods rely only on static measurements that are poor at describing trends over time, especially if the samples are analyzed only for sediment chemistry and not benthos (Cooper and Barmuta 1993). Field experiments should be an integral part of biomonitoring since there is greater control over relevant variables, to determine the pathways of interaction among biotic and abiotic factors that produce the observed responses (Cooper and Barmuta 1993).

The spatial scale of field experiments ranges from microcosms through mesocosms to entire lake manipulations (Cooper and Barmuta 1993). In experimental design, ecologists are often faced with difficult choices; small enclosures are easy to replicate and manipulate but environmentally unrealistic, while whole lakes and streams have high realism, but are difficult to manipulate and replicate (Cooper and Barmuta 1993). An example of this issue of spatial scale was identified when evaluating the roles of various nutrients in controlling phytoplankton growth. Early experiments done in small jars consistently showed that carbon was limiting to algal growth; however, a large-scale whole lake manipulation at the Experimental Lakes Area in the Canadian Shield showed that phosphorus was the major limiting nutrient (Schindler 1977). On a temporal scale, two aspects need attention for field experiments involving treatments of an environmental stressor: the duration of manipulations (press or pulse), and the length of monitoring to assess the impact of those manipulations (Cooper and Barmuta 1993). Different

conclusions may be reached if the treatment is continually applied (press), or applied in a single dosage (pulse) and monitored. The design of the experiments will affect the results, as well as the interpretation of the data, so caution must be used when selecting the spatial and temporal scale of the experiment.

According to Hurlbert (1984), pseudoreplication, or the misidentification of replicates, is a common error in designing experiments. Spatial pseudoreplication involves comparisons of a single control with a single treatment unit (Hurlbert 1984). Researchers must take into account that two areas are likely to diverge over time, even without experimental manipulation. This method will provide unclear results since the experimenter will not know if the difference would have occurred in the absence of the impact (Cooper and Barmuta 1993; Hurlbert 1984; Green 1979). Committing temporal pseudoreplication involves taking samples at different points through time, rather than through space, within the same replicate (Hurlbert 1984). Samples are likely to be correlated and therefore not independent which may lead to the incorrect use of statistical analysis (Cooper and Barmuta 1993; Hurlbert 1984). Sacrificial pseudoreplication occurs when researchers have a number of replicate units per treatment, but then inappropriately analyze units nested within experimental units as independent replicates or ignore the replicates by pooling the data (Cooper and Barmuta 1993; Hurlbert 1984). It is important to take nested samples within treatment units provided they are analyzed properly, as they provide additional information about variability within an experiment (Cooper and Barmuta 1993; Hurlbert 1984). Understanding the sample sizes required to achieve

desired levels of power is an important component of experimental design to maximize human resources and project expenditures (Kennedy *et al.* 1999).

With adequate comprehension of field processes and proper experimental design, the experimenter can compare the multispecies lab bioassay with field responses (Livingston and Meeter 1985). One method is to measure the field biological response along a known gradient of disturbance and to compare such effects with a bioassay that simulates stressor concentrations along the field gradient (Livingston and Meeter 1985). This was the approach of my research project on the effect of aquaculture solid waste on benthic invertebrates.

1.22. Integrated strategies in assessing environmental effects

When assessing sediment quality is the objective, authors often conclude that laboratory and field tests must be combined before one can reasonably estimate the effects of stressors (Chapman 2007; Cairns and Pratt 1989; Rosenberg *et al.* 1986; Kimball and Levin 1985). Combining resident biotic community data together with sediment chemistry data is essential to address the consistency of association between stressor and effect and the spatial or temporal dose/concentration-response relationship (Chapman 2007). Physical and chemical measurements provide information only on conditions that exist when the samples are collected, whereas biological surveys reflect conditions that have been integrated over a period of time (Rosenberg *et al.* 1986). There are no perfect methods in sediment quality assessment, hence the importance of several types of data for use in decision making (Chapman 2007).

Assessments of sediment quality often include descriptions of sediment chemistry, geochemical factors that affect bioavailability, benthic community structure, and direct measures of toxicity during biological assays (Ireland and Ho 2005). The interpretation of these factors depends upon the 'weight of evidence', by forming conclusions based on all available information (Chapman and Anderson 2005; Chapman 1996). The Sediment Quality Triad approach, developed by Chapman (1986), was based on the assumption that the biological responses observed in sediment bioassays and *in situ* studies are due to the concentration of stressors (*e.g.*, heavy metals, nutrient loading) in the sediments of the study area (Chapman 1986). The Triad involves three components: sediment chemistry, a measure of the concentration of the stressor itself, lab toxicity tests in which effects are measured under standardized conditions, and assessments of resident community alteration to measure field conditions (Chapman 1996). The Triad was developed as a result of the realization that all three components complement each other and are necessary to provide evidence of environmental degradation (Chapman *et al*. 1987). Bioavailability should be added to the Triad since the cause of toxicity cannot otherwise be identified with certainty (Borgmann *et al*. (2001). In a later study, Chapman (2007) agreed, stating that sediment chemistry provides information only on presence of the stressor, not its bioavailability or toxicity. The 'weight of evidence' approach provides some confidence when extrapolating these predictions to natural systems. There are always arguments that can be made against only using one approach to examine the response of an environmental stressor. For example, lack of genetic diversity in lab cultures does occur and could lead to biased results in bioassays that investigate the life-

history responses of model species in the lab (Nowek *et al.* 2007). As well, resident benthic communities can have very different responses to a stressor because of the possibility of prior exposure to a similar stressor.

Several methods of estimating the effects of waste loading have been discussed in this literature review. Each type of analysis has its benefits and limitations, so for the most comprehensive look at the effects, biological assays, sediment chemistry and benthic community surveys should be included, as was suggested by the Sediment Quality Triad (Chapman 1986; Chapman 1996). For this particular study, sedimentation rates are also critical to measure near aquaculture operations. These rates will provide information regarding exposure to the primary cause of effect, the sedimentation of solid waste. Examining the alterations to sediment chemistry will describe the effects of the stressor on the habitat for benthos beneath aquaculture operations. Benthic community surveys in the field will reveal community responses to waste loading. In the laboratory, using biological endpoints such as growth and reproduction during bioassays subjecting benthos to a gradient of waste loading, would link these responses to the stressor to effects at the population and community level.

1.23. Conclusion

Similar to other food-producing sectors, aquaculture relies upon the use of natural resources such as land and water (FAO 2006). Expansion in the aquaculture industry worldwide has brought attention to the range of potential impacts associated with organic enrichment derived from the deposition of wastes (Sutherland *et al.* 2007). Reducing the

effect on the benthic community has been a central element in the monitoring and regulation of aquaculture operations in many countries (Chamberlain and Stucchi 2007). There is a need to understand and be able to quantify these effects, and to develop guidelines for the rational exploitation of aquatic resources (Droppo *et al.* 2007; Sutherland *et al.* 2007; Naylor *et al.* 2000; Beveridge 1987).

Aquaculture and capture fisheries are linked economically through competition in world markets, and biologically through stocking of fish, exotic species introductions and possible disease transmissions (Naylor *et al.* 2000). As the human population continues to expand, its reliance on farmed fish production as a source of protein will also increase (Naylor *et al.* 2000). The ability to provide fish to meet future demands will likely be due to coordinated partnerships between aquaculture and managed wild fisheries, in addition to the protection and management of aquatic ecosystems (Tidwell and Allan 2002).

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Chapter II: Effect of carbon loading from aquaculture waste on benthic invertebrates and sediment chemistry at three freshwater net-pen farms

2.1. Abstract

The rates and effects of waste loading from aquaculture were examined along distance transects from two commercial and one experimental net-pen rainbow trout (*Oncorhynchus mykiss* Walbaum*)* farms in Ontario, Canada. Sedimentation of waste was measured by deploying sedimentation traps at each site and sediment cores were collected to determine sediment chemistry and benthic invertebrate community composition. Waste sedimentation rates declined exponentially with distance resulting in limited dispersal. At a distance of 30 m from the cages, carbon deposition was reduced by 90 to 95 % at Commercial Farm 1 (CF1) and by 88 to 92 % at Commercial Farm 2 (CF2). Carbon sedimentation rates returned to background levels at 5 m and 15 m from CF1 and CF2, respectively. At the experimental fish farm, carbon sedimentation was not significantly different from background 15 m and 10 m distant from the cage in 2006 and 2007, respectively. The carbon, nutrient and metal concentrations in the sediment were elevated within 30 m of the cages at all three farms. In order of abundance, the five major invertebrate taxa collected at both commercial farms were Oligochaeta, Harpacticoida, Nematoda, Chironomidae and Ostracoda. Tubificinae and Naidinae (Oligochaeta) individual mean biomass at both commercial farms peaked at moderate levels of carbon sedimentation (3.07 - 6.97 g C m^{-2} day^{-1}). Invertebrate density peaked between 1.0 and 2.0 g C m^{-2} day^{-1}, and then exponentially declined between 2.0 and 3.0 g C m^{-2} day^{-1}.

Dispersion modeling tools are available (*e.g.,* DEPOMOD) and carbon loading thresholds of effect have been described for marine farms; however, Ontario does not

have an equivalent process for the management of the freshwater cage aquaculture industry. Based upon the observations of this study, the predicted threshold of effect on invertebrate abundance and richness is between 2.0 and 3.0 g C m^{-2} day^{-1}. This intensity of carbon sedimentation was observed within 5 m from CF1, 10 -15 m from CF2, and at the edge of the experimental cage farm.

2.2. Introduction

Worldwide growth of the aquaculture industry is expected over time due to overexploitation of wild fish stocks (Tidwell and Allan 2002; FAO 2010). It is therefore important to understand the environmental implications of this industry. Open net-pen aquaculture is a system in which fish are reared within a net-pen or cage that is suspended in the water column and permits the free flow of water (Beveridge 1987). A commercial farm typically consists of many net-pens secured together, each ranging in size from 100 to 300 m^2 in surface area (Beveridge and Stewart 1998). Nutrition for finfish aquaculture is usually supplied by formulated feeds, and wastage at modern farms is typically less than 5% (Cromey *et al*. 2002). In Ontario, 78% (approximately 2964 tonnes) of the total rainbow trout (*Oncorhynchus mykiss*) production comes from eight cage culture operations (Moccia and Bevan 2007; Northern Ontario Aquaculture Association (NOAA), personal communication, January 19, 2012). These farms are near Manitoulin Island in the North Channel of Lake Huron, and in Georgian Bay.

Net-pen farming has gained attention as an environmental issue due to the release of nutrient-rich, untreated wastes directly into the receiving environment (Beveridge 1984; Elberizon and Kelly 1998). The amount of waste released depends on farm size, fish density, and husbandry practices (Mente *et al.* 2006). Farm wastes are both

particulate and dissolved. Dissolved wastes will be diffused into the entire body of water, whereas particulate wastes will be deposited beneath and immediately surrounding the farm (Sanz-Lázaro *et al*. 2011). The impact of a farm is based on the ability of the surrounding environment to assimilate farm waste in relation to the loading rate (Carroll *et al.* 2003) and the degree of waste dispersal (Kutti *et al*. 2007; Holmer *et al.* 2007). Well-flushed sites can have little to no accumulation on the bottom (Tlusty *et al.* 2000). When farm wastes do accumulate, this material is likely to produce alterations to the sediment chemistry and benthic communities (*e.g.*, Brown *et al.* 1987; Weston 1990; Karakassis *et al*. 2000; Rooney and Podemski 2009, 2010).

The highest sedimentation rates are typically observed directly beneath cage farms (Giles 2008). Troell and Berg (1997) reported a rate beneath fish cages of 20 - 28 g C m^{-2} day^{-1}, compared with less than 1.0 g C m^{-2} day^{-1} at reference sites. Sedimentation rates decline quickly with increasing distance, and water depth and current velocity are the two main factors determining the pattern of sedimentation around cage sites (Cromey *et al.* 2002). Holmer *et al.* (2007) reported that sedimentation was reduced by up to 91% by 40 m away from three Mediterranean fish farms. These farms were in 16 to 28 m of seawater, with current speeds of 5 to 20 cm s^{-1} (Holmer *et al.* 2007). At deep water locations (>30 m), the sedimentation rate of farm waste is typically lower, but the material may disperse over a larger area (Giles 2008). Kutti *et al.* (2007) estimated the annual carbon loading rate under a Norwegian marine farm located over 230 m of water depth and with a mean current speed ranging from 0.8 - 2.8 cm s^{-1} to be 1 g C m^{-2} day^{-1}. The majority of fish waste settled within 250 m from the farm; however, some farm waste was transported 550 - 900 m from the operation (Kutti *et al.*2007). At relatively

shallow farms with minimal current, such as those in sheltered freshwater lakes, the dispersal of waste material should be limited (Cromey *et al.* 2002). Sedimentation rates from marine cage culture operations are well-studied (*e.g.*, Weston 1990; Cromey *et al.* 2002; Van Biesen and Parrish 2005; Rapp *et al*. 2007; Kutti *et al*. 2007, 2008; Holmer *et al.* 2007; Sanz-Lázaro *et al*. 2011); however, there is very limited literature on this subject in freshwater (*e.g.,* Troell and Berg 1997). There have been no published studies describing sedimentation rates for commercial cage farms in Ontario.

Accumulated farm wastes may have a significant impact on the sediment beneath and surrounding fish farm operations. The accumulation of nutrient-rich farm waste in sediment increases carbon, nitrogen, and phosphorus content (Rooney and Podemski 2010). Enrichment of the sediment below and surrounding aquaculture operations has been documented at farms all over the world (*e.g.,* Rooney and Podemski 2010; Gao *et al.* 2005; Edgar *et al*. 2005; Guo and Li 2003; Lin and Yi 2003; Wildish *et al*. 2003; Hargrave *et al*. 1997; Ackefors and Enell 1994; Cornel and Whoriskey 1993; Johnsen *et al.* 1993; Penczak *et al.* 1982). In the Bay of Fundy, Canada, Hargrave *et al.* (1997) reported the average value for sediment carbon was 40% higher under the farm than the value for reference sites as a result of deposition of farm wastes. At a marine farm in Hong Kong, the total carbon, nitrogen, and phosphorus levels at the sampling station closest to the fish cages were 82.8%, 128.5% and 1315.7% higher, respectively than those of reference stations (Gao *et al*. 2005). Depleted oxygen, reduced pH, and the accumulation of ammonia are commonly reported to result from the breakdown of organic wastes (Enell and Lof 1983; Troell and Berg 1997; Brooks and Mahnken 2003; Veenstra *et al.* 2003). Troell and Berg (1997) reported that the sediment beneath a cage

farm in Lake Kariba, Zimbabwe consumed 305 mg O_2 day^{-1} compared with approximately 200 O_2 mg day^{-1} at control sites. Development of anoxia beneath the cages may occur if the oxygen demand exceeds the supply. Elevations in total ammonia concentrations in the sediment near freshwater fish farms have been reported (*e.g.*, Rooney and Podemski 2010; Troell and Berg 1997). Ammonia is released during decomposition of fish waste at the sediment–water interface, as well as from anaerobic bacterial mineralization occurring deeper in the sediment (Hargreaves 1998). Elevated sediment copper (Cu) and zinc (Zn) concentrations have also been described (*e.g.*, Parker and Aubé 2002; Rooney and Podemski 2010). Zinc is a component of fish feed, and the concentration would be expected to increase where wastes accumulate (Parker and Aubé 2002). Increased sediment copper concentration may be due to anti-fouling coatings applied to net-pens to reduce periphyton growth, though this practice is less common in freshwater (Brooks and Mahnken 2003).

The effects of sediment enrichment with organic waste can result in changes in benthic community abundance, species richness, and biomass (Pearson and Rosenberg 1978; Carroll *et al.* 2003). The Pearson-Rosenberg (1978) model predicts the response of the benthic invertebrate community along temporal and spatial gradients of organic enrichment. With increasing organic enrichment, invertebrate abundance will gradually increase to a peak as a result of increasing numbers of opportunistic species. At high levels of organic loading, invertebrate abundance will decline and an azoic zone may be formed (Pearson and Rosenberg 1978). Species richness will peak at moderate loading in an area where tolerant and sensitive species are both present, known as the ecotone point (Pearson and Rosenberg 1978). If organic enrichment is further increased, richness

declines as a few opportunistic species come to dominate the community after the loss of those species sensitive to low dissolved oxygen levels and increased ammonia concentrations (Pearson and Rosenberg 1978).

The addition of organic waste can also influence both the total community biomass of invertebrates and mean individual biomass. Individuals that are exposed to moderate amounts of organic enrichment tend to grow larger than those in remote, control locations (Dobrowolski 1987), suggesting that this material acts as a food subsidy. Total biomass of the invertebrate community will be influenced by changes in individual size, changes in species composition and changes in abundance. The Pearson-Rosenberg (1978) model predicts two peaks in total invertebrate biomass: the first occurring in the same region as the peak in species richness, resulting from the stimulatory effects of low levels of organic enrichment (Peeters *et al.* 2004; Vos *et al.* 2004). The second peak in biomass occurs where the benthic community has become composed of high numbers of tolerant, opportunist species (Pearson and Rosenberg 1978). An opportunist is generally defined as a small-bodied species that can thrive in a wide range of environmental conditions, with short lifespans, and a highly variable population size (Warwick 1986). However, taxa that are most commonly cited as opportunist species in organically enriched freshwater profundal sediments zone can larger-bodied in comparison to some sensitive taxa such as Harpacticoida and Ostracoda (Strayer 1985; Peeters *et al.* 2004; Vos *et al.* 2004). Examples in freshwater, include *Tubifex tubifex* (Müeller) and chironomid larvae, especially Chironomini. The mean biomass of a 4th instar Chironomidae larvae is 39.1µg , compared with an ostracod and a harpacticoid copepod at 17.3 µg, and 1.5 µg, respectively (Strayer 1985). To understand

position along the Pearson-Rosenberg enrichment gradient, it is necessary to determine the cause of a change in community biomass.

The Pearson-Rosenberg biomass predictions are not observed consistently in field studies. Weston (1990) examined five stations near a marine cage farm located in Puget Sound, Washington: one at the cage edge, and at 45, 90, 150, and 450 m from the cage edge. He did not observe the peak in total biomass predicted by Pearson and Rosenberg (1978), but did observe that mean individual biomass within a taxon was directly correlated with distance from the farm. Individuals grew larger when exposed to organic waste, supporting the theory that aquaculture waste loading has a stimulatory effect on benthic invertebrates (Weston 1990). In one of the few studies of the effect of freshwater aquaculture on invertebrate biomass, Dobrowolski (1987) reported that mean individual biomass was elevated beneath a farm in Lake Letowskie, Poland. It is unclear whether the results were due to increased individual size of the opportunistic species, or if there was a shift in the community composition. Rooney and Podemski (2009) reported an increase in total biomass that was likely caused by a community shift beneath the farm. After two years of production, 50% of the invertebrates under the cage were Chironomini larvae (Rooney and Podemski 2009). The few chironomids in a sample had a higher biomass than hundreds of Harpacticoida, or dozens of Ostracoda (Rooney and Podemski 2009). It is important to distinguish an increase in total biomass due to the shift from small-bodied profundal taxa to larger-bodied chironomids and oligochaetes, from an increase in individual biomass due to stimulation from increased food quality and quantity (Rooney and Podemski 2009).

The Pearson-Rosenberg model has been used in marine studies to describe benthic communities near net-pen farms (*e.g.,* Gowen and Bradbury 1987; Weston 1990; Karakassis *et al.* 1999, 2000; Brooks and Mahnken 2003; Kalantzi and Karakassis 2006; Kutti *et al.* 2007). Few researchers have examined the effect of aquaculture waste loading on benthic invertebrates in freshwater systems, but published results provide some support for the predictions of the Pearson-Rosenberg (1978) model (*e.g.,* Dobrowolski 1987; Rooney and Podemski 2009). Rooney and Podemski (2009) reported that initial invertebrate responses closely resembled the model's predictions at high levels of loading, but peaks at moderate levels of loading were never observed. Since the study was completed shortly after the establishment of the experimental farm, dense populations of enrichment specialists may not have had time to develop (Rooney and Podemski 2009). In addition, due to extremely localized waste deposition from the farm there was a steep gradient between affected and unaffected invertebrate communities (Rooney and Podemski 2009). This transition occurred between sampling sites at 5 and 10 m from the cage edge, so peaks at moderate levels of loading would not have been detected (Rooney and Podemski 2009).

Sediments in oligotrophic water bodies are of relatively poor quality as a food resource for invertebrates. The organic material within these lakes is normally not easily decomposed nor digested (Wetzel 2001). It is therefore reasonable to expect that at small to moderate amounts of waste loading, the highly labile waste material may act as a food subsidy to the invertebrate community. Kullman *et al.* (2009) and Wellman (2011) used stable isotope analysis to determine that the assimilation of aquaculture-derived carbon was evident in profundal invertebrates near an experimental cage farm. However, the

ability of invertebrates to utilize this additional food source also depends on their functional feeding group. Hargrave *et al.* (1997) reported that the mean biomass of deposit feeders beneath net-pens was significantly higher ($p<0.05$) than the mean biomass at reference sites while that of suspension feeders was not significantly different.

This is the first study to describe the effects of aquaculture waste loading on benthos and sediment chemistry at commercial and experimental net-pen farms in Ontario. The first objective of this study was to document the spatial and temporal variation in sedimentation rates at commercial and experimental rainbow trout cage farms. The second objective was to describe alterations in the sediment chemistry surrounding these farms, and the third was to identify any stimulation or reduction in invertebrate density and biomass with increasing levels of waste loading from the farms. A fourth objective was to determine which measure of carbon sedimentation (concurrent, mean or maximum) would best describe the relationship with effects on sediment chemistry and the benthic community for the commercial farms. Knowledge of this relationship would be useful during the design of a monitoring program to be used when establishing a new farm or expanding an existing operation.

2.3. Methods

2.3.1. Study locations

This study was conducted at three rainbow trout farms in Ontario: two commercial farms in the Manitoulin Island/North Channel area of Lake Huron (Commercial Farm 1, Commercial Farm 2) and one experimental cage farm at the Experimental Lakes Area, located in northwestern Ontario, Canada. Commercial Farm 1 (CF1) was located in Lake Wolsey, on Manitoulin Island, Ontario, Canada (45° 48'

6.28"N, 82° 32' 53.60"W). The farm consisted of eighteen, 15.24 m x 15.24 m steel-framed net-pens with a maximum of 12 in use at any one time. Average annual production during 2006-2007 was approximately 294 835 kilograms of rainbow trout with a feed conversion ratio (FCR) of 1.20 -1.25 (M. Meeker, Owner, MTM Aquaculture, personal communication). Commercial Farm 2 (CF2) was located off Little La Cloche Island, in the North Channel of Lake Huron (45°59'28.4"N, 81°43'56.671"W). The farm contained twelve, 15.24 m x 15.24 m steel-framed net-pens. In 2007, annual production was 356 604 kilograms of rainbow trout, with a biological FCR ranging from 1.02 – 1.53 during May to November (T. Horne, Operations Manager, Coldwater Fisheries, personal communication). Each of the farm operators provided monthly feed records for the 2006-2007 production seasons. The transect at each farm extended from a cage to 100 m away in the dominant current direction, as identified by the farm operator. Cages were selected that would contain fish throughout the sampling period. At the commercial farms, sampling transects were established over 14 m of water depth. During sampling, a measured line was attached from the boat to the cage and constant tension was maintained on this line to set the sampling distances. The 100 m sampling location was used as a reference point to compare with near-farm sites. In these freshwater farms, the effects from the cages are not likely to be observed up to 100 m from the farms (Giles 2008). Giles (2008) examined spatial trends in impacts from cage aquaculture and reported that significant effects were confined to within approximately 40 – 70 m around the farms. Due to comparably low current speeds, the effects of freshwater fish culture are typically more localized (Beveridge *et al.* 1997) than marine operations (*e.g.*, Kutti *et al.* 2007).

In addition to the two commercial farms, we also sampled along a transect at an experimental cage farm located in the north basin of Lake 375 (L375) at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (49°44'40"N, 93°47'20"W). The effects of the experimental farm have been previously reported in Rooney (2006), Bristow (2006), Hille (2008), Bristow *et al.* (2008), Kullman *et al.* (2007, 2009), Rooney and Podemski (2009, 2010), Blanchfield *et al.* (2009), Findlay *et al*. (2009), Azevedo *et al.* (2011), Paterson *et al.* (2010, 2011), and Wellman (2011). One, steel-framed fish cage with a net that measured approximately 10 m x 10 m x 10 m was installed in 2003 (Rooney and Podemski 2009). The bottom of the net was suspended 5 - 7 m above the sediment (Rooney and Podemski 2009). L375 is dimictic, although spring turnover is often incomplete (Rooney and Podemski 2009). Midsummer current speed measured 1 m above the bottom averaged less than 0.2 cm s^{-1} (C.L. Podemski, Fisheries & Oceans Canada, unpublished data). The experimental farm thus simulated a poorly sited farm with minimal current and waste dispersion (Rooney and Podemski 2010). Each spring (2003-2007), this experimental farm was stocked with approximately 10 000 female rainbow trout. Many of the net-pen farmers in Ontario used Martin Mills Profishent © (Martin Mills, Elmira, Ontario) fish feed, so this feed was provided at the experiment farm (Rooney and Podemski 2009). The farm was operated according to commercial practices, assisted by training and regular advice from the Northern Ontario Aquaculture Association. The FCR ranged from 1.10 – 1.46, with a mean for all production cycles (2003-2007) of 1.28. More details about farm operation are available in Rooney and Podemski (2009, 2010).

2.3.2. Sedimentation

The most direct way to measure particulate loading is to catch the material in sedimentation traps as it descends through the water column (Rapp *et al.* 2007). Sedimentation traps were set up directly under and at nine distances from a cage at each farm (1 m, 3 m, 5 m, 10 m, 15 m, 20 m, 30 m, 50 m and 100 m). Each trap consisted of four tubes (L= 26 cm, I.D.= 4.4 cm, V= 450 mL) on a tray suspended 2.5 m above the sediment. The traps were anchored to the bottom with a cinder block and were marked on the surface with a buoy. Bloesch and Burns (1980) suggested the best design for sedimentation traps to be used in non-turbulent water is a cylinder with a minimum diameter of 5 cm with an aspect ratio (height:diameter) of greater than 5. Although the diameter of the traps in this study was smaller than suggested (4.4cm ID), the aspect ratio of 5.9 agreed with that advocated by Bloesch and Burns (1980). The sedimentation traps were retrieved after 5-7 days and washed into acid-washed polyethylene bottles. Samples were collected no more than one week after deployment to minimize decomposition of the highly organic material caught in the trap (Tlusty *et al.* 2000). Preservatives were not used because of the short deployment time of the traps, as recommended by Bloesch and Burns (1980) and Bloesch (1996). Due to the large amount of suspended material in the samples, a 100 mL homogenized sub-sample from each replicate was vacuum-filtered through a pre-combusted 0.47 μm GF/C filter for analysis at the Freshwater Institute, Fisheries and Oceans Canada, Winnipeg. Sedimentation rates were determined at CF1 once in September 2006 and then once in June, July and September of 2007. In 2007, sedimentation traps were similarly deployed in July and September at CF2. We were not able to measure sedimentation rates at the following stations along the transects because

the traps were lost during storms: CF1 (Sept 2007): 1 m, 15 m, and 30 m; CF2 (July 2007) 1 m, 3 m, 5 m and 50 m; (Sept 2007) 1 m, 15 m, 50 m and 100 m. At the experimental cage farm, sedimentation rates along the transect were determined weekly from July to October in 2006, and May to October in 2007. In addition, three sedimentation traps were deployed in the north and south basins, and at the centre of Lake 375 to measure background sedimentation. The distances from the cage farm of these traps were 95.90 m, 562.01 m and 362.09 m, respectively. These reference sedimentation traps were also collected weekly over the same time period.

2.3.3. Sediment Chemistry

At the commercial farms, core samples were collected for analysis of sediment chemistry during the same sampling periods and at the same distances as sedimentation rates. Core samples were collected after the removal of the sedimentation traps from the water column. At the experimental farm, sediment core samples were collected in November 2006 and October 2007. Sediments near the farms were fine-grained and therefore a Kajak-Brinkhurst corer was used. The core tubes were 4.80 cm in diameter and 30 cm in length, and the samples contained approximately 20-25 cm of sediment with 5-10 cm of overlying water. Samples were visually inspected to ensure that the sediment-water interface was undisturbed. If a layer of fish waste was present on the sediment, the depth of the material was recorded at four points around the core sample (3, 6, 9 and 12 o'clock). Overlying water and sediment subsamples were removed by extrusion. The top 0-2 cm layer of sediment was placed into a Whirl-Pak® bag and frozen for transportation to the laboratory.

Sediment subsamples were freeze-dried and ground until homogenized. Nutrient analysis was completed by the Northwest Atlantic Fisheries Centre (Fisheries and Oceans Canada, St. John's, Newfoundland) in 2006 and at the University of Alberta Biogeochemical Analytical Service Laboratory in 2007. Total nitrogen and total carbon were measured using an Exeter Analytical Model CE-440 Rapid Analysis Elemental Analyzer with a detection limit of 0.70 µg N g^{-1} and 7.06 µg C g^{-1} dry-weight, respectively. In 2006, total phosphorus was measured with a Spectronic Genesys 5 (Milton Roy), using methods described by Andersen (1976). In 2007, total phosphorus was measured with a Lachat QuikChem 8500 FIA automated ion analyzer, (Lachat Instruments, Colorado, USA) after samples were ashed at 500˚C. More detailed methods are available in American Water Works Association (1999). Sediment concentrations of a variety of metals were measured by Acme Analytical Laboratories, Ltd. in 2006 and 2007. Samples were digested by the addition of a 10 mL aliquot of the acid solution (2:2:1:1 H_2O-HF-$HClO_4$-HNO_3), and heated until fuming on a hot plate. A 4 mL aliquot of 50% HCl was added to the residue and heated using a mixing hot block. After cooling, the solutions were transferred to polypropylene test-tubes and adjusted to a 10 mL volume with 5% HCl. These solutions, aspirated into a Spectro Ciros Vision or Varian 735 ICP emission spectrometer, were analyzed for 35 elements: Ag, Al, As, Au, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Sb, Sc, Sn, Sr, Th, Ti, U, V, W, Y, Zn and Zr.

2.3.4. Benthos

Core samples were collected for invertebrate community analysis using a Kajak-Brinkhurst gravity corer at CF1 in September 2006 and 2007, at CF2 in September 2007,

and at the experimental farm in November 2006 and October 2007. Ten core samples were collected at each distance and washed through a 250 µm sieve to remove fine particles. The remaining material was preserved in 10% formalin for two weeks, transferred to 90% ethanol, and then to 70% ethanol for sorting. Invertebrates were enumerated by sorting the samples in gridded Petri dishes under 6-50X stereomicroscope. Ten per cent of the samples were randomly chosen for a quality assurance check in which the residue was reprocessed to ensure that sorting efficiency exceeded 90%. The organisms were identified to subfamily for Oligochaeta, tribe for Chironomidae, and to order for all other taxa. The number of individuals of each taxon was determined from each core sample taken, and then converted to density per m^2 using the surface area sampled by the Kajak-Brinkhurst core tube.

Mean individual biomass for Tubificinae and Naidinae (Oligochaeta, Naididae) was determined by placing all individuals from one sample in pre-weighed aluminum foil weigh boats and drying at 40°C for 72 hours. Some of these individuals were fragmented and these segments were too small to determine individual mass. Therefore, dry weight was divided by the total number of individuals in each core sample to determine mean individual biomass. The total dry weight of the contents of each weigh boat was determined using a Fisher Scientific Accu-124D dual range balance. Total numbers of Tubificinae and Naidinae per sample were determined by enumerating heads. Chironomidae head capsule length and width and total body length were measured using a Leica MZ12 stereomicroscope and an attached Leica DFC320 camera. Using AxioVision Rel 4.6, measurements were taken from a digital photograph by tracing the head capsule with a curved spline.

2.3.5. Statistical Analysis

GraphPad Prism v. 5.04 (GraphPad Software, Inc 2010) statistical software were used for linear, polynomial, and exponential regression analysis. MINITAB v. 12.1 (MINITAB 1998) was used for analysis of variance (ANOVA). Normality and homogeneity of residuals were tested using Anderson-Darling and Bartlett's tests, respectively. Statistical analysis of sedimentation rates and effects on sediment chemistry and the benthic community will be described separately.

a) Sedimentation rate with distance

A plot of sedimentation rate with distance was reviewed to determine if the relationship between the variables appeared linear or curvilinear. The regression model was then chosen based on a balance between goodness of fit (r^2 value) and what could be explained biologically. Exponential regression was used to describe carbon, nitrogen and phosphorus sedimentation with distance from all three farms. Tukey's pairwise comparisons were used in conjunction with significant one-way ANOVAs ($p<0.05$) to determine how far from the cage waste sedimentation was elevated, compared with the reference site. The exponential regression equations of carbon sedimentation rate with distance indicated little variation around the regressions ($r^2=0.70 – 0.97$) and were therefore used to predict missing sedimentation values resulting from the loss of traps (Table 2.1) in order to create a more complete dataset.

b) Sediment chemistry with carbon sedimentation

Depending upon the shape of the response and by comparison of r^2 and p-values, linear or polynomial regression analyses were used to describe the influence of carbon sedimentation on sediment chemistry (Table 2.6, 2.7). Predicted carbon sedimentation

values were used in the dataset to replace missing values resulting from trap loss. To determine which measure of sedimentation best described the relationship with sediment chemistry, concurrent, mean and maximum rates were compared (Appendix 2.1, 2.2). At CF1 in September 2006 and in June, July and September 2007, linear regression was used to describe sediment carbon, nutrient and metal concentration with carbon sedimentation (Table 2.6). The effect of concurrent carbon sedimentation on sediment chemistry at CF2 during July and September 2007 was analyzed by polynomial regressions (Table 2.7).

c) Effects of carbon sedimentation on benthos

The polynomial relationships between benthic invertebrate density and concurrent, mean and maximum carbon sedimentation rates were compared to determine which measure of sedimentation was the better predictor of effects on benthos at CF1 and CF2 in 2007 (Appendix 2.3, 2.4). Predicted carbon sedimentation values were used in the dataset to replace missing values resulting from trap loss. After a comparison of r^2 and p-values, mean carbon sedimentation along the distance transect was used for further analysis. Polynomial regression was used to describe the relationship between mean carbon sedimentation and benthic invertebrate density (total invertebrate, Oligochaeta, Chironomidae, Nematoda, Harpacticoida, Ostracoda) at CF1 and CF2. Exponential regressions were used to quantify the rate of decline only for invertebrate density (total invertebrate, Oligochaeta, Chironomidae, Nematoda, Harpacticoida) with carbon sedimentation at CF1 and CF2 (Table 2.8). Polynomial regressions were used to describe the influence of mean carbon sedimentation on mean individual biomass (Tubificinae and Naidinae) and head capsule length and width and total body length (Chironomidae) at both commercial farms.

2.4. Results

2.4.1. Sedimentation rates of aquaculture waste

The sedimentation rates of carbon, nitrogen and phosphorus were highest immediately beneath the commercial farms. In September 2006, sedimentation rates under the cage at CF1 were 11.6 g C m^{-2} day^{-1}, 4.9 g N m^{-2} day^{-1}, and 1.8 g P m^{-2} day^{-1}, respectively (Figure 2.1). In 2007, sedimentation under the cage at CF1 ranged from 9.47 - 23.19 g C m^{-2} day^{-1}, 0.94 - 2.24 g N m^{-2} day^{-1}, and 0.83 - 1.27 g P m^{-2} day^{-1} (Figure 2.2). There was an exponential decline in carbon sedimentation with distance from the farm in all sampling periods (Table 2.1). Mean carbon sedimentation in 2007 at the reference site 100 m from the cage was 0.88 g C m^{-2} day^{-1}, 0.12 g N m^{-2} day^{-1}, and 0.02 g P m^{-2} day^{-1}. At CF2 in 2007, sedimentation beneath the cage ranged from 21.41- 23.07 g C m^{-2} day^{-1}, 0.96 - 2.25 g N m^{-2} day^{-1}, and 0.26 - 2.73 g P m^{-2} day^{-1} (Figure 2.3). There was an exponential decline in carbon sedimentation with distance from the farm in July (r^2= 0.96) and September 2007 (r^2=0.97) (Table 2.1). Mean carbon sedimentation at the reference site 100 m from the cage was 1.38 g C m^{-2} day^{-1}, 0.18 g N m^{-2} day^{-1}, and 0.05 g P m^{-2} day^{-1}. Mean, median, and a range of carbon sedimentation rates at each site along the transects at both commercial farms are listed in Table 2.2. Carbon sedimentation was not significantly different from the 100 m reference site at 1 m from CF1 in 2006 ($F_{9,7}$=61.33, p<0.001), and at 3 m ($F_{9,27}$= 34.41, p<0.001), 5 m ($F_{9,21}$= 389.72, p<0.001) and 1 m ($F_{9,16}$= 12.68, p<0.001) from the cage in June, July, and September 2007, respectively (Table 2.2). At CF2 in 2007, carbon sedimentation was not significantly different from the 100 m reference site at 10 m ($F_{5,15}$= 143.18, p<0.001) and 1 m from the cage ($F_{5,15}$= 83.99, p<0.001) in July and September 2007, respectively (Table 2.2).

Carbon, nitrogen and phosphorus sedimentation was also highest directly beneath the experimental fish farm. Sedimentation rates were measured from July to October in 2006 and from May to October in 2007. The mean carbon sedimentation rate was highest under the cage at 25.8 and 34.1 g C m^{-2} day^{-1} in 2006 and 2007, respectively (Table 2.3). Carbon sedimentation decreased rapidly with distance and was described by an exponential regression (Table 2.1). Mean, median, minimum, maximum carbon sedimentation rates at each site along the transect, in addition to the background sedimentation rate in the north basin of L375 are listed in Table 2.3. Mean carbon sedimentation rates declined to 0.42 g m^{-2} day^{-1} in 2006, and 0.46 g m^{-2} day^{-1} in 2007 at distances of 15 m and 10 m, respectively (Table 2.3). Background sedimentation rate in the north basin of L375 was 0.369 g C m^{-2} day^{-1}, 0.040 g N m^{-2} day^{-1} and 0.003 g P m^{-2} day^{-1} in 2006,and 0.409 g C m^{-2} day^{-1} , 0.045 g N m^{-2} day^{-1} and 0.003 g P m^{-2} day^{-1} in 2007.

2.4.2. Sediment chemistry with carbon sedimentation

Visible accumulations of fish waste did not extend far from the cages. At CF1, there was a visible layer of fish waste beneath the cage and up to 1 m from the cage edge. In 2007, the layer of fish waste was up to 28.90mm thicker than in 2006 (Table 2.4). At CF2, there was a measurable layer of fish waste up to 15 m from the farm (Table 2.4). At both farms the depth of accumulated fish waste was highest immediately below cages to 1 m from the cage edge. In July 2007, the layer of fish waste at CF2 exceeded the length of the core liner (30 cm) so we were not able to record the actual accumulation of fish waste.

Carbon, nitrogen, and phosphorus concentrations in the sediment were highest immediately beneath the cages at all three farms (Tables 2.5a, 2.5b). The mean concentrations of TC, TN and TP in the surface 2 cm of sediment at CF1 (2006 and 2007), CF2 (2007) and the experimental farm (2006 and 2007) are listed in Table 2.5a and 2.5b, respectively. At CF1 in September 2006, the carbon concentration in the sediment was 31% higher under the cage where there was a mean sedimentation rate of 17.6 g C m^{-2} day^{-1}, compared with 100 m away from the farm where the mean sedimentation rate was 0.88 g C m^{-2} day^{-1} (Table 2.5a). At CF1 in September 2007, the carbon concentration in the sediment under the cage, where it was exposed to 22.2 g C m^{-2} day^{-1}, was 68% higher than 100 m away from the farm where the mean sedimentation rate was 2.0 g C m^{-2} day^{-1} (Table 2.5a). At CF2 in 2007, sediment carbon concentration was up to 88% higher under the cage where sediments were exposed to a mean sedimentation rate of 22.2 g C m^{-2} day^{-1}, as compared with 0.84 g C m^{-2} day^{-1} at 100 m from the cage (Table 2.5a).

Concurrent sedimentation rates were those collected during the same week as the sediment samples, whereas the mean and maximum carbon sedimentation rates were determined by comparing all sampling periods within the same year. In September 2006, sediment carbon and nitrogen concentration were not related to concurrent carbon sedimentation; however, there was a significant linear relationship between sediment phosphorus concentration and carbon sedimentation (Table 2.6, Appendix 2.1). In June, July and September 2007, there was a significant linear relationship between concurrent carbon sedimentation and carbon concentration in the sediment (Table 2.6). Nitrogen concentration in the sediment was also related to carbon sedimentation in all three

sampling periods. Phosphorus concentration in the sediment was related to concurrent carbon sedimentation in June and September 2007; however, in July 2007 the relationship was not significant. Concurrent, mean and maximum carbon sedimentation were linearly related to sediment carbon, nitrogen and phosphorus concentrations (Appendix 2.1). In June 2007, concurrent sedimentation rates best described the relationship with sediment chemistry surrounding CF1 (Appendix 2.1). For July and September 2007, mean and concurrent sedimentation rates were nearly equivalent at describing the relationship with sediment chemistry (Appendix 2.1).

The effect of carbon sedimentation on sediment nutrient concentration at CF2 was best described using polynomial regression when all sampling stations from under the cage to 100 m away were included (Table 2.7). This non-linear relationship was due to the influence of a >2cm thick layer of accumulated fish waste under and immediately surrounding the cage. Between 1m and 100 m from the cage, the relationship between sediment chemistry and carbon sedimentation was linear. The linear relationships between concurrent, mean and maximum carbon sedimentation with sediment chemistry from 1-100 m away from the cage are listed in Appendix 2.2. The linear relationship between sediment nutrient concentration and carbon sedimentation was always stronger in July than September. Concurrent and mean sedimentation rates were the best predictors of sediment chemistry in July 2007; whereas for September, the mean and maximum sedimentation rates had a stronger linear relationship with sediment chemistry than the concurrent rate (Appendix 2.2).

At the experimental cage farm, the strong relationship between carbon sedimentation and sediment carbon concentration was explained by polynomial

regression in both 2006 ($y= 122.3 + 57.78x – 2.050x^2$, $r^2=0.76$, $p<0.05$) and 2007 ($y= 148.8 + 6.55x – 0.018x^2$, $r^2=0.78$, $p<0.05$). Sediment carbon concentration was 42 - 60% higher under the cage where it was exposed to a mean sedimentation rate of 25.8 to 34.1 g C m^{-2} day^{-1}, as compared with 50 m away where the mean sedimentation rate was 0.40 g C m^{-2} day^{-1}.

In addition to carbon and nutrients, elevated sediment copper (Cu) and zinc (Zn) concentrations were observed in the vicinity of the three cage farms (Tables 2.5a, 2.5b). At CF1, copper concentrations in the sediment were 2-14 times higher at sites with carbon sedimentation over 20 g C m^{-2} day^{-1}, compared with sites receiving less than 2 g C m^{-2} day^{-1}. Zinc levels were up to five times higher at sites with carbon sedimentation over 20 g C m^{-2} day^{-1}, compared with those at less than 2 g C m^{-2} day^{-1}. In 2006, there was a significant linear relationship between carbon sedimentation and the copper ($p<0.001$) and zinc ($p<0.0001$) in the sediment. The r^2 values were low: 0.22 and 0.30, respectively, so the concentration of metals in the sediment cannot be well predicted from carbon sedimentation. The results were very similar in June 2007, with significant regressions for metal concentration with carbon sedimentation, but relatively low r^2 values observed (Table 2.6). In July and September 2007, linear regressions between copper and carbon sedimentation were not significant (Table 2.6). At CF2, polynomial regressions best described the relationship between metal concentration and carbon sedimentation. In July and September 2007, sediment copper and zinc concentrations were highest at 8.03 g C m^{-2} day^{-1} and 3.39 g C m^{-2} day^{-1}, respectively. These carbon sedimentation rates were measured at 1 m from the edge of the farm. Carbon sedimentation was a better predictor of copper and zinc concentration in July 2007 ($r^2=0.51$, 0.59), compared with September

2007 (r^2=0.20, 0.19) (Table 2.7). At the experimental fish farm, the relationship between copper and zinc concentrations and carbon sedimentation was explained using polynomial regression. There was a curvilinear relationship between sediment copper concentration and carbon sedimentation in 2006 ($y= 22.51 + 49.52x – 1.812x^2$, r^2=0.69) and 2007 ($y= 27.61 + 19.72x – 0.524x^2$, r^2=0.48). There was a similar relationship for carbon sedimentation and zinc in 2006 ($y= 63.50 + 407.0x – 14.26x^2$, r^2=0.87) and 2007 ($y= 142.3 + 237.9x – 6.272\ x^2$, r^2=0.53).

2.4.3. Invertebrate response to carbon sedimentation

Polynomial regressions were used to describe benthic invertebrate density with carbon sedimentation at CF1 (Appendix 2.3) and CF2 (Appendix 2.4). At CF1 in 2006, total invertebrate density was more closely related to concurrent carbon sedimentation (r^2=0.54) than any of the individual taxa (r^2=0.14 - 0.27) (Appendix 2.3). In 2007, mean carbon sedimentation, rather than concurrent or maximum, was the best predictor of total invertebrate density, as well as for individual density for the following taxa: Oligochaeta, Chironomidae, Nematoda, Harpacticoida and Ostracoda (Appendix 2.3). At CF2 in 2007, mean carbon sedimentation was the also best predictor of total invertebrate density, as well as for predicting individual density for Oligochaeta, Chironomidae, Nematoda, and Harpacticoida (Appendix 2.4). For Ostracoda at CF2 in 2007, concurrent, mean and maximum carbon sedimentation were equally poor at predicting density.

Invertebrate density varied with the rate of mean carbon sedimentation. At both commercial farms, total invertebrate density was highest at mean carbon sedimentation rates less than 3.0 g C m^{-2} day^{-1} (Figures 2.6, 2.7). At CF1 in 2006, the density of benthic invertebrates was lowest under the cage where carbon sedimentation was 11.6 g C m^{-2}

day^{-1} (Figure 2.6a). Two peaks in total invertebrate density were found: one, at a concurrent carbon sedimentation rate of 1.7 g C m^{-2} day^{-1}, and a second, smaller peak at 2.9 g C m^{-2} day^{-1} (Figure 2.6a). At the same farm in 2007, invertebrate density peaked at a mean sedimentation rate of 1.21 g C m^{-2} day^{-1}, and was reduced at sedimentation rates beyond 1.81 g C m^{-2} day^{-1} (Figure 2.6b). At CF2, there were also two peaks in total invertebrate density in 2007: one, at a mean carbon sedimentation rate of 2.0 g C m^{-2} day^{-1}, and a second, smaller peak at 2.4 g C m^{-2} day^{-1} (Figure 2.7). At each farm, the total invertebrate density was highest at mean carbon sedimentation rates $\leq$3.0 g C m^{-2} day^{-1} and then declined with increasing carbon sedimentation. The rapid decline in invertebrate density that occurred immediately following the peak in density was described using exponential regression (Table 2.8). Total invertebrate density was reduced at CF1 beginning at 2.9 g C m^{-2} day^{-1} in 2006, and at 1.2 g C m^{-2} day^{-1} in 2007. At CF2, total invertebrate density was reduced beginning at 2.0 g C m^{-2} day^{-1} in 2007. The exponential decline in density of the four most abundant taxa collected, Oligochaeta (Tubificinae), Chironomidae, Nematoda and Harpacticoida, is described in Table 2.8. Ostracoda and Chironomidae were the first taxa to be reduced, followed by Nematoda, Harpacticoida and Oligochaeta (Figures 2.6, 2.7). At the experimental farm (L375), mean sedimentation rates from 2006 and 2007 were compared with total invertebrate densities measured by Rooney and Podemski (2009). A polynomial regression of total invertebrate density (in 2005) with mean carbon sedimentation demonstrated a strong exponential relationship in 2006 ($y= 5301 \cdot e^{-6.947x} + 234.0$, $r^2=0.74$), and 2007 ($y= 3296 \cdot e^{-7.395x} + 234.0$, $r^2=0.75$). Invertebrate density declined beginning at 2.65 g C m^{-2} day^{-1} and 2.85 g C m^{-2} day^{-1} based on the 2006, and 2007 sedimentation rates, respectively.

Carbon sedimentation had an effect on size of individuals, measured as mean individual biomass (Tubificinae, Naidinae) and head capsule size (Chironomini). At CF1 in 2006, mean individual biomass for Tubificinae was greatest at 6.97 g C m^{-2} day^{-1} (Figure 2.8a). At this same farm in 2007, there was a peak in the mean individual biomass of Tubificinae exposed to a mean carbon sedimentation rate of 4.05 g C m^{-2} day^{-1} (Figure 2.8b). At CF2 in 2007, mean individual biomass for Tubificinae (Figure 2.9a) and Naidinae (Figure 2.9b) were greatest at 3.07 g C m^{-2} day^{-1}. The relationship between Chironomini head capsule size (length and width) and carbon sedimentation was best described using polynomial regression (Table 2.9). At CF1, Chironomini head capsule length was significantly related to mean carbon sedimentation in 2007 (Table 2.9). At CF2 in 2007, Chironomini head capsule width was significantly related to mean carbon sedimentation (Table 2.9). The peak in Chironomini head capsule length and width occurred between 1.72 - 10.03 g C m^{-2} day^{-1}. Of the Chironomidae larvae collected, the majority of the individuals were Chironomini. There were small numbers of Tanypodinae and Orthocladiinae larvae collected at each of the commercial farms, but there were not enough for statistically significant comparisons of individual size with carbon sedimentation. The effect of carbon sedimentation on Nematoda, Ostracoda, and Harpacticoida biomass was not determined due to the likely influence of different tolerances masked by high-level identification.

2.5. Discussion

2.5.1. Sedimentation rates of aquaculture waste

There is limited information on dispersion and rates of sedimentation of particulates near freshwater fish farms. This study is the first to describe these trends for

commercial cage farms in Ontario. Sedimentation rates at both commercial farms ranged from 9.47 - 23.19 g C m^{-2} day^{-1}, 0.94 - 4.92 g N m^{-2} day^{-1}, and 0.26 - 1.76 g P m^{-2} day^{-1} directly under the cages. The range of measured sedimentation rates was wider at CF1, compared with CF2. This was due to additional sampling periods in September 2006 and June 2007, where mean sedimentation rates were generally lower compared with July and September 2007. At the experimental cage farm, sedimentation rates ranged from 2.9 - 87.2 g C m^{-2} day^{-1}, 0.4 - 20.0 g N m^{-2} day^{-1}, and 0.4- 9.3 g P m^{-2} day^{-1}. The range of measured sedimentation rates at the experimental cage farm was larger than at the commercial farms because every week of the production cycle over two seasons was sampled, compared with only 2-3 weeks of the production cycle at the commercial farms. Bristow (2006) measured carbon, nitrogen and phosphorus sedimentation during the first year of production under the same experimental rainbow trout cage farm. He reported sedimentation rates ranging from 1.0 - 26.0 g C m^{-2} day^{-1}, 0.1 - 3.2 g N m^{-2} day^{-1}, and 0.1 -2.1 g P m^{-2} day^{-1} (Bristow 2006), which are much lower than the current estimates. Bristow (2006) deployed sediment traps between June and October, for periods ranging from 8 to 29 days, whereas this study collected traps weekly. It is likely that mineralization of the organic material occurred in the traps deployed for longer periods (Tlusty *et al.* 2000). Loss of organic matter may have occurred through diffusion into the water column, through consumption, or decay (Tlusty *et al.* 2000). Tlusty *et al.* (2000) reported that *O. mykiss* faecal matter lost approximately 50% of its organic matter in 12 days. The estimates in this study are based on data from sediment traps deployed for seven days; therefore, the loss of labile compounds due to decomposition should be less than in Bristow (2006).

At the three net-pen farms in this study, carbon sedimentation rates fell exponentially as distance from the fish farm increased. At the commercial farms, carbon deposition was reduced by 90 to 95 % at 30 m from the cages at CF1, and 88 to 92 % at CF2. At 5 m from the experimental net-pen farm, deposition was reduced by 96.7% in 2006, and 97.8% in 2007. An exponential decline in particulate waste sedimentation with increasing distance from farms has been reported by many authors (*e.g.,* Weston 1990; Holmer *et al.* 2007; Kutti *et al.* 2007; Rapp *et al.* 2007). Holmer *et al.* (2007) reported high rates of sedimentation (38 - 64 g C m^{-2} day^{-1}) directly beneath three fish farms in the Mediterranean, diminishing by up to 91% at 5 to 40 m away from the cages. Rapp *et al.* (2007) measured sedimentation of waste feed around a cobia (*Rachycentron canadum* Linnaeus) farm in Puerto Rico, moored in 27.2 m of seawater. Ninety per cent of the material fell within 30 m of the cage mooring block (Rapp *et al.* 2007). Unfortunately, the authors were only concerned with the dispersion of waste feed, and not fish faecal material which was sieved out of the samples before analysis. (Rapp *et al.* 2007). It is likely that the majority of fish waste would have also been deposited within this range. The spread of waste material is influenced by the settling velocity of the faecal pellets (Cromey *et al.* 2002; Moccia *et al.* 2007). Cromey *et al.* (2002) measured the mean settling velocity for salmon faeces in seawater as 3.2 $cm \cdot sec^{-1}$. Moccia *et al.* (2007) reported the mean settling velocity of rainbow trout faeces in freshwater as 5.2 $cm \cdot sec^{-1}$. The more rapid settling in freshwater would result in more limited dispersion as compared with marine environments.

Water depth is a critical factor for determining patterns of sedimentation around cage sites (Cromey *et al.* 2002). For example, Kutti *et al*. (2007) compared sedimentation

rates at a deep-water (230 m) marine net-pen Atlantic salmon (*Salmo salar* Linnaeus) farm in Norway, with rates reported by Hansen *et al.* (1991) at a farm located at a sheltered site in less than 20 m of water depth. Sedimentation rates under the cages at the shallow water site were two to ten times higher (3 - 30 g m^{-2} day^{-1}) than at reference sites and there was significant accumulation of wastes on the sediment beneath the cages (Hansen *et al.*1991), whereas Kutti *et al*. (2007) measured no significant increase in organic matter or waste accumulation in the sediment surrounding the deepwater farm. Kutti *et al.* (2007) reported particulate organic carbon sedimentation rates of between 2.5 - 3.8 g m^{-2} day^{-1} beneath the farm at peak production. Current speed may alter the pattern of sedimentation around the farm. Total particulate matter was consistently higher in the traps 6 m off the bottom, compared with the traps 150 m from the surface (Kutti *et al*. 2007). This may be due to resuspension from pulses of higher current speed (10-20 cm s^{-1}) that occur up to three times a day, compared with the average current speed of 0 -1.0 cm s^{-1} (Kutti *et al*. 2007).

Sedimentation of particulate waste surrounding the cage farms examined in this study was not consistent throughout the year. Sedimentation of net-pen farm waste is highly correlated with intensity of feeding. Feeding generally increases throughout the open water season with the growth of the fish. At cage farms in Ontario, feed is applied at a rate of 1-3% of body weight per day, depending on surface water temperature (Rooney and Podemski 2010). The rate of aquaculture waste sedimentation has been linked to feeding intensity at marine farms. Kutti *et al*. (2007) examined temporal variations in the sedimentation pattern near a Norwegian salmon farm. Sedimentation rates within the footprint of the farm followed the feeding pattern (Kutti *et al*. 2007) while sedimentation

traps located 150 m from the surface showed evidence of increased sedimentation occurring during settling of the spring algal bloom (Kutti *et al*. 2007). Background sedimentation rates can fluctuate due to wind-driven resuspension, ice cover, algal blooms and peripheral wave action (Douglas *et al*. 2003), in addition to anthropogenic inputs such as agricultural runoff and residential use.

There was a large difference in annual fish production and waste accumulation on the sediment beneath the two commercial farms. One would expect to see increased sedimentation at CF2 where production and waste accumulation were higher; however, the rates at CF1 and CF2 were quite similar (Table 2.2). The timing of the September sampling period may have influenced the measured sedimentation rates. At CF2, feed input to the farm declined in September (Figure 2.4b), whereas at CF1 feed input declined in August (Figure 2.4a). If sedimentation at CF2 had been measured in August or October, (in addition to the July sampling) the mean rate would likely have been much higher. Farm location may have also influenced the deposition rate. Commercial Farm 1 was more sheltered, located in Lake Wolsey, on Manitoulin Island, whereas Commercial Farm 2 was more exposed, located off Little La Cloche Island, in the North Channel of Lake Huron. Increased water movement in the North Channel could have spread the material more than at CF1 in Lake Wolsey. Mean sedimentation rate was slightly higher at each of the stations along the transect at CF2, compared with CF1 (Table 2.2). Current speed was not recorded during this study but would have been useful for comparison and is recommended for future work.

2.5.2. Effects on sediment chemistry

The accumulation of fish waste alters the sediment chemistry surrounding cage farms. In this study, nutrients (TN, TP), metals (Cu, Zn) and total carbon (TC) were more concentrated in the sediment under the three *O. mykiss* farms, compared with the reference sites (Tables 2.5a, 2.5b). At the commercial farms, mean sediment TC, TN, TP and zinc concentrations were higher at CF2, compared with CF1 (Table 2.5a). The differences in carbon and nutrient outputs between farms will largely depend on production levels. Both commercial farms were 0.28 hectares in surface area but CF2 produced approximately 450 000 kilograms of rainbow trout in 2007, compared with approximately 300 000 kilograms at CF1. Higher production at CF2 was reflected in the increased accumulation of fish waste beneath the farm (Table 2.4). At CF2, the depth of fish waste under the cages in 2006 and 2007 was always greater than 2 cm, so the sediment sample was composed entirely of fish waste. Fish faeces contain higher concentrations of carbon and nutrients than natural sediment (Moccia *et al.* 2007; Rooney and Podemski 2010). Moccia *et al.* (2007) determined the composition of faecal waste collected from commercial *O. mykiss* farms in Ontario to be 41.43 % TC, 3.97 % TN, and 2.87 % TP. These values are very similar to the TC, TN, and TP concentrations under the cage at CF2 in 2007 (43.6 - 44.3 % TC, 3.9 - 4.4 % TN, and 2.7 - 2.9 % TP). At CF1, the sediment samples were composed mostly of natural sediment with some fish waste accumulation (Table 2.4), so the sediment TC, TN, and TP concentrations in the top 2 cm were generally lower (5.1 - 21.9 % TC, 0.6 - 1.7 % TN, and 2.1 - 3.7 % TP). Similar to CF2, at the experimental farm, entire sediment samples (*i.e.*, top 2 cm) from under the cage in 2006 and 2007 were composed of fish waste (25.0 - 35.4 % TC, 4.1 - 5.9% TN,

and 3.6 - 4.4 % TP) (Table 2.5b). The amount of fish waste accumulation, in conjunction with the depth of sediment collected influenced the reported sediment concentrations under these farms.

In one of the few studies examining impacts of commercial rainbow trout cage aquaculture on sediments, Alpaslan and Pulatsü (2008) reported elevated TC, TN and TP concentrations under a farm in a Turkish reservoir compared to a control site approximately 60 m away. Under the cage, sediment concentrations ranged from 5.41-8.59 % TC, 0.26 - 0.44 % TN and 0.06 - 0.13 % TP, which is an order of magnitude lower than TC, TN, and TP concentrations recorded under the Ontario commercial farms in 2007 (Table 2.5a), and similar to the TC, TN, and TP sediment concentrations at the reference sites 100 m from the Ontario commercial farms. The elevation in sediment TC, TN, and TP under the cages was also far less than that observed between cage and reference sites at the Ontario farms. In Alpaslan and Pulatsü (2008), sediment TC, TN and TP concentrations were 1.2 - 27.5%, 13.3 - 25.7 % and 14.3 - 61.5 % greater than at control sites, respectively. In comparison, at CF1 sediment TC was 68.1 - 83.5% higher under the cage, compared with 100 m away (Table 2.5a) and at CF2, the difference in TC concentration between under the cage and the reference site was even greater, ranging from 86.9 - 88.5% (Table 2.5a). The Turkish farm was similar to CF1 and CF2 in spatial scale (12 net-pens, 100 m^3 per cage); however production was only 20 000 kg $year^{-1}$, compared with 300 000 - 450 000 kg $year^{-1}$ at the commercial farms in this study. The authors suggested that short water renewal time (0.04 years) of this facility likely was a key factor in reducing the effects of the cages (Alpaslan and Pulatsü 2008). Water renewal time may be important for the removal of dissolved wastes, but is unlikely to

affect the accumulation of particulate waste unless current speeds that exceed the sinking rates are attained. It is more likely that the low annual production of the farm was the primary factor responsible for the minimal effects to the sediment chemistry. Other authors have reported elevations in sediment TC, TN, and TP concentrations near the cages at freshwater rainbow trout farms. Rooney and Podemski (2010) studied the effects of the experimental farm on sediment chemistry during the first two production cycles (2003-2004). Sediment TC, TN, and TP concentrations measured at the experimental farm during this study in September 2006, and October 2007 were similar to those observed after nearly two seasons of production in September 2004 (Rooney and Podemski 2010). The production intensity (approximately 10 000 rainbow trout raised from May to October) and feeding regime (Martin Mills Profishent© at a rate of 1-3% of body weight per day) had not changed, so the carbon and nutrient output to the sediment should have been similar in 2003-2004 and 2006-2007. Organic material was also elevated under a rainbow trout farm in Lake Letowskie, Poland compared with reference sites (Korzeniewski and Salata 1982; Dobrowolski 1987). Unfortunately, there is no pre-farm versus operational comparison available for the farm in Lake Letowskie as sampling did not occur until seven years after the start of production. It is difficult to compare the Lake Letowskie findings to those at the experimental and commercial farms in this study because the farm was located over littoral sediments in <10 m of water (Dobrowolski 1987), whereas the farms in Ontario in this study are all over the profundal zone. In addition, the FCR for the farm was 2.5 (Korzeniewski and Salata 1982), which indicates higher waste sedimentation than would be expected under modern operating practices (≤1.2) (Cho and Bureau 2001). In the marine environment, organic carbon, nutrients and

metals are also commonly observed to be elevated in sediments beneath and in the near vicinity of cage farms (*e.g.,* Johnsen *et al*. 1993; Kempf *et al*. 2002; Brooks and Mahnken 2003; Chou *et al*. 2004; Kalantzi and Karakassis 2006; Giles 2008; Sanz-Lázaro *et al.* 2011).

Effects on sediment chemistry beneath the Ontario farms were localized to within 20-30 m from the commercial cages, and only 3 m from the edge of the experimental cage farm. Studies conducted at other freshwater farms have reported impacts detected to 25 m (Doughty and McPhail 1995) and up to 50 m away from the cages (Guo and Li 2003). In the marine environment, farms appear to affect a larger area. Kutti *et al.* (2007) reported that the majority of fish waste from a Norwegian salmon farm settled within 250 m from the farm; however, some farm waste was transported 550 - 900 m from the operation, likely due to resuspension. The species-specific settling rate of fish faeces will influence the effect of sedimentation on sediment chemistry. For example, faecal pellets for cobia (*R. canadum*) sink at an estimated rate of 1 cm s^{-1} (Rapp *et al.* 2007); compared with > 6 cm s^{-1} for rainbow trout (Reid *et al.* 2009). The higher density of seawater (1.025 $g.cm^{-3}$) compared with freshwater (1.000 $g.cm^{-3}$) also may affect fish waste dispersal distances. Due to increased buoyancy in seawater, the fish waste has the potential to disperse greater distances from the cages.

In addition to the waste loading rate, the size of a farm's footprint will be influenced by lake morphometry, flow rates, and farm size so these must be considered when comparing impacts on sediment chemistry (Rooney 2006). The age of a farm also may affect the size of its footprint on sediment chemistry. At the experimental farm, Rooney and Podemski (2010) found detectable impacts on the sediment out to 1 m from

the cage after two production cycles (2003-2004). During this study, the experimental farm was on its fourth and fifth years of operation, and impacts were observed up to 3 m away. The commercial farms in this study have been in operation for approximately 15 - 30 years. Determining the boundary of effects is important to regulators since permits are granted to farmers based on a specific area beyond which their farm must not significantly affect the receiving environment (Thorburn *et al.* 2004).

Clear temporal trends in sediment carbon and nutrient concentrations were not evident at the cage farms in Ontario. Sediment TC, TN and TP concentrations under CF1 were higher in 2007 than in 2006 (Table 2.5a) likely resulting from greater accumulation of fish waste in 2007 (Table 2.4), which greatly elevates sediment TC, TN and TP concentrations above that of natural sediment. The biggest contributing factor to effects on sediment TC, TN and TP is likely feed input. Seasonally, feed input varies with the size of the fish and water temperatures. In general, feed input at all three *O. mykiss* farms increased from spring through the fall each year and then was reduced from December to March, according to feed records supplied by the farms (Figure 2.4). A temporary, late summer reduction in feed input was associated with high water temperatures. The reduction in feeding at CF1 in August 2007 was reflected in the sediment chemistry, as TC concentration decreased significantly from mid-July to the September sampling period (Figure 2.4a). At CF2, TC, TN and TP concentrations were consistent from July to September 2007 (Figure 2.4b). Since sediment samples collected under the cage at CF2 were entirely composed of fish waste, seasonal changes in sediment chemistry would likely not occur. At the experimental cage farm, feeding ceased from the time of harvest in October (2007) or November (2006) until restocking during the following May. As at

CF2, sediment samples were composed entirely of fish waste under the cage so a clear seasonal trend was not evident. A correlation between the feeding regime and effects on sediment chemistry has been noted in numerous studies, primarily marine (*e.g.,* Ackefors and Enell 1994; Guo and Li 2003; Kalantzi and Karakassis 2006; Alpanslan and Pulatsü 2008). Alpanslan and Pulatsü (2008) observed temporal changes in sediment chemistry at a commercial freshwater rainbow trout farm in Turkey; feeding was increased in the spring each year, which corresponded with increased sediment TN and TP concentrations.

Elevated concentrations of heavy metals such as zinc (Zn) and copper (Cu) have been reported from sediments near cage culture facilities (*e.g.,* Kempf *et al.* 2002; Brooks and Mahnken 2003; Chou *et al.* 2004). This study found similar effects in freshwater, with the highest concentrations of sediment Zn and Cu observed beneath the three farms and then decreasing with distance. At the experimental farm, Rooney and Podemski (2010) reported elevated sediment Cu and Zn concentrations to 1 m beyond the cage edge after two production cycles. In the Broughton Archipelago, British Columbia, Sutherland *et al*. (2007*b*) collected sediment samples near marine finfish sites to determine the spatial extent of aquaculture-derived zinc and copper in the sediment. Significant elevations in zinc and copper concentrations were restricted to within 30 m from the operations (Sutherland *et al*. 2007*b*).

Since copper and zinc are constituents of fish feed (Parker and Aubé 2002), with minimal accumulation of fish waste, one would expect the temporal pattern of sediment chemistry to vary with feeding. Sediment copper and zinc concentrations at CF1 decreased over the sampling period with the addition of fish waste (Figure 2.5a). The

sediment underlying these farms was more concentrated with copper and zinc in the spring. The decline in feeding over the winter (December to March) and the decomposition of organics over the winter may have increased the proportion of metals in the sediment. However, at both commercial farms there was no statistically significant difference in copper or zinc concentration from July to September 2007 due to high variability (Figure 2.5). The sediment under CF2 was not sampled in June 2007, so it is unclear if the concentration of metals would have been higher in the spring.

Some authors have reported a positive correlation between sedimentation rates and sediment chemistry near cage culture facilities (*e.g.,* Troell and Berg 1997; Sanz-Lázaro *et al.* 2011). Typically, both sedimentation rate and effects on sediment chemistry decline exponentially with distance from the farm and these relationships have also been described by exponential decay curves in the present study. At marine sites, significant water depth and/or high current speed may modify the relationship between sedimentation rate and sediment chemistry (*e.g.,* Holmer *et al*. 2007; Kutti *et al.* 2007). In this study, the shape of the relationship between carbon sedimentation rate and sediment chemistry at CF2 was modified as a result of an accumulation of >2 cm of fish waste up to 15 m from the farm. This thick layer of fish waste resulted in sediment nutrient, carbon and metal concentrations being reflective of manure composition rather than sedimentation rate. Despite the large changes in sedimentation rates with distance that occurred in this area, sediment nutrient, metal and carbon concentrations did not change. The relationship between sediment chemistry and carbon sedimentation was linear between stations from 1 m to 100 m away from the farm.

In general, sediment carbon, nutrient and metal concentrations were better predicted early in the season from concurrent sedimentation rates (Appendix 2.1, 2.2). Less fish waste had accumulated in the spring, so the concurrent carbon sedimentation had a stronger effect on the relationship with sediment chemistry. By midsummer, mean and concurrent sedimentation were equally useful for predicting effects on sediment chemistry. Feed input increased throughout the season, peaking between August and October (Figure 2.5). In September, the mean and maximum sedimentation rates were both good predictors of sediment chemistry due to the timing of peak farm production.

Despite similar sedimentation rates, the increased accumulation of material under CF2 suggests that waste breakdown and/or removal of waste material are different between the two farms. While coring for sediment samples using SCUBA at CF1, the divers observed an abundance of round gobies (*Neogobius melanstomus* Pallas) beneath the farm, in addition to schools of yellow perch (*Perca flavescens* Linnaeus) and an extensive population of zebra mussels (*Dreissena polymorpha* Pallas). Consumption of fish waste material by wild biota may be a factor contributing to the lack of accumulation under CF1. Future work could include isotopic analysis similar to Kullman *et al*. (2009), and Wellman (2011) to determine if these organisms are using the aquaculture waste as a food source.

2.5.3. Invertebrate response to carbon sedimentation

Until recently, researchers have conducted very few studies addressing the effects of aquaculture waste on freshwater zoobenthos (*e.g.,* Kullman *et al*. 2007, 2009; Rooney and Podemski 2009; Wellman 2011). In general, changes in benthic community composition, richness, and density can be expected along gradients of enrichment

(Hutchinson 1957; Pearson and Rosenberg 1978; Carroll *et al.* 2003). In Pearson and Rosenberg's (1978) seminal review of zoobenthic responses to organic enrichment in marine systems, the authors developed a model to predict community response along spatial and temporal gradients. The Pearson-Rosenberg (1978) model described the faunal change as the community goes from an unperturbed, species-rich community, to a perturbed, species-poor one. It is at the grossly polluted stage, where the fauna is expected to be completely eliminated (Pearson and Rosenberg 1978). The model provided three major predictions regarding invertebrate density, richness, and biomass along an organic enrichment gradient (Pearson and Rosenberg 1978).

The first prediction of Pearson and Rosenberg's (1978) model is that along a gradient in increasing organic enrichment, invertebrate density will increase to a maximum. This maximum will be the result of a peak in the abundance of opportunistic species that are tolerant of organic loading (Pearson and Rosenberg 1978). The Pearson-Rosenberg model predicted that if loading continues to increase, abundance will abruptly decline. This prediction applied well to the results at the commercial *O. mykiss* farms. At both farms, total invertebrate density peaked at carbon sedimentation rates $\leq$3.0 g C m^{-2} day^{-1} and then exponentially declined (Figures 2.4, 2.5). Compared with the peak density along the gradients, invertebrate density was reduced by >95% under the cages. There was not an azoic zone in the sediment under the cages, which were subjected to sedimentation rates between 9.5 – 23.2 g C m^{-2} day^{-1}. The peaks in invertebrate density were mainly due to an increase in Tubificinae (Oligochaeta), followed Harpacticoida and Nematoda. Oligochaetes (*e.g.*, Tubificinae) are the most often cited enrichment opportunist in freshwaters receiving organic waste (Milbrink 1980; Dobrowolski 1987;

Camargo 1992; Doughty and McPhail 1995; Loch *et al.* 1996; Guo and Li 2003; Clerk *et al.* 2004; Kirkagac *et al.* 2004). Harpacticoid copepod density peaked at carbon sedimentation rates ≤2.0 g C m^{-2} day^{-1} at CF1 (Figure 2.6) and near 2.5 g C m^{-2} day^{-1} at CF2 (Figure 2.7). Harpacticoid copepods cannot endure anoxic conditions and are therefore sensitive to high levels of organic loading (Särkkä 1992). Little is known about the response of freshwater nematodes to aquaculture waste loading. Sutherland *et al.* (2007*a*) observed a slight decline in nematode abundance with increasing organic enrichment at a marine salmonid farm. Lower-level identification of nematodes would be required to reduce variability due to species-specific tolerances. Without the consideration of differences in size and feeding morphologies, the authors reported that nematodes were not suitable as an indicator of organic enrichment in their study (Sutherland *et al.* 2007*a*).

While only Rooney and Podemski (2009) explicitly tested the Pearson-Rosenberg model against their data, studies from several other freshwater cage farms also fit the predicted pattern in invertebrate abundance. Doughty and McPhail (1995) examined the effects of cage farming on the zoobenthos at sixteen Scottish commercial farms. At the three largest *O. mykiss* cage farms, oligochaete abundance increased by as much as two orders of magnitude between control sites and those 25 m from the farms and then declined directly beneath the farms, producing the peak in abundance predicted by Pearson and Rosenberg (1978). Guo and Li (2003) reported a decline in invertebrate density beneath a freshwater cage farm in China. The authors compared the benthic community beneath and beside the farm, and sampled before farming began and at harvest (Guo and Li 2003). The exact distance between the sampling station beside the

farm and the cages was not specified. Total density beneath the farm declined from 120 to individuals $\cdot m^{-2}$ to 16 individuals $\cdot m^{-2}$ below the farm, and from 255 individuals $\cdot m^{-2}$ to 165 individuals $\cdot m^{-2}$ beside the operation (Guo and Li 2003). Guo and Li (2003) did not describe how the sediment was sampled, so it is unclear if these are reasonable estimates of the benthic community. Under the cages, Guo and Li (2003) might have observed the point in Pearson and Rosenberg's (1978) model where density will abruptly decline. However, the authors sampled only two points, and had no distant reference station within the lake, so it is difficult to describe the results using the non-linear Pearson-Rosenberg model.

According to Pearson and Rosenberg's (1978) second prediction, species richness will exhibit a peak and then a decline along a gradient in organic enrichment. The peak in species richness is predicted to occur at the ecotone point; *i.e.*, the region at which the invertebrate community transitions from the background community to only the tolerant taxa, and therefore species from both are present. In the present study, the peak in richness occurred at carbon sedimentation rates $\leq$2.0 g C m^{-2} day^{-1} (Figures 2.6, 2.7). At this sedimentation rate, the community contained both sensitive, (*e.g.*, Harpacticoida, Ostracoda) and tolerant taxa such as Oligochaeta, Nematoda and Chironomidae. As predicted, richness peaked at a lower intensity of waste loading than the maximum peak in abundance (*i.e.*, $\leq$3.0 g C m^{-2} day^{-1}) (Pearson and Rosenberg 1978). Invertebrates were identified only to subfamily for Oligochaeta, tribe for Chironomidae, and Order for all other taxa. Without lower-level identification, many different taxonomic groups will have been clumped together and thus the effects of genus or species-specific tolerances on richness will not have been observable in the data set. At a newly-established *O. mykiss*

farm, Rooney and Podemski (2009) reported reduced species richness at high levels of waste loading, as predicted by the Pearson-Rosenberg model; however, significant peaks in richness at moderate loading were not observed. The study was completed during the first two production cycles of the farm, and the authors predicted it would take time for an equilibrium to be reached in response to farm waste loading (Rooney and Podemski 2009). Other authors have described reductions in taxon richness in the vicinity of cage farms (*e.g.,* Dobrowolski 1987; Doughty and McPhail 1995; Guo and Li 2003; Rooney and Podemski 2009). As invertebrate taxa are differentially sensitive to the stresses associated with organic enrichment, they are progressively eliminated as enrichment increases and the community becomes less diverse with a higher proportion of deposit feeders (Sanz-Lázaro and Marín 2011). Near the Ontario cage farms, in areas receiving sedimentation rates greater than 3.0 g C m^{-2} day^{-1}, deposit-feeding oligochaetes and chironomids dominated the benthic community while the abundance of filter-feeding ostracods was reduced by up to 99% as compared with the 100 m reference sites.

Pearson and Rosenberg's (1978) third prediction is that total biomass will exhibit two peaks along the organic enrichment gradient. The first peak will occur in the same region as the peak in species richness, resulting from the stimulatory effects of low levels of organic enrichment (Peeters *et al.* 2004; Vos *et al.* 2004). The second peak will occur in the same region as the peak in species abundance, and will result from the high density of opportunists (Pearson and Rosenberg 1978). Mean individual, rather than total biomass, was determined in this study for selected taxa so direct comparisons to the model's predictions regarding total community biomass cannot be made. In this study, mean individual biomass was determined for Tubificinae and Naidinae at the commercial

fish farms. At CF1, two peaks in biomass were observed (Figure 2.8). Species-specific preferences may have been observed, though could not be described due to family-level identification of oligochaetes. The peak at 2.0 g C m^{-2} day^{-1} corresponds to the peak in richness; however, this increase was non-significant (Figure 2.8a). A second peak at 6.97 g C m^{-2} day^{-1} in 2006 (Figure 2.8a) and 4.05 g C m^{-2} day^{-1} in 2007 (Figure 2.8b) was significant, and occurred at the carbon sedimentation rate at which other taxa had been nearly eliminated. A density dependent response may explain why the peak at 2.0 g C m^{-2} day^{-1} was not significant. Monti (1987) reported that a high density field population of *T. tubifex* (up to 150 000 individuals per m^2) exhibited decreased fecundity and smaller mean individual weight. Populations of oligochaetes over 150 000 individuals m^{-2}, mostly Tubificinae, were found in 2007 at both commercial farms at carbon sedimentation rates less than 2.5 g C m^{-2} day^{-1} (Figures 2.6b and 2.7). Individual biomass of worms at these lower sedimentation rates may have been reduced due to increased competition for food.

In this study, individual size of Chironomini was measured as head capsule length and width. Variation in both was related to carbon sedimentation; however, there was a great deal of variability around the regression lines (r^2=0.22 – 0.78). Many species could have been present at the commercial farms; however, since the chironomids were identified only to tribe, all Chironomini data were pooled for analysis which would have increased variability. The growth and survival of benthic invertebrates, including Chironomini, were examined after a 21-day exposure to various rates of carbon sedimentation from aquaculture waste (Wetton, Chapter III). For *Chironomus riparius* (Meigen), mean individual biomass was stimulated by the increased waste sedimentation

but there was no significant effect on larval body length or head capsule length or width (Wetton, Chapter III). Mean individual biomass for the Chironomini larvae from the commercial farms was not determined, but it would have been a useful measure to determine if these larvae were utilizing the farm waste. Stable isotopic evidence from recent studies at the experimental cage farm suggest that chironomid larvae do consume farm waste (Kullman *et al.* 2009; Wellman 2011).

Much of the previous work identifying effects on freshwater benthic fauna has been descriptive, rather than predictive. Some marine studies have shown that changes in sediment chemistry resulting from increased rates of organic matter sedimentation can be used to derive quantitative relationships to predict effects on benthic community structure (*e.g.*, Hargrave *et al*. 2008). Sedimentation rates >1 g C m^{-2} day^{-1} resulted in the formation of hypoxic sediments around salmon farms (Hargrave 1994). Findlay and Watling (1997) identified a similar threshold (1–5 g C m^{-2} day^{-1}) close to salmon pens in coastal Maine where the biodiversity of macrofauna was reduced due to sediment enrichment. Thresholds of effect on benthos based on rates of sedimentation are important for developing regulations to use in conjunction with depositional models. The present study is the first to identify thresholds of effect due to freshwater net-pen aquaculture. Based on the evidence described in this study, the threshold of effect on benthic abundance and richness is likely 2.0 - 3.0 g C m^{-2} day^{-1}. This sedimentation rate was measured approximately 5 m from CF1, 10 -15 m from CF2, and at the edge of the experimental cage farm (Tables 2.2 - 2.3).

Habitat alterations from aquaculture waste

The distribution of organisms will depend in part on their habitat requirements (Hutchinson 1957). The accumulation of farm waste alters the benthic environment, affecting the invertebrates on the basis of their specific tolerances. There are numerous environmental changes associated with fish farm waste deposition that could account for the observed changes in the zoobenthos such as oxygen depletion, the build-up of ammonia from decomposition, the accumulation of heavy metals, alterations in sediment texture and food availability. Oxygen depletion could be responsible for some of the observed effects on the benthos, as oxygen is consumed in the decomposition of organic farm waste (Loch *et al.* 1996; Findlay and Watling 1997; Kirkagac *et al.* 2004). Fish farm waste has a high biological oxygen demand (BOD) (Loch *et al.*1996; Kirkagac *et al.* 2004). Troell and Berg (1997) found that sediment below a freshwater cage farm in Zimbabwe consumed 30 - 40% more oxygen than sediments at control sites.

Oxygen depletion can affect invertebrate respiration and cause changes in the benthic community, resulting in increased density of more tolerant species. Evidence of such community changes in response to reduced dissolved oxygen in the vicinity of cage farms has been reported by Dobrowolski (1987), Doughty and McPhail (1995), Guo and Li (2003), and Clerk *et al.* (2004). These changes included an increase in abundance of Tubificinae and Chironomini larvae. Chironomids with haemoglobin-like respiratory pigments are capable of surviving at levels > 2 mg O_2 L^{-1} and oligochaetes of the Tubificidae family to levels > 1 mg O_2 L^{-1} (Hamburger *et al.* 2000). These taxa also store glycogen to provide energy during anoxic periods, allowing them to survive for months at oxygen levels >0.2 mg O_2 L^{-1} (Hamburger *et al.* 2000). Dissolved oxygen concentration

within the sediment was not measured during this study, but reduced sediment oxygen concentrations may explain the loss of some taxa and the dominance of Tubificinae with increasing farm waste deposition.

Ammonia may also be responsible for effects on the benthic community. The anoxic breakdown of proteins produces ammonia and ammonium, which can be found in elevated concentrations with the accumulation of organic wastes (McCahon *et al.* 1991; Hickey *et al.* 1999). Ammonia concentrations are commonly elevated in the sediment beneath and immediately surrounding fish farms (*e.g.,* Axler *et al.* 1996; Troell and Berg 1997; Veenstra *et al.* 2003; Rooney and Podemski 2010). Ammonia exists in two forms: a relatively non-toxic ionized (NH_4^+) and a toxic unionized form (NH_3) (Emerson *et al.* 1975). The NH_4^+ : NH_3 ratio in freshwater is regulated primarily by temperature and pH, with the unionized form dominating at high pHs (Emerson *et al.* 1975). Rooney and Podemski (2010) described the development of changes in sediment and pore-water chemistry at a newly established *O. mykiss* farm in Ontario. Total pore-water ammonia was elevated under the cage after one month of production (Rooney and Podemski 2010). After sixteen months, the concentration was over 300 times the pre-farm value and elevated ammonia could be detected up to 5 m beyond the cage edge (Rooney and Podemski 2010). Troell and Berg (1997) measured pore-water total ammonia concentrations at a freshwater tilapia farm in Zimbabwe. The authors reported significantly higher total ammonia concentrations in sediment pore-water beneath cages (0.69 to 4.750 mg L^{-1}) than at reference sites (0.303 to 1.409 mg L^{-1}) (Troell and Berg 1997). Unionized ammonia (NH_3) is toxic to invertebrates and its accumulation in pore-water could therefore affect the benthic community (Monda *et al.* 1995). As with oxygen

requirements, taxa will be differentially sensitive to unionized ammonia. For example, *Chironomus tentans* Fabricius has a 96 hr LC_{50} of 1.65 mg NH_3 L^{-1} (Williams *et al*. 1984); whereas, the 96 hr LC_{50} for *Chironomus riparius* Meigen is much higher, 6.60-9.40 mg NH_3 L^{-1} (Monda *et al*. 1995) For the invertebrates found beneath the commercial farms, chronic exposure is assured; however, the effects of these exposures on growth, survival and reproduction are significantly less well studied than acute (McCahon *et al.* 1991; Kuhn *et al.* 2000). Pore-water ammonia was not measured at the commercial farms, but was likely a factor in the reduced invertebrate density beneath the cages. Future sediment monitoring programs should include pore-water ammonia as recommended by Rooney and Podemski (2010).

The accumulation of heavy metals such as copper and zinc may have caused a toxic response in the benthos exposed to high concentrations of farm waste. Zinc and copper concentrations have previously been reported to increase in sediment beneath freshwater (*e.g.*, Rooney and Podemski 2010) and marine cage farms (*e.g.*, Brooks and Mahnken 2003). Copper and zinc are of concern as they often persistent in the environment and can be toxic to invertebrates (Birge *et al.* 1987; Brooks and Mahnken 2003). These metals reach the sediment underlying cage farms being bound to the solid, settleable fraction of farm waste (Parker and Aubé 2002). Benthic invertebrates are most likely exposed to these metals through dietary uptake of metal-contaminated particles in the sediment (Gillis *et al*. 2006). The federal and the Ontario provincial governments have developed maximum allowable levels for Cu and Zn in freshwater sediments to protect aquatic fauna. Based on extensive empirical evidence, levels in excess of governmental guidelines are likely to cause toxic responses among most benthic

invertebrates (CCME 2002; Jaagumagi and Persaud 1999). The first threshold, called the Lowest Effects Level (LEL), is the maximum level of a toxicant considered safe for exposed taxa in all their aquatic life stages (CCME 2002). The LEL value for sediment zinc concentration is 0.12 mg g^{-1} (CCME 2002), which was exceeded at every sampling station at CF1, CF2 and at the experimental farm in October 2007 (Tables 2.5a, 2.5b). The second threshold, known as the Severe Effects Level (SEL) is set at the level at which pronounced effects can be expected for the benthic community (CCME 2002). At CF2 (July and September 2007), and at the experimental farm (November 2006 and October 2007), the sediment zinc concentration at 1 m from the cage exceeded the SEL of 0.82 mg g^{-1} (CCME 2002). There was a benthic invertebrate community at all three farms despite copper and zinc concentrations far beyond the LEL, and in some cases SEL, thresholds. Bioassays could be used to assess the availability of metals in farm waste to invertebrates and at what levels toxic responses could be anticipated (*e.g.*, Kullman *et al*. 2007).

Sediment texture and stability can be altered by aquaculture waste. Droppo *et al*. (2007) evaluated the stability of sediment containing aquaculture waste from a discontinued cage farm near Great La Cloche Island in the North Channel of Lake Huron. This material was low density, had an increased water content and significant microbial activity (Droppo *et al*. 2007). Some caution should be applied when applying these results to the waste material under active farms in Lake Huron. The researchers collected the fish waste three years after the farm had closed, and indicated that the waste deposit had been thinning at a rate of 5.9 cm y^{-1} and declining in organic content (7% reduction in 3 years) (Droppo *et al*. 2007). The deposit of fresh fish waste under active farms will

be subject to constant deposition, and therefore a higher organic content would be expected. Although some fungus was present while coring on SCUBA, the extensive mats described by Droppo *et al*. (2007) were not observed beneath the Ontario commercial farms. Decreased sediment density and increased water content may impede the survival of some benthic invertebrates, such as sphaeriid clams and ostracods. Sphaeriid clams burrow into the sediment and access the water column through a small hole in the sediment (Gilmore 1917). If the sediment is of low density, the hole may not be maintained for water exchange through the siphon which would effectively smother the clam. During an *in situ* bioassay, *S. simile* exposed to sediment from directly under an experimental cage farm experienced 100% mortality, and this was attributed to the low bulk density of the sediment (Kullman *et al.* 2007). Mean sedimentation rates in 2006 and 2007 ranged from 25.78 - 34.09 g C m^{-2} day^{-1} at the cage, compared with 1.64 - 2.85 g C m^{-2} day^{-1} one metre away (Table 2.3). The large difference in sedimentation rates would have been a major factor influencing conditions in the sediment used for the *S. simile* bioassay, and demonstrates the limited dispersal of the waste material. Sphaeriids were not collected within 100 m from the commercial farms during this study, but have been recorded in the North Channel of Lake Huron (Nalepa *et al.* 2007). Ostracoda abundance is generally a function of water depth, food availability and substrate (Delorme 2001). Based on their feeding habits, the food availability for these detritivore-herbivores (Strayer 1985) would be expected to increase from the deposition of organic waste underlying a cage farm. However, at CF1 in 2007, ostracod density peaked at 22 526 individuals $\cdot m^{-2}$ at 0.88 g C m^{-2} day^{-1} (Figure 2.6). At CF2 in 2007, ostracod density was far less, peaking at only 379 individuals $\cdot m^{-2}$ at 2.3 g C m^{-2} day^{-1} (Figure 2.7). Deposition

of low density organic waste could be responsible for the reduced abundance of this taxon as they require a stable sediment water interface (Delorme 2001). At CF1, ostracod density under the cage farm was reduced by 89.3% in 2006 and 99.3% in 2007 compared with the 100 m reference sites. At CF2 in 2007, ostracods were eliminated at carbon sedimentation rates greater than 3.3 g C m^{-2} day^{-1} which corresponds to 3 m away from the farm (Figure 2.7).

Evidence suggests benthic invertebrates consume fish waste from aquaculture (Kullman *et al.* 2007, 2009; Wellman 2011) although the dispersal of this novel source of energy is limited in freshwater. Stable isotopic analysis was used to describe the assimilation of *O. mykiss* waste on native biota during (Kullman *et al.* 2009) and after (Wellman 2011) the operation of the experimental cage farm. A significant shift towards the $\delta^{13}C$ signature of the aquaculture-derived carbon was reported for profundal chironomids, *Mysis relicta* (Lovén) and minnows in the second year of fish production (Kullman *et al.* 2009). Wellman (2011) reported the $\delta^{13}C$ signature in fish and benthic invertebrates had returned to pre-aquaculture levels by the second year of recovery; however, for profundal chironomids this shift had already occurred by the third year of fish production. The author later indicated that chironomids were sampled further away from the cage in later years, where they may not have had direct access to settled fish waste from the cage (Wellman 2011). The exact location of chironomid sampling over time is unclear in Wellman (2011). However, if they were collected more than 5 m from the cage it is highly likely that they were not able to feed directly on *O. mykiss* waste given the very limited dispersal of this material. Carbon sedimentation was reduced by 96.7% in 2006 and 97.8% in 2007 at 5 m from the cage (Table 2.3).

Summary

The literature regarding the impact of waste loading from cage aquaculture can be divided into two closely related topics (Sanz-Lázaro *et al*. 2011): (1) the prediction of dispersion by waste deposition modelling (*e.g.*, Elberizon and Kelly 1998; Cromey *et al.* 2002; Jusup *et al*. 2009), and (2) assessing the changes in the benthic community and sediment chemistry around farms (*e.g.*, Brown *et al.* 1987; Weston 1990; Kullman *et al*. 2007, 2009; Rooney and Podemski 2009, 2010; Wellman 2011). Few studies have integrated the two areas of research in the marine environment to link measure of deposition rate with impacts to the benthic environment (*e.g.,* Pusceddu *et al.* 2007; Hargrave *et al.* 2008, Kutti *et al.* 2008; Díaz-Almela *et al*. 2008) and prior to this study, no one has merged the two subjects in freshwater.

Open net-pen aquaculture releases wastes directly into the water body; however, the deposition of this material declines exponentially with distance. Sedimentation rates measured at two commercial and an experimental rainbow trout cage farm in Ontario were only elevated within the near vicinity (<30 m) of the farm. .Low current velocity and lower water density can result in more local deposition of settleable organic waste in freshwater than in marine systems (Elberizon and Kelly 1998). The exponential decline in deposition can result in distinct zonation in the benthic environment around farms that has been reported by several authors (*e.g.*, Enell and Lof 1983; Dobrowolski 1987; Cornel and Whoriskey 1993; Doughty and McPhail 1995; Elberizon and Kelly 1998; Diaz *et al.* 2001; Guo and Li 2003; Rooney and Podemski 2010), with a heavily altered community in close proximity to an area that is not significantly impacted.

The linking of deposition patterns with measures of the benthic environment allows researchers to proposed thresholds of waste deposition beyond which adverse impacts may occur. Prior to this study there were no predictions for thresholds of effect at freshwater cage farms in the literature. Based on the results described in this study, the predicted threshold of effect on benthos is 2.0 - 3.0 g C m^{-2} day^{-1}, beyond which both abundance and richness of the benthic community will be reduced. Hargrave (1994) suggested that in marine systems, deposition exceeding 1.0 g C m^{-2} day^{-1} is likely to cause adverse changes in the benthic community. Similarly, Kutti *et al.* (2008) reported an exponential increase in benthic invertebrate production with increased carbon loading up to a threshold somewhere between 0.82 – 1.36 g C m^{-2} day^{-1}, and that invertebrate biomass and abundance declined at carbon deposition rates exceeding 0.74 g C m^{-2} day^{-1} at a deep-water (230 m) marine fish farm. The identification of these thresholds of effect are important tools for regulation of the industry as they can be used in conjunction with predictive dispersion modelling (e.g., DEPOMOD) to suggest the spatial scale and intensity of impacts at proposed sites.

Despite high demand, North American aquaculture production has grown by only 1.2% per year since 2000 (FAO 2010). Decisions affecting the expansion of the industry have been based upon the precautionary principle as there has not been a lot of data to aid regulators in making informed decisions (Podemski and Blanchfield 2006). Modeling tools available for the marine environment (*e.g.,* DEPOMOD) have been applied in a regulatory context in Scotland by establishing allowable zones of effect (Cromey *et al.* 2002; Mayor *et al.* 2010). Ontario does not have an equivalent formal process for the management of the freshwater cage aquaculture industry (MOE 2008). The relationship

described in this study between sedimentation rates and effects on the sediment chemistry, as well as benthic communities could be used to predict changes occurring with the establishment of a new farm, or when an existing farm is to be expanded and facilitate informed rather than precautionary regulatory decisions in the future.

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Table 2.1. Exponential regression equations to describe carbon sedimentation with distance from three open net-pen aquaculture operations in Ontario, Canada.

Farm	Equation	r^2
Commercial Farm 1 (2006) - September	$y=4.779 \cdot e^{-0.1546x}+1.456$	0.93
Commercial Farm 1 (2007) - June	$y=4.494 \cdot e^{-0.1424x}+ 0.3248$	0.91
Commercial Farm 1 (2007) - July	$y=5.286 \cdot e^{-0.2537x}+ 1.190$	0.97
Commercial Farm 1 (2007) - September	$y=9.434 \cdot e^{-0.1679x}+ 1.729$	0.70
Commercial Farm 2 (2007) - July	$y=7.823 \cdot e^{-0.1863x}+ 1.535$	0.97
Commercial Farm 2 (2007) - September	$y=1.295 \cdot e^{-0.5464x}+ 3.165$	0.96
L375, ELA (2006) - Mean	$y=2.150 \cdot e^{-0.4933x}+ 0.4602$	0.63
L375, ELA (2007) - Mean	$y=8.754 \cdot e^{-0.2755x}-0.2252$	0.65

Table 2.2. Carbon sedimentation (g C m^{-2} day^{-1}) below and surrounding commercial farms in Ontario, Canada (2006 and 2007). Sedimentation rates were determined over a one-week period in September 2006; therefore, the minimum, maximum and mean rates could not be calculated. In 2007, mean, minimum and maximum sedimentation rates were calculated using the rates determined from three 5-day sampling periods in June, July and September at CF1, and over two 5-day sampling periods in July and September at CF2. Observed and predicted rates were used for 2007 minimum, maximum, mean and median values. Letters denote significant Tukey's groupings for among-site differences at $\alpha = 0.05$.

Commercial Farm 1

	Distance from cage edge (m)									
	Under cage	**1**	**3**	**5**	**10**	**15**	**20**	**30**	**50**	**100**
2006										
September	11.58[a]	6.97[b]	4.05[c]	2.45[c]	1.93[c]	0.88[c]	2.90[c]	1.53[c]	1.58[c]	1.72[c]
2007										
June	9.47[a]	4.11[b]	3.69[b]	2.17[b]	1.48[b]	0.79[b]	0.57[b]	0.49[b]	0.36[b]	0.24[b]
July	20.13[a]	4.04[b]	4.82[b]	3.19[b]	2.07[c]	1.72[c]	1.22[c]	1.06[c]	0.76[c]	0.47[c]
September	23.19[a]	-	10.03[b]	3.20[b]	1.90[c]	-	2.60[b]	-	2.52[b]	1.92[c]
Minimum	9.47	4.04	3.69	2.17	1.48	0.79	0.57	0.49	0.36	0.24
Maximum	23.19	9.71	10.03	3.20	2.07	2.49	2.60	1.79	2.52	1.92
Mean	17.60	5.95	6.18	2.86	1.81	1.67	1.46	1.11	1.21	0.88
Median	20.13	4.11	4.82	3.19	1.90	1.72	1.22	1.06	0.76	0.47

Commercial Farm 2

2007	Distance from cage edge (m)									
	Under cage	**1**	**3**	**5**	**10**	**15**	**20**	**30**	**50**	**100**
July	21.41[a]	-	-	-	2.53[b]	2.28[b]	1.91[b]	2.00[b]	-	0.84[b]
September	23.07[a]	-	3.34[b]	3.39[b]	3.62[b]	-	2.73[b]	3.08[b]	-	-
Minimum	21.41	3.92	3.34	3.39	2.53	2.28	1.91	2.01	1.54	0.85
Maximum	23.07	8.03	6.01	4.62	3.62	3.17	2.73	3.08	3.17	3.17
Mean	22.24	5.97	4.67	4.00	3.08	2.72	2.32	2.54	2.35	2.01

Table 2.3. Carbon sedimentation (g C m^{-2} day^{-1}) below and surrounding an experimental cage farm on L375, at the ELA. Rates were determined every seven days over the entire sampling period (July - Oct 2006, May - Oct 2007). Background sedimentation values are listed as North Basin (NB) and were measured approximately 95 m away from the farm.

	Distance from cage edge (m)										
	Under cage	**cage edge**	**1**	**3**	**5**	**10**	**15**	**20**	**30**	**50**	**NB**
2006											
MIN	2.94	0.60	0.58	0.50	0.22	0.26	0.25	0.24	0.25	0.24	0.17
MAX	67.07	6.30	3.72	2.52	2.14	1.17	0.66	0.65	0.60	0.64	0.84
MEAN	25.78	2.65	1.64	1.00	0.86	0.55	0.42	0.39	0.39	0.40	0.39
MEDIAN	24.38	2.43	1.59	0.79	0.66	0.45	0.37	0.36	0.35	0.36	0.33
STD ERR	5.07	0.44	0.22	0.14	0.13	0.06	0.03	0.03	0.03	0.03	0.04
2007											
MIN	6.32	0.28	0.24	0.26	0.23	0.18	0.15	0.14	0.15	0.14	0.11
MAX	87.27	37.18	7.68	3.36	1.66	0.90	0.61	0.61	0.63	0.63	1.10
MEAN	34.09	14.01	2.85	1.24	0.74	0.46	0.32	0.31	0.29	0.29	0.39
MEDIAN	29.07	14.72	2.30	1.21	0.61	0.36	0.27	0.27	0.27	0.27	0.28
STD ERR	4.32	2.20	0.43	0.16	0.09	0.05	0.03	0.03	0.03	0.03	0.05

Table 2.4. Mean depth of fish waste measured from core samples taken at two commercial farms (n= 8-10) in Ontario, Canada. Only those sites with a visible layer of fish waste are listed.

Farm	Date	Site	Mean fish waste depth (mm)	Standard Error
1	Sep-06	cage	7.70	4.72
1	Sep-06	1m	36.60	5.35
1	Jun-07	cage	16.88	2.62
1	Jun-07	1m	22.25	2.83
1	Jul-07	cage	28.53	2.92
1	Jul-07	1m	30.71	2.84
1	Sep-07	cage	23.75	3.68
1	Sep-07	1m	28.00	3.96
2	Jul-07	cage	n/a*	-
2	Jul-07	1m	n/a*	-
2	Jul-07	3m	62.44	8.60
2	Jul-07	5m	116.44	43.80
2	Jul-07	10m	39.38	8.77
2	Jul-07	15m	30.63	7.49
2	Sep-07	cage	88.78	4.67
2	Sep-07	1m	89.69	5.21
2	Sep-07	3m	40.50	5.04
2	Sep-07	5m	25.00	6.71
2	Sep-07	10m	34.29	3.31
2	Sep-07	15m	26.08	5.43

*Farm waste exceeded the length of the core sample (>200mm)

Table 2.5a. Mean concentration (±SE) of carbon, nitrogen, phosphorus and metals (mg g^{-1} dry weight) in the top 2 cm of sediment beneath the commercial (CF1 and CF2) farms in Ontario, Canada, with a reference site 100 m away (n=5 at CF1, n=3 at CF2).

CF1	**2006**		**2007**					
	September		June		July		September	
Variable	Under cage (n=5)	100 m from farm (n=5)	Under cage (n=5)	100 m from farm (n=5)	Under cage (n=5)	100 m from farm (n=5)	Under cage (n=5)	100 m from farm (n=5)
Carbon	50.99 ± 4.99	35.09 ± 0.73	137.77 ± 33.22	32.20 ± 1.60	219.47 ± 23.61	36.43 ± 1.25	100.8 ± 21.03	32.20 ± 1.90
Nitrogen	6.42 ± 0.99	3.67 ± 0.13	13.23 ± 3.77	2.20 ± 0.12	17.47 ± 0.80	3.00 ± 0.15	12.73 ± 4.16	2.83 ± 0.27
Phosphorus	37.43 ± 2.92	2.26 ± 0.09	28.83 ± 4.98	1.93 ± 0.13	21.23 ± 5.04	2.03 ± 0.17	29.83 ± 9.45	1.57 ± 0.33
Copper	0.21 ± 0.12	0.03 ± 0.00	0.32 ± 0.26	0.02 ± 0.00	0.06 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00
Zinc	0.80 ± 0.07	0.15 ± 0.00	0.64 ± 0.35	0.12 ± 0.00	0.60 ± 0.08	0.12 ± 0.00	0.46 ± 0.07	0.14 ± 0.01

CF2	**2007**			
	July		September	
Variable	Under cage (n=3)	100 m from farm (n=3)	Under cage (n=3)	100 m from farm (n=3)
Carbon	435.60 ± 9.89	57.30 ± 3.70	443.17 ± 4.24	51.37 ± 3.37
Nitrogen	39.43 ± 1.15	7.23 ± 0.61	44.60 ± 0.86	5.10 ± 0.38
Phosphorus	26.67 ± 1.76	2.63 ± 0.23	28.9 ± 1.90	2.27 ± 0.15
Copper	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Zinc	0.73 ± 0.04	0.19 ± 0.00	0.85 ± 0.02	0.19 ± 0.00

Table 2.5b. Mean concentration (±SE) of carbon, nitrogen, phosphorus and metals (mg g^{-1} dry weight) in the top 2 cm of sediment beneath the experimental farm (ELA, L375) in Ontario, Canada, with a reference site 100 m away.

	2006		**2007**	
	November		October	
	Under cage (n=4)	100 m from farm (n=4)	Under cage (n=3)	100 m from farm (n=3)
Carbon	249.44 ± 0.06	144.97 ± 0.02	354.87 ± 30.91	141.40 ± 4.39
Nitrogen	59.07 ± 0.01	25.60 ± 0.00	41.37 ± 2.53	11.03 ± 0.88
Phosphorus	43.50 ± 5.00	2.13 ± 0.07	36.81 ± 2.22	1.80 ± 0.06
Copper	0.10 ± 0.01	0.02 ± 0.00	0.09 ± 0.00	0.03 ± 0.00
Zinc	0.90 ± 0.07	0.11 ± 0.00	0.96 ± 0.03	0.12 ± 0.02

Table 2.6. Linear regression equations and r^2 values for sediment carbon, nutrient and metal concentration with current carbon sedimentation at CF1 (n=5) in Lake Wolsey, Ontario, Canada.

Farm #	Date Sampled	Variable	Type of Regression	Equation	r^2	P-value
1	Sep-06	Carbon	Linear	y= 46.19 + 0.3789x	0.01	0.6198
1	Jun-07	Carbon	Linear	y= 55.46 + 9.444x	0.45	<0.0001
1	Jul-07	Carbon	Linear	y= 50.79 + 8.728x	0.83	<0.0001
1	Sep-07	Carbon	Linear	y= 37.30 + 2.956x	0.55	<0.0001
1	Sep-06	Nitrogen	Linear	y= 4.67 + 0.1370x	0.03	0.2244
1	Jun-07	Nitrogen	Linear	y= 5.16 + 1.022x	0.46	<0.0001
1	Jul-07	Nitrogen	Linear	y= 5.53 + 0.6619x	0.64	<0.0001
1	Sep-07	Nitrogen	Linear	y= 3.31 + 0.4885x	0.50	<0.0001
1	Sep-06	Phosphorus	Linear	y= 2.75 + 2.551x	0.59	<0.0001
1	Jun-07	Phosphorus	Linear	y= 11.06 + 2.648x	0.39	0.0002
1	Jul-07	Phosphorus	Linear	y= 13.94 + 0.6840x	0.10	0.0883
1	Sep-07	Phosphorus	Linear	y= 10.62 + 1.340x	0.30	0.0019
1	Sep-06	Copper	Linear	y= 14.18 + 13.58x	0.22	0.0007
1	Jun-07	Copper	Linear	y= 29.25 + 28.72x	0.28	0.0024
1	Jul-07	Copper	Linear	y= 53.26 + 1.113x	0.01	0.5304
1	Sep-07	Copper	Linear	y= 70.04 - 0.1554x	0.00	0.9417
1	Sep-06	Zinc	Linear	y= 241.20 + 38.40x	0.30	<0.0001
1	Jun-07	Zinc	Linear	y= 273.40 + 42.26x	0.25	0.0052
1	Jul-07	Zinc	Linear	y= 312.50 + 16.79x	0.28	0.0031
1	Sep-07	Zinc	Linear	y= 241.70 + 11.62x	0.23	0.0071

Table 2.7. Polynomial regression equations and r^2 values for sediment carbon, nutrient and metal concentration with carbon sedimentation at CF2 (n=3) in Ontario, Canada.

Farm #	Date Sampled	Variable	Type of Regression	Equation	r^2	p-value
2	Jul-07	Carbon	Polynomial	$y= 62.97 + 75.71x - 2.736x^2$	0.79	0.000
2	Sep-07	Carbon	Polynomial	$y= -494.3 + 251.3x - 9.130x^2$	0.48	0.000
2	Jul-07	Nitrogen	Polynomial	$y= 11.83 + 5.762x - 0.2105x^2$	0.61	0.000
2	Sep-07	Nitrogen	Polynomial	$y= -64.83 + 31.98x - 1.181x^2$	0.36	0.003
2	Jul-07	Phosphorus	Polynomial	$y= 6.484 + 4.067x - 0.1472x^2$	0.52	0.000
2	Sep-07	Phosphorus	Polynomial	$y= -46.31 + 22.85x - 0.8492x^2$	0.23	0.030
2	Jul-07	Copper	Polynomial	$y= 36.54 + 13.64x - 0.6314x^2$	0.51	0.000
2	Sep-07	Copper	Polynomial	$y= -60.14 + 50.08x - 1.970x^2$	0.20	0.003
2	Jul-07	Zinc	Polynomial	$y= 288.1 + 216.0x - 9.134x^2$	0.59	0.000
2	Sep-07	Zinc	Polynomial	$y= -1663 + 911.3x - 34.78x^2$	0.19	0.002

Table 2.8. Exponential equations to describe the declines in invertebrate density at CF1 and CF2 in Ontario, Canada (2006 and 2007).

Farm #	Year	Density	Type of Regression	Equation	r^2
1	2006	Total invertebrate	Exponential	$y= 147529 \cdot e^{-0.5113x} - 22.35$	0.38
1	2007	Total invertebrate	Exponential	$y= 1.858e+008 \cdot e^{-5.676x} + 4939$	0.94
2	2007	Total invertebrate	Exponential	$y= 4.836e+007 \cdot e^{-2.376x} - 8670$	0.75
1	2006	Tubificinae	Exponential	$y= 3.032e+007 \cdot e^{-0.5113x} - 22.35$	0.49
1	2007	Tubificinae	Exponential	$y= 451721^{-2.187x} + 3174$	0.71
2	2007	Tubificinae	Exponential	$y= 1.240e+012 \cdot e^{-6.498x} - 340.8$	0.78
1	2006	Chironomidae	Exponential	$y= 491227 \cdot e^{-2.284x} + 47.07$	0.37
1	2007	Chironomidae	Exponential	$y= 22996^{-1.480x} + 91.07$	0.72
2	2007	Chironomidae	Exponential	$y= 1.798e+012 \cdot e^{-9.039x} + 109.2$	0.51
1	2006	Nematoda	Exponential	$y= 4.225e+007 \cdot e^{-2.751x} + 565.00$	0.80
1	2007	Nematoda	Exponential	$y= 1.029e+007 \cdot e^{-4.147x} + 300.2$	0.77
2	2007	Nematoda	Exponential	$y= 9.638e+010 \cdot e^{-5.909x} + 18.30$	0.65
1	2006	Harpacticoida	Exponential	$y= 2.038e+022 \cdot e^{-23.88x} + 929.30$	0.69
1	2007	Harpacticoida	Exponential	$y= 1.892e+014 \cdot e^{-17.40x} + 230.4$	0.95
2	2007	Harpacticoida	Exponential	$y= 2.230e+010 \cdot e^{-5.504x} - 35.98$	0.56

Table 2.9. Polynomial regression equations and r^2 values for Chironomini head capsule length and width with carbon sedimentation at CF1 and CF2 in Ontario, Canada. P-values in bold are significant.

Farm	Year	Measure of head capsule size	Regression Equation	r^2	p-value
CF1	2006	length	$y= 81.78 + 519.65x – 125.60x^2$	0.60	0.249
CF1	2007	length	$y= 326.78 + 67.25x – 3.86x^2$	0.67	**0.037**
CF2	2007	length	$y= 420.86 – 53.27x + 2.12x^2$	0.62	0.089
CF1	2006	width	$y= 114.50 + 384.84x – 93.51x^2$	0.61	0.239
CF1	2007	width	$y= 440.01 - 29.33x + 2.17x^2$	0.62	0.057
CF2	2007	width	$y= 317.20 – 54.97x + 2.16x^2$	0.90	**0.032**

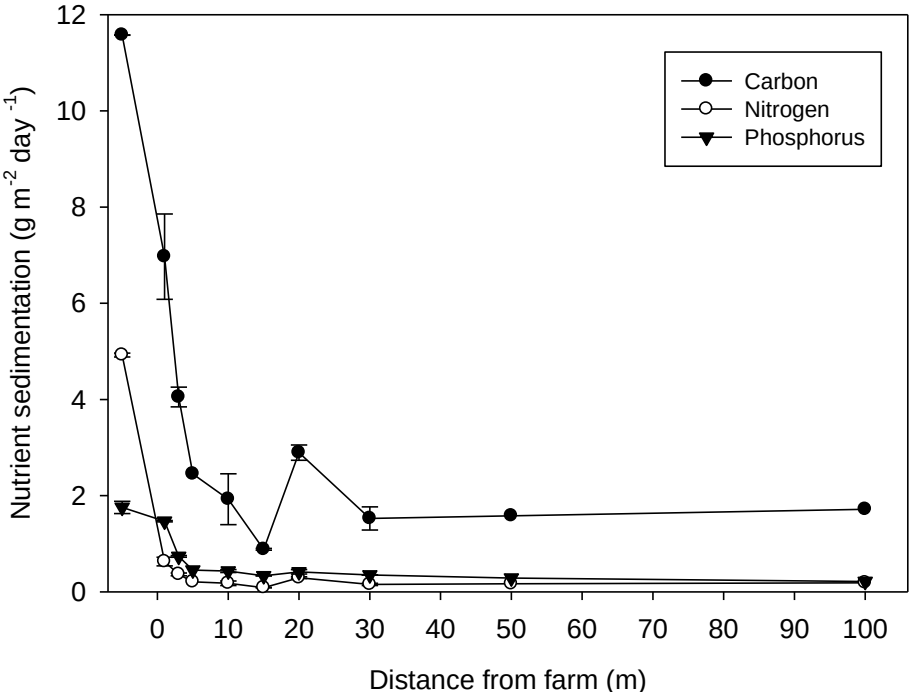

Figure 2.1. Sedimentation (carbon, nitrogen and phosphorus) rates (±SE) along a 100 m transect from Commercial Farm 1 (CF1) in Lake Wolsey, Ontario during September 2006 (n=4).

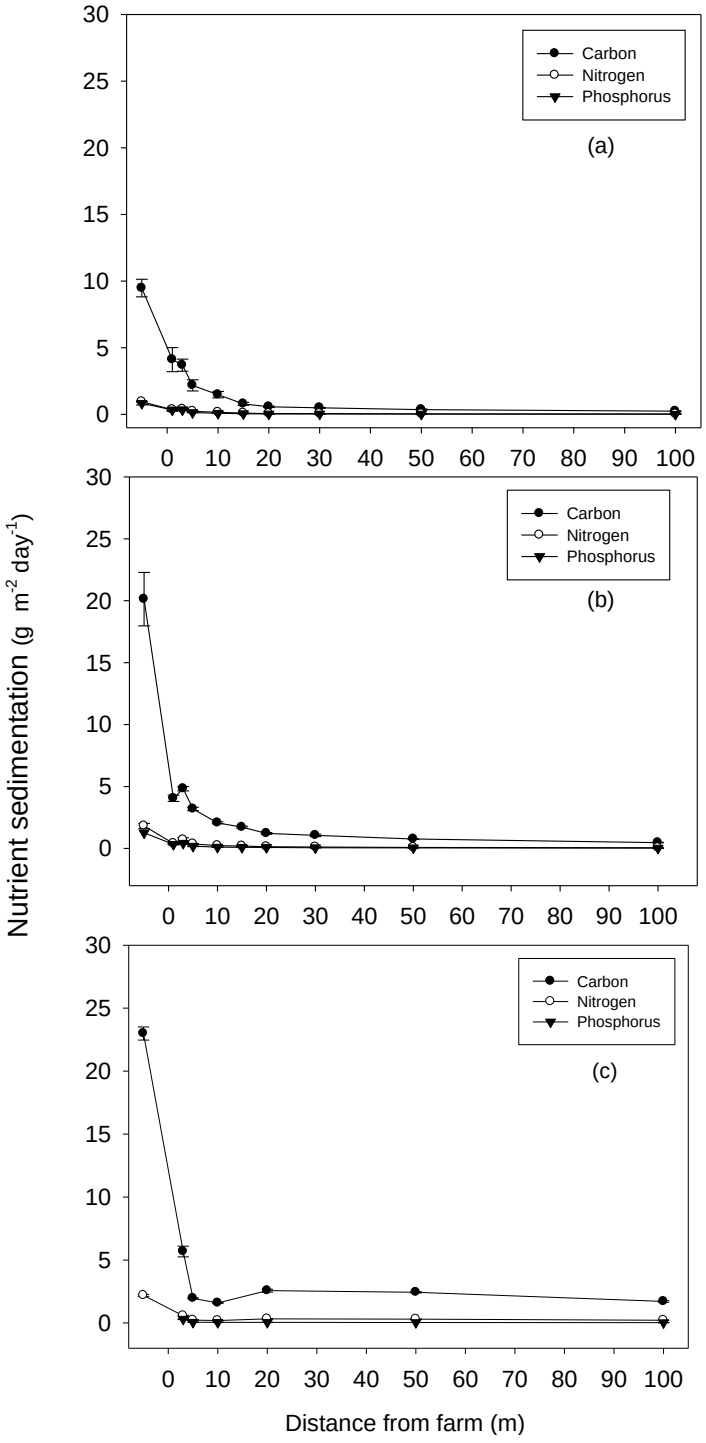

Figure 2.2. Sedimentation (carbon, nitrogen and phosphorus) rates (±SE) along a 100 m transect from Commercial Farm 1 in Lake Wolsey, Ontario during June (a), July (b) and September (c) of 2007 (n=4).

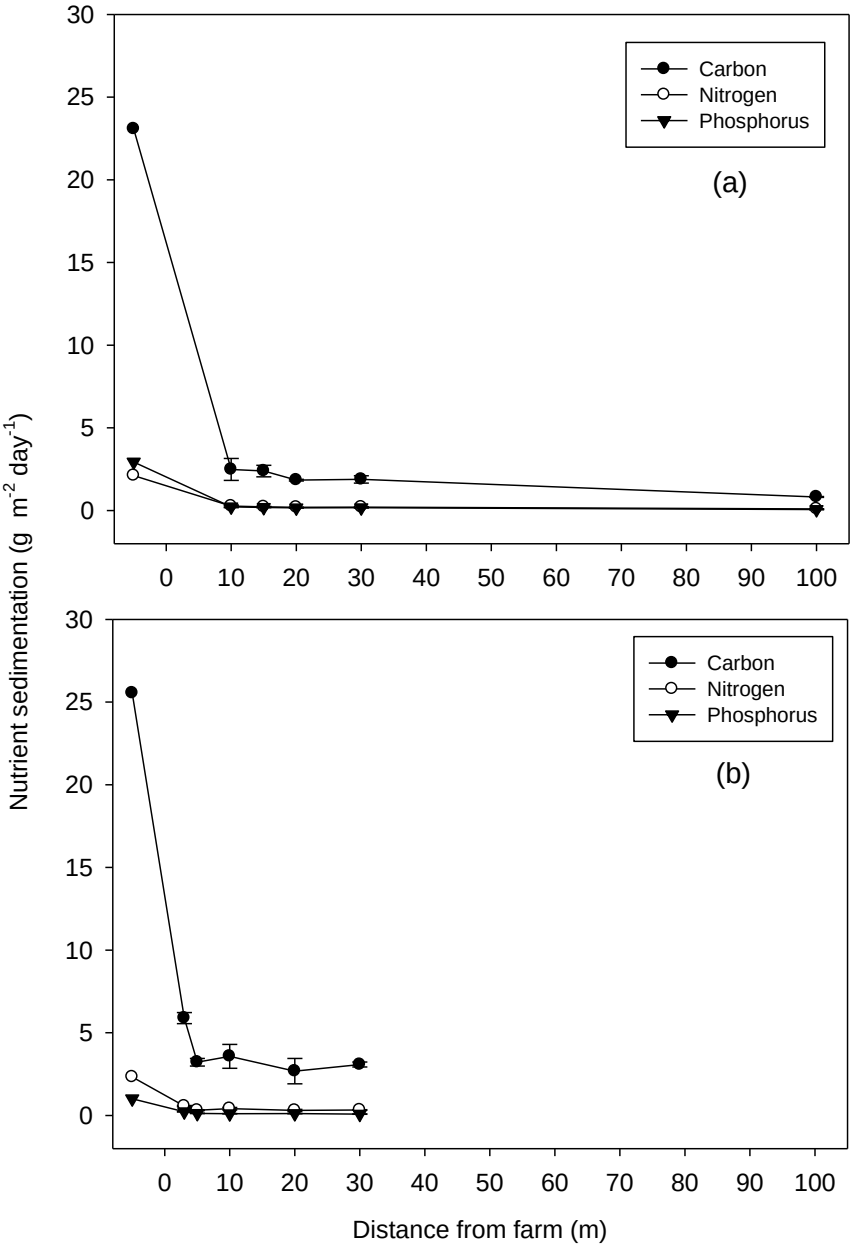

Figure 2.3. Sedimentation (carbon, nitrogen and phosphorus) rates (±SE) along a 100 m transect from Commercial Farm 2 in Ontario, Canada during July (a) and September (b) 2007 (n=4).

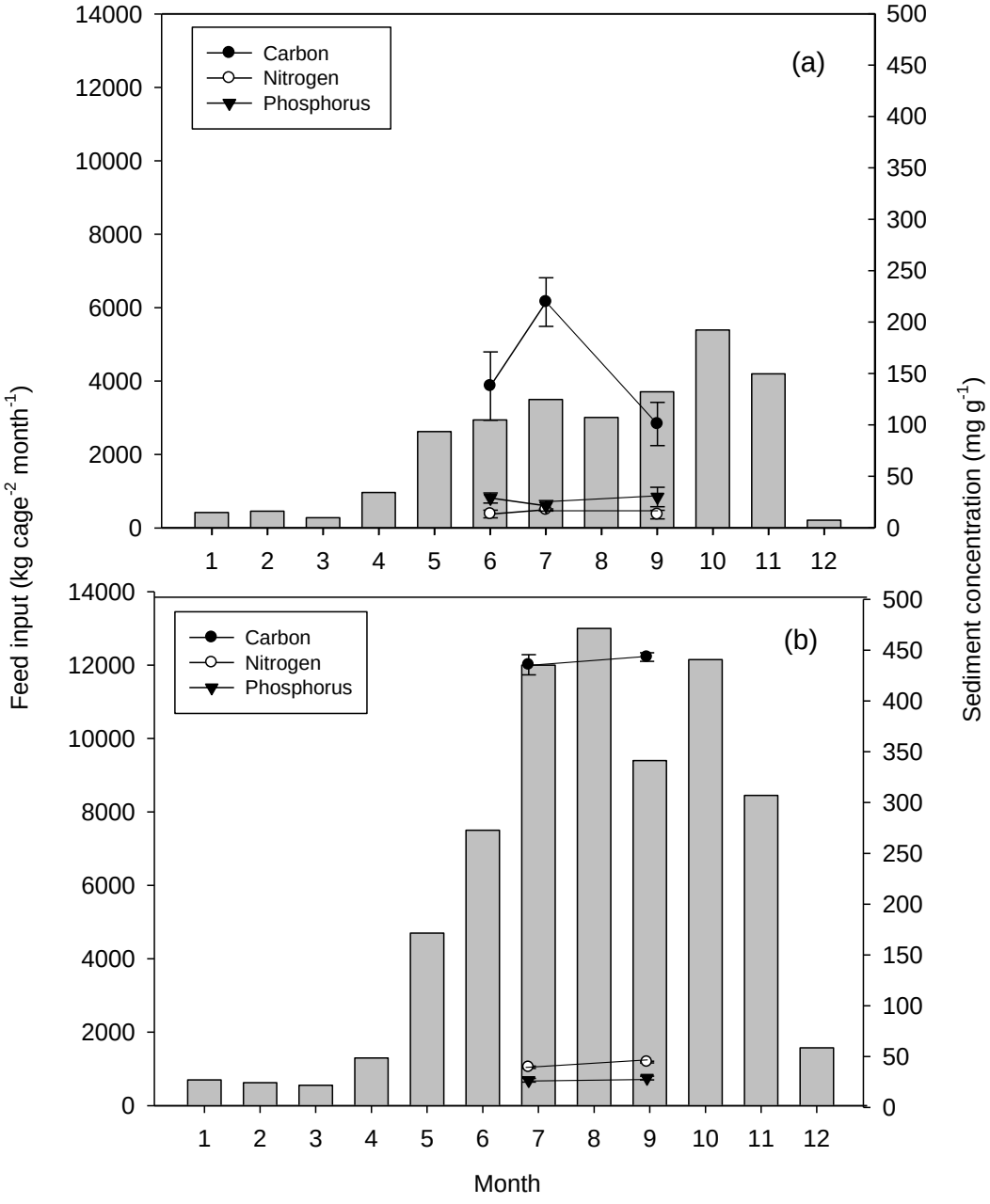

Figure 2.4. Feed input (kg cage^{-2} month^{-1}) (bars) and sediment TC, TN, TP concentrations (lines) under the cage at Commercial Farm 1 (a) and 2 (b) in Ontario (2007). Error bars are standard error, n=5 (CF1) and n=3 (CF2) for sediment concentrations.

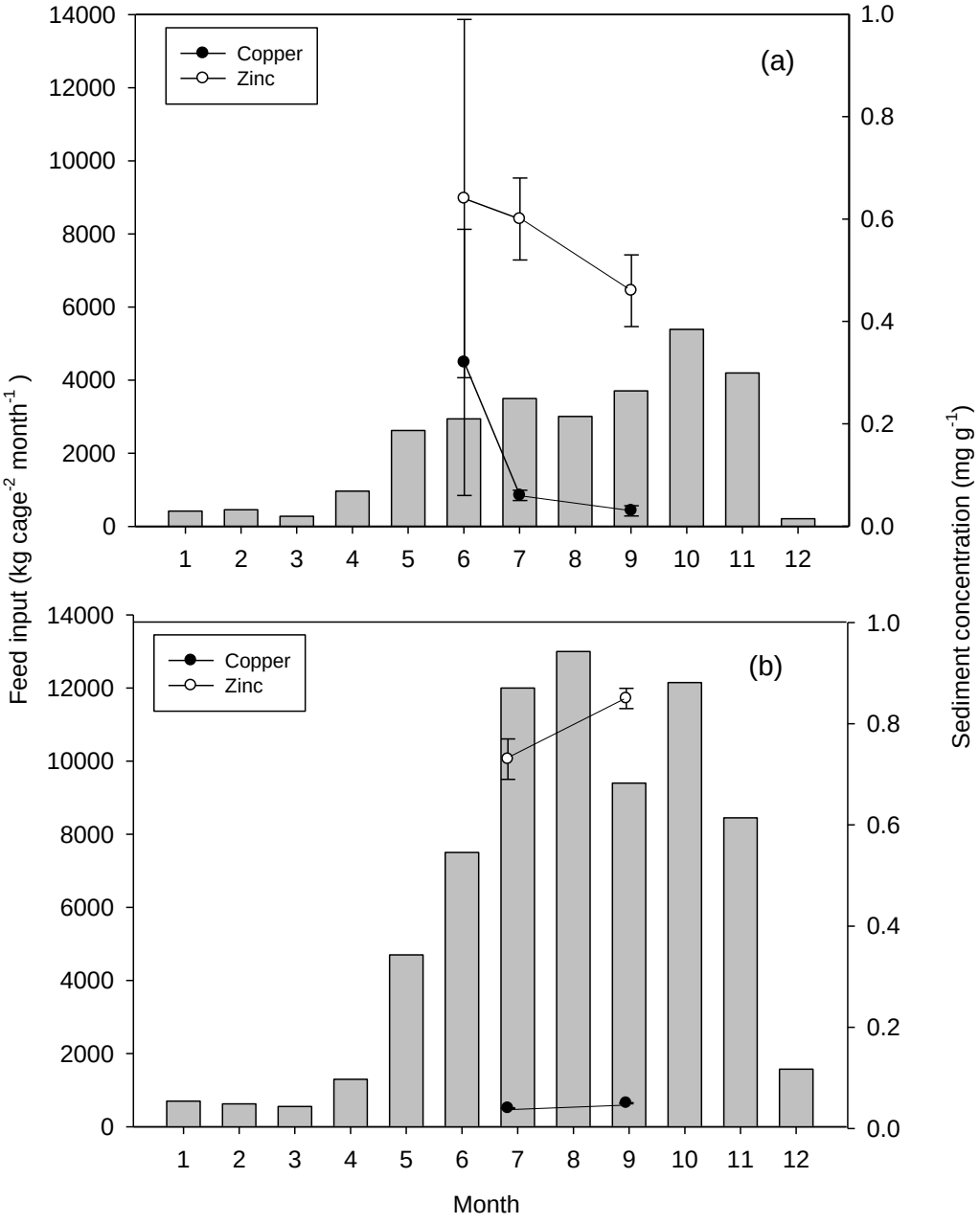

Figure 2.5. Feed input (kg cage^{-2} month^{-1}) (bars) and sediment copper and zinc concentrations (lines) under the cage at Commercial Farm 1 (a) and 2 (b) in Ontario (2007). Error bars are standard error, n=5 (CF1) and n=3 (CF2) for sediment concentrations.

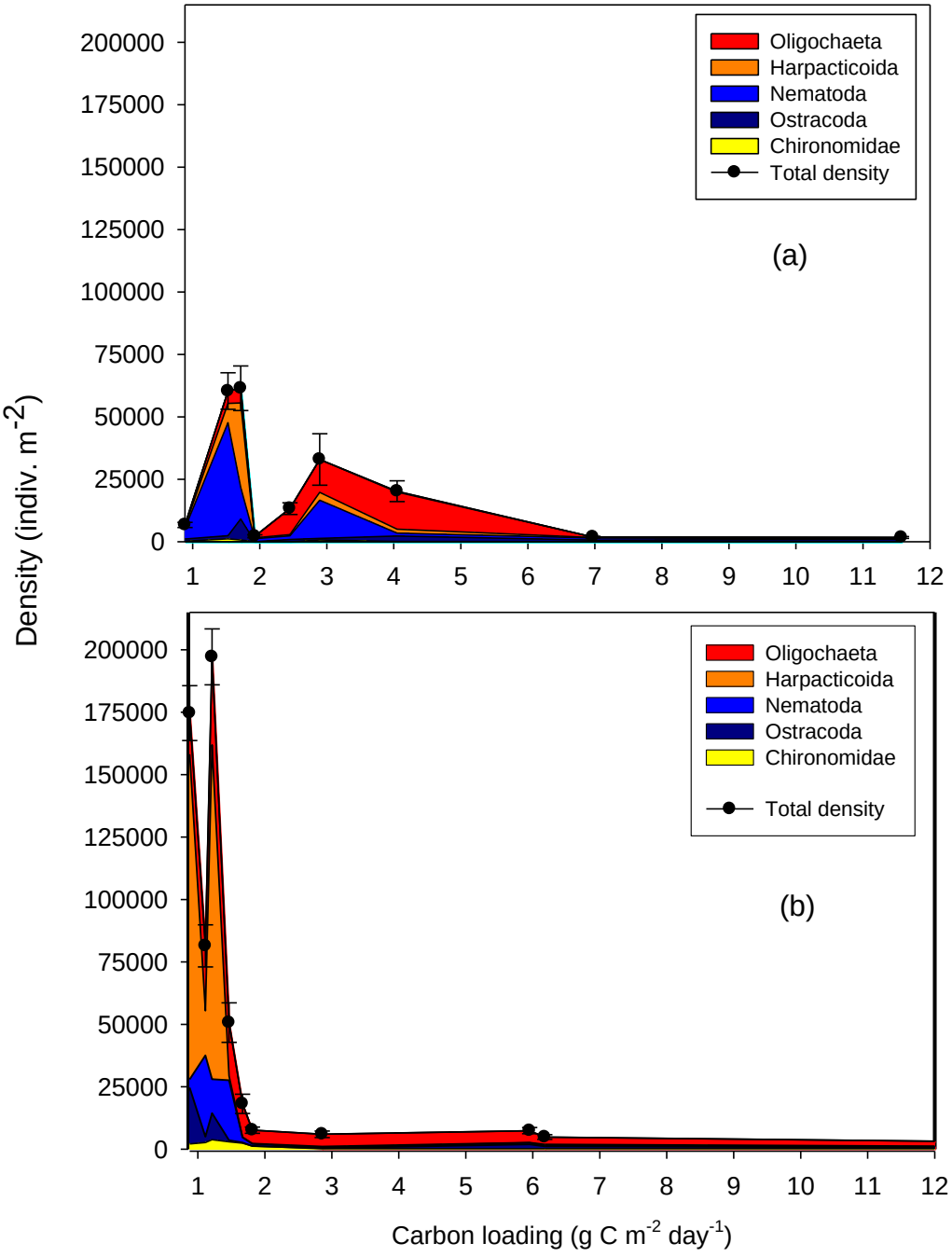

Figure 2.6. Invertebrate density (±SE) with concurrent carbon loading at CF1 in 2006 (a) and mean carbon loading in 2007 (b) in Lake Wolsey, Ontario. n=10.

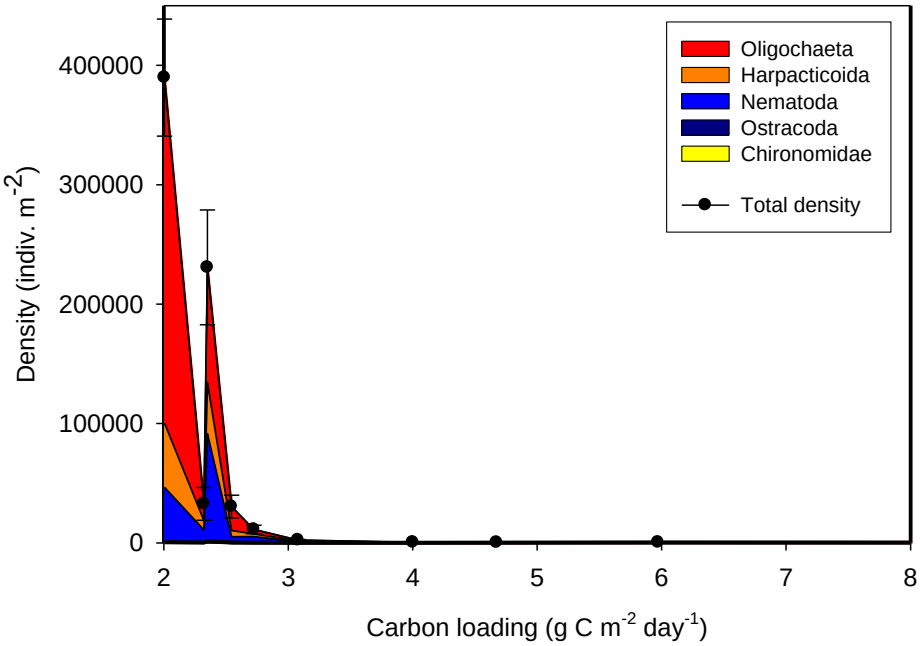

Figure 2.7. Invertebrate density (±SE) with mean carbon loading at CF2 in Ontario, Canada (2007). n=10.

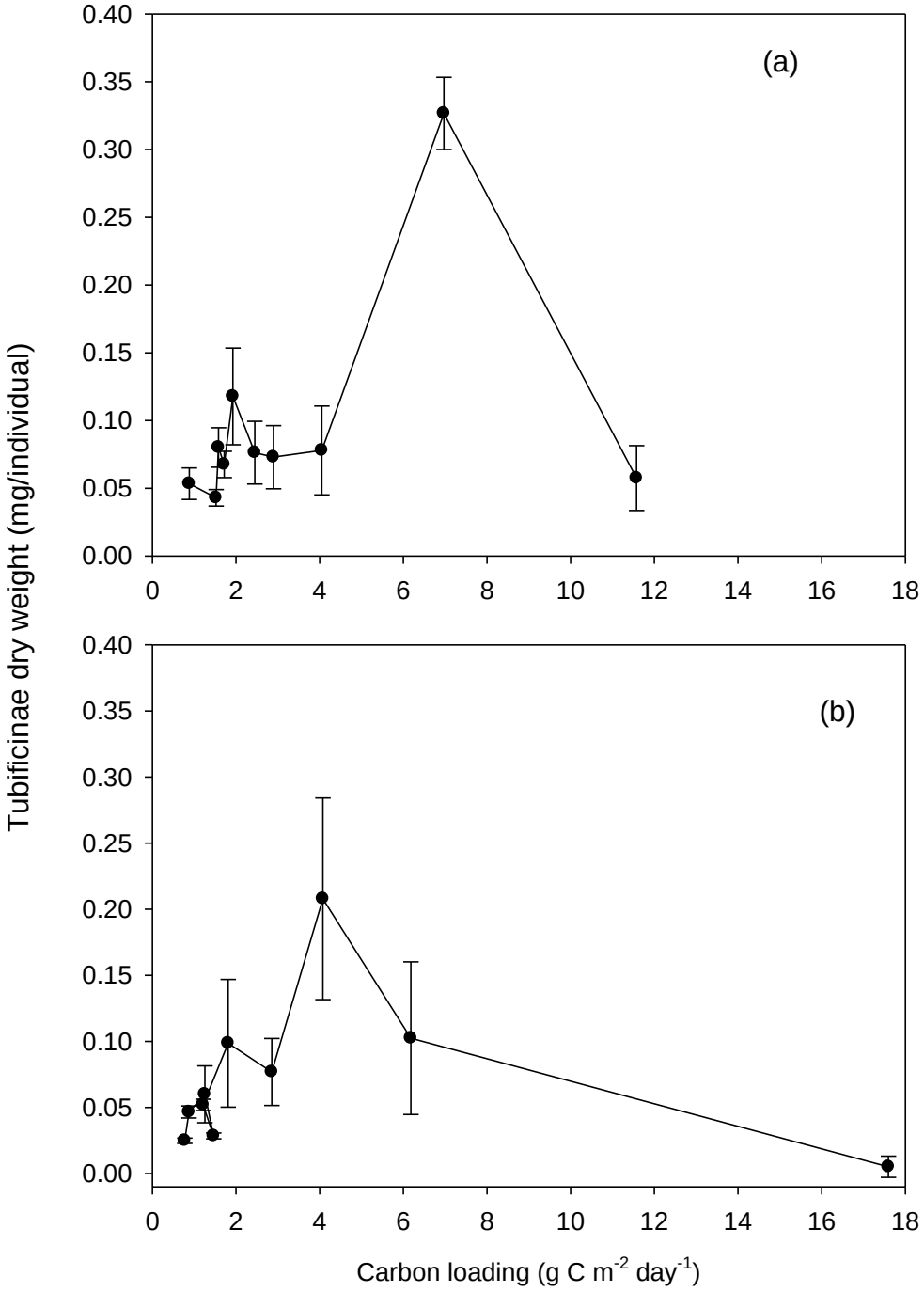

Figure 2.8. Mean individual dry weight (±SE) of Tubificinae worms with carbon loading at CF1 in 2006 (a) and 2007 (b) in Lake Wolsey, Ontario.

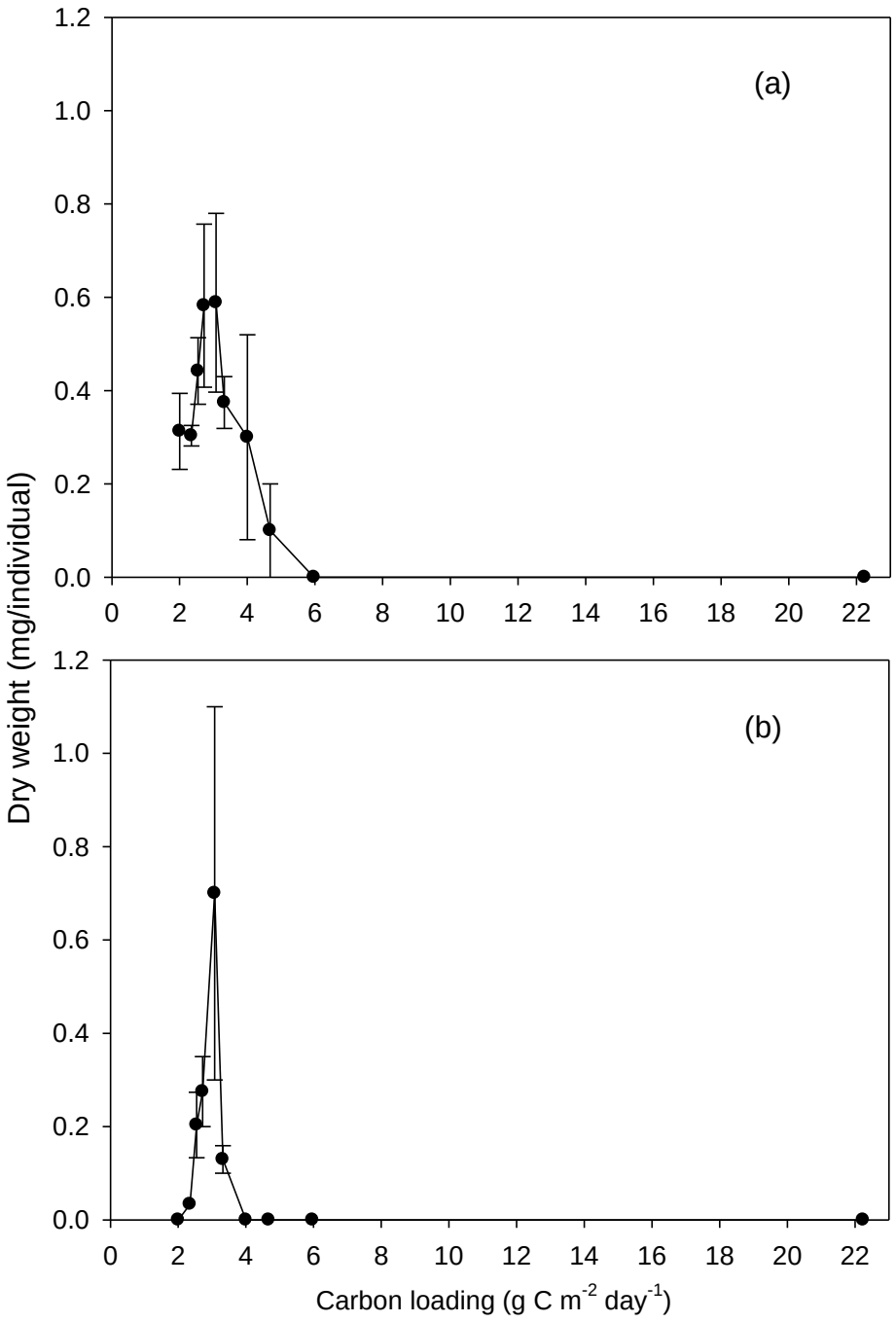

Figure 2.9. Mean individual dry weight (±SE) of Tubificinae (a) and Naidinae (b) worms with carbon loading at CF2 in Ontario, Canada (2007).

Appendix 2.1. Linear and polynomial regression equations to describe waste loading with sediment (C, N, P) concentrations at CF1 in Lake Wolsey, Ontario (2006 and 2007). Concurrent sedimentation rates were collected during the same week as the sediment chemistry samples. Mean and maximum sedimentation rates were determined from all the sampling periods in 2007.

Variable	Month	Measure of Sedimentation	Type of Regression	Regression Equation	r^2	p-value
2006						
Carbon	Sept	Concurrent	Linear	y= 46.19 + 0.3789x	0.01	0.6198
Nitrogen	Sept	Concurrent	Linear	y= 4.67 + 0.1370x	0.03	0.2244
Phosphorus	Sept	Concurrent	Linear	y= 2.75 + 2.551x	0.59	<0.0001
2007						
Carbon	June	Concurrent	Linear	y=55.46 + 9.444x	0.45	<0.0001
Carbon	June	Mean	Linear	y= 59.43 + 4.749x	0.36	0.0004
Carbon	June	Maximum	Linear	y=60.26 + 3.293x	0.31	0.0015
Carbon	July	Concurrent	Linear	y=50.79 + 8.728x	0.83	<0.0001
Carbon	July	Mean	Linear	y=47.45 + 9.923x	0.82	<0.0001
Carbon	July	Maximum	Linear	y=48.26 + 7.187x	0.77	<0.0001
Carbon	Sept	Concurrent	Linear	y=37.30 + 2.956x	0.55	<0.0001
Carbon	Sept	Mean	Linear	y=38.85 + 3.925x	0.54	<0.0001
Carbon	Sept	Maximum	Linear	y=37.17 + 2.968x	0.55	<0.0001
Nitrogen	June	Concurrent	Linear	y= 5.159 + 1.022x	0.46	<0.0001
Nitrogen	June	Mean	Linear	y=5.647 + 0.4987x	0.36	0.0005
Nitrogen	June	Maximum	Linear	y=5.739 + 0.3450x	0.30	0.0018
Nitrogen	July	Concurrent	Linear	y= 5.533 + 0.6619x	0.64	<0.0001
Nitrogen	July	Mean	Linear	y=5.244 + 0.7619x	0.65	<0.0001
Nitrogen	July	Maximum	Linear	y=5.347 + 0.5438x	0.59	<0.0001
Nitrogen	Sept	Concurrent	Linear	y=3.308 + 0.4885x	0.50	<0.0001
Nitrogen	Sept	Mean	Linear	y=3.637+ 0.6308x	0.47	<0.0001
Nitrogen	Sept	Maximum	Linear	y=3.289 + 0.4903x	0.50	<0.0001
Phosphorus	June	Concurrent	Linear	y=11.06 + 2.648x	0.39	0.0002
Phosphorus	June	Mean	Linear	y=12.68 + 1.199x	0.26	0.0044
Phosphorus	June	Maximum	Linear	y=12.98 + 0.8143x	0.21	0.0116
Phosphorus	July	Concurrent	Linear	y=13.94 + 0.6840x	0.10	0.0883
Phosphorus	July	Mean	Linear	y=13.41+ 0.8459x	0.12	0.0637
Phosphorus	July	Maximum	Linear	y=13.78+ 0.5552x	0.09	0.1067
Phosphorus	Sept	Concurrent	Linear	y=10.62 + 1.340x	0.30	0.0019
Phosphorus	Sept	Mean	Linear	y=11.63 + 1.704x	0.27	0.0033
Phosphorus	Sept	Maximum	Linear	y=10.56+ 1.346x	0.30	0.0018

Appendix 2.2. Linear and polynomial regression equations to describe waste loading with sediment (C, N, P) concentrations at CF2 in 2007 from 1 – 100 m away from the farm (cage data has been removed from analysis). Concurrent sedimentation rates were collected during the same week as the sediment chemistry samples. Mean and maximum sedimentation rates were determined from all the sampling periods in 2007.

Variable	Month	Measure of Sedimentation	Type of Regression	Regression Equation	r^2	p-value
Carbon	July	Concurrent	Linear	y= -69.20 + 148.70x	0.77	<0.0001
Carbon	July	Mean	Linear	y= -56.87 + 128.80x	0.75	<0.0001
Carbon	July	Maximum	Linear	y= -4.63 + 99.92x	0.53	0.0002
Carbon	Sept	Concurrent	Linear	y= -402.80 + 193.10x	0.30	0.0028
Carbon	Sept	Mean	Linear	y= -13.10 +96.69x	0.82	<0.0001
Carbon	Sept	Maximum	Linear	y= 4.34 + 82.88x	0.72	<0.0001
Nitrogen	July	Concurrent	Linear	y= -6.31 + 15.98x	0.82	<0.0001
Nitrogen	July	Mean	Linear	y= -2.01 + 12.08x	0.79	<0.0001
Nitrogen	July	Maximum	Linear	y= 1.57 + 9.83x	0.62	<0.0001
Nitrogen	Sept	Concurrent	Linear	y= -52.55 + 24.33x	0.28	0.0048
Nitrogen	Sept	Mean	Linear	y= -7.26 + 13.81x	0.82	<0.0001
Nitrogen	Sept	Maximum	Linear	y= -2.55 + 11.03x	0.62	<0.0001
Phosphorus	July	Concurrent	Linear	y= -8.32 + 12.42x	0.85	<0.0001
Phosphorus	July	Mean	Linear	y= -4.59 + 9.07x	0.78	<0.0001
Phosphorus	July	Maximum	Linear	y= - 2.44 + 7.59x	0.65	<0.0001
Phosphorus	Sept	Concurrent	Linear	y= -36.97 + 17.19x	0.19	0.0241
Phosphorus	Sept	Mean	Linear	y= -11.78 + 12.71x	0.71	<0.0001
Phosphorus	Sept	Maximum	Linear	y= -5.80 + 9.56x	0.47	<0.0001

Appendix 2.3. Polynomial regression equations to describe waste loading with benthic invertebrate density at CF1 in Lake Wolsey, Ontario (September 2006 and 2007). Concurrent sedimentation rates were collected during the same week as the benthos samples. Mean and maximum sedimentation rates were determined from all the sampling periods in 2007.

Density	Measure of Sedimentation	Regression Equation	r^2	p-value
2006				
Total invertebrate	Concurrent	$y=-334809 + 562148x - 199520x^2$	0.54	0.000
Oligochaeta	Concurrent	$y=-10299 + 11943x + 1490x^2$	0.14	0.054
Chironomidae	Concurrent	$y=-1314 + 2641x - 881.9x^2$	0.17	0.012
Nematoda	Concurrent	$y=-46086 + 87121x + 28509x^2$	0.27	0.000
Harpacticoida	Concurrent	$y=-25109 + 41995x - 11662x^2$	0.14	0.008
Ostracoda	Concurrent	$y=-5006 + 9321x - 2638x^2$	0.14	0.080
2007				
Total invertebrate	Concurrent	$y=112697 - 16121x + 490.5x^2$	0.24	0.000
Total invertebrate	Mean	$y=131193 - 33579x + 1501x^2$	0.39	0.000
Total invertebrate	Maximum	$y=114527 - 16648x + 510.6x^2$	0.24	0.000
Oligochaeta	Concurrent	$y=23729 - 2825x + 79.83x^2$	0.25	0.000
Oligochaeta	Mean	$y=26267 - 5558x + 235.8x^2$	0.35	0.000
Oligochaeta	Maximum	$y=24054 - 2918x + 83.38x^2$	0.26	0.000
Chironomidae	Concurrent	$y=3136 - 438.0x + 13.36x^2$	0.32	0.000
Chironomidae	Mean	$y=3393 - 789.3x + 34.51x^2$	0.42	0.000
Chironomidae	Maximum	$y=3170 - 447.0x + 13.69x^2$	0.32	0.000
Nematoda	Concurrent	$y=16422 - 2443x + 75.46x^2$	0.17	0.000
Nematoda	Mean	$y=17872 - 4404x + 194.0x^2$	0.23	0.000
Nematoda	Maximum	$y=16694 - 2521x + 78.43x^2$	0.17	0.000
Harpacticoida	Concurrent	$y=61793 - 9414x + 292.4x^2$	0.14	0.001
Harpacticoida	Mean	$y=74443 - 20515x + 933.5x^2$	0.25	0.000
Harpacticoida	Maximum	$y=62864 - 9723x + 304.1x^2$	0.14	0.000
Ostracoda	Concurrent	$y=7614 - 1000x + 29.51x^2$	0.1	0.006
Ostracoda	Mean	$y=9218 - 2313x + 103.2x^2$	0.18	0.004
Ostracoda	Maximum	$y=7745 - 1039x + 30.99x^2$	0.1	0.010

Appendix 2.4. Polynomial regression equations to describe the relationship between waste loading and benthic invertebrate density at CF2 (September 2007). Concurrent sedimentation rates were collected in September 2007. Mean and maximum sedimentation rates were determined from all the sampling periods in 2007.

Density	Measure of Sedimentation	Regression Equation	r^2	p-value
Total invertebrate	Concurrent	$y=485520 - 141443x + 5220x^2$	0.08	0.020
Total invertebrate	Mean	$y=313316 - 83324x + 3119x^2$	0.26	0.000
Total invertebrate	Maximum	$y=237609 - 45808x + 1542x^2$	0.13	0.001
Oligochaeta	Concurrent	$y=287272 - 83242x + 3068x^2$	0.05	0.000
Oligochaeta	Mean	$y=197581 - 53196x + 1996x^2$	0.2	0.000
Oligochaeta	Maximum	$y=144288 - 27799x + 935.1x^2$	0.09	0.000
Chironomidae	Concurrent	$y=1720 - 482.9x + 17.78x^2$	0.08	0.923
Chironomidae	Mean	$y=811.1 - 169.8x + 6.09x^2$	0.11	0.918
Chironomidae	Maximum	$y=697.5 - 104.8x + 3.32x^2$	0.08	0.928
Nematoda	Concurrent	$y=113907 - 33468x + 1237x^2$	0.07	0.031
Nematoda	Mean	$y=65278 - 16902x + 629.5x^2$	0.18	0.012
Nematoda	Maximum	$y=53766 - 10413x + 351.6x^2$	0.11	0.003
Harpacticoida	Concurrent	$y=82991 - 24441x + 903.6x^2$	0.07	0.025
Harpacticoida	Mean	$y=49463 - 13054x + 487.7x^2$	0.22	0.008
Harpacticoida	Maximum	$y=38733 - 7507x + 253.1x^2$	0.12	0.003
Ostracoda	Concurrent	$y= -237.8 + 139.3x - 5.593x^2$	0.04	0.156
Ostracoda	Mean	$y=185.3 - 7.979x - 0.01149x^2$	0.03	0.212
Ostracoda	Maximum	$y= 85.48 + 18.94x - 0.9781x^2$	0.03	0.227

Chapter III: Effect of carbon loading from aquaculture waste on benthic invertebrates: a microcosm study

3.1. Abstract

The main goal of this research was to establish the relationship between waste loading from freshwater aquaculture operations and the growth and survival of benthic invertebrates. A community comprised of *Tubifex* (Oligochaeta, Naididae), *Chironomus* (Diptera, Chironomidae) and *Sphaerium* (Bivalvia, Pisidiidae) enclosed in a microcosm was subjected to daily additions of fish waste to simulate the environment beneath a new cage culture operation. During this 21-day bioassay, carbon (r^2=0.28, $p<0.05$) and phosphorus (r^2=0.82, $p<0.01$) concentration in the sediment increased with waste loading, as did the concentration of zinc (r^2=0.63, $p<0.01$) and pore-water ammonia (r^2=0.66, $p<0.01$). Sediment copper concentration did not change with increasing fish waste (r^2=0.09, p=0.225). Invertebrate survival and growth were variable and species-specific. Survival (r^2=0.50, $p<0.01$) and growth (r^2=0.57, $p<0.01$) of *S. simile* were highest at intermediate levels of waste loading. For *C. riparius*, mean individual biomass was stimulated by the increased waste loading (r^2=0.58, $p<0.01$), but body length was unaffected (r^2=0.09, p=0.230). Mean individual biomass of *Tubifex tubifex* increased with exposure to fish waste, compared with the control ($F_{1, 16} = 4.90$, $p<0.05$). *Tubifex tubifex* survival (r^2=0.03, p=0.472) and cocoon production (r^2=0.11, p=0.178) were not related to waste loading, which suggests that a bioassay of longer duration (>21 days) would be required for significant effects to be observed. The sedimentation rates used in this study were based on preliminary sampling of rates at a commercial farm in the North Channel of Lake Huron.

3.2. Introduction

Open net-pen aquaculture primarily affects the aquatic environment through the production and release of organic wastes (Carroll *et al.* 2003; Bureau and Cho 1999). Open net-pen farming is a system where fish are reared within a net that is suspended in the water column (Beveridge 1987). The amount of waste released from these farms will vary with individual management practices such as stocking density, cage configuration and feeding methods and rates (Arthington and Bluhdorn 1996). In lakes containing net-pen farms, sedimentation rates have been reported between 5 to 200 times background levels (Enell and Lof 1983; Merican and Phillips 1985; Troell and Berg 1997). The primary component of solid wastes released from aquaculture operations is faecal material (Podemski and Blanchfield 2006; Ackefors and Enell 1994). Faecal production is estimated at approximately 15-30% of applied feed (Costello *et al.* 1996; Bureau *et al.* 2003). Technology to trap and remove solid waste is costly and ineffective (Behmer *et al*. 1993; Angel *et al.* 2002), so few commercial farms in Canada use a waste collection system. Cage culture is estimated to release 240 to 318 kg of solid waste per 1000 kg of fish produced (Bureau *et al.* 2003); the majority of this solid waste settles to the lake bottom where it is consumed by biota or decomposes (Johansson *et al*. 1998).

When solid wastes accumulate, they may have a significant impact on the sediment beneath fish farm operations. These effects are usually confined to a relatively small area directly beneath and adjacent to fish cages (*e.g.*, Brown *et al.* 1987; Cornel and Whoriskey 1993; Podemski and Blanchfield 2006; Rooney 2006). The primary concerns associated with the release of aquaculture solid wastes are the potential for nutrient-

induced stimulation of local algal blooms and the creation of oxygen-depleted waters and enriched sediments beneath open net-pen farms (Podemski and Blanchfield 2006). Excess readily decomposable organics settling on the sediment will increase oxygen demand at the sediment-water interface. If oxygen consumption from faunal respiration and aerobic bacterial decomposition exceeds the supply from photosynthesis and vertical mixing, then hypoxia or even anoxia may occur (Mulsow *et al.* 2006, Gray *et al*. 2002).

Sediments beneath and surrounding fish farms may become enriched with increased concentrations of carbon, nitrogen and phosphorus. At marine salmon farms off the coast of Norway, Carroll *et al*. (2003) found that sediments immediately adjacent to the cages had significantly higher concentrations of total organic carbon compared to reference sites. At 50 -100 m from the farm, there was no detectable difference in carbon concentrations compared with sediment from the same reference site (Carroll *et al*. 2003). Rooney and Podemski (2010) studied alterations in sediment chemistry beneath and surrounding an experimental *Oncorhynchus mykiss* (Walbaum) farm in a freshwater lake in Ontario, Canada. Total organic carbon, total nitrogen and total phosphorus were elevated in the sediment below the cage 60 days after farming had begun compared to sediments beyond 5 m from the edge of the cage. Increases in phosphorus concentration in the sediment are a concern due to the risk of eutrophication (Yan 2005). The majority of phosphorus in fish waste settles quickly (≥6 cm/s) to the sediment in solid form (Azevedo *et al.* 2011; Reid *et al.* 2009; Phillips *et al.* 1995). Phosphorus that sinks out of the euphotic zone is not available to pelagic algae; however, internal phosphorus loading

during periods of hypolimnetic anoxia could eventually promote algal growth in the lake basin (Rooney and Podemski 2010).

Correlations between organic enrichment and alterations in measures of community structure such as biomass, abundance, and taxa richness have been reported in many parts of the world (*e.g.*, Brown *et al*. 1987, Weston 1990, Loch *et al.* 1996, Gao *et al*. 2005, Rooney 2006, Sutherland *et al*. 2007). Benthic invertebrate biomass may increase because of the increased food supply from the sedimentation of solid waste. Organic waste from aquaculture is labile, with a low C:N ratio and a high content of nutrients (Kelly 1992; Kristensen 1990). Hargrave *et al*. (1997) measured the response of the benthos to a salmon farm off the coast of New Brunswick, Canada. Mean biomass of suspension feeders at the cage sites and reference sites was not significantly different; however, average biomass of deposit feeders at cage sites (22 g m^{-2}) was significantly higher than at reference sites (13 g m^{-2}). The high rates of organic matter sedimentation under the cages may have enhanced the food supply for this trophic group (Hargrave *et al*. 1997). The changes in benthic community structure due to aquaculture wastes usually follow an organic enrichment gradient comparable with those from domestic sewage and wood pulp effluent (Pearson 1972, Swartz *et al.* 1985, Whitehurst and Lindsey 1990). Pearson and Rosenberg (1978) created a model to describe the structural changes that occur in benthic communities along a gradient of organic enrichment. In areas that are heavily enriched, the number of species, their abundance, and the total biomass of the community will be very low. As loading declines, abundance of the opportunistic species will peak. These species are those that are able to survive in areas with reduced oxygen

availability, increased pore-water ammonia concentrations and high sedimentation rates. This assemblage of opportunists is separated by an "ecotone point" where an assemblage of greater species richness and biomass gradually changes through a "transition zone" to become more characteristic of an unimpacted community (Pearson and Rosenberg 1978). At a marine salmon farm on the western coast of Scotland, Brown *et al.* (1987) found invertebrate abundance was lowest 120-1400 m from the cages, slightly higher 3 m from the farm, and at 15 m from the edge of the farm was two to three times greater than all the other stations (Brown *et al*. 1987). Opportunistic polychaete worms made up 99% of the total invertebrate abundance at the 3 m station, and taxa richness increased with distance from the farm. Responses to organic loading in freshwater environments include a decrease in taxa richness due to the loss of sensitive species, such as Ephemeroptera, Plecoptera, and Tricoptera, and an increase in the abundance of resistant organisms such as Tubificinae, Naidinae and Chironomidae (Hynes 1963; Johnson *et al.* 1993).

Benthic organisms living in the vicinity of the cage farms may be exposed to increased concentrations of nutrients and metals in the sediment, as well as elevated levels of pore-water ammonia (*e.g.*, Rooney and Podemski 2010). The addition of organic material may also lead to alterations in food quality (*e.g.*, Kullman *et al*. 2009), or physical smothering due to the build-up of material on the sediment surface (*e.g.*, Kullman *et al.* 2007). These conditions may cause changes in invertebrate abundance near aquaculture operations. Rooney and Podemski (2009) observed that benthic impacts responded very quickly to waste outputs from a new experimental farm operation. Abundance was the first invertebrate metric detectably affected by the farm. After two

months, invertebrate abundance was reduced under the fish cage (2 542 ± 569 individuals m^{-2}) compared with samples collected 45 m away (16 137 ± 2 624 individuals m^{-2}) (Rooney and Podemski 2009).

The majority of the studies examining alterations in benthic communities resulting from aquaculture waste describe effects by the distance from the farm (*e.g*. Rooney 2006; Weston 1990; Brown *et al.* 1987). This makes it difficult to compare between farms, as site-specific characteristics will affect how far the waste material will travel (*e.g.*, water velocity, depth, farm size, and other nutrient sources). A more useful way to compare effects of aquaculture waste on invertebrates is to examine the effects based on the associated carbon loading rate (g C m^{-2} day^{-1}). In an associated study, we measured sedimentation rates at two commercial rainbow trout farms in Lake Huron, and at an experimental operation in Ontario (Wetton, Chapter II). Using these rates of carbon deposition, effects on invertebrate survival and growth were studied in laboratory microcosms.

The main objective of this study was to investigate the effect of aquaculture solid waste on survival and growth of benthic invertebrates. Test organisms were chosen to be representative of the benthic community beneath net-pen operations in Ontario. Another objective of this study was to observe alterations in sediment characteristics within these microcosms. By studying the response of the invertebrates and the alterations to sediment characteristics at known levels of waste loading, I hoped to compare these results with measured loading rates at the commercial and experimental farms from a concurrent

study to help set meaningful guidelines for the release of farm waste from open net-pen operations.

3.3. Methods

A series of eighteen (141 L) insulated fibreglass microcosms (60.96 cm x 30.48 cm x 76.2 cm) was connected to refrigerated head tanks to create a partial flow-through system. The refrigeration units cooled the water from 22°C to 10°C (± 3°), a temperature typical of benthic environments in Lake Huron (Barton and Griffiths 1984). Each of the microcosms received a constant gravity-fed flow rate of 0.3 L min^{-1} of dechlorinated water, or just over two volume replacements per day. If using daily water renewal during a bioassay, overlying water should be replaced at a rate of two volume additions per day (US EPA 2000; Environment Canada 1997). Each microcosm had a 425 μm screen placed over the main drain to prevent the escape of test organisms. A smaller emergency drain was also installed to prevent overflow in the event of main drain blockage. A low density polyethylene liner was installed in each microcosm, to allow for easier removal of sediment and to assure that microcosm material did not alter the results of the test. Each microcosm was covered with a polystyrene lid to further insulate each microcosm.

Natural sediment was collected in the North Channel of Lake Huron with a 15.24 cm x 15.24 cm x 15.24 cm Ekman dredge. The sediment was heated to >35°C to eliminate any viable zebra mussel (*Dreissena polymorpha* Pallas) larvae. This sediment was also thoroughly mixed and sieved to remove any large indigenous organisms. Organic content of the sediment was measured as %LOI by placing five randomly

selected, dried samples in a muffle furnace for three hours at 550°C (%LOI 550) and determining weight loss. The organic content of the sediment was only 5%, which is less than typical background levels (8%) collected from the upper 10 cm of sediment from the North Channel of Lake Huron (M. Charleton, Environment Canada, unpublished data). Therefore, the organic content of the sediment was adjusted by mixing in ground Tetramin® to ensure that the microcosms more closely represented the organic content of the sediment in Lake Huron. Without a food supply in the sediment, it is possible there would have been decreased growth of the organisms in the control microcosms and the results may have been confounded. A 10 cm layer of this amended sediment was placed in each microcosm, creating a system with a surface area of 0.186 m^2 and a water volume to sediment volume ratio of 7:1. After the addition of the sediment and filling the microcosms with water, the entire system was allowed to equilibrate for 72 hrs prior to the addition of any test organisms.

Laboratory cultures of *Tubifex tubifex* Müller (Oligochaeta, Naididae) and *Chironomus riparius* Meigen (Diptera, Chironomidae) were kept in a controlled environment room at 23°C (± 2°), under a 16h light: 8h dark photoperiod. *Tubifex tubifex* were obtained from a certified commercial source (Aquatic Research Organisms), and culturing protocols were based on Paoletti (1989). Eight-week old *T. tubifex* were used in the assay. *Tubifex tubifex* was chosen for the bioassay because of the abundance of Naididae near commercial operations in Ontario, as well as the availability of culturing protocols. Egg ropes for *C. riparius* were provided from an established culture from Environment Canada (Burlington, ON). The chironomid larvae were raised in an

environmentally controlled chamber within glass aquaria that contained Exo-terra® Riverbed sand (Hagen Exo Terra®) and dechlorinated water. The larvae were exposed to a 16 h light: 8 h dark photoperiod, and fed ground TetraMin® Tropical Flakes twice daily (Anderson 1980). Second instar larvae were used in the assay, as these would be large enough to be retained within the 425 µm drain screen, but were unlikely to emerge as adults during the bioassay. Chironomini larvae were present near commercial operations in Ontario (Figures 2.6 - 2.7, Chapter II). *Sphaerium simile* Say (Bivalvia, Pisidiidae) were collected on 27 October 2007 from Lake 222 at the Experimental Lakes Area, Ontario (49° 41' 47.12" N , 93° 43' 21.39" W). This species has been used previously in a field bioassay testing survival and growth responses to sediment collected at varying distances from an *O. mykiss* cage farm (Kullman *et al*. 2007). Clams were collected in 0.5-1.0 m water depth by using 1 mm mesh D-frame dip nets. Clams were transported back to the laboratory in aerated buckets of Lake 222 water, and then slowly acclimated (2°C per day) to 23°C in an incubator, before transfer into the controlled environment room. The clams were held in several aerated aquaria, containing Lake 222 sediment, mixed with Riverbed sand and dechlorinated water. Rearing procedures for *S. simile* were based on several preliminary growth tests with various substrates and food sources (Wetton, unpublished data). Only individuals measuring 8-12 mm in length were used in the experiment. Length was defined as the distance between the anterior and posterior margins, when measured perpendicularly to the umbo (Kullman *et al*. 2007). Individual clams were numbered using an indelible marker, and the initial length was measured to 0.01 mm using digital calipers. The number of invertebrates in each microcosm was selected to mimic naturally-occurring densities in Lake Huron (Barton and Griffiths

1984). Invertebrates to be used in the bioassay were acclimated (2°C per day) from 23°C to 10°C in an incubator and then randomly assigned into 19 groups. Eighteen groups were randomly assigned to experimental microcosms, and one group was immediately preserved in 10% formalin to be used for initial length and dry weight comparisons.

To simulate open net-pen farm deposition, fish faecal matter was added to the microcosms daily during the 21-day bioassay. Each of the microcosms was randomly assigned to one of six treatment groups. Each treatment group had three replicate microcosms, each receiving identical amounts of solid waste (Table 3.1). The range of concentrations was set based on a concurrent study measuring the waste loading rates from commercial fish farms in Lake Huron (Wetton, Chapter II). The waste was collected twice weekly during the experiment from a commercial land-based Arctic charr (*Salvelinus alpinus* Linnaeus) farm. The concentration of fish waste was determined individually for each 25 L bucket of waste that was collected. The waste was mixed by aeration for five minutes per bucket, and then three, 30 mL aliquots of the liquid waste were placed in pre-weighed pans, dried at 40°C for 48hrs and weighed. After determining the dry weight of this material, the mass of dry fish waste (mg) per mL of water was calculated. The wet fish waste was kept at 4°C until needed for the experiment, and was stored no longer than 96 hours before use.

Water quality measurements were recorded once daily in all 18 microcosms, one hour before the addition of any fish waste. Temperature and dissolved oxygen were measured using a HACH HQ10 meter and DO probe. Conductivity and pH were recorded

using a HACH HQ 40d multi meter. In all cases, the probe was suspended vertically in the water column, 20 cm from the surface. The temperature did fluctuate within the microcosms (±3°C), which can be attributed to a poorly functioning water cooling system, despite all efforts to test it before the experiment.

Alterations of sediment quality were assessed by comparing nutrient and metal concentration, and organic content before and after the bioassay. Before the addition of waste, five random core samples were collected for sediment analysis. After 21 days of fish waste addition, three replicate core samples were collected from each microcosm. All sediment samples were 4.80 cm in diameter and 30 cm in length, containing approximately 10 cm of sediment with 20 cm of overlying water. The top 0-2 cm of each sediment core was extruded and placed into a Whirl-Pak® bag. Sediment nutrient sub-samples were freeze-dried, ground and homogenized then sent for analysis: C, N and P analysis by the University of Alberta biogeochemical laboratory and at the Freshwater Institute, Winnipeg, and elemental composition by Acme Analytical Laboratories, Ltd. Total nitrogen and total carbon were measured using an Exeter Analytical Model CE-440 Rapid Analysis Elemental Analyzer with a detection limit of 0.70 µg N g^{-1} and 7.06 µg C g^{-1} dry-weight, respectively. Total phosphorus was measured with a Lachat QuikChem 8500 FIA automated ion analyzer, (Lachat Instruments, Colorado, USA) after samples were ashed at 500˚C. More details are available from American Water Works Association (1999). Organic content of the sediment was measured by calculating %LOI 550. Methods for determining %LOI 550 were adapted from Reiners and Reiners (1972). Samples for elemental composition were digested by the addition of a 10 mL aliquot of

the acid solution (2:2:1:1 H_2O-HF-$HClO_4$-HNO_3), and heated on a hot plate until fuming. A 4 mL aliquot of 50% HCl was added to the residue and heated using a mixing hot block. After cooling the solutions were transferred to polypropylene test-tubes and made up to a 10 mL volume with 5% HCl. These solutions were aspirated into a Spectro Ciros Vision or Varian 735 ICP emission spectrometer and then analyzed for numerous elements: Ag, Al, As, Au, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, La, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Sb, Sc, Sn, Sr, Th, Ti, U, V, W, Y, Zn and Zr.

On day 21, three replicate sediment cores were collected from each microcosm for the purposes of measuring pore-water ammonia. In each core, pore-water pH and temperature was measured using an IQ Scientific Instruments ion selective field effect transistor probe. Temperature and pH must also be measured to assess the risk of elevated pore-water ammonia to benthos, as only the unionized fraction of total ammonia is toxic (Emerson *et al.* 1975). The proportion of unionized ammonia depends on temperature and pH (Emerson *et al.* 1975). The probe was calibrated by creating a three point curve using 4.0, 7.0, and 10.0 standard solutions. The tip of the electrode was inserted into the center of the upper two sections of each core (0-2 cm and 2-4 cm) and a reading was taken after stabilization criteria were met (< 0.1 change in pH per 45 seconds.). The top 0-2 cm and 2-4 cm sections of the sediment cores were quantitatively transferred into 0.02 μm GF/F filter unit to extract the pore water by suction filtration. The pH of the porewater samples was made basic through the addition of 200 μL Orion Ionic Strength Adjuster, which converts ammonium ions to ammonia. The total ammonia for each sample was then determined using a Fisher Accumet AR25 meter and a Thermo Orion

ion selective ammonia probe calibrated with a 10:1 dilution series made from a 0.1 M ammonium chloride standard. In addition to determining pore-water ammonia concentrations, total ammonia in the water column was measured on Day 7, 14 and 21 in each of the microcosms for all treatments. A 10 mL sample of water was tested for three replicates of each treatment using the same method as above for the sediment pore-water.

Survival for all species was determined by a count of the invertebrates in each microcosm at the completion of the 21-day experiment. The entire contents of each microcosm were passed through a 425 μm sieve. The retained material was preserved in a 10% formalin solution before further processing, with the exception of adult *S. simile*. The numbered fingernail clams were removed during sieving and placed in dechlorinated water, and then the individual length of each individual was measured using digital callipers for paired comparisons of body length. After initially fixing in formalin, the sieved sediment was transferred to 95% ethanol ten days later. This sediment was sorted using a gridded Petri dish under a 6-50X stereomicroscope, and the remaining *C. riparius*, *T. tubifex* (including cocoons) and *S. simile* young were counted and then stored in vials containing 70% ethanol. Voucher specimens are located at the Freshwater Institute (Fisheries and Oceans Canada) in Winnipeg, Manitoba, Canada.

Growth of the surviving individuals was determined as the increase in body length (with the exception of *T. tubifex*) and/or dry weight. Initial weight was determined from the sample of organisms that were immediately preserved rather than placed into a microcosm. The dry weight of invertebrates was determined using a Fisher Scientific

Accu-124D dual range balance. Body length was measured for *C. riparius* using a Leica MZ12 stereomicroscope and an attached Leica DFC320 camera. Using AxioVision Rel 4.6, the body length of each organism was measured from a digital photograph by tracing the body with a curved spline. Body length of *T. tubifex* could not be reliably determined due to breakage of these delicate organisms, and their tendency to contract when preserved. Therefore the growth of *T. tubifex* was determined by a comparison of dry weight of pre- and post-exposure organisms. The entire mass of worms from each microcosm (or initial group) was placed in a pre-weighed tin foil weigh boat and dried at 40°C for 72 hours. The total dry weight was divided by the number of *T. tubifex* per weigh boat. The total number of individuals was determined by enumerating heads (before drying). The difference in dry weight (pre- and post-exposure) was also determined for *C. riparius*; however, these organisms were weighed individually.

MINITAB v. 12.1 (MINITAB 1998) statistical software was used for linear and polynomial regressions of sediment chemistry and benthos variables with carbon loading. A plot of the data was reviewed to determine if the relationship between the variables appeared linear or curvilinear. Normality and homogeneity of residuals were tested using Anderson-Darling and Bartlett's tests, respectively. Confidence limits (95 %) were calculated for the slope and intercept of the regression lines, as well as the peak on polynomial regressions. If the assumptions were met, a linear or polynomial regression was performed based on the pattern of the plotted data. The relationship between carbon (TC), nutrient (TN, TP), metal (Cu, Zn) and pore-water ammonia (2-4 cm) concentration with carbon loading was described using linear regression analysis. Total phosphorus was

log_{10}-transformed to meet the assumptions of the linear regression. *Tubifex tubifex* and *C. riparius* survival and growth with carbon loading was also described using linear regression. The relationship between *S. simile* survival and growth, as well as water column and pore-water ammonia (0-2 cm) concentration with carbon loading was described using polynomial regression analysis. MINITAB was also used for a one-way ANOVA with an alpha significance of 0.05 to test for among-treatment differences in growth for *T. tubifex* that were exposed to fish waste, compared with those in the control group.

3.4. Results

3.4.1. Sediment and pore-water chemistry

As expected, the carbon concentration in the top 2 cm of sediment increased with increasing carbon loading rates (Figure 3.1) (Eqn. 1; Table 3.3). Carbon concentration was lowest in the control microcosms receiving no fish waste, and highest in the microcosms receiving 46.7 g C m^{-2} day^{-1} (Table 3.2). The relationship between carbon loading and the concentration of nitrogen in the sediment was not significant (Figure 3.2) (Eqn. 2; Table 3.3). Sediment phosphorus concentration increased in the sediment with the rate of carbon loading after the data were log_{10}-transformed (Figure 3.3) (Eqn. 3; Table 3.3). The concentration of zinc in sediment was significantly related to carbon loading (Figure 3.4) (Eqn. 5; Table 3.3). The relationship between sediment copper concentration and carbon loading was non-significant (Eqn. 4; Table 3.3).

The concentration of pore-water ammonia extracted from the top 2 cm of sediment in each microcosm increased with increasing carbon loading to a maximum at 42.18 g C m^{-2} day^{-1} (Figure 3.5) (Eqn. 6; Table 3.3). In pore-water collected from the 2-4 cm layer of sediment, the concentration of ammonia also increased with increased carbon loading, though the relationship was not as strong (Figure 3.6) (Eqn. 7; Table 3.3). For samples collected mid-water in the microcosms, ammonia levels on day 21 were closely related to the addition of solid waste (Figure 3.7) (Eqn. 8; Table 3.3). Ammonia levels increased with carbon loading to a calculated maximum value of 55.64 g C m^{-2} day^{-1} (Eqn. 8; Table 3.3).

3.4.2. Invertebrate survival and growth

For *C. riparius* and *T. tubifex*, there was no relationship between carbon loading and survival (Figure 3.8) (Eqn.10, 11; Table 3.3). The significant polynomial regression suggests survival of *S. simile* was dependent on waste addition (Eqn. 9; Table 3.3). Up to 20 g C m^{-2} day^{-1}, the survival rate was between 80-90%. Using the polynomial regression equation, a calculated loading rate of 19.25 g C m^{-2} $day^{-1}$was determined to be the maximum loading that *S. simile* could tolerate. Beyond that value, survival of *S. simile* decreased with increased waste loading.

The largest increase in mean body length of *S. simile* (0.09 mm) was recorded for clams exposed to an intermediate loading rate of 18.7 g C m^{-2} day^{-1} (Figure 3.9). At the highest loading rate, 46.7 g C m^{-2} day^{-1}, inhibition of growth was observed compared to control microcosms. A polynomial regression best described the relationship (Eqn. 12;

Table 3.3). Based on this equation, a loading rate of 22.46 g C m^{-2} day^{-1}would have been optimal for growth of *S. simile*. For *C. riparius*, a significant linear relationship with carbon loading was observed when using dry weight as a measure of growth (Figure 3.10) (Eqn. 14; Table 3.3). When using length as a measure of growth, the relationship was not significant (Eqn. 13; Table 3.3). Using linear regression analysis, there was no relationship between carbon loading and growth of *T. tubifex* using dry weight as a measure of increased size (Eqn. 15; Table 3.3). However, the results of the ANOVA conducted between all *T. tubifex* exposed to fish waste and those that were not, showed a significant increase in dry weight for those animals exposed to fish waste ($F_{1, 16} = 4.90$, $p<0.05$). Cocoon production was also compared among the different intensities of carbon loading. There was no relationship between the loading rate, and the number of cocoons produced (Eqn. 16; Table 3.3).

3.5. Discussion

The waste loading rates for this experiment were set based on those observed at a commercial fish farm in Lake Huron (Wetton, Chapter II). The rates were comparable to those measured by Holmer *et al*. (2007) and Kutti *et al.* (2007) near marine aquaculture operations, and within the range sampled by Troell and Berg (1997) at a freshwater operation in Zimbabwe. Sediment carbon concentration could not be entirely predicted from the rate of carbon loading ($r^2=0.28$) but it did increase with 21 days of carbon loading (Figure 3.1). The concentration of carbon was quite variable, even within microcosms exposed to the same intensity of loading. The labile carbon added to the microcosms in the form of fish waste may have been a food source for *T. tubifex*, *C.*

riparius, and *S. simile* (Kullman *et al.* 2009; Wellman 2011) which could affect the remaining sediment carbon concentration. In addition, the sediment samples collected from each microcosm were of the top 2 cm. The distribution of the fish waste on the sediment surface may have been heterogeneous, leading to variability in the sediment chemistry results. The concentration of nitrogen in the top two centimetres of sediment increased with carbon loading; however, the relationship was not very strong (r^2=0.21, Figure 3.2). The aqueous solution of fish waste collected from tanks at the land-based Arctic charr farm may have depleted concentrations of nitrogen due to the water filtration system to remove ammonia. Ammonia is the primary nitrogenous waste released from the organic decomposition by heterotrophic bacteria (Wetzel 2001). As the fish waste began to decompose within the tanks, ammonia was released into the water column and filtered out. Land-based aquaculture operations often use tanks with a recirculating water supply so the removal of nitrogenous wastes is crucial for fish survival (Meade 1985). Sediment phosphorus concentration in the sediment after the 21-day bioassay was related to carbon loading (r^2=0.82, Figure 3.3). The majority of phosphorus from aquaculture is lost to the sediments as solid wastes (Ackefors and Enell 1994). Degradation of the accumulated solid waste could have also resulted in the release of labile P back into the water column which could account for some of the variability (Kelly 1993).

Copper (Cu) and zinc (Zn) and are two heavy metals associated with aquaculture facilities (Brooks and Mahnken 2003). Copper is used in the anti-fouling coatings applied to some net-pens to minimize the growth of periphyton (Brooks and Mahnken 2003). Although copper can accumulate in the sediments surrounding the cages, the use

of anti-fouling net coatings is less common in freshwater farms than at marine operations (Rooney 2006). As the fish waste collected for this experiment was from a land-based farm, there was no additional copper added in the faeces from net coatings. As expected, there was no significant relationship between sediment copper concentration and waste loading (Table 3.2). However, the sediment copper concentrations in this experiment exceeded the lowest effects level (LEL) of the Ontario provincial sediment quality guideline (0.016 mg g^{-1}) (CCME 2002) in all of the microcosms (Table 3.2). This was not due to the addition of fish waste, as the sediment copper concentration of the natural sediment used in the bioassay was 18 ug g^{-1} (unpublished data). It is unclear why the control microcosms showed an increased in copper concentration, as no fish waste was added to these enclosures. The dechlorinated water source should be analyzed to determine if copper was added to the system.

Zinc is a component of fish feed and reaches the sediment beneath cage farms bound to solid farm waste (Parker and Aubé 2002). In this study, Zn concentration in the sediment rose significantly with increased faecal matter addition (Table 3.2). As a component of the feed, one would expect the concentration of zinc to rise with increasing waste addition to the microcosms. It is unknown if the organisms were able to acclimate to increasing zinc concentrations during the bioassay. Miller and Hendricks (1996) subjected third and fourth instar *C. riparius* larvae to a range of zinc concentrations over a series of acute exposures. The authors noted 'acclimation-based resistance' to zinc in fourth instar *C. riparius* larvae that was not passed down to their progeny (Miller and Hendricks 1996). *Chironomus riparius* larvae may have been able to acclimate to the

sediment zinc concentration in this bioassay, as their growth and survival was not adversely affected at high levels of waste loading. Bouché *et al.* (2000) described mucus secretion forming detoxifying complexes in *T. tubifex* for physiological resistance from heavy metals. *Tubifex tubifex* survival was not related to carbon loading in this bioassay, so these worms may have used physiological mechanisms to avoid toxicity from zinc. The sediment concentrations of zinc in this experiment only exceeded the LEL of the Ontario provincial sediment quality guideline (0.120 mg g^{-1}) (CCME 2002) in the microcosms subjected to 46.7 g C m^{-2} day^{-1} (Table 3.2). The risk of toxicity from sediment zinc concentration should therefore be low.

The decomposition of organic waste from aquaculture can promote chemical reactions that may be detrimental to the benthos, such as the stimulation of bacterial activity (*e.g.,* Droppo *et al.* 2007) resulting in oxygen depletion and the production of ammonia (*e.g.,* Rooney and Podemski 2010). Pore-water ammonia was measured on day 21 of the bioassay, in both the 0-2 cm (Figure 3.5) and 2-4 cm (Figure 3.6) layers. The concentration of ammonia did increase with carbon loading, though this relationship was more strongly linked in the 0-2 cm depth layer. Based on these data, up to a calculated maximum carbon loading of 42.18 g C m^{-2} day^{-1}, the concentration of ammonia would be expected to increase. In a study at the Experimental Lakes Area in Ontario, Canada, pore-water ammonia was the first variable to respond after the initiation of an experimental fish farm in May 2003 (Rooney 2006). In the sediment under the cage, ammonia concentrations reached over 480 times the level in the reference sediment by September 2004 (Rooney 2006). Rooney and Podemski (2010) suggested that measurement of pore-

water ammonia be included in sediment monitoring programs because of its rapid rate of response, sensitivity and biological significance. The ammonia concentration was also measured in the water column on Day 21 (Figure 3.7), and was significantly related to waste loading (r^2=0.95). However, all of the test organisms were infaunal so the sediment pore-water concentration is a more useful predictor of exposure to ammonia.

Survival of fingernail clams (*S. simile*) exceeded 70% in all treatments. Fingernail clams are particularly resistant to hypoxia as they use ventilatory and circulatory strategies to regulate dissolved oxygen transport (Joyner-Matos *et al.* 2011). In moderate hypoxia, molluscs react to the decreasing partial pressure of oxygen by increasing ventilations and raising their heart rate to improve hemolymph circulation (Grieshaber *et al.* 1994). According to the polynomial regression (Figure 3.8), the optimum carbon loading rate for *S. simile* survival would have been 19.25 g C m^{-2} day^{-1} in this experiment. The calculated optimal carbon loading rate for growth of *S. simile* was slightly higher, at 22.46 g C m^{-2} day^{-1}. Deposit feeding provides approximately 75% of the energy budget for Pisidiidae (Hornbach *et al*. 1984). Filter-feeding has also been documented for this group (Joyner-Matos *et al.* 2011; Vaughn and Hakenkamp 2001). These clams would have been able to make use of fish waste that had been mixed into the sediments by *T. tubifex* and *C. riparius*, as well as the waste that was settling on the sediment surface. Growth was low with no additional waste added (0 g C m^{-2} day^{-1}) and also at the highest level of loading (46.7 g C m^{-2} day^{-1}). It is possible that in the control microcosms, not enough food was provided, and that at the highest loading intensity, the clams may have been smothered by the excess of organics. In an *in situ* bioassay by Kullman *et al.* (2007),

fingernail clams exposed to sediment from directly under the cage experienced 100% mortality; however, those clams exposed to sediment 1 m from the cage had the greatest growth, and most embryos after the six-week bioassay (Kullman *et al*. 2007). The authors concluded that settled aquaculture waste may have been a beneficial food source for benthos living just beyond the edge of the cage (Kullman *et al*. 2007). Similarly, in the present study clam mortality was highest in those individuals exposed to the highest concentration of faecal material and increased growth was observed with moderate levels of waste loading. There was no difference in embryo counts after 21 days of exposure.

Chironomus riparius larvae are able to survive in areas of low oxygen and high organics (Charles *et al*. 2004); so waste loading likely had little effect on their ability to survive throughout the experiment. Survival in this experiment ranged from 40-90% of the total number that was added to the microcosms (Figure 3.8). *Chironomus riparius* pupae were found when sorting through the sieved material from the microcosms. The life cycle of *C. riparius* at 20°C is completed in about 25 days (Anderson 1980). The microcosms were kept at 10°C (± 3°) but due to the temperature fluctuation, it is possible that the chironomids emerged before day 21 of the bioassay, though no emerged imagos were observed beneath the polystyrene lid. Mean midge larval biomass increased when exposed to higher rates of waste deposition (Figure 3.10). The heaviest larvae were collected from the microcosms that received the most solid waste, 46.7 g C m^{-2} day^{-1}. *Chironomus riparius* larvae are often collected in organically enriched waters, and will feed on detritus that has been deposited on the sediment (Charles *et al.* 2004). Dobrowolski (1987) found high densities of chironomids in close proximity to a cage

culture farm, specifically those of the tribe Chironomini. Chironomini larvae feed on organic detritus (Zieba 1984) and may benefit from moderate increases in organic loading. The body cuticle is membranous, which allows the larval body to grow continuously (Gullan and Cranston 2005). There was no significant relationship between waste loading and total body length (Table 3.3). *C. riparius* has a well described growth pattern that is independent of nutrient availability (Watts and Pascoe 2000). Increases in body length are only limited to the period immediately after molting (Gullan and Cranston 2005).

Tubifex tubifex survival and growth were not related to the intensity of carbon loading in this experiment (Figure 3.8); however, *T. tubifex* exposed to fish waste were significantly heavier that those that were not (control group). These worms are able to thrive in the vicinity of organic effluents due to the adaptation of their respiratory physiology to operate at very low oxygen concentrations, or even in anaerobic conditions (Pateris *et al.*1996; Aston 1973). Palmer and Chapman (1970) reported that the haemoglobin of *T. tubifex* is able to load at very low oxygen concentrations, when the carbon dioxide content of the water is high. In addition, survival in anaerobic conditions for up to four weeks is known to occur in *T. tubifex* (Dausend 1931; Alsterberg 1922; Aston 1973). The numbers of cocoons produced by *T. tubifex* were also counted at the end of this bioassay; however, this reproductive output was also not related to the intensity of carbon loading (Table 3.3). It is likely that the duration of the bioassay was not long enough for statistically significant results.

Comparing the results from laboratory bioassays to responses in natural systems is only effective if the bioassay is a close representative of conditions in the field (Cairns and Pratt 1989). In this study, the temperature of the microcosms was kept at approximately 10° C, representative organisms were tested using realistic densities, and the faecal material was obtained from an aquaculture facility in order to best re-create the benthic environment in Lake Huron, the location of the majority of cage culture in Canada. The sedimentation rates used in this study were based on a concurrent study at commercial open net-pen farms in the North Channel of Lake Huron (Wetton, Chapter II). However, because reference sediment was used, this bioassay was likely more representative of effects along a waste loading gradient from a new cage culture operation, rather than a well-established farm.

Although sediment concentrations were an order of magnitude higher at commercial farms compared with the microcosms, there were some similar trends in the response to carbon loading. During the microcosm experiment, sediment carbon, nutrient (TN, TP) and zinc concentration increased linearly with increasing carbon loading (Table 3.3). In the field study, these variables also increased linearly at Commercial Farm 1 (Table 2.6, Chapter II), but the relationship was curvilinear at Commercial Farm 2 (Table 2.7, Chapter II). The varying build up of faecal matter at the net-pen farms affected sediment chemistry; whereas in this bioassay, there was limited accumulation (<5 mm) on the reference sediment after only 21 days. Sediment quality guidelines for phosphorus, zinc and copper were exceeded at the commercial and experimental cage farms, but in this study, the only LEL exceeded was for zinc at 46.7 g C m^{-2} day^{-1}.

The invertebrates within the microcosms were able to withstand higher loading rates since they only had to survive in these conditions for 21 days. In the field study, total invertebrate density began to decline at a carbon loading rate beyond 3.0 g C m^{-2} day^{-1}. If the bioassay had gone on for a longer period of time, there would likely be decreased survival, especially at higher rates of sedimentation. They were not able to escape the conditions by moving further away from the source of the stressor as some invertebrates can in the natural community. Stimulation in invertebrate growth with carbon loading was observed in both the bioassay, and in the field. Mean individual biomass of *T. tubifex* was greater for those worms exposed to fish waste during the bioassay, compared with those that were not. In the field study, moderate levels of waste loading were linked to an increase in Oligochaeta (Tubificinae and Naidinae) biomass at both commercial farms (Figures 2.8- 2.9, Chapter II). In addition, growth and survival of *S. simile* was greatest at moderate levels of sedimentation. Stimulation of growth in *S. simile* using sediment from an experimental net-pen was also observed in Kullman *et al.* (2007).

Minimization of effects on the benthic environment is one of the main goals of monitoring and regulation of aquaculture operations (Chamberlain and Stucchi 2007). As the majority of effects can be linked to the deposition of fish waste, there is a need to develop meaningful guidelines for sedimentation rates from cage culture operations (Droppo *et al.* 2007; Sutherland *et al.* 2007; Naylor *et al.* 2000). This study was the first to measure benthic invertebrate growth and survival when exposed to a gradient of realistic aquaculture waste loading rates in laboratory microcosms. These results will be

used in conjunction with depositional modelling tools in the development of regulations for the freshwater net-pen aquaculture industry in Canada.

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Table 3.1. Loading rates of Arctic charr (*Salvelinus alpinus*) faecal material by treatment group for a 21-day multispecies microcosm experiment.

Experimental Group	Loading Rate ($g\ C\ m^{-2}\ day^{-1}$)
1	46.7
2	28.0
3	18.7
4	11.2
5	5.6
control	0.0

Table 3.2. Mean concentration (mg g^{-1}) (±SE) of carbon, nutrients and metals in sediment after exposure to fish faecal waste at varying loading rates during a 21-day multispecies microcosm experiment, n=3.

	Carbon loading rates (g C m^{-2} day^{-1})					
Variable	**0**	**5.6**	**11.2**	**18.7**	**28**	**46.7**
Carbon	52.1 ± 5.82	51.0 ± 5.02	52.1 ± 2.58	48.6 ± 3.70	59.0 ± 7.87	75.1 ± 8.05
Nitrogen	7.4 ± 1.06	7.0 ± 0.83	7.1 ± 0.47	6.7 ± 0.65	8.6 ± 1.43	10.4 ± 1.40
Phosphorus	0.9 ± 0.09	1.0 ± 0.06	1.2 ± 0.04	1.3 ± 0.07	2.0 ± 0.32	3.1 ± 1.58
Copper	0.025 ± 0.001	0.024 ± 0.001	0.028 ± 0.001	0.027 ± 0.003	0.027 ± 0.003	0.030 ± 0.012
Zinc	0.070 ± 0.003	0.073 ± 0.003	0.088 ± 0.005	0.073 ± 0.003	0.089 ± 0.008	0.124 ± 0.011

Table 3.3. Statistical results from regression analysis after a 21-day multispecies microcosm experiment (α=0.05, n=3 for each of 6 treatment groups) P-values in bold are significant. Equation numbers are referred to within the text.

Comparison (with carbon loading)	Type of Regression	Regression Equation	F	r^2	P-value	Equation Number
[Carbon]	Linear	$y=47.20 + 0.505x$	6.11	0.28	**0.025**	(1)
[Nitrogen]	Linear	$y=6.57 + 0.071x$	4.27	0.21	0.055	(2)
[Phosphorus] *	Linear	$y=-0.05 + 0.0108x$	72.95	0.82	**0.000**	(3)
[Copper]	Linear	$y=25.3 + 0.0938x$	1.59	0.09	0.225	(4)
[Zinc]	Linear	$y=66.50 + 1.08x$	27.38	0.63	**0.000**	(5)
Pore-water ammonia (0-2cm)	Polynomial	$y=4.26 + 1.51 \times 10^{-2}x - 1.79 \times 10^{-4}x^2$	14.83	0.66	**0.000**	(6)
Pore-water ammonia (2-4cm)	Linear	$y=4.53 + 0.00497x$	7.03	0.31	**0.017**	(7)
Water column ammonia	Polynomial	$y=0.645 + 5.23 \times 10^{-2}x - 4.70 \times 10^{-2}x^2$	148.54	0.95	**0.000**	(8)
S. simile survival	Polynomial	$y=82.76 + 1.01265x - 2.63 \times 10^{-2}x^2$	7.48	0.50	**0.006**	(9)
T. tubifex survival	Linear	$y= 79.8 - 0.246x$	0.54	0.03	0.472	(10)
C. riparius survival	Linear	$y= 61.9 + 0.339x$	3.36	0.17	0.085	(11)
S. simile growth (length)	Polynomial	$y=3.55 \times 10^{-2} + 3.32 \times 10^{-3}x - 7.39 \times 10^{-5}x^2$	10.13	0.57	**0.002**	(12)
C. riparius growth (length)	Linear	$y=7046 - 20.2x$	1.56	0.09	0.230	(13)
C. riparius growth (dry wt)	Linear	$y=0.721+ 0.0053x$	22.05	0.58	**0.000**	(14)
T. tubifex growth (dry wt)	Linear	$y=0.323 + 9.14 \times 10^{-4}x$	1.10	0.06	0.311	(15)
# cocoons (*Tubifex*)	Linear	$y=11.9 + 0.124x$	1.98	0.11	0.178	(16)

*Data for the concentration of phosphorus in sediment were $\log_{10}$ transformed.

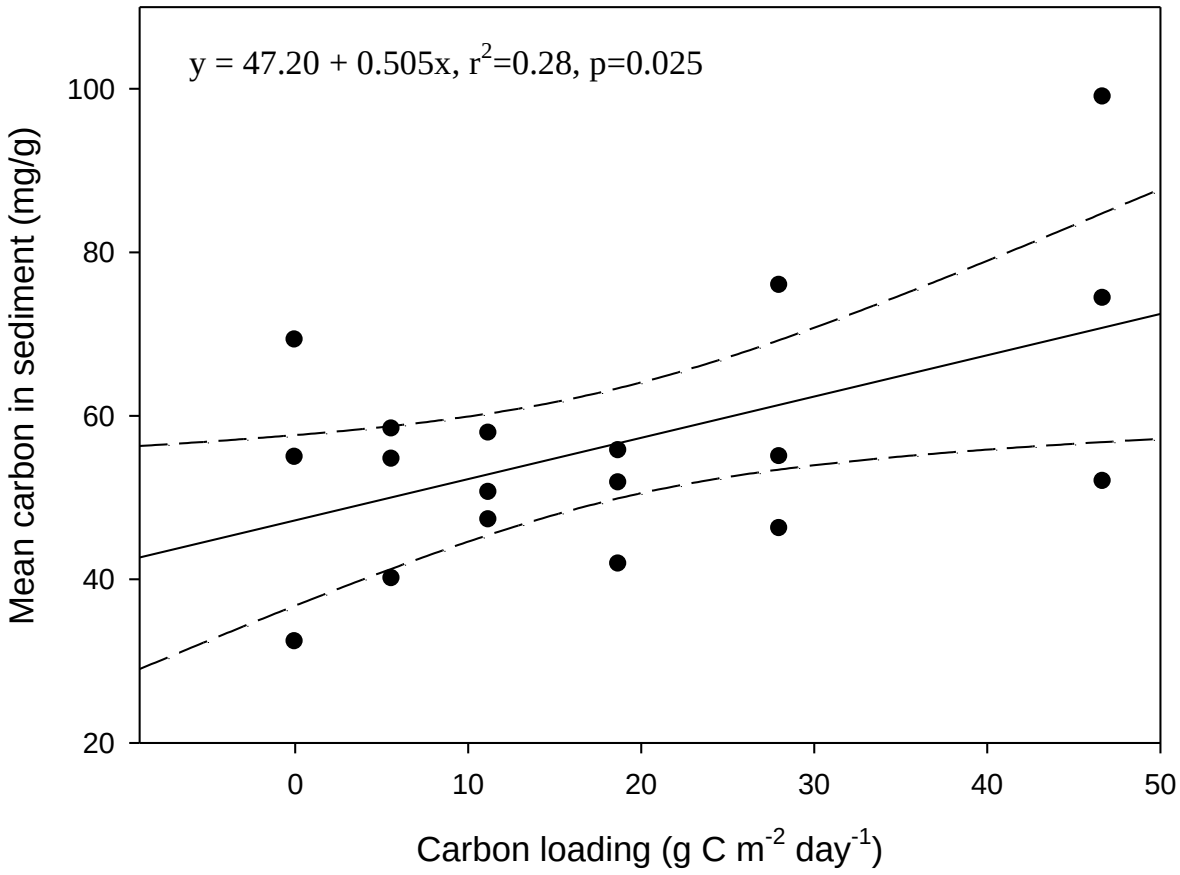

Figure 3.1. Concentration of carbon (mg/g) in the top 2 cm of sediment after a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

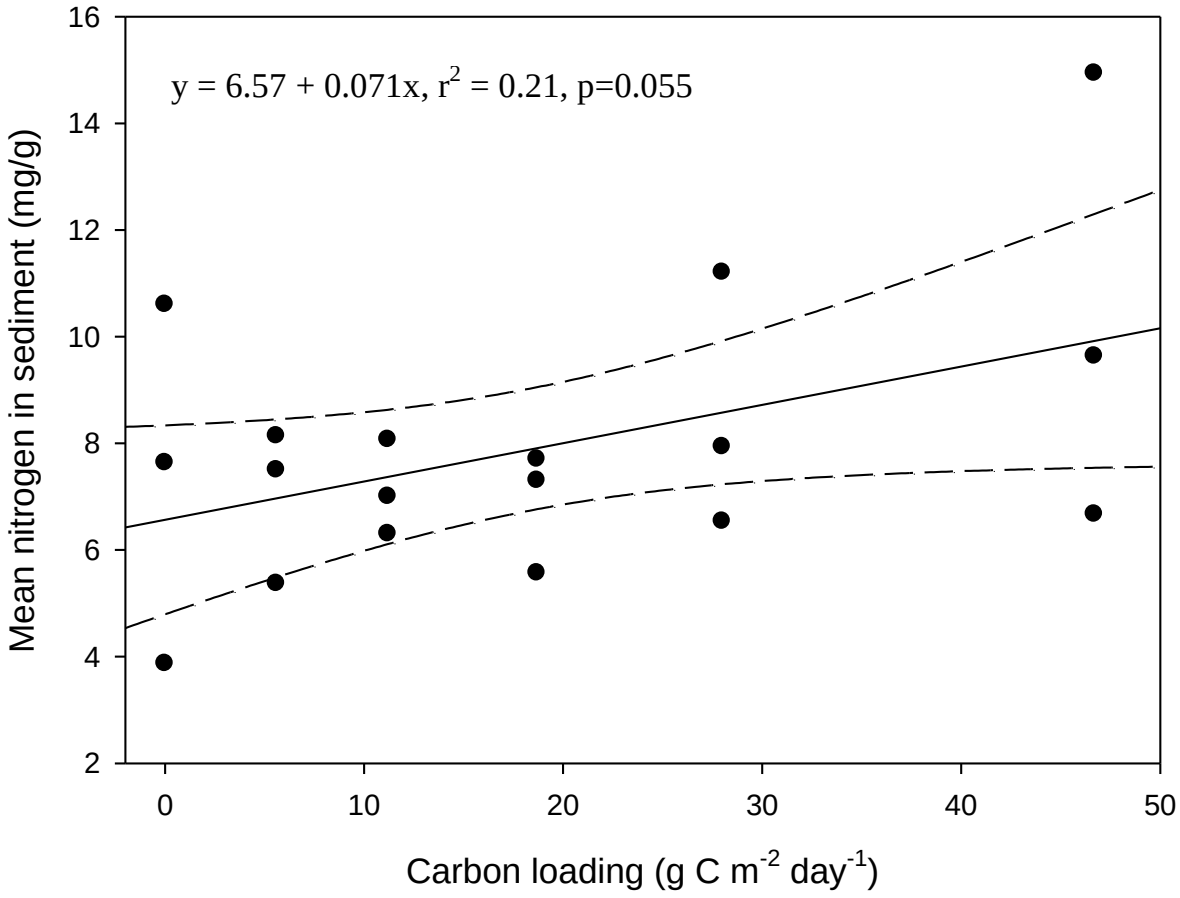

Figure 3.2. Concentration of nitrogen (mg/g) in the top 2 cm of sediment after a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

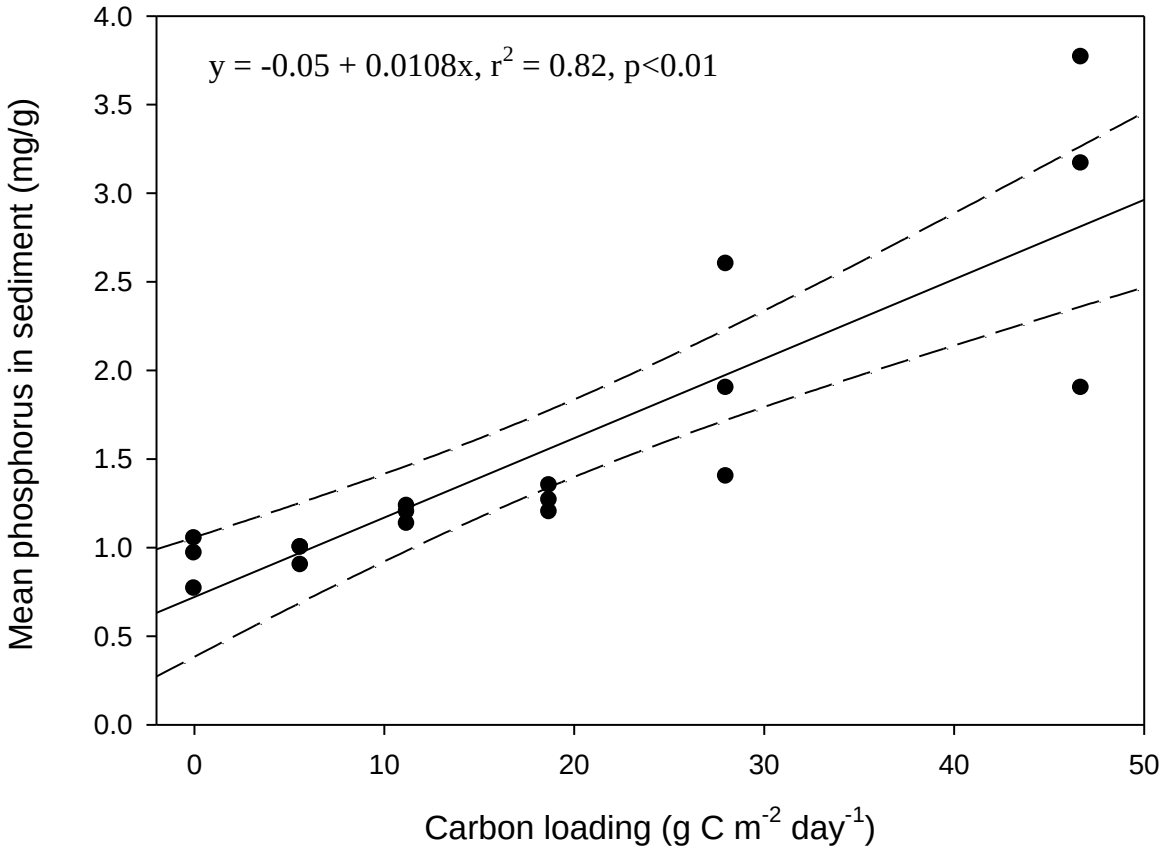

Figure 3.3. Concentration of phosphorus (mg/g) in the top 2 cm of sediment after a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines. Mean phosphorus concentration was $\log_{10}$ transformed.

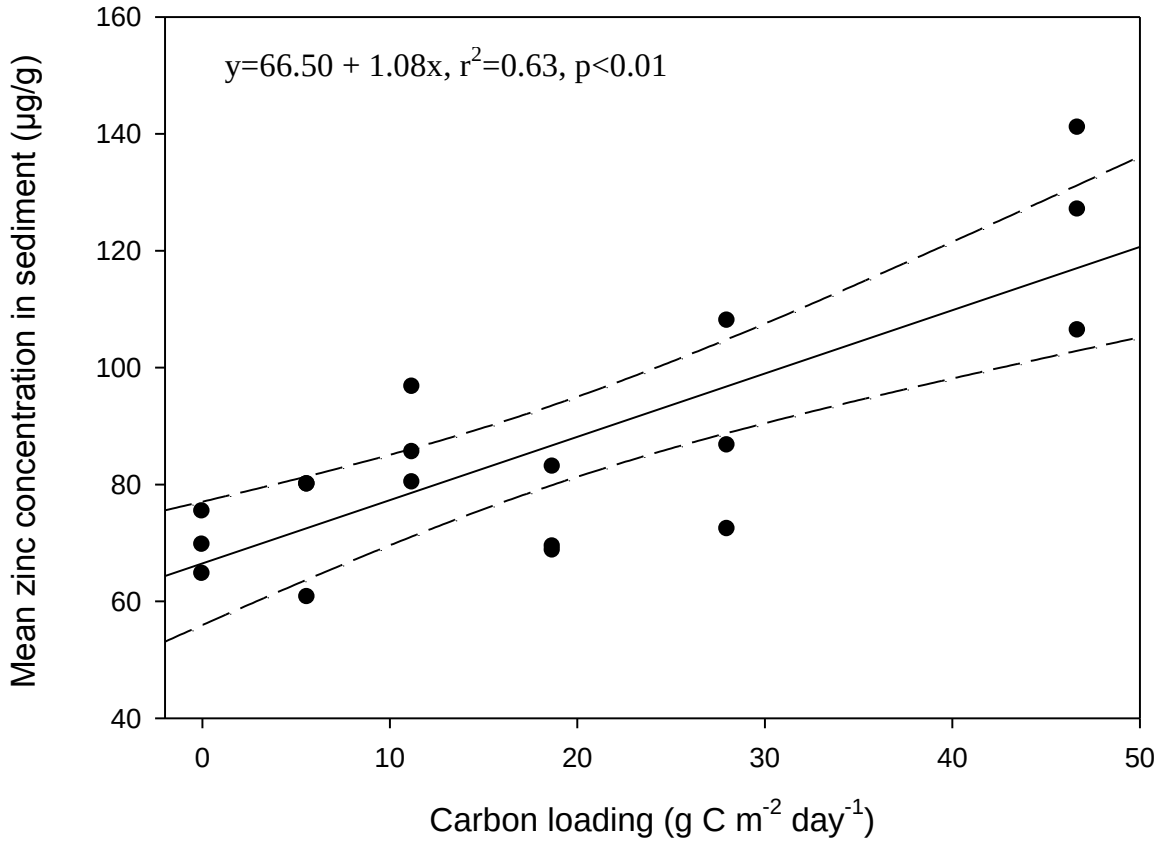

Figure 3.4. Concentration of zinc (µg/g) in the top 2 cm of sediment after a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

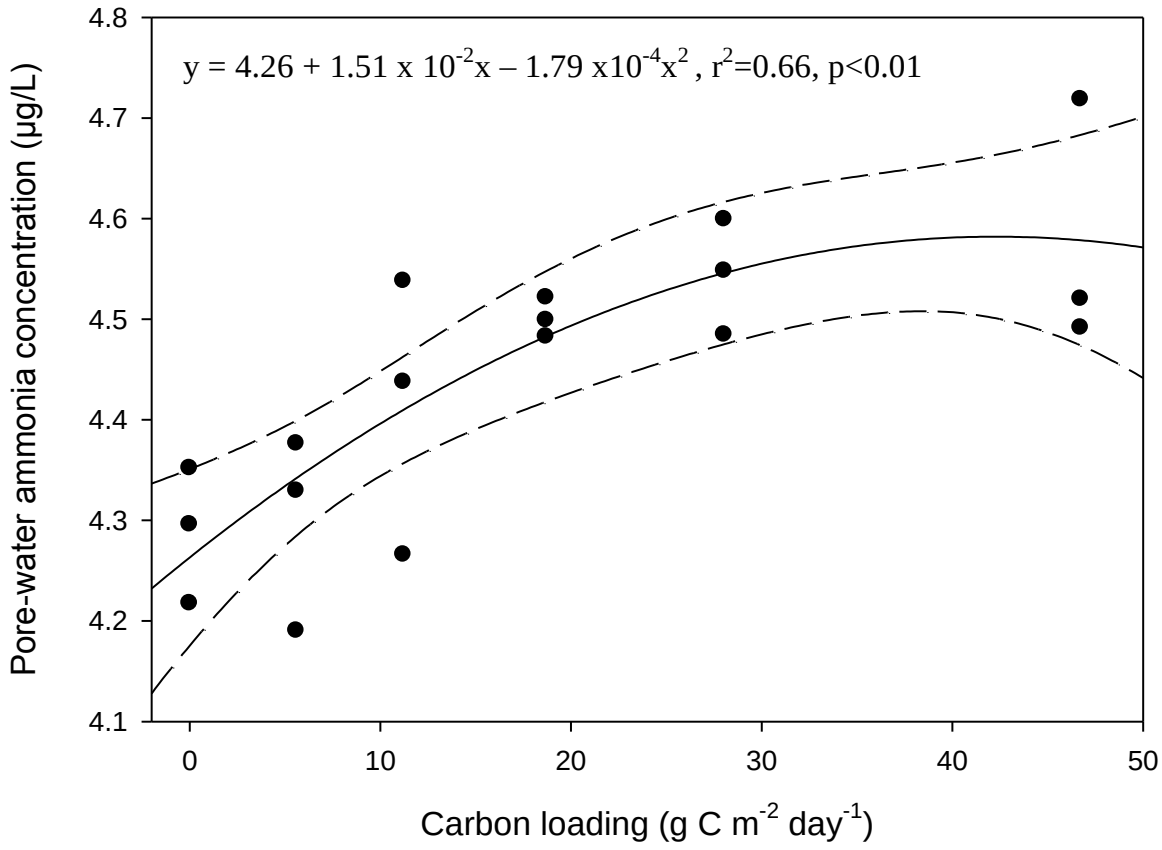

Figure 3.5. Pore-water ammonia (µg/L) in the top 2 cm of sediment after a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

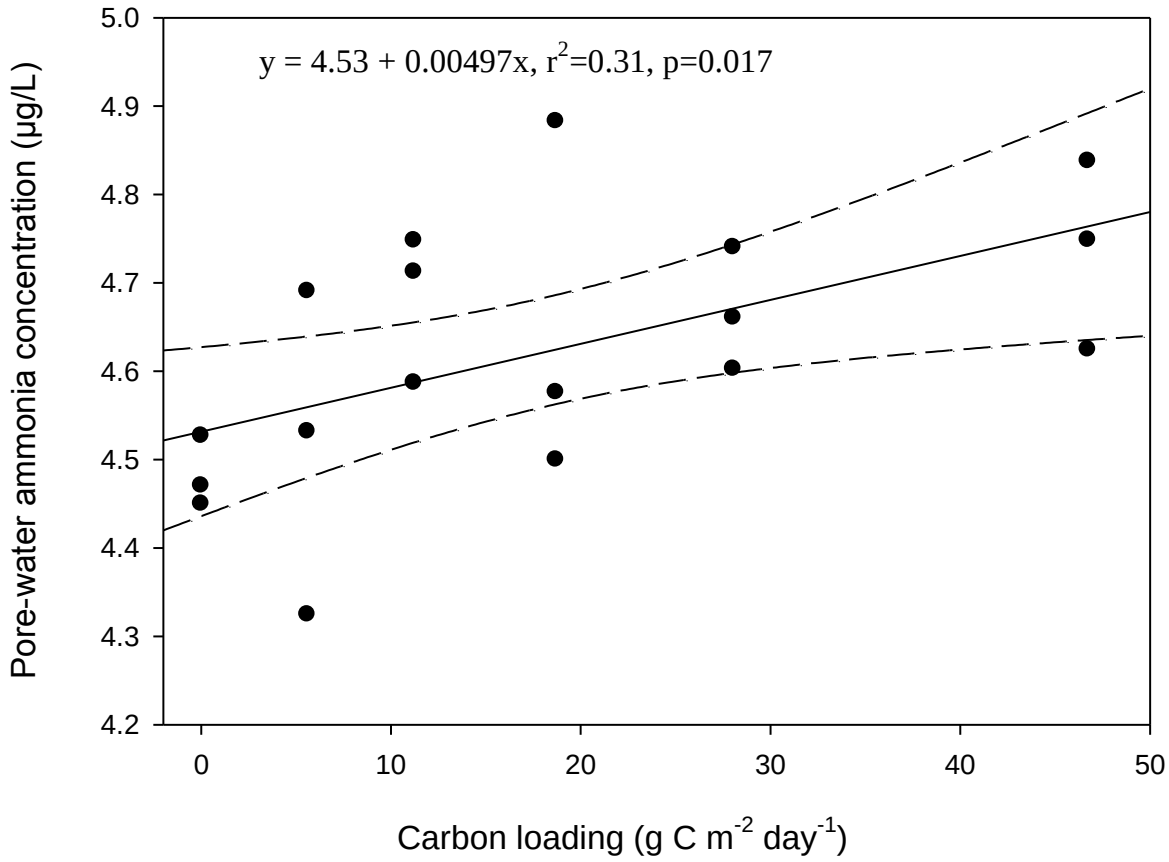

Figure 3.6. Pore-water ammonia concentration (µg/L) in the 2-4 cm layer of sediment after a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

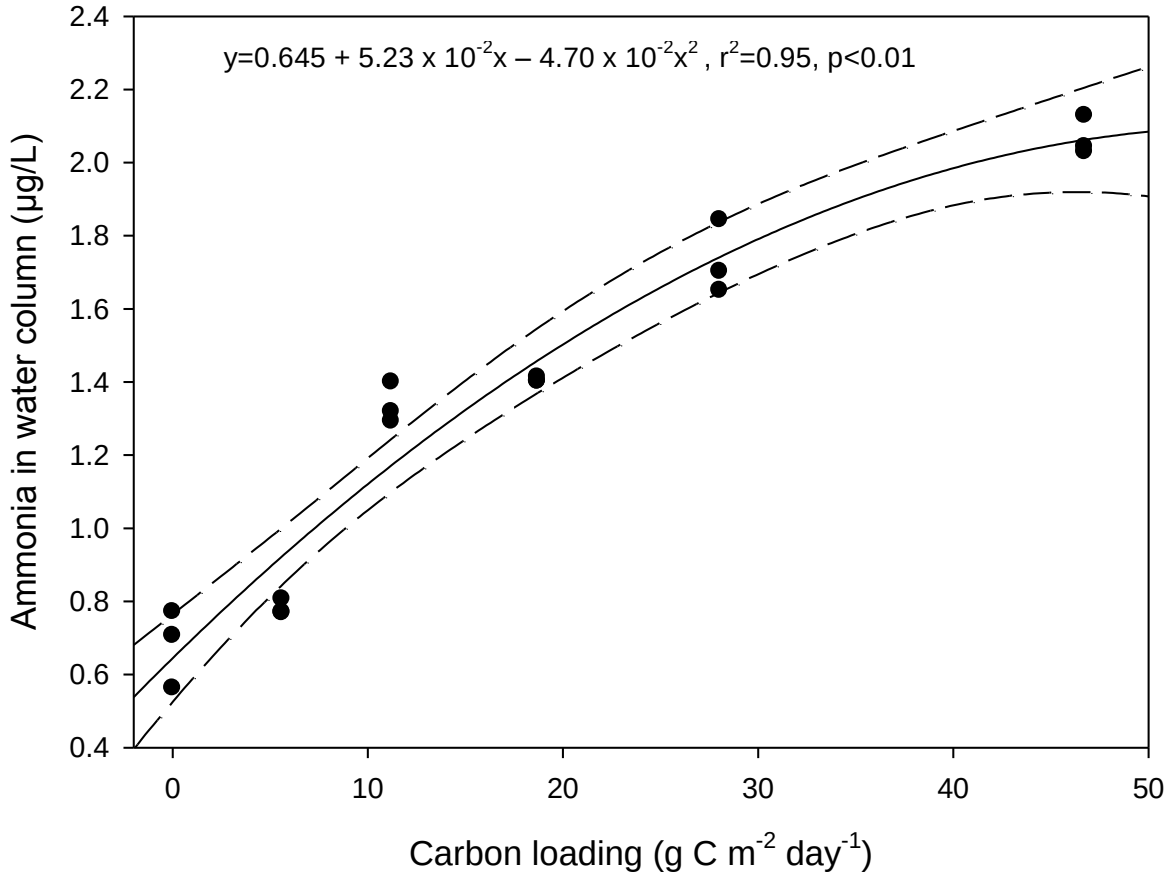

Figure 3.7. Ammonia (µg/L) in the water column with carbon loading on Day 21 of a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

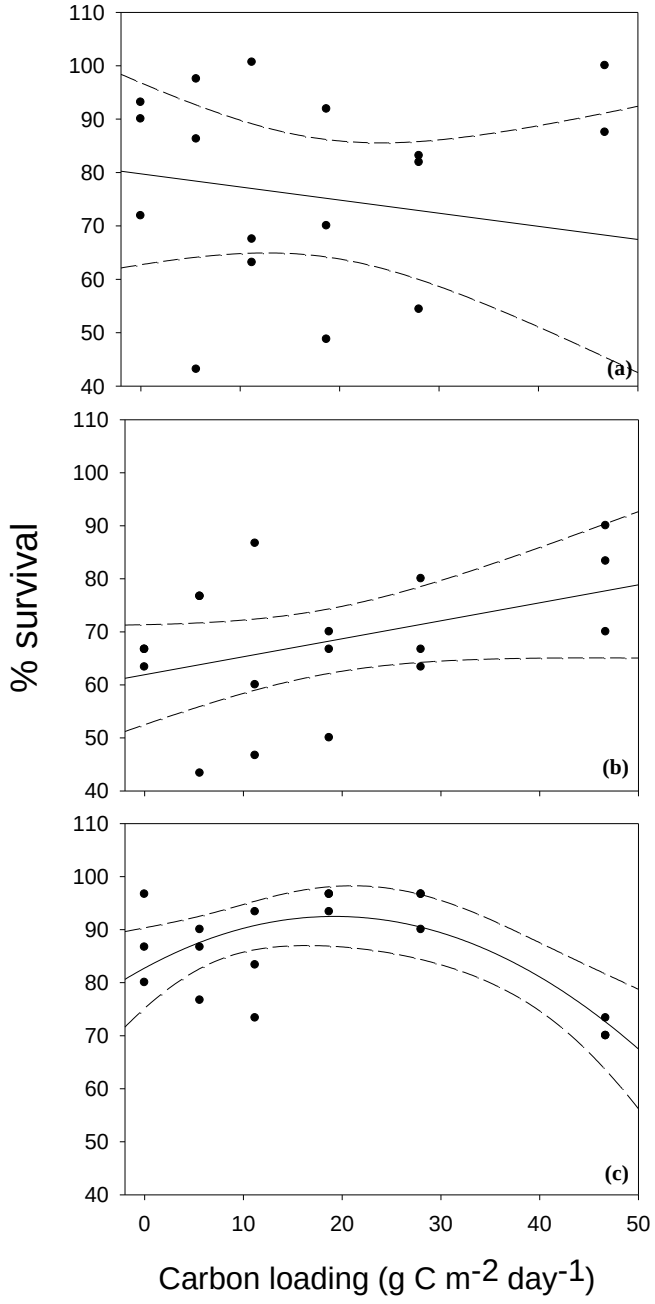

Figure 3.8. Invertebrate survival for *T. tubifex* (a), *C. riparius* (b), and *S. simile* (c) after a 21-day multispecies microcosm experiment, n=3. Regression lines are solid, 95% confidence limits are dashed lines. Regression equations are: (a) $y = 79.80 – 0.246x$, $r^2 = 0.03$, $p=0.472$; (b) $y = 61.90 + 0.339x$, $r^2=0.17$, $p=0.085$; (c) $y = 82.76 + 1.013x - 2.63 \times 10^{-2}x^2$, $r^2 = 0.50$, $p=0.006$.

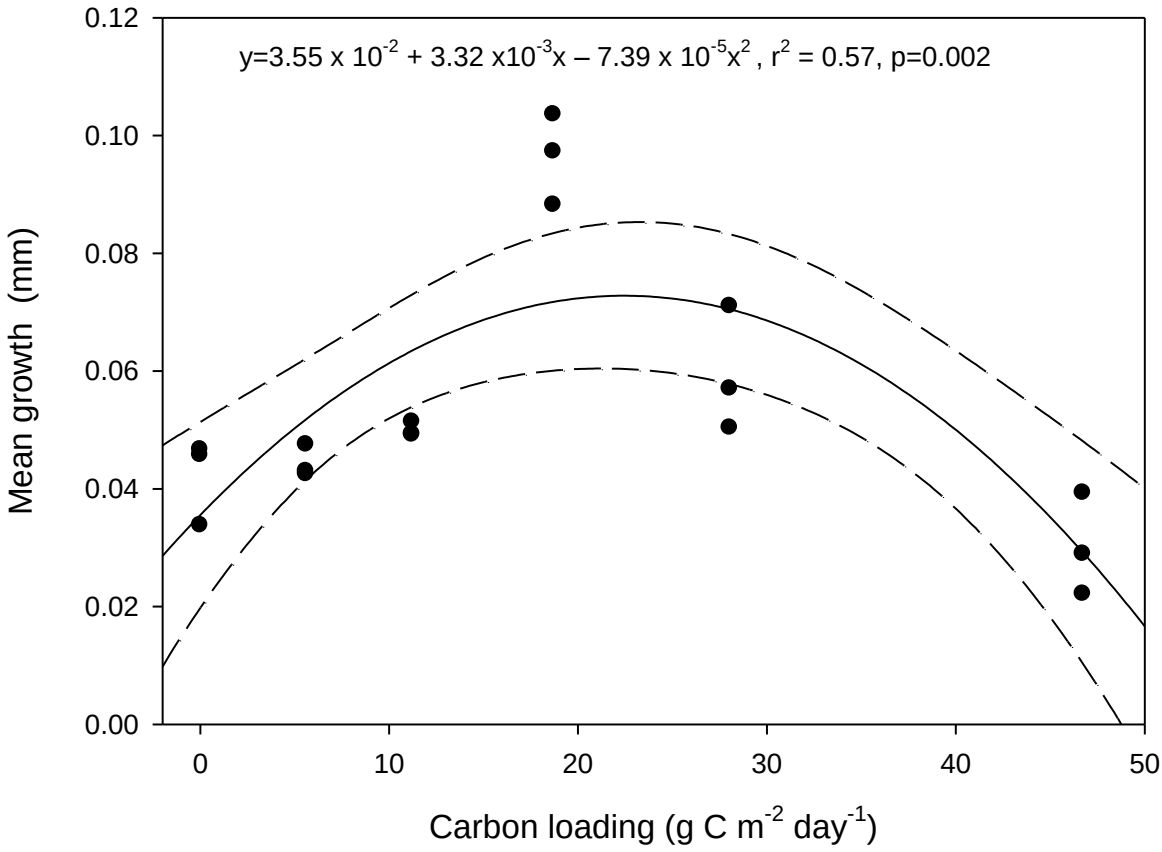

Figure 3.9**.** Growth measured as increase in length (mm) of *S. simile* with carbon loading during a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dotted lines.

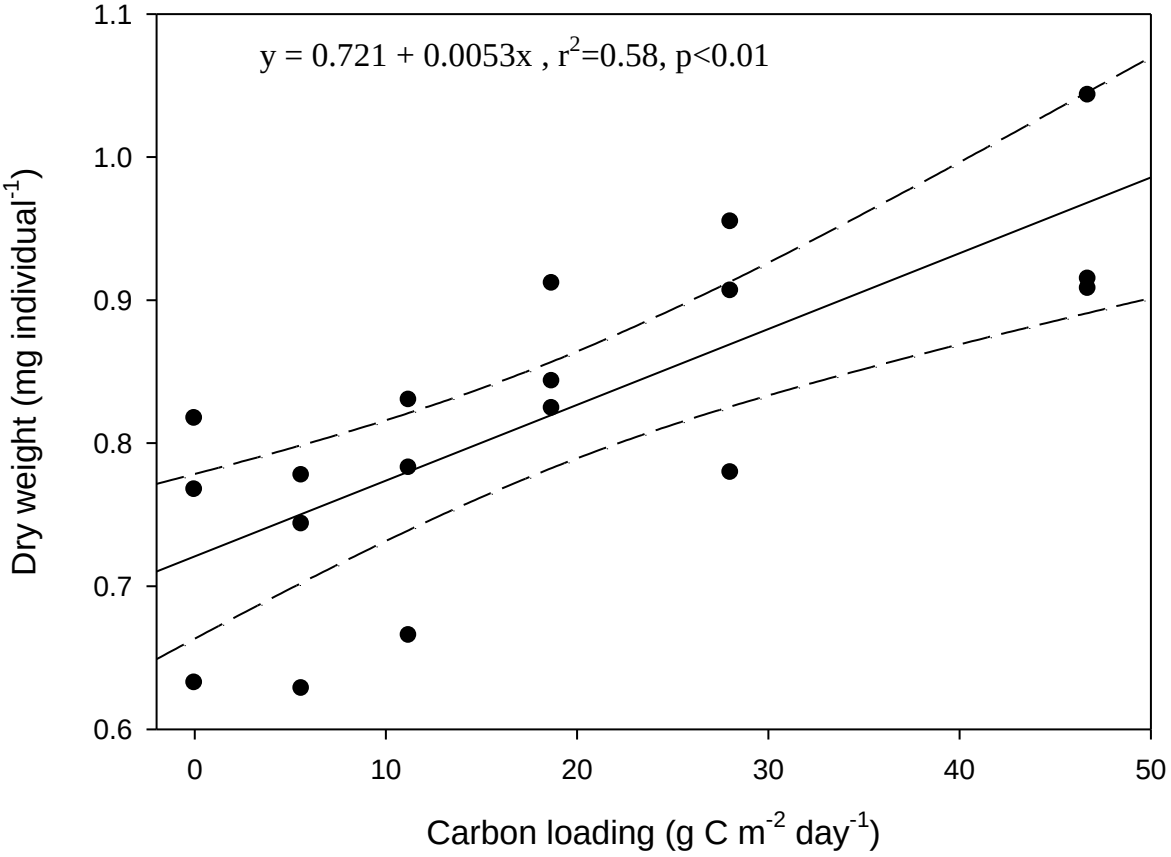

Figure 3.10. Mean *C. riparius* growth, measured as increase in dry weight (mg) with carbon loading during a 21-day multispecies microcosm experiment. Regression lines are solid, 95% confidence limits are dashed lines.

Chapter IV: Summary

4.1. Introduction

In 2010, marine and freshwater aquaculture supplied just over half of the world's food fish supply (FAO 2010). The demand for food fish is expected to continue rise as the world's population increases, but over 80% of commercial capture fisheries are already overexploited (FAO 2010). The difference between demand and production from capture fisheries will be met by aquaculture (FAO 2010). While production from capture fisheries stopped increasing in the mid-1980s, the aquaculture sector has continued to grow at an average of 8.3% per year worldwide from 1970 to 2008 (FAO 2010). Freshwater fishes represented the largest proportion of worldwide aquaculture production at 28.8 million tonnes (FAO 2010).

With our vast freshwater resources, the potential for growth of the freshwater aquaculture industry in Canada is considerable, but expansion of the highly-regulated industry has been limited (Podemski and Blanchfield 2006). Making informed decisions regarding new licence applications, or expansion plans for existing farms has been difficult when the effects of aquaculture waste loading on freshwater ecosystems were largely unknown. Research into the impacts of freshwater aquaculture in Canada has greatly increased in recent years, *e.g.,* Rooney (2006), Bristow (2006), Hille (2008), Bristow *et al.* (2008), Kullman *et al.* (2007, 2009), Rooney and Podemski (2009, 2010), Blanchfield *et al.* (2009), Findlay *et al*. (2009), Azevedo *et al.* (2011), Paterson *et al.* (2010, 2011), and Wellman (2011).

The greatest impact of freshwater aquaculture is the release of nutrient-rich, untreated solid waste directly into the receiving environment (Beveridge 1984; Elberizon and Kelly 1998). The highest rates of waste sedimentation rates are typically observed directly beneath marine and freshwater cage farms and decline rapidly with increasing distance from the farm (Giles 2008). Freshwater lakes generally have much lower current speeds than in the ocean (Nixon 1988); therefore, deposition of farm waste occurs in closer proximity to the operation (Elberizon and Kelly 1998). The spread of waste material is also influenced by the settling velocity of the faecal pellets (Cromey *et al.* 2002; Moccia *et al.* 2007). The settling velocity of salmonid faecal pellets is higher in freshwater, 5.2 $cm \cdot sec^{-1}$ (Moccia *et al.*2007), than marine, 3.2 $cm \cdot sec^{-1}$ (Cromey *et al.* 2002), which will also limit the spatial distribution of waste.

Accumulated farm wastes may have a significant impact on the sediment beneath and surrounding fish farm operations. Sediments beneath freshwater net-pens are typically enriched in organic carbon, TN and TP (Rooney and Podemski 2010; Podemski and Blanchfield 2006; Cornel and Whoriskey 1993), as well as zinc and copper (Rooney and Podemski 2010). Depleted oxygen, reduced pH, and the accumulation of ammonia are commonly reported to result from the breakdown of organic wastes (Enell and Lof 1983; Troell and Berg 1997; Brooks and Mahnken 2003; Veenstra *et al.* 2003). Sediment texture can also be altered by aquaculture waste. Droppo *et al.* (2007) evaluated the stability of sediment containing aquaculture waste from a discontinued cage farm near Great La Cloche Island in the North Channel of Lake Huron. This material was low density, had an increased water content and significant microbial activity compared with sediment from a reference site (Droppo *et al.* 2007). Mortality of *S. simile* in placed in

sediment from directly under an experimental cage farm during an *in situ* bioassay was attributed to the low bulk density of the sediment Kullman *et al.* (2007).

Effects of fish farm wastes on the benthic community are similar to those associated with other forms of organic loading, such as pulp mill effluent (Pearson 1972; Gray *et al.* 1979). These responses usually include a decrease in richness due to the loss of sensitive taxa, and an increase in the abundance and dominance of organisms resistant to sedimentation and reduced oxygen concentration in the sediment (Podemski and Blanchfield 2006). Pearson-Rosenberg (1978) described a model for the changes in benthic community richness, biomass and abundance that occur along a gradient in organic enrichment. This model has been used in marine and freshwater studies to describe benthic communities near net-pen farms (*e.g.,* Gowen and Bradbury 1987; Weston 1990; Karakassis *et al.* 1999, 2000; Brooks and Mahnken 2003; Kalantzi and Karakassis 2006; Kutti *et al.* 2007; Rooney and Podemski 2009). The first prediction of Pearson and Rosenberg's (1978) model is that along a gradient in increasing organic enrichment, invertebrate density will increase to a maximum. If loading continues to increase, abundance will abruptly decline (Pearson and Rosenberg 1978). Pearson and Rosenberg's (1978) second prediction is that species richness will exhibit a peak and then a decline along the gradient in organic enrichment. The peak occurs at a lower level of enrichment than the maximum in density and is in the ecotone area where species characteristic of both enriched and pristine environments co-occur. The third prediction is that total biomass will exhibit two peaks along the organic enrichment gradient. The first peak will occur in the same region as the peak in species richness, and results from the stimulatory effects of low levels of organic enrichment on individual growth rates. The

second peak will occur in the same region as the peak in species abundance, and will result from the high density of opportunists (Pearson and Rosenberg 1978). At an experimental freshwater cage farm, Rooney and Podemski (2009) reported that initial invertebrate responses closely resembled the Pearson-Rosenberg (1978) model's predictions at high levels of loading, but peaks at moderate levels of loading were never observed. Since the study was completed shortly after the establishment of the experimental farm, dense populations of enrichment specialists may not have had time to develop (Rooney and Podemski 2009). In addition, due to extremely localized waste deposition from the farm there was a steep gradient between highly affected and unaffected invertebrate communities (Rooney and Podemski 2009). This transition occurred between sampling sites at 5 and 10 m from the cage edge, so peaks at moderate levels of loading would not have been detected as they likely occurred between sampling locations (Rooney and Podemski 2009) At a marine cage farm in the Western Mediterranean, Sanz-Lázaro *et al*. (2011) similarly did not observe peaks in abundance and species richness at intermediate levels of organic loading. The authors suggested that this was due to differences in the physico-chemical features of the Mediterranean, such as higher temperature and carbonate sediments, as compared with areas culturing salmon where most of the marine studies have been performed (Sanz-Lázaro *et al*. 2011). However, Sanz-Lázaro *et al*. (2011) had only four stations for sedimentation traps and sediment sampling: directly under the cage and at 20, 120 and 600 m away. Similar to Rooney and Podemski (2009), the number of sampling stations along the enrichment gradient may have been too limited to detect intermediate peaks in abundance and species richness.

Waste loading, and its subsequent effects on sediment chemistry, have been used at marine farms to predict effects on the benthic community by identifying thresholds of effect (*e.g.,* Hargrave *et al*. 2008). Hargrave *et al*. (2008) developed a nonogram to describe the interrelated changes in redox, pH, and measures of dissolved sulfides and benthic invertebrate community characteristics that occur in response to increasing carbon sedimentation in marine environments. The authors were able to identify thresholds of organic carbon deposition rates that resulted in characteristic changes in the state of the benthic environment (Hargrave *et al*. 2008). The identification of thresholds of effect based on carbon sedimentation is important for developing regulations to use in conjunction with depositional models. Modeling tools (*e.g.,* DEPOMOD) have been applied in a regulatory context in Scotland (Cromey *et al.* 2002; Mayor *et al.* 2010). There are no recognized thresholds of effect in the literature for freshwater aquaculture. This is the first study to propose a carbon deposition threshold, based on observed effects of aquaculture waste loading on sediment chemistry and the benthic community at freshwater open net-pen farms.

4.2 Objectives and main findings of research

Understanding the relationship between sedimentation rates and potential effects on the benthic community is important for the purpose of setting meaningful guidelines for freshwater net-pen aquaculture. Modeling tools are available for the marine environment (*e.g.,* DEPOMOD) to predict the spatial distribution and intensity of effects (Cromey *et al.* 2002). An equivalent formal process for the management of the freshwater cage aquaculture industry in Ontario is not currently available (MOE 2008). The objective of this study was to characterize the gradient in waste exposure around freshwater farms and

the resulting effects on sediment chemistry and benthic invertebrate populations. Thresholds of effect identified in this study could then be applied within a regulatory framework to predict impacts beneath and surrounding other farms. There were two studies completed for this thesis: one was field-based, sampling sedimentation rates, sediment chemistry and the benthic community at three *O. mykiss* farms in Ontario; and the second, a laboratory multispecies microcosm experiment to study invertebrate growth and survival at various intensities of waste loading.

1) Effect of carbon loading from aquaculture waste on benthic invertebrates and sediment chemistry at three freshwater net-pen farms

The field survey of Ontario net-pen farms (Chapter II) was designed to investigate the following questions:

1. What is the rate and spatial distribution of waste sedimentation in the receiving environment resulting from freshwater net-pen rainbow trout farms?
2. Is there an elevation in carbon, nutrients and metal concentrations in sediment surrounding the farms? How far from the farms are effects on sediment chemistry observed?
3. Do observations of the benthic invertebrate community along the gradient in waste loading from the three farms agree with predictions of the Pearson-Rosenberg (1978) model?
    a. Does invertebrate abundance follow the expected polynomial response along the gradient in organic enrichment?
    b. Is there a peak in taxonomic richness and does it occur at the same point along the deposition gradient as a peak in biomass?

c. Are there two peaks in invertebrate biomass, and do these peaks co-occur with the peak in opportunist abundance and the peak in richness?

In general, the rates of carbon, nitrogen and phosphorus sedimentation were highest beneath the cages and declined quickly with increasing distance. The dispersal of waste was spatially limited and the decline in loading with distance from the farm was exponential. At the commercial farms, carbon deposition was reduced by 90 to 95 % at 30 m from the cages at CF1, and 88 to 92 % at CF2. At 5 m from the experimental net-pen farm, deposition was reduced by 96.7% in 2006, and 97.8% in 2007. Sedimentation rates returned to background levels only 5 m from CF1 and within 15 m from CF2 and the experimental farm. An exponential decline in particulate waste sedimentation with increasing distance from marine farms has been reported by many authors (*e.g.*, Weston 1990; Troell and Berg 1997; Holmer *et al.* 2007; Kutti *et al.* 2007; Rapp *et al.* 2007). There is potential for greater dispersal at marine farms, especially at deep water sites and those that are well flushed by currents. Kutti *et al.* (2007) estimated that the majority of fish waste from a Norwegian marine farm situated over 230 m of water depth had settled within 250 m from the farm; however, some farm waste was transported 550 - 900 m from the operation (Kutti *et al.*2007). At relatively shallow farms with minimal current, such as those in sheltered freshwater lakes, the dispersal of waste material should be limited (Cromey *et al.* 2002).

Sediment carbon, nutrient and metal concentrations were highest beneath the cages and decreased exponentially with distance. Elevations in sediment nutrient and metal concentrations did not entirely follow the pattern in sedimentation. Sedimentation

rates were significantly elevated within 15 m of CF2 and the experimental cage farm, and within 5 m from CF1, compared with the reference sites. However, at the commercial farms significantly elevated sediment concentrations were observed further from the farms than detectable deposition, as far as 30 m from the nets.. The commercial farms had been in operation for over 25 years at the time of sampling, compared with only 4-5 production seasons at the commercial farm. Elevated sediment metal and nutrient concentrations at distances where the sedimentation rate was not significantly higher than background was likely the cumulative effect of many years of deposition of very small amounts of farm waste. In addition, variability in background sedimentation rate was higher at the commercial farms than at the experimental operation (Table 2.3, Chapter II). Therefore, the difference in sedimentation rates had to be larger at the commercial farms as compared with the experimental in order to be statistically significant and therefore elevated deposition would only have been detectable closer to the farm.

Invertebrate density, richness and biomass patterns along the sedimentation gradient from the commercial cage farms were similar to the model described by Pearson and Rosenberg (1978). As predicted, with increased waste loading there was first a peak in invertebrate density followed by an exponential decline. There was no azoic zone in the sediment under any of the cages described in this study; however, invertebrate density was reduced by >95%, compared to the reference sites. At both commercial farms, total invertebrate density peaked at carbon sedimentation rates between 1.0 and 2.0 g C m^{-2} day^{-1}, and then exponentially declined between 2.0 and 3.0 g C m^{-2} day^{-1} (Figures 2.6 - 2.7, Chapter II). The density of different benthic taxa peaked in different locations along the enrichment gradient; first an increase in Harpacticoida, then Nematoda, followed by a

peak in density of Tubificinae (Oligochaeta) that occurred at the peak in total invertebrate density. Pearson and Rosenberg (1978) predicted that the peak in invertebrate density along the organic enrichment gradient would be due to increased density of opportunists. Oligochaetes (*e.g.,* Tubificinae) are the most often cited enrichment opportunist in freshwaters receiving organic waste (Milbrink 1980), and therefore these results support the model.

The Pearson-Rosenberg (1978) model predicts that the peak in taxonomic richness will occur at a lower intensity of waste loading than the maximum peak in density, in an area where both tolerant and sensitive taxa co-occur. However, across the three farms there was much overlap in the carbon sedimentation rates where the peaks in total invertebrate density (1.0 - 2.0 g C m^{-2} day^{-1}) and species richness (1.72 and 2.01 g C m^{-2} day^{-1}) occurred. At these sedimentation rates, the communities contained both sensitive, (*e.g.*, Harpacticoida, Ostracoda) and tolerant taxa such as Oligochaeta, Nematoda and Chironomidae. The relatively coarse level of taxonomy that was used resulted in a low number of total taxa (5), and peaks in richness measured at a species level could therefore not be revealed. If lower-level identification had occurred, the predictions for richness in the Pearson-Rosenberg (1978) model may have been supported.

Pearson and Rosenberg (1978) predicted that total biomass will exhibit two peaks along a gradient in organic enrichment: the first resulting from increased individual size and occurring in the same region as the peak in richness, and the second peak in total biomass resulting from the peak in density of opportunists. Total biomass was not measured in this study, so it is unknown if these peaks were present. Mean individual

biomass was determined for Tubificinae, and two peaks were observed (Figure 2.8, Chapter II). The peak at 2.0 g C m^{-2} day^{-1} corresponded to the peak in richness; whereas, the second peak between 4.05 - 6.97 g C m^{-2} day^{-1} occurred at the carbon loading rate where Tubificinae dominated the community. The increase in mean individual biomass for deposit-feeding Tubificinae at the peak in richness was likely due to the increase in food quantity and quality. The second peak in individual size could have been the result of a shift in species composition to larger-bodied taxa, but family-level taxonomy requires that this remain a speculation.

At the experimental cage farm, Rooney and Podemski (2009) did not observe the peaks predicted by the Pearson-Rosenberg (1978) model at moderate levels of loading. The study was completed after only two production cycles (May – November) of the experimental farm (Rooney and Podemski 2009). Dense populations of enrichment specialists may not have had time to develop (Rooney and Podemski 2009). In the present study, peaks in biomass at moderate loading were only observed for Tubificinae oligochaetes in Lake Huron; this taxa was not collected in L375, at the Experimental Lakes Area (C. L. Podemski, unpublished data). In addition, there was a steep gradient between affected and unaffected invertebrate communities due to extremely localized waste deposition from the farm (Rooney and Podemski 2009). Using 2006 – 2007 sedimentation data, waste loading rates were likely 25.78 – 34.09 g C m^{-2} day^{-1} under the cage, compared with 1.64 – 2.85 g C m^{-2} day^{-1} only 1 m from the cage edge (Table 2.3, Chapter II). Rooney and Podemski (2009) were not able to observe the moderate peaks described in the Pearson-Rosenberg (1978) model due to the limited spread of waste

material and the resulting inability to place multiple sampling sites along this very steep gradient.

2) Effect of carbon loading from aquaculture waste on benthic invertebrates: a microcosm study

Observing invertebrate responses to a realistic gradient in waste exposure in an experimental setting allowed the measurement of population endpoints that could not be assessed in the field. Population and community level endpoints are linked, as community structure changes when population-level impacts are expressed (Attrill and Depledge 1997). The 21-day laboratory multispecies microcosm experiment (Chapter III) was designed to investigate the following questions:

1. What are the effects of a gradient in exposure to aquaculture waste on population parameters of three species of test organisms? Specifically:
    a. effects on survival of *Chironomus riparius, Tubifex tubifex, Sphaerium simile*;
    b. effects on growth, as measured by individual biomass of *T. tubifex,* as both size and individual biomass of *C. riparius,* and as size of *S. simile*; and
    c. effects on reproduction of *T. tubifex.*
2. How are sediment and water chemistry affected along an experimental gradient of exposure to aquaculture waste? More specifically, how does the gradient of waste exposure affect those variables that have been reported in the literature and observed in the field component of this research to change near fish farms:
    a. nutrient (N and P) and carbon content (as measured by %LOI);

b. the metals, zinc and copper;

c. pore-water ammonia; and

d. water column ammonia.

This study is the first to measure freshwater benthic invertebrate growth and survival when exposed to realistic aquaculture waste loading rates in laboratory microcosms. *Chironomus riparius, Tubifex tubifex and Sphaerium simile* were chosen for this study as representatives of taxa typical of profundal environments in the North Channel of Lake Huron and for their ease of culture in the laboratory. Invertebrate response to waste loading during the 21-day bioassay was taxon-specific. Two of the species, *C. riparius and T. tubifex,* were representative of taxa that were collected in high densities near the commercial cage farms. Additionally, Tubificinae oligochaetes and Chironomini larvae are the most often cited enrichment opportunists in freshwater (*e.g.*, Guo and Li 2003; Doughty and McPhail 1995). Therefore, it was not surprising that survival of *C. riparius and T. tubifex* during the 21-day experiment was not adversely affected by waste loading. Growth of *C. riparius*, as measured by increase in dry weight, was positively and linearly related to carbon loading. The mean individual dry weight of *T. tubifex* did not increase with increasing carbon loading; however, worms exposed to all rates of waste loading weighed more than those in the control group. Both taxa were able to utilize the waste as a food subsidy. Sphaeriidae was not collected near the cage farms in this study, though this taxa has been observed in proximity to aquaculture operations in Lake Huron (Nalepa *et al*. 2007). This species represented taxa more sensitive to organic loading. Bivalves tolerate transient, unfavorable changes in conditions by closing their shell valves (Kramer *et al.*1989). However, valve closure leads to a gradual decline of the

oxygen in the mantle cavity water, cessation of feeding, and the accumulation of excretory products (Heinonen *et al*. 1997). Heinonen *et al*. (1997) observed that low oxygen concentrations of 2.8 - 4.1 mg O_2 L^{-1} caused valve closure in *Sphaerium corneum* Linnaeus. High rates of organic loading can result in reduced oxygen concentrations in the sediment (Loch *et al.* 1996; Findlay and Watling 1997; Kirkagac *et al.* 2004), which may be detrimental to the survival and growth of sphaeriids. In addition, when exposed to high rates of sedimentation these clams may be smothered by waste material, hampering the ability of these organisms to obtain oxygen (Gilmore 1917). During the microcosm experiment, survival and growth of *S. simile* was dependent on carbon loading. Survival and growth of *S. simile* peaked at 11.2 g C m^{-2} day^{-1} and 18.7 g C m^{-2} day^{-1}, respectively, followed by a decline. This suggests that a moderate amount of carbon loading was beneficial to these clams, but higher loading was detrimental. Microcosms subjected to 28.0 and 46.7 g C m^{-2} day^{-1} had an extremely flocculent surface. This likely made burrowing and feeding difficult for these suspension and deposit feeders (Hornbach *et al*. 1984). Clams exposed to 46.7 g C m^{-2} day^{-1} had significantly higher mortality than all other treatments. According to the Pearson-Rosenberg (1978) model, total invertebrate biomass should peak at moderate levels of waste loading due to the presence of both tolerant and sensitive taxa, and the stimulatory effect of the increased quantity of food. Although total invertebrate biomass was not determined after the microcosm experiment, there was increased growth (by dry weight) for *C.riparius* and *T. tubifex*, as well as *S. simile* (by length) at moderate levels of waste loading. It is likely that Pearson-Rosenberg (1978)'s prediction regarding invertebrate biomass was supported in this experiment.

Sediment chemistry variables (*e.g.*, carbon, nutrients, metals) were affected by the addition of aquaculture waste; however, the concentrations were low compared to those measured in the field. Sediment carbon and phosphorus concentration increased with elevated waste loading, while sediment nitrogen concentration was not linked to the intensity of waste loading. This was likely due to fish waste collection methods at the land-based farm. The fish waste settled in the bottom of the tanks for up to 3 – 4 days prior to collection. As the fish waste began to decompose, proteins in faecal matter would have been converted to ammonia and subsequently lost from the particulate fraction to the water column (McCahon *et al.*1991; Hickey *et al.* 1999). Sediment zinc concentration was correlated with carbon loading, whereas copper concentration was not. Zinc is a common fish feed supplement and so the sediment concentration would be expected to rise with increased waste loading, whereas elevated copper is most often attributed to net coatings and therefore not associated with land-based aquaculture waste.

Unlike at the cage farms, sediment phosphorus and copper concentrations by the end of the bioassay did not exceed the Ontario provincial sediment guidelines for Lowest Effects Level (LEL). Sediment zinc concentration only exceeded the LEL in the microcosms exposed to the highest rate of waste application, 46.7 g C m^{-2} day^{-1} (Table 3.2, Chapter III). At this loading rate, the survival and growth of *S. simile* was reduced, and the increase in dry weight of *C. riparius* over the 21-day experiment was greatest (Figure 3.10; Chapter III). *Chironomus riparius* larvae may have been more tolerant of increased zinc sediment concentrations as compared with *S. simile.* Malaj *et al*. (2012) ranked macroinvertebrate species according to their physiological sensitivity to heavy metals. Bivalves were more sensitive to zinc than *Chironomus riparius* using toxicity

data from acute laboratory assays (Malaj *et al*. 2012). The reduced potential toxicity of sediments in the microcosm experiment and the shorter duration of exposure in the microcosm versus field may explain why invertebrates in the bioassay were able to tolerate higher levels of waste loading without adverse effects. Total invertebrate density began to exponentially decline between 2.0 – 3.0 g C m^{-2} day^{-1} at commercial fish farms in Ontario; whereas, survival in the bioassay was not reduced until the test organisms were exposed to 46.7 g C m^{-2} day^{-1}.

Out of all the chemical and biological variables measured during the bioassay, water column and pore-water ammonia reacted most quickly to the addition of farm waste. Pore-water ammonia is a biologically significant variable due to its toxicity to organisms living within the sediment (Rooney and Podemski 2010). However, by day 21 of the bioassay, the water column and pore-water ammonia concentrations only reached a maximum of 0.002 and 0.005 mg L^{-1}, respectively. Significant growth reductions are likely to result in most aquatic animals exposed to 0.05 mg NH_3 L^{-1} (Fries and Bowles 2002), so ammonia toxicity was likely not a factor affecting invertebrate survival and growth in this bioassay.

4.3. Comparing the microcosm experiment and field research

There were similar trends in sediment chemistry from the laboratory experiment and at the Ontario cage farms. Sediment total carbon (TC), total phosphorus (TP) and zinc concentration were elevated in the sediment with increased carbon loading. Sediment copper concentration increased with waste deposition at the farms, but was not related to carbon loading after the 21- day bioassay. This was expected, as the fish waste

came from a land-based facility that did not use copper-coated nets to contain the fish. Sediment concentrations of TC, TN, TP and zinc peaked at the highest waste loading rates in the field and the laboratory, with the field sediment concentrations being an order of magnitude higher in most cases (Table 2.5, Chapter II, Table 3.2, Chapter III).

It is not surprising that sediment concentrations in the bioassay did not attain the levels observed in field sediments because sediment concentrations at the three farms reflected conditions that had developed over many years of exposure to aquaculture waste. In the microcosms, pore-water ammonia, as well as sediment carbon, nutrient and zinc concentrations would be more similar to the results after the first few weeks at a newly-established farm (*e.g.*, Rooney 2006). Rooney and Podemski (2010) reported that within one month of farm activity at the experimental farm, pore-water ammonia concentrations were significantly elevated in sediment beneath the farm. Likewise, during the bioassay, relationship between carbon loading and the concentration of pore-water ammonia was significant after 21 days (Figures 3.5 - 3.7, Chapter III). Nutrient concentrations were also significantly elevated in sediment beneath the experimental farm within one month of production, compared to reference sites (Rooney 2006). Based on mean sedimentation rates measured in 2006-2007, these sites were exposed to between 25.78 – 34.09 g C m^{-2} day^{-1} and 0.29 – 0.40 g C m^{-2} day^{-1} at the under cage and 50 m sites, respectively (Table 2.3, Chapter II).The microcosms were exposed to a similar, but slightly larger range of waste loading rates (0.0 - 46.7 g C m^{-2} day^{-1}) during the experiment (Table 3.1, Chapter III).

The rate of carbon loading at which invertebrate density began to decrease in the field was lower than in the laboratory experiment. This was due to longer-term exposure

to waste deposition, and elevated sediment carbon, nutrient and metal concentrations in the field. Reduced survival due to the constant presence of a stressor over time may lead to the exclusion of some taxa. During the laboratory experiment, survival rates of *S. simile* were reduced at waste loading rates greater than 11.2 g C m^{-2} day^{-1}, *C. riparius* survival increased with waste loading, and *T. tubifex* survival was unaffected (Figure 3.8, Chapter III). In the field, the decline of benthic abundance and richness occurred between 2.0 - 3.0 g C m^{-2} day^{-1} (Figures 2.6-2.7, Chapter III). Although we could not measure survival in the field, carbon loading rates of approximately 11.2 g C m^{-2} day^{-1} were observed at the edge of the cage, where invertebrate density was greatly reduced compared to reference sites (Figures 2.6 and 2.7, Chapter II). At these sites, Tubificinae dominated the community, though some Chironomidae were present; all other taxa had been excluded. Rooney and Podemski (2009) collected sphaeriid clams at the experimental cage farm prior to operation, but after two years of production, sphaeriids had been excluded from within 5 m of the cage edge. Based on loading rates measured in 2006-2007 (Table 2.3, Chapter II) these sites would have been receiving waste loading rates exceeding 11.2 g C m^{-2} day^{-1}.

There is field and lab evidence that benthic invertebrates utilize aquaculture waste as a food source (Kullman *et al.* 2007; Wellman 2011). While *T. tubifex* growth was not directly related to the increase in carbon loading during the bioassay, the worms exposed to fish waste ≥5.60 g C m^{-2} day^{-1} were significantly heavier that those in the control group. In the field, Tubificinae biomass peaked at moderate loading rates between 4.05 and 6.97 g C m^{-2} day^{-1}. Tubificinae are the most often cited enrichment opportunist in freshwater receiving organic waste (Dobrowolski 1987; Doughty and McPhail 1995; Guo

and Li 2003; Clerk *et al.* 2004; Kirkagac *et al.* 2004). These deposit-feeding worms were likely able to use fish waste as a food source, demonstrated by the increase in mean individual dry weight. During the microcosm bioassay, mean *C. riparius* larval biomass increased when exposed to higher rates of waste deposition. *Chironomus riparius* larvae are often collected in organically enriched waters, and will feed on detritus that has been deposited on the sediment (Charles *et al.* 2004). We would expect to see increased biomass of larvae close to the cage operations. During the bioassay, *S. simile* showed increased growth when exposed to 18.7 g C m^{-2} day^{-1}, compared with clams exposed to the other sedimentation rates (Figure 3.9, Chapter III). Compared with the peak in field-collected Tubificinae biomass (4.05 - 6.97 g C m^{-2} day^{-1}), the loading rate at which a peak in *S. simile* growth was observed was higher and this was unexpected. These deposit-feeding clams were likely able to withstand higher waste loading rates in the microcosms because the nutrient and metal concentrations in the sediment did not exceed LEL or SEL thresholds. Similar results were found by Kullman *et al.* (2007) who reported increased growth and reproduction of *S. simile* exposed to sediment from 1 m from the cage during a six-week *in situ* bioassay. In the microcosm experiment, there was no difference in *S. simile* embryo counts after 21 days of exposure, though this may have been too short a time frame for significant differences to be observed.

Ecological thresholds are points or zones at which relatively rapid change occurs from one "ecological condition" to another (Huggett 2005; Bennett and Radford 2003). The cause of this change is due to a change in one or more key factors (Huggett 2005). In this study, higher waste loading rates led to increased sediment nutrient and metal concentrations, as well as decreased oxygen availability. These changes to the habitat

brought about alterations in the invertebrate community. Based on the results described in this study, the proposed threshold of effect on freshwater benthos is 2.0 - 3.0 g C m^{-2} day^{-1}. Above this deposition rate, adverse effects on freshwater benthic invertebrate abundance and richness rapidly increase. This intensity of carbon sedimentation was observed within 5 m from CF1, 10 -15 m from CF2, and at the edge of the experimental cage farm. At marine farms, thresholds of effect for waste loading from aquaculture have previously been reported. Sedimentation rates >1 g C m^{-2} day^{-1} resulted in the formation of hypoxic sediments around salmon farms (Hargrave 1994). Findlay and Watling (1997) identified a similar threshold (1–5 g C m^{-2} day^{-1}) close to salmon pens in coastal Maine where the abundance of macrofauna was reduced by up to 94.9% due to sediment enrichment. Thresholds of effect on benthos based on rates of sedimentation are important for developing regulations to use in conjunction with depositional models. Modeling tools are available (*e.g.*, DEPOMOD) for marine farms; however, Ontario does not have an equivalent process for the management of the freshwater cage aquaculture industry. Identification of a carbon deposition threshold for freshwater will be an important tool that regulators can use in the future for reviewing applications for new sites or site modifications.

4.4. Limitations of research and recommendations for future work

Although this study was the first to determine sedimentation rates at a variety of distances from commercial open net-pen farms in Ontario, there are some limitations in the data. Sedimentation rates at the commercial farms were only measured two or three weeks per year during the entire open water season. Variation in waste sedimentation is related mainly to feed input and also the variation in natural sedimentation (*e.g.*, surface

runoff, erosion). Monthly sampling of sedimentation rates would provide a better estimation of mean and maximum loading. Sedimentation traps were located up to a maximum of 100 m from the commercial operations, but there were no traps located beyond that for comparison. The mean carbon loading at 100 m from CF2 (2.01 g C m^{-2} day^{-1}) was higher than 100 m from CF1 in 2006 (1.72 g C m^{-2} day^{-1}) and 2007 (0.88 g C m^{-2} day^{-1}). These commercial farms are in two different types of water bodies (*i.e.*, open channel and sheltered bay), and were measured during two different years. Having distant reference stations in multiple locations would have allowed the characterization of natural sedimentation rates enabling a better comparison between the two farm sites. In addition, a more sturdy design is recommended for deploying sedimentation traps in Lake Huron, as the anchoring systems were often not robust enough to survive rough water conditions which limited the collection of sedimentation data. The sampling transects were located in line with the dominant water current direction as identified by the farm operators. Measuring the actual current speed and direction of flow at these commercial farms using a current meter would have been useful to describe the horizontal transport of waste loading. Current velocity, along with water depth, are critical factors determining patterns of sedimentation around cage sites (Cromey *et al.* 2002). Instead of just doing one transect from each farm, multiple transects of sediment traps should be used to collect sedimentation, and the associated sediment chemistry and benthic community data. Since the sedimentation rate at each sampling location would be known, data from multiple transects could be compiled to increase sample size. In addition, waste loading is likely not symmetrical around the perimeter of the farm so multiple transects are useful to describe the spatial scale of deposition. Pore-water ammonia concentrations were not

measured at the commercial fish farms, but they could be an excellent indicator of the decomposition of farm waste. Rooney and Podemski (2010) recommended the measurement of pore-water ammonia be included in freshwater aquaculture monitoring programs.

In order to extrapolate the results of a bioassay to benthic communities, field surveys must be conducted to have some idea of the intensity of the disturbance. Laboratory bioassays may overestimate or underestimate the response seen in the field by not testing the appropriate intensity of the stressor (Ireland and Ho 2005). In 2006, during the design of the microcosm experiment, sedimentation rates were assessed from CF1 to ensure that realistic loading rates were used. A range of loading rates was chosen (0.0 - 46.7 g C m^{-2} day^{-1}) that was slightly higher than what we observed in 2006, but would represent the loading observed at the commercial and experiment open net-pen farms. In the field at the commercial fish farms, we found that total invertebrate density began to decline at a carbon loading rate between 2.0 and 3.0 g C m^{-2} day^{-1} and that very few invertebrates were collected at locations exposed to loading over 25.0 g C m^{-2} day^{-1}. If this experiment were to be run again, lower loading rates between 0.0 and 25.0 g C m^{-2} day^{-1} should be tested to focus attention on the area of the exposure gradient with the greatest response.

The incorporation of field and microcosm research leads to stronger cause-effect linkages between exposure to a stressor (*i.e.*, aquaculture waste) and associated effects on the benthic community (Culp *et al.* 2000). However, the failure of the bioassay to result in sediment concentrations reflective of long term exposure to farm waste means that the bioassay was unable to test invertebrate response to realistic sediment exposures. This is

supported by the observation that sediment concentrations did not exceed these guidelines after 21 days of loading, whereas LEL guidelines were exceeded at the majority of farm sites. Direct correlation to field effects is important for bioassays such as this one, which was designed to address site-specific questions (Giesy and Hoke 1989). The results of the bioassay are therefore best used to understand the consequences of an introduction of waste loading to an area of sediment, as would occur from the commissioning of a new farm operation. In order to understand the long term consequences of living at a particular loading rate, sediment from close to the cage farms could be used along with the daily waste additions in a future bioassay rather than using sediment from a reference site. This would better simulate the effects of a well-established farm, rather than a new operation.

4.5. Conclusion

The major contribution of this work is the description of the relationship between aquaculture waste loading and invertebrate growth, survival and community composition through laboratory experiments and field sampling. Until this study, there were no predictions for thresholds of effect at freshwater cage farms in the literature. Based on the results described in this study, the proposed threshold of effect on freshwater benthos is 2.0 - 3.0 g C m^{-2} day^{-1}. A number of models have been used to link fish farming production, depth and current regime to sedimentation of waste material with distance from marine fish farms (e.g., AWATS, DEPOMOD) (Dudley *et al.* 2000; Cromey *et al.* 2002). These models have not yet been validated for freshwater open net-pen aquaculture but that work is currently underway for DEPOMOD (C. Podemski, pers. comm.). The identification of thresholds of effect for waste loading on profundal benthic invertebrates

will be important for developing regulations that might be used in conjunction with depositional models when they become available.

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