

Freshwater rainbow trout (*Oncorhynchus mykiss*) farming affects sediment and pore-water chemistry

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Abstract. Marine aquaculture has come under scrutiny, whereas little is known about the nature and extent of the effects of cage aquaculture in freshwater. We describe the development of changes in sediment and pore-water chemistry caused by an experimental *Oncorhynchus mykiss* farm located in freshwater Lake 375 (Experimental Lakes Area, north-western Ontario, Canada) during its first two production cycles along a distance transect from the cage. Significant changes in sediment chemistry were quick to develop; pore-water ammonia was elevated under the cage after 1 month and sediment nutrients were elevated 1 month later. The effects on the benthic environment were spatially localised, although variables responded to different extents. Within 16 months, nutrient concentrations in surface sediment reached an asymptote, whereas concentrations of metals and ammonia continued to increase. Copper (Cu) and zinc (Zn) concentrations under the cage reached levels that exceed sediment quality guidelines and may cause adverse biological impacts. An anti-fouling coating applied to the net pen was the major source of Cu, whereas Zn originated also from fish feed. Ammonia and pH are recommended for inclusion in monitoring programs because they were sensitive to fish farming and are biologically relevant. We also suggest inclusion of Cu and Zn in monitoring programs at farms with treated nets.

Additional keywords: benthic impacts, cage farming, freshwater, monitoring tools, sediment quality.

Introduction

The North American marine aquaculture industry has undergone significant growth in the past decade, whereas freshwater aquaculture lags behind. For example, between 1996 and 2006, Canadian finfish production increased from 53 499 to 132 262 t, whereas trout production, the dominant freshwater product, went from 6615 to 5033 t in the same period (Statistics Canada 2007). In Canada, we have <15 freshwater cage farms in comparison with hundreds of land-based farms (>200 in Ontario, alone), and yet, cage farms are responsible for the majority of table production in freshwater. Most of these farms grow rainbow trout, *Oncorhynchus mykiss* (Walbaum), in oligotrophic Lake Huron. The largest Canadian freshwater farm occurs in a mesotrophic reservoir, Lake Diefenbaker. Because freshwater cage aquaculture is an industry with considerable capacity for expansion, its potential environmental impacts are of public concern.

The predominant effects of freshwater aquaculture, like those of marine farming, are a consequence of the release of nutrient-rich, untreated solid waste directly into the receiving environment. Intensive freshwater farms are estimated to release 240–318 kg dry weight of solid waste per tonne of fish produced (Bureau *et al.* 2003). This waste consists primarily of fish faecal material, with some uneaten feed, scales, mucus and mortalities (Walker *et al.* 2003).

The magnitude of the impact of a farm is determined by the capacity of its site to incorporate farm waste, and this will be controlled by both the loading rate and the degree of waste dispersal. Loading rate is a product of the farm size, fish density, husbandry practices and the feed conversion ratio (FCR) (Mente *et al.* 2006). Dispersal is the consequence of water depth, flow rates and other hydrographic features (Kempf *et al.* 2002). In addition, certain invertebrate communities will be better able to process organic waste than others. These factors are the same as those controlling waste accumulation in the marine environment; however, the range of loading rates and dispersal distances can be expected to differ between fresh and marine environments, as will invertebrate communities. For example, flow rates are likely to be lower in thermally stratified lakes than in the ocean.

Where it accumulates, solid waste from aquaculture may have a significant impact on the receiving environment. The nutrient-rich waste may increase the content of organic carbon (C), nitrogen (N) and phosphorus (P) in the sediment and the decomposition of organic matter will consume oxygen. For example, Troell and Berg (1997) found that sediment beneath a tilapia (*Tilapia* sp.) cage farm in tropical Lake Kariba consumed 30–40% more oxygen than sediments at control sites, and hypolimnetic oxygen depletion in large, temperate Lake Texoma in Oklahoma and in the small, deep Twin-City South quarry pit of Minnesota was attributed to aquaculture (Axler *et al.* 1998; Veenstra *et al.*

2003). Oxygen depletion may lower the sediment redox potential (Eh), resulting in numerous indirect chemical impacts including internal P loading and sulfide reduction. The accumulation of farm waste may also affect sediment pH, with consequences for sediment toxicity. Farm-waste decomposition products include ammonia, which is weakly basic, and weak acids, such as fatty and carbonic acids (Cook *et al.* 1986). The net effect of these competing processes on sediment pH beneath the freshwater cage farms is unknown. Ammonia exists in equilibrium between its relatively non-toxic ionised (NH_4^+) and its toxic unionised (NH_3) forms, mediated by pH and temperature (Emerson *et al.* 1975). Thus, without pH buffering, increasing ammonia can result in a larger fraction of total ammonia present in the toxic form. Changes in pH may also influence the toxicity of metals present in the farm waste (Campbell and Stokes 1985). All the following chemical changes have been observed at various farms: depleted oxygen, Eh, and pH changes, and the accumulation of ammonia, sulfide, nutrients and heavy metals (e.g. Troell and Berg 1997; Veenstra *et al.* 2003).

These chemical variables are of ecological importance and consequently of public concern. Changes to the chemistry of the benthic environment will alter its suitability for invertebrates and are associated with community-scale changes (Rooney and Podemski 2009). They may also pose a risk to wild fish communities, e.g. as a result of hypolimnetic oxygen depletion. Nutrient inputs coupled with increased internal P loading could result in eutrophication, a major concern of many stakeholders (Yan 2005). Measurement of selected chemical variables may, therefore, provide reliable and inexpensive tools for monitoring the nascent impacts of aquaculture waste in freshwater.

The impacts of freshwater cage farms have received little study relative to those in the marine environment, particularly in temperate regions. Chemical variables are commonly used in effects monitoring of marine aquaculture facilities (Costal Smith 2006); however, their indicator potential has not been tested at freshwater farms. Because of the substantial differences in the chemistry of freshwater and marine systems, it cannot be assumed that chemical variables will respond similarly to farm-waste additions. Results from research conducted in the marine environment cannot be uncritically applied to the freshwater industry; however, they do provide a useful point from which to launch investigations in freshwater.

The knowledge gaps regarding the effects of freshwater cage culture present serious challenges to those charged with assessing the potential impacts of industry growth (Yan 2005). Historical-impact studies (e.g. Cornel and Whoriskey 1993) offer little assistance, because improvements in fish feed and farming practice have dramatically reduced the amount of waste released per tonne of fish produced at modern commercial farms (Cho and Bureau 2001). Even observational studies carried out at modern commercial farms are problematic. All Canadian freshwater cage farms have been in operation longer than 20 years and no pre-farming data exist for them. The effects observed at farms in Lake Huron cannot be attributed unequivocally to aquaculture, because numerous confounding commercial, domestic and recreational activities with related impacts are also carried out in the lake and its watershed. The absence of pre-farming data and the inability to assign responsibility of observed environmental impacts exclusively to

commercial aquaculture operations means that observational studies conducted at commercial farms alone are inadequate to develop our understanding of how farm waste influences the freshwater-receiving environment.

Study of an experimental farm in a controlled environment where reference data are available and all nutrient inputs are accounted for presents the most rigorous approach to assessing the environmental impacts of freshwater cage farming (Yan 2005) and to identifying potential chemical monitoring tools. This was the approach we took, and our three objectives were (1) to document the development of impacts on sediment and pore-water in the vicinity of a new freshwater cage farm, (2) to examine the relationship between the distance from the cage and any observed impacts and (3) to identify chemical variables that could be early indicators of aquaculture impacts in freshwater systems.

Materials and methods

Study site

The experiment was conducted in Lake 375 (L375, 49°44'40"N, 93°47'20"W); a research lake at the Experimental Lakes Area (ELA), north-western Ontario, Canada (Cleugh and Hauser 1971) (Fig. 1). ELA is located in the boreal forest and is home to the Rawson Lake meteorological station. The mean annual daily temperature between 1971 and 2000 was 2.6°C, with a maximum mean daily temperature of 19.2°C in July and a minimum of -17°C in January (Environment Canada 2002). Mean annual precipitation during that period was 688.6 mm, with the majority falling between June and September, and mean annual wind speed was 10.4 km h⁻¹ (Environment Canada 2002).

L375 is an oligotrophic double-basin lake, measuring 0.232 km². Mean depth of the lake is 11.6 m and residence time averages 5.7 years. The dominant wind direction is north-south and the maximum fetch is small (Fig. 1). L375 is dimictic, although spring turnover is often incomplete. L375 is typically ice-free from the last week of April to the third week of November. The inflow and outflow are intermittent and mid-summer current speed measured 1 m above the bottom averages <0.2 cm s⁻¹ (C. L. Podemski, unpubl. data). The littoral zone consists of areas of exposed granitic bedrock, interspersed with patches of glacial till, whereas the profundal region consists of flocculent organic material. The lake supports a fish community including lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersoni*), slimy sculpin (*Cottus cognatus*) and several cyprinid species (Blanchfield *et al.* 2009).

Farm operation

The farm consisted of a single 10-m deep net pen supported by a square, steel floating frame, measuring ~10 m × 10 m. The bottom of the net was ~5–7 m above the sediment, depending on lake water levels. The frame was anchored by two weights at every corner, weighing ~160 kg each. The anchors were deployed at an angle to minimise movement of the cage. Movement of the cage during the study was <3 m around a central point, which was within a GPS measurement error. The cage was assembled on the winter ice in 2003. Farming began the following June, with a single production cycle lasting from stocking in spring to harvest that fall. Stocking and harvest dates

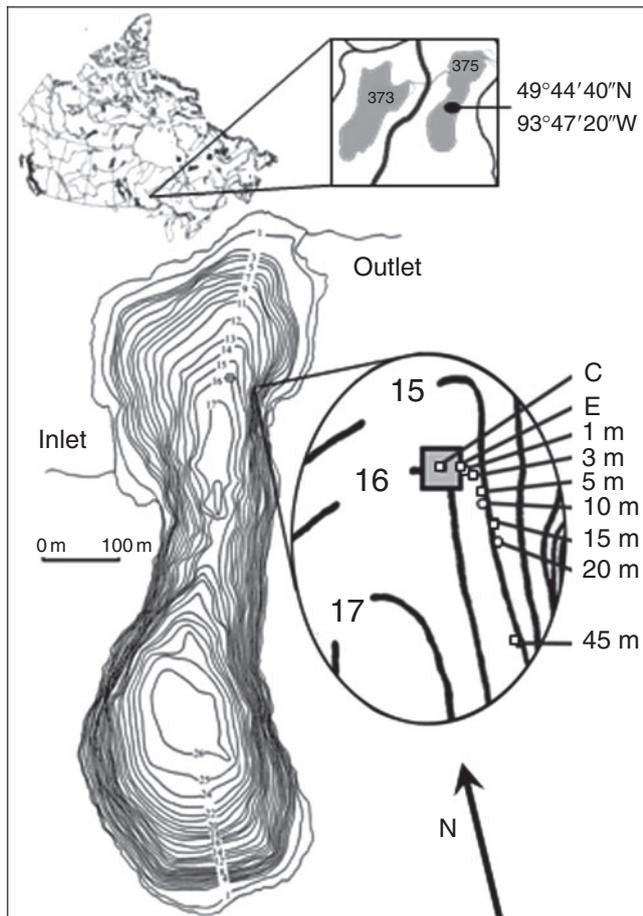


Fig. 1. Bathymetric map of the L375 cage farm at the Experimental Lakes Area, showing the location of the cage (large square) and an example of the sampling transect (□, sites sampled on all sampling dates; ○, sites sampled only in Months 14–16). Sample label C is beneath the cage centre, E is beneath the cage edge. Numbers represent the distance from the cage edge in metres.

Table 1. Summary of farming operation information from the experimental *Oncorhynchus mykiss* farm in L375 at the Experimental Lakes Area during its first two production cycles

Stocking and harvest dates, number of fish and mean fish weight are given

Stocking or harvest	Date	No. of fish	Mean fish weight (g)
Stocking	5 June 2003 (Month 1)	10 640	94
Harvest	18 November 2003 (Month 6)	9988	850
Stocking	31 May 2004 (Month 12)	10 249	101
Harvest	1 November 2004 (Month 18)	9830	996

in 2003 and 2004 are provided in Table 1, along with the number of fish stocked and harvested and their mean weights.

The farm operated according to commercial practices, assisted in this objective through regular off-site supervision by the Northern Ontario Aquaculture Association. We raised ~10 000 fingerling rainbow trout annually, equivalent to a 10-t

aquaculture operation. The scale of the operation was small compared with commercial cage farms; however, our stocking density was relatively high to ensure that we could detect a signal from the farm and to match P loading rates of previous eutrophication studies conducted at the ELA. To provide some sense of the scale, aerial loading rates of P from the farm were 0.27 g m⁻² in 2003 and 0.32 g m⁻² in 2004, loading rates close to those responsible for the eutrophication of Lakes 226 and 227 in earlier ELA research (Schindler *et al.* 1971; Schindler 1980).

The trouts were fed Martin Mills Profishent[®] (Martin Mills, Elmira, Ontario) high-energy feed at a rate of 1–3% of bodyweight per day, depending on the surface water temperature and fish satiation. Fish were fed in two feedings, divided between dawn and dusk. Total wet-weight feed inputs were 8714 kg and 9672 kg in 2003 and 2004, respectively, and the FCR, the ratio of feed provided to the wet weight of fish produced by the farm, was 1.14 and 1.09 in 2003 and 2004, respectively. The feed used throughout each season was produced in a single batch to ensure a consistent composition. On the basis of an analysis by the Fish Nutrition Research Laboratory in Guelph in 2003 by standard methods (AOAC 1995), the feed consisted of 95.5% dry matter, 7.1% ash, 44.3% crude protein and 22.5% lipids. The composition of the feed in 2004 was similar, with 92.6% dry matter, 6.2% ash, 43.8% crude protein and 22.1% lipids (P. Azevedo, unpubl. data).

Study design

In May 2003 (Month 0), before the experimental farm became operational, and approximately monthly thereafter during the open water season, sediment core samples were obtained at multiple distances from the cage (Fig. 1). Here, we focus on samples collected in Months 0, 2, 5, 12 and 16. Samples were taken in a direction that allowed a consistent 15-m water depth, because water depth is known to affect the characteristics of the sediment and benthic invertebrate community. This direction proved to be at an approximately 70° angle from the inlet to the outlet in the north basin, being generally westward and perpendicular to the dominant wind direction. The distances sampled included beneath the centre of the cage, at the edge of the cage, and 1, 3, 5, 15 and 45 m beyond the edge of the net pen. In Month 14, stations were added at 10 m and 20 m from the edge of the cage. These distances were sampled in addition to the original distances on all subsequent sampling dates.

Sampling and analysis

On each sample date, a measuring line was attached to the cage and samples were taken along this line at the appropriate distances while the boat was driven in reverse to maintain tension on the line and thereby ensure that the distances were measured accurately. Because the movement of the cage in response to wind action was generally slight (<3 m) and perpendicular to the transect direction, such movement was not considered to have a significant impact on sampling. Samples from beneath the cage were obtained by scuba divers who also made visual observations of the pile of farm waste accumulating beneath the cage. Samples from the edge of the cage and beyond were obtained with a Kajak–Brinkhurst gravity corer, suitable for soft, fine-grained sediments. A depth sounder was used to measure water depth. All cores were 4.80 cm in diameter and

retrieved ~25 cm of sediment with 5 cm of overlying water. Cores were visually inspected to ensure that the sediment–water interface was undisturbed. Overlying water and sediment subsamples were removed with a silicon cork extruder inserted at the base of the core to push the sediment up into 2-cm-thick removable sections of the core liner.

Pore-water chemistry

In 2003, both sulfide and ammonia were measured in sediment pore water. Three replicate cores were taken at each sampling station for pore-water analysis. Cores were maintained at ~4°C during transport to the laboratory. The upper 2 cm of each core was subsampled by extrusion in a glove box under a nitrogenous atmosphere. Pore water was then removed from the subsamples by suction-filtration through a 0.45- μm cellulose nitrate filter, and separate aliquots were taken for sulfide and ammonia determination. After 14 months, sulfide remained below detection limits, although other response variables had by that time changed considerably. Measurements were therefore discontinued. During months when only total ammonia was measured, extrusions and filtering were conducted in open air.

Sulfide aliquots were stabilised by addition of sulfide anti-oxidant buffer (at a ratio 1 : 1) before measurement with a solid-state silver/sulfide ion-selective electrode (ISE, Thermo-Orion, Waltham, MA, USA). The electrode was calibrated with a six-point 10 : 1 dilution series. The concentration of sulfide in the standard was determined by titration with lead perchlorate.

The concentration of total ammonia was measured with a gas-sensing NH_3 ISE (Thermo-Orion), after the pH was adjusted to be >12 by the 2% by volume addition of Thermo-Orion ionic strength adjustor solution. The electrode was calibrated with a six-point 10 : 1 dilution series from a 0.1-mol L⁻¹ ammonium chloride standard.

In May 2005, five replicate core samples were obtained from all sampling locations. For each core, pore-water pH was measured in the field with an ion-selective field effect transistor probe with automatic temperature compensation (IQ Scientific Instruments, San Diego, CA, USA). The probe was calibrated with a three-point curve determined with standard solutions of pH 4.0, 7.0 and 10.0. The probe was inserted into the upper 2 cm of each core and a reading was taken after stabilisation criteria were met (<0.1 change in pH per 45 s). During the same sampling period, pore-water total ammonia was also determined from the upper 2 cm of four replicate cores, following the method outlined above. On the basis of the mean pH and total ammonia values observed in Month 24 and hypolimnetic temperature measured with a HOBO temperature logger (Onset, Cape Cod, MA, USA), the concentration of unionised ammonia in sediment pore water was inferred by using the equation of Emerson *et al.* (1975).

Sediment chemistry

Core samples obtained for sediment analyses were stored on ice in a cooler during transport. In the laboratory, the upper 2 cm of each core was subsampled by extrusion and quantitatively transferred to acid-washed polyethylene specimen jars. The subsamples were freeze-dried and homogenised. Two to four sediment subsamples obtained from replicate core samples on selected dates were analysed for nutrient and metal content.

Nutrient analyses were performed at the Freshwater Institute in Winnipeg, Manitoba, Canada. Total N (TN) and total C (TC) were measured with an Exeter Analytical Model CE-440 rapid analysis elemental analyser (Exeter Analytical Inc., Chelmsford, MA, USA), with a detection limit of 0.70 $\mu\text{g N g}^{-1}$ and 7.06 $\mu\text{g C g}^{-1}$ dry weight, respectively. Concentrations of total organic C (TOC) were obtained by subtracting total inorganic C (TIC) from TC. TIC was measured by acidifying the sample to dissolve the carbonate and then measuring dissolved inorganic C with an O.I. Corporation model 700 TOC analyser (O.I. Corporation, College Station, TX, USA), with a detection limit of 0.6 mg C L⁻¹. Total P (TP) was measured with a Technicon Autoanalyzer® II colourimeter (Technicon Corporation, Bran+Luebe, Delavan, WI, USA), measuring the concentration of arsenate–phosphate compounds produced under acidic conditions at a detection limit of 1 $\mu\text{g P L}^{-1}$.

Sediment concentrations of a suite of metals were measured by the Saskatchewan Research Council (SRC, Saskatoon, Saskatchewan, Canada). Following an aqua regia–perchloric acid digestion, concentrations of the following elements were measured by inductively coupled plasma atomic emission spectroscopy: aluminium (Al), barium (Ba), beryllium (Be), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), Cu, iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), potassium (K), sodium (Na), strontium (Sr), titanium (Ti) and Zn.

On 16 July 2004 (Month 14), a collection trap was suspended beneath the cage to obtain a sample of settling farm waste. The trap was retrieved after 24 h, and the collected farm waste was frozen, freeze-dried and homogenised. Three replicate samples of the feed were collected from separate bags in both 2003 and 2004. Faeces and feed samples were submitted to SRC for metals determination.

Twenty-four months after the study began, we obtained a sample of the nylon net used to contain the fish in 2003 and 2004. The concentrations of Cu and Zn in the net were determined at the Freshwater Institute, Winnipeg, with a Varian Flame AA-55 spectroscopy instrument (detection limit of 0.120 $\mu\text{g g}^{-1}$ dry weight; Varian Inc., Palo Alto, CA, USA) following digestion with nitric acid and hydrogen peroxide.

Statistical analyses

One-way model-I ANOVAs were run on each chemical variable on each date with distance from the cage edge as the treatment. Validity of ANOVA assumptions of normality and homogeneity of error were confirmed with Anderson–Darling and Levene's tests, respectively. It was often necessary to log-transform the data before analysis to meet ANOVA assumptions. When transformations were inadequate, non-parametric Kruskal–Wallis tests were performed. When there was a significant difference in the mean variable concentration between stations for a given date, Tukey's multiple comparisons were performed. Where the assumptions of parametric tests were violated, visual comparison of error-bar overlap was used to assess how the concentration of variables differed among the sampled distances. To assess whether changes in the nutrient composition of sediments were reaching an asymptote after the first production cycle, two-tailed Student's *t*-tests were used to assess whether

TOC, TN or TP concentrations in sediment from beneath the cage obtained in Month 5 differed from those sampled in Month 16. MINITAB v. 12.1 software was used to perform the Student's *t*-tests, ANOVAs, and Anderson–Darling, Levene's and Tukey's tests (MINITAB 1998).

Principal components analysis (PCA) was performed on the correlation matrix of log-transformed mean total ammonia, nutrients and metals in sediment obtained at the end of the second production cycle because this represented the most complete dataset. The data were averaged to eliminate problems associated with unequal numbers of replicates among variables. Linearity among the variables was confirmed with bivariate scatter plots and PCA axis significance was assessed by the heuristic broken-stick model (McCune and Grace 2002). This test assesses whether the amount of variance explained by an axis is greater than could be explained by a random chance. PCA was run on PC-ORD v. 4.0 software (McCune and Mefford 1999). The Spearman rank correlation coefficient was calculated between significant PCA axes scores and distance from the cage. Correlation coefficients were calculated using MINITAB v. 12.1 software (MINITAB 1998).

Results

Pore-water chemistry

Throughout the study, pore-water chemistry was a sensitive indicator of farm activity, although not all of the variables measured were detectably altered. Pore-water total ammonia and pH were significantly affected by farming activity in L375 (Figs 2, 3), whereas pore-water sulfide was not.

Pore-water total ammonia concentration responded rapidly to farm-waste inputs, showing a significant increase beneath

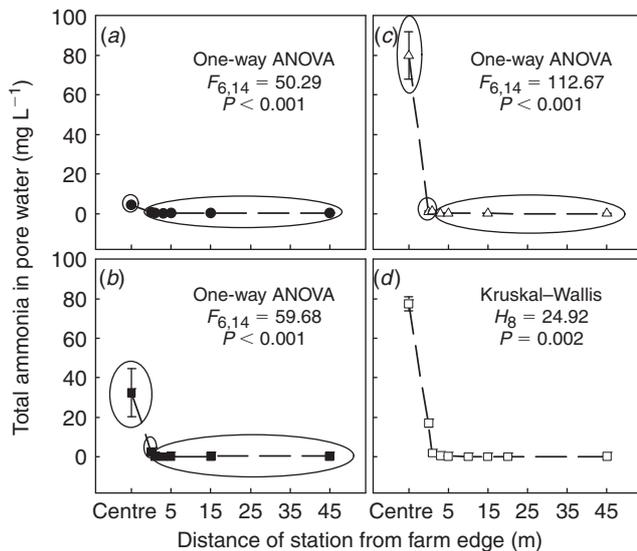


Fig. 2. Mean (\pm s.e.) total ammonia (mg L^{-1}) in sediment pore water obtained from the distance transect. The data are from (a) 1 month after the cage was stocked in 2003, (c) before the fish were stocked in 2004 and (b, d) before the fish were harvested each year. Circles reflect Tukey's groupings. Data from Month 16 (d) did not meet ANOVA assumptions and, consequently, Kruskal–Wallis test was performed in place of ANOVA and Tukey's comparisons were not performed.

the cage within 1 month of farming, relative to the rest of the transect (Fig. 2a). Its concentration continued to increase beneath the cage throughout the study and had not stabilised by Month 16. By the end of the study, it had reached $>77 \text{ mg L}^{-1}$, being >300 times the levels found in sediment obtained from that location before farming.

The distance along the transect exhibiting significantly elevated pore-water total ammonia expanded over time. In Month 3, it was first detectably elevated beyond the perimeter of the cage to a distance of 1 m. In Month 14, it was elevated 3 m beyond the edge of the cage. By Month 16, pore-water ammonia was detectably elevated up to 5 m beyond the edge of the cage (Fig. 2).

Sulfide never exceeded the detection limit of $0.008 \mu\text{g L}^{-1}$ in pore water from any sample. In Month 14, it remained below the detection limit, although significant quantities of waste ($>2 \text{ cm}$ deep) were visible in the core samples from the cage centre and there were significant changes in pore-water ammonia and sediment nutrients. By this time, the benthic invertebrate community was severely affected (Rooney and Podemski, in press). We discontinued sulfide measurement because it clearly had no potential as an early indicator.

Results of the ANOVA conducted on the May 2005 pH measurements indicated that pH values differed significantly among distances ($F_{8,36} = 48.05$, $P < 0.001$). Tukey's multiple comparisons revealed that pH was significantly depressed from beneath the centre of the cage to 10 m beyond its edge, relative to remote stations (Fig. 3). However, the change in pH with distance from the cage was non-linear. A significant difference was also detected among the total ammonia measurements (ANOVA: $F_{8,26} = 214.77$, $P < 0.001$). Tukey's results indicated that this was due to elevated levels within 5 m of the edge of the cage, the same distance to which it was elevated the previous fall. Inferred unionised ammonia also varied significantly among stations ($F_{8,26} = 5.25$, $P = 0.001$); however, because of the effects of pH, the concentration was about the same beneath the cage as at the 45-m station, where the mean values were 0.0025 mg L^{-1} and 0.0020 mg L^{-1} , respectively (Fig. 3).

Sediment chemistry

Before farming began (Month 0), there were no significant differences in the mean TOC, TN or TP among sampling distances, and at this time the concentration of TN was nearly an order of magnitude larger than the concentration of TP. By Month 5, TOC, TN and TP were all significantly elevated beneath the cage centre relative to other transect stations (TOC: $F_{6,14} = 431.06$, $P < 0.001$; TN: $F_{6,14} = 849.77$, $P < 0.001$; TP: $F_{6,30} = 248.77$, $P < 0.001$), and the concentration of TP was of the same order of magnitude as that of TN beneath the cage (Fig. 4b, c). During the winter between production cycles, the concentration of TP declined beneath the cage; however, it rebounded once production resumed (Fig. 4f). During 2004, the area exhibiting elevated nutrient concentrations expanded, reaching the edge of the cage for TOC and TP by Month 16 (Fig. 4d–f). The mean concentrations of TOC, TN and TP beneath the cage in Month 5 were not significantly different from those obtained at the end of Month 16 ($P > 0.05$, in all cases).

In Month 5, total Cu and Zn in the upper two centimetres of the sediment were significantly elevated beneath the cage

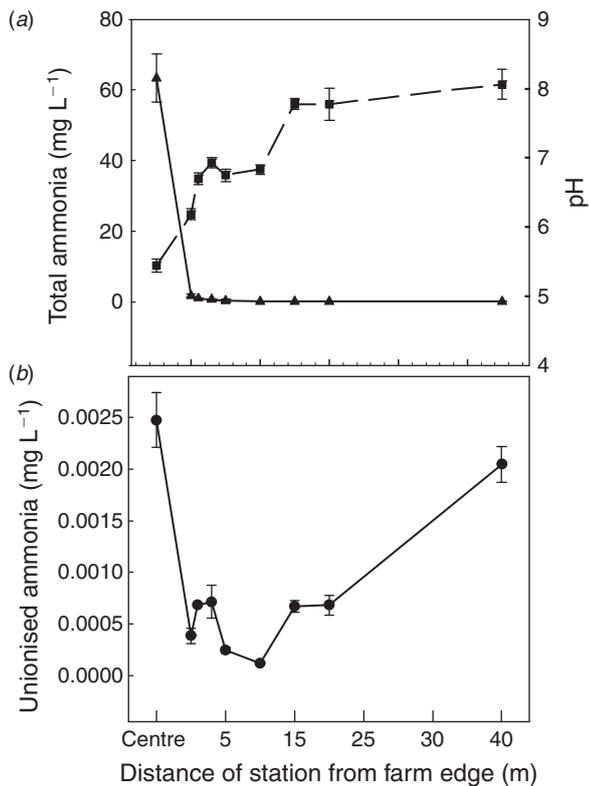


Fig. 3. Mean concentrations of (a) total ammonia (▲) and pH (■) and (b) inferred unionised ammonia (●) in pore water at stations along the distance transect in May 2005 (24 months after the farming began). The fraction of total ammonia present in the unionised form was inferred on the basis of pH and temperature (Emerson *et al.* 1975). Error bars represent 1 s.e. of the mean.

relative to the reference sediment (Fig. 5a, b). At the end of Month 16, the stations at the cage edge and 1 m beyond it also exhibited elevated Cu and Zn (Fig. 5c, d). Unexpectedly, the concentration of Cu at the cage edge was significantly higher than beneath the centre of the cage.

Solid waste, feed and net-pen material

Feed and faeces exhibited higher concentrations of Ca, Cu, TP, Na, Sr, TN, TOC and Zn than did sediment from 45 m beyond the cage edge (Table 2). The net-pen material had higher concentrations of Cu and Zn than did the feed, faeces and sediment from the 45-m station (Table 2).

Principal components analysis

Principal components analysis of sediment and pore-water chemistry from Month 16 produced one significant axis, with an eigenvalue greater than its broken-stick eigenvalue, i.e. PC1 18.8 v. 3.7 (Fig. 6) (McCune and Grace 2002). This axis explained 85.3% of the total variance in the dataset. The eigenvalue of PC2 was only 1.6, compared with its broken-stick eigenvalue of 2.6, and it explained only 7.4% of the total variance in the dataset. A strong Spearman rank correlation was found between the distance from the cage and the PC1

coordinates of sampled sites ($r = -0.733$, $P = 0.025$). This correlation indicates that those chemical variables with negative eigenvector values (Group 1) were greater in concentration nearest the cage, whereas the concentration of those with positive eigenvector values (Group 2) increased with distance from the cage. No eigenvector exceeded $|0.23|$. Rather, most variables exerted approximately equal weight on PC1, ranging from $|0.21|$ to $|0.23|$. PC1 primarily reflected differences in concentration of pore-water total ammonia (-0.22), Ca (-0.23), Cu (-0.21), TP (-0.22), Na (-0.23), Sr (-0.22), Zn (-0.22), TN (-0.23) and TOC (-0.23) as members of Group 1, and Al (0.22), Ba (0.21), Cd (0.23), Co (0.23), Fe (0.23), Pb (0.22), Ni (0.21) and K (0.23) as members of Group 2.

Discussion

The major advantage of the present study is that the data were generated by establishing a model-scale farm in a previously pristine lake. This enabled us to examine the effects of aquaculture on the freshwater ecosystem in the absence of confounding nutrient inputs or other disturbances present in large lakes in populated areas. Additionally, we were able to collect pre-farming data, and have been able, for the first time, to document the development of sediment changes arising from farming activities during the first two production cycles of a new farm following modern farming methods.

It is important to recognise that results of the present study likely simulate a worst-case scenario, e.g. a poorly sited farm. Lake 375 is a relatively small, sheltered lake that stratifies quickly after ice-out, has only a small inflow and outflow, short fetch and a long residence time. Shifting of the cage about its anchors as a result of wind action was <3 m. This suggests that currents, and therefore waste dispersion, within the lake are minimal. However, the ELA was established to provide a setting in which to examine water-quality issues relevant to the Great Lakes at a scale sufficiently reduced to permit controlled experimentation analogous to field plots in an agricultural study (Johnson and Vallentyne 1971). The climate, water chemistry and sediment chemistry of L375 are sufficiently similar to Lake Huron (Armstrong and Schindler 1971; Brunskill *et al.* 1971) so that our observations are relevant to understanding sediment changes at commercial farms and are of value in informing the development of monitoring strategies.

Temporal and spatial development of effects

The first two objectives of our research were to document the development of impacts on pore-water and sediment chemistry caused by a new cage farm over time and across distance. No previous research has tracked the development of sediment and pore-water impacts from freshwater aquaculture, and evidence from marine farms is of questionable applicability because of the differences in hydrology and water chemistry. The expansion of benthic impacts beyond the boundaries of a farm is important to regulators if effects are not to extend beyond farm-turbulence boundaries. Also, if different variables are detectably altered to different distances from the cage, it will have bearing on which variables might constitute useful monitoring tools.

The spatial extent of benthic impacts was relatively small for all variables, although the distance beyond the cage to which

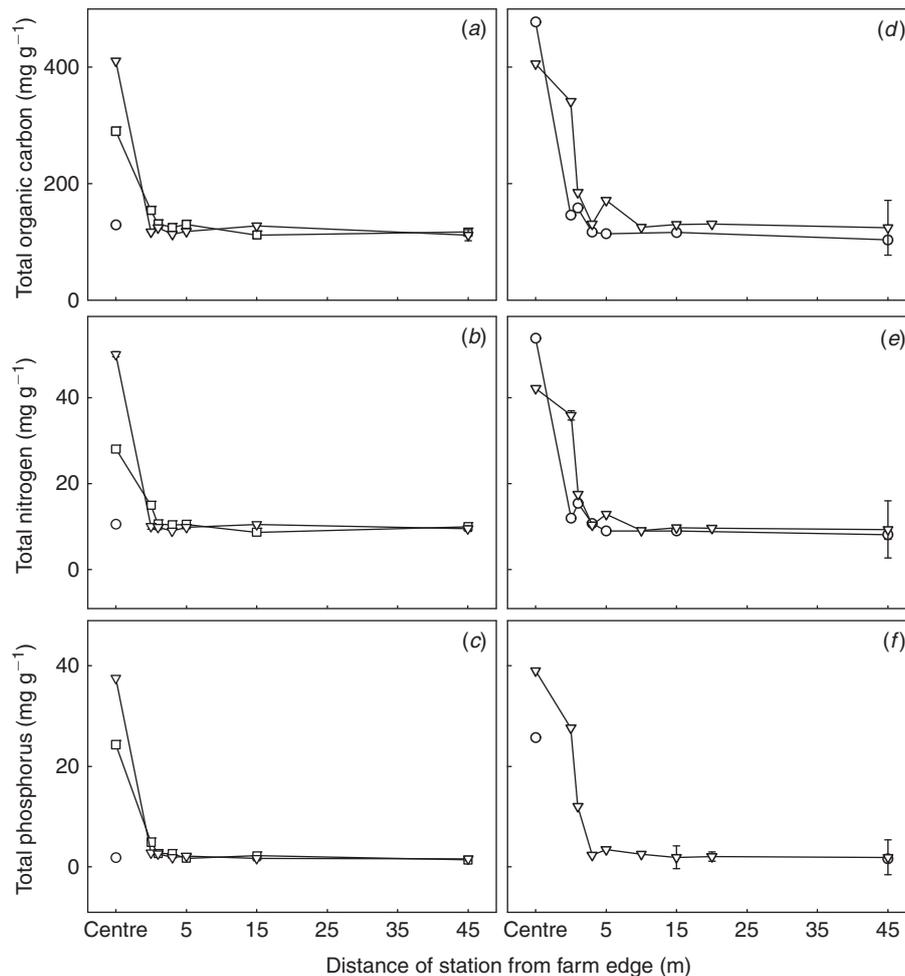


Fig. 4. Mean concentration (mg g^{-1} dry weight \pm s.e.) of (a, d) total organic carbon (TOC), (b, e) total nitrogen (TN) and (c, f) total phosphorus (TP) in sediment at different distances from the L375 cage farm. The distance transect was sampled at the beginning (\circ) and at the end (∇) of each production cycle in (a–c) 2003 and (d–f) 2004. In 2003, the transect was also sampled in Month 2 (\square). In Month 0, TOC, TN and TP were measured only in samples taken under the cage. In Month 12, TP was measured only in samples at the under-cage station and at the reference station 45 m away.

each was detectably altered differed. Even after the second production cycle, all sediment and pore-water effects had dissipated within 10 m of the cage edge. The speed with which benthic impacts were detected after farming began was rapid in all cases where changes became evident within the study period. However, the rate of expansion beyond the edge of the cage at which impacts were detected was not always linear and differed among variables. Consequently, the different variables are considered separately.

Ammonia is of biological significance because of its potential toxicity and the likelihood that it will accumulate where farm waste does. Protein is a major component of fish feed, and consequently the organic-waste released from cage farms is nitrogen-rich. Its decomposition thus releases dissolved ammonia out of the sediment via ammonification. The ammonia produced is toxic to aquatic life, particularly its unionised fraction (Dion 2003), which dominates at high pH and temperature (Emerson *et al.* 1975).

Elevated pore-water ammonia and elevated rates of ammonia flux from sediments have been reported in the vicinity of

both marine and freshwater farms (e.g. Troell and Berg 1997; Merceron *et al.* 2002; Veenstra *et al.* 2003). In the present study, pore-water ammonia concentrations responded very rapidly to fish farming, did not asymptote as quickly as many of the sediment chemistry variables, and showed the largest footprint of all the chemical variables we measured. Its concentration was significantly elevated under the cage after only a month of farming and the area affected continued to grow throughout the study, ending with elevated pore-water ammonia detectable between 5 and 10 m away from the edge of the cage after two production seasons. There is no information available to compare the rate at which this impact developed to that in other freshwater systems or at marine farms; unfortunately, those authors who examined pore-water ammonia levels at freshwater cage farms looked only at stations directly below the cages *v.* remote reference sites, and thus the extent of the impact of the farm in their studies cannot be determined. However, the distance beyond the L375 cage to which pore-water ammonia was elevated can be compared with the footprint of the farms reported in marine studies. In the marine environment, farms

appear to affect a much larger area, i.e. ~40–70 m surrounding cages (Giles 2008). Given that the L375 farm was designed to be a smaller-scale analogue for commercial farms in large lakes, it is not surprising that its footprint on the receiving environment was more restricted in size. The area exhibiting elevated

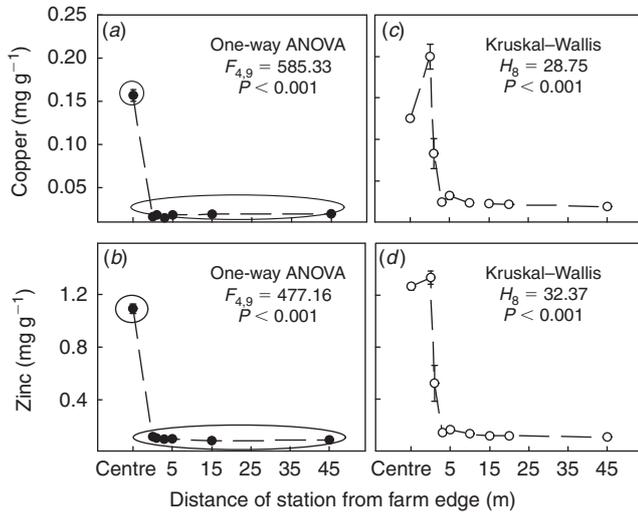


Fig. 5. Mean (\pm s.e.) concentration (mg g^{-1} dry weight) of (a, c) copper (Cu) and (b, d) zinc (Zn) in sediment from transect stations sampled in Months 5 (●) and 16 (○), at the ends of the (a, b) first and (c, d) second production cycles. Circles denote Tukey's groups. ANOVA assumptions were violated when analysing the data from Month 16; thus, Kruskal–Wallis test was performed instead.

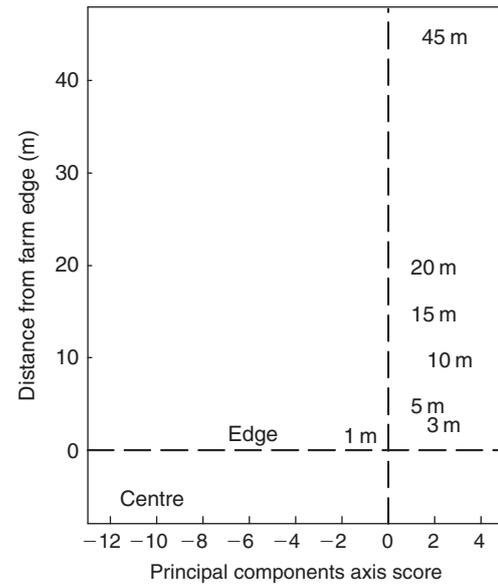


Fig. 6. Relationship between the distance from the cage and mean score on the first and the only significant principal components analysis axis for sediment collected along the transect during Month 16, at the end of the second production cycle. The axis explained 85.3% of the variation in the dataset. Variables with coefficients $>|0.20|$ included total pore-water ammonia (-0.22), total organic carbon (-0.23), total nitrogen (-0.23), zinc (-0.22), strontium (-0.22), sodium (-0.23), phosphorus (-0.22), copper (-0.21) and calcium (-0.23) with negative coefficients, and potassium (0.23), nickel (0.21), lead (0.22), iron (0.23), cobalt (0.23), cadmium (0.23), barium (0.21) and aluminum (0.22) with positive coefficients.

Table 2. Mean concentration (mg g^{-1} dry weight) of metals and nutrients in remote sediment (45 m from the cage edge), sediment beneath the cage, feed, farm waste, and the net-pen material
n.a., not analysed

Variable	Remote sediment, Sep. 2004 ($n=4$)	Under-cage sediment, Sep. 2004 ($n=4$)	Feed, 2003 ($n=3$)	Feed, 2004 ($n=3$)	Farm waste, 2004 ($n=1$)	Net-pen material ($n=1$)
Aluminum	14.275	0.385	0.047	0.046	0.150	n.a.
Barium	1.3925	0.028	0.0018	0.0036	0.026	n.a.
Cadmium	0.0069	0.0002	0.00007	0.00025	0.00019	n.a.
Calcium	6.018	77.975	13.567	13.533	65.900	n.a.
Chromium	0.0092	0.0079	0.0008	0.0011	0.0033	n.a.
Cobalt	0.0101	0.0013	0.0002	0.0005	0.0010	n.a.
Copper	0.019	0.125	0.028	0.033	0.073	16.4258
Iron	108.100	3.185	0.405	0.537	0.688	n.a.
Lead	0.0345	0.0045	0.0005	0.0005	0.0043	n.a.
Magnesium	1.850	1.575	1.267	1.467	1.430	n.a.
Manganese	45.200	1.118	0.103	0.123	1.160	n.a.
Molybdenum	0.1066	0.0017	0.0001	0.0009	0.0006	n.a.
Nickel	0.0135	0.0042	0.0008	0.0015	0.0024	n.a.
Potassium	1.675	0.810	6.933	5.967	0.800	n.a.
Sodium	0.230	2.275	6.767	4.933	0.920	n.a.
Strontium	0.042	0.135	0.022	0.026	0.107	n.a.
Zinc	0.108	1.268	0.223	0.200	0.930	6.0542
Organic carbon	124.6	406.0	n.a.	482.0	439.0	n.a.
Nitrogen	9.4	42.3	n.a.	70.5	42.4	n.a.
Phosphorus	1.90	38.95	10.667	10.733	29.800	n.a.

ammonia continued to expand away from the cage throughout the study. Consequently, the spatial extent of the impact of the L375 farm on pore-water ammonia levels could approach the range reported in the literature as the farm ages. Giles (2008) suggested an alternate explanation. He reviewed 24 studies that reported the spatial extent of benthic impacts of marine cage farms and noted that at shallower sites (<15 m), the area of impact was much smaller whereas the intensity of impact was greater. Thus, the affected area in L375 is likely to be smaller than that observed in larger, deeper lakes because the settling waste in L375 reaches the sediment before it disperses very far and there is little in the way of currents to transport the waste or its decomposition products once it has settled.

On the basis of this correlation with depth, we might predict that the intensity of impact beneath the L375 farm would be greater than observed in other freshwater studies. By Month 16, the concentration of ammonia in pore-water retrieved from beneath the L375 cage was 77.249 mg L^{-1} (s.e. = ± 3.585) and it did not appear to have reached a plateau. This dramatically exceeded concentrations reported by other authors. Troell and Berg (1997) found that the concentration of total ammonia was significantly higher in the pore-water of sediments beneath a tilapia (*Tilapia* sp.) cage farm in monomictic Lake Kariba, a 5000-km² human-made lake on the border between Zambia and Zimbabwe, than at associated reference sites, with a maximum concentration of 4.8 mg L^{-1} . In an earlier study, Enell and Lof (1983) reported pore-water ammonia ranging from 6.1 to 8.4 mg L^{-1} beneath salmonid cage farms in two large dimictic Swedish lakes. Lake Kariba is exposed to a different climate than L375, and differences in fish-waste properties between *Tilapia* sp. and *O. mykiss* species are expected; however, these differences are not at play between the L375 and the Swedish lakes. Farming practices including stocking density and feed type may influence waste loading (Wu 1995), although in general, these changes have reduced loading rates at modern farms. It is likely that pore-water ammonia is an order of magnitude higher beneath the L375 farm than beneath the farms in these other stratified lakes because of the greater dispersal of settling waste in the epilimnion of the larger and deeper Zimbabwean and Swedish lakes. Measures of fish waste depth on the sediment bottom taken through the ice in March 2006 and 2007 confirmed that the deposit was restricted to directly under the cage (C. L. Podemski, unpubl. data) and the water depth under the L375 farm was 5–15 m less than reported by these other authors.

Previous research into the benthic impacts of freshwater cage aquaculture did not suggest that pore-water pH would be significantly affected by farming (e.g. Cornel and Whoriskey 1993), and Cook *et al.* (1986) remarked on the enormous buffering capacity of sediment in lakes at the Experimental Lakes Area; thus, we expected that the natural buffering capacity of L375 would prevent changes in pore-water pH. However, pH in L375 varied significantly with distance from the cage, up to the same distance as the changes in ammonia were observed (Fig. 3). Unfortunately, the timeline of this development is unknown, as we sampled pore-water pH only towards the end of the study. Although we were unable to find other freshwater aquaculture studies that observed a decline in pore-water pH, corroborating evidence is available from the marine aquaculture literature (e.g. Pawar *et al.* 2001; Carroll *et al.* 2003)

and measurement of the sediment pH profiles has been suggested as a way to measure benthic enrichment from marine aquaculture (Giles 2008).

The decomposition of organic matter to yield organic and carbonic acids is likely to be responsible for the observed decrease in the pH in the vicinity of the farm (Cook *et al.* 1986). To determine the specific processes involved, examination of acid–base equilibria, irreversible kinetic reactions and transport processes is necessary (Jourabchi *et al.* 2005). The presence of organic and carbonic acids and alkalinity-affecting cations and anions should be measured in future studies.

Pore-water pH will affect the equilibrium concentrations, and thus the toxicity, of other important chemicals in the sediment, such as heavy metals (Campbell and Stokes 1985) and ammonia (Emerson *et al.* 1975). For example, the reduction in pH counteracted the potential toxicity of the increased pore-water ammonia by reducing the proportion of total ammonia present in its more toxic form precisely where ammonia levels were highest (Fig. 3). Despite total ammonia being orders of magnitude higher beneath the cage than at the remote sampling station, the concentration of unionised ammonia was not significantly different from the concentration 45 m beyond the cage. Consequently, unionised ammonia did not exceed the Canadian Environmental Quality Guideline (CEQG) of 0.019 mg L^{-1} beneath the cage (Dion 2003). This highlights the importance of including pH measurements wherever substances with pH-dependent speciation are measured.

Sulfide is of concern because of its toxicity and its capacity to form complexes with Fe to permanently reduce the phosphorus-binding capacity of the sediment (Holmer and Storkholm 2001). Pore-water sulfide concentration has been suggested as a primary indicator for aquaculture effects monitoring programs in marine systems (Wildish *et al.* 1999; DFO 2005) and has been recognised as one of the most sensitive response variables at marine sites (Giles 2008); however, our results suggest it has limited utility in freshwaters. The production of H₂S in response to salmonid farm-waste loading was observed in a freshwater system by Axler *et al.* (1998); however, the lake in question was a deep, amictic, mine-pit lake with a chemocline, very unlike the environments typical of commercial freshwater aquaculture. During our study period, sulfide did not accumulate to detectable levels beneath the L375 farm or any sampled distance from it, despite the visible accumulation of organic waste.

Differences in the chemistry of marine v. freshwaters and their sediments are likely to explain the difference in the reliability of sulfide as an indicator of degradation of organic waste. Sulfide is the by-product of sulfate reduction in the sediment and sulfate concentrations in freshwater are typically lower than in marine water by an order of magnitude (Holmer and Storkholm 2001). Additionally, any sulfide that did form as a consequence of decomposition in L375, likely immediately complexed with Fe and precipitated as either FeS or FeS₂ because iron levels in L375 were high, with reference sediment averaging $98.115 \text{ mg Fe g}^{-1}$ (s.e. = ± 5.832 , $n = 7$) in Months 5 and 16. Lake Huron, the site of most freshwater cage farming in Canada, also has abundant sediment iron (mean $26.708 \text{ mg Fe g}^{-1}$ dry weight, s.e. = ± 1.460 , $n = 24$) (Thorburn *et al.* 2004). This should caution against uncritically importing knowledge gained about marine aquaculture, because the two systems differ in their chemical response to

fish waste inputs. Sulfide may become elevated in sediment pore-water over time as a result of declining Fe availability via burial and reduction; however, it is clearly a poor early indicator of the effects of aquaculture in freshwater.

Observations of elevated sediment nutrients are expected in both marine and freshwater aquaculture because fish faeces and waste feed contain higher concentrations of these elements than does natural sediment (e.g. Table 2). Elevated sediment P (TP) and N (TN) have been reported by authors studying freshwater farms (e.g. Karakassis *et al.* 1998; Temporetti and Pedrozo 2000), and the concentrations of TN and TP observed in the sediment beneath the L375 farm agree with those reported in the more recent literature.

The degree of increase in the L375 sediment TP exceeded that of TN, resulting in a much lower TN : TP ratio in the under-cage sediment than in the pre-farming or remote-station sediment; however, this is due to the relatively higher concentration of TN in the reference sediment rather than to a higher concentration of TP in the farm waste. In fact, the concentration of TN in the L375 farm waste exceeded that of TP ($\sim 42 \text{ mg g}^{-1}$ v. 30 mg g^{-1}). Thus, the rate of loss of TN from the sediment must exceed that of TP, likely being caused by a combination of denitrification and flux of ammonia or other dissolved nitrogen out of the sediment.

The increase in the sediment TOC we observed is mirrored in the marine and freshwater literature (e.g. Tlustý *et al.* 2000; Carroll *et al.* 2003), although the magnitude of the impact has lessened with improvements in fish feed and farming practice (Cho and Bureau 2001). For example, Cornel and Whoriskey (1993) observed a maximum TOC concentration beneath a salmonid cage farm in Lac-du-Passage, Quebec, of 690 mg C g^{-1} dry weight, whereas the highest value observed in the present study was 477 mg C g^{-1} dry weight (s.e. = ± 3.8 , $n = 2$). The FCR of the farm studied by Cornel and Whoriskey (1993) was 3.7, whereas that of L375 was in keeping with modern commercial farm FCRs, i.e. 1.14 in 2003 and 1.09 in 2004. It is important to consider the increase in palatability and digestibility of feeds, and thus reduced waste production, when applying published results to modern farms.

Detectable sediment nutrient impacts developed rapidly, although the changes in sediment TOC, TN and TP did not follow the pattern of continuous increase demonstrated by ammonia. No other freshwater study has examined the development of changes in sediment nutrients during the first production cycles of a new farm, and so the rapidity of these changes cannot be compared with other studies. Sediment TOC, TN and TP exhibited elevated concentrations beneath the cage by Month 2, which was 1 month after changes were first observed in pore-water ammonia. TP declined beneath the cage during the fallow winter period, indicating that sediment TP may swiftly be incorporated into the lake ecosystem after farming ceases. The concentration of sediment TP then rebounded once the fish were reintroduced in Month 12 and organic inputs resumed, such that TP beneath the cage did not differ significantly between the ends of the first and second production cycles. The concentrations of TOC and TN were also the same in sediments collected in Months 5 and 16, indicating that the sediment concentration of TOC, TN and TP reached some stable level by the end of each production cycle (Fig. 4).

The plateau in sediment TOC, TN and TP concentration beneath the cage was likely to be a consequence of our sampling protocol (top 2 cm). By Month 3, an orange layer of farm waste had accumulated up to 2 cm thick beneath the cage, and continued to thicken throughout the study. Thus, the entire 2-cm-thick sediment subsample that was used to measure the nutrient content consisted of farm waste. Once this occurred, a change in nutrient composition of the fish feed would be required to observe a change in sediment nutrient concentrations.

During the first production cycle, the increases in TOC, TN and TP were limited to beneath the centre of the cage; however, by the end of the second production cycle, the effect had spread. The increase in sediment TOC and TP extended further from the cage than the increase in TN (Fig. 4), namely up to 1 m beyond the cage v. up to the cage edge, respectively. We observed high variability in TN measurements and a lower-magnitude increase than in TOC and TP, resulting in low statistical power to detect changes in TN. Another factor contributing to this disparity is that TN was lost from the sediment in the form of soluble ammonia and gaseous nitrogen at a rate greater than the loss of TOC and TP. This is in agreement with the rationale presented above for why the increase in sediment TP exceeded that in sediment TN, despite their concentrations in farm waste being nearly equal. Karakassis *et al.* (1998) examined sediment nutrients at different distances from a cage sea bream farm in Cephalonia Bay, Greece, that was located in water between 16 and 20 m deep, similar to the farm in L375. They noted that only the sampling station directly beneath the cage consistently exhibited increased nutrients from farming, which agrees well with our findings. This supports the concept that shallower farms will influence a smaller area.

Increases in sediment TP are of particular concern because of the risk of eutrophication (Yan 2005). Bound in the sediment, TP is not available to pelagic algae in the euphotic zone; however, internal phosphorus loading during periods of hypolimnetic anoxia could eventually promote algal growth in the lake basin. Indeed, a four-fold increase in the total phytoplankton biomass was observed in L375 after the farming began, although similar increases in periphyton were not observed (Findlay *et al.*, in press). It is generally agreed that TP flux from sediment to the water column in freshwater lakes is controlled by Fe and Al complexation (e.g. Dorin-Sorin 1993); however, Temporetti and Pedrozo (2000) found that beneath a salmonid cage farm in the Alicura Reservoir, Argentina, calcium and organic matter were more influential in TP release. It is, therefore, unclear which factors control TP release, and they may vary among lakes, depending on alkalinity, pH and redox potential. In L375, farm-waste loading increased the concentration of Ca but decreased the concentration of Fe beneath the cage; however, without understanding the mechanisms governing the flux of P from the sediment in L375, the consequences of this chemical change are unclear. Our results suggest that the flux of TN out of the sediment exceeds that of TP; however, the biochemical processes controlling the flux of P underlying cage farms require further study before the risk of eutrophication posed by waste accumulation under commercial farms can be assessed.

Cu and Zn are heavy metals of concern around marine aquaculture sites (e.g. Mendiguchía *et al.* 2006). Heavy metals are often persistent in the environment (Birge *et al.* 1987) and

can be toxic in their free ion form (Huggett *et al.* 2001). It has consequently been recommended that Canadian marine finfish aquaculture monitoring programs include these metals (DFO 2005). However, their indicator potential at freshwater cage farms has not been assessed before.

The timeline by which sediment Cu and Zn were affected by farming is unknown, because metals were measured only at the beginning and towards the end of each production cycle; however, they were significantly elevated under the cage centre by Month 5 (Fig. 5). By Month 16, both Cu and Zn were significantly elevated up to the cage edge (Fig. 5). This corresponds with the distance from the cage centre to which elevated TN was observed, although it is much less than observed in marine studies. For example, Brooks and Mahnken (2003) observed elevated Zn in the top 2 cm of the sediment from 30 to 75 m down-current of seven salmonid cage farms in British Columbia. This is most likely because of the relative shallowness of the L375 farm and the consequent reduced dispersal of the wastes.

By the end of the study, concentrations of Cu and Zn at the L375 farm were higher than observed at marine farms, further corroborating the theory that a greater intensity of effects is correlated with less dispersal of waste. Concentrations exceeded the empirically derived Canadian Probable Effects Level (PEL) ($0.197 \text{ mg Cu g}^{-1}$ and $0.315 \text{ mg Zn g}^{-1}$, respectively) and Ontario Severe Effects Level (SEL) ($0.110 \text{ mg Cu g}^{-1}$ and $0.820 \text{ mg Zn g}^{-1}$, respectively) in sediment underlying the cage edge. Thus, the concentrations in sediment beneath the cage were high enough to provoke acute toxicity in exposed invertebrates (CCME 1995; Jaagumagi and Persaud 1999), depending on sediment pH and organic content. Concentrations of Cu and Zn observed in the sediment under the cage in L375 exceeded the concentrations reported from beneath a group of marine salmonid cages by Brooks and Mahnken (2003) and Chou *et al.* (2002). Unfortunately, water depth where the samples were taken and the area of impact was not reported in either study, so we cannot assess whether depth and waste dispersal were a factor in this discrepancy.

There are two potential sources of Cu and Zn to sediment underlying the cage farms. Copper and Zn are present in fish feed (Table 2) and can therefore be expected to occur in farm waste and the net pen itself may be a source of Cu and Zn. In the marine environment, plants and animals colonise cage nets, restricting water flow and retaining fish wastes. An anti-fouling coating containing Cu is often applied to cage nets to reduce colonisation (Brooks and Mahnken 2003) and our net had a coating with high levels of Cu and Zn (Table 2). In freshwater systems, fouling by colonising organisms is less problematic, and coated nets were rarely used at the time of the study. However, the use of coated nets is legal in freshwater and one freshwater farm is increasing their use to reduce maintenance (C. L. Podemski, unpubl. data). Thus, contributions of Cu and Zn from treated nets are of potential concern in freshwater.

Determining the origin of Cu and Zn observed in the sediment underlying freshwater cage farms is critical. If Cu and Zn originate exclusively from treated nets, then eliminating their use at freshwater farms would eliminate Cu and Zn loading. Conversely, if Cu and Zn originate primarily from fish faeces and waste feed, they could be important contaminants at all farms and should be included in monitoring programs, as

recommended for marine aquaculture by Sutherland *et al.* (2007) among others. In L375, the concentration of Cu and Zn was higher in the sediment beneath the cage and the cage edge than in either the feed or faeces (Table 2), but organic matter is known to concentrate heavy metals (Mendiguchía *et al.* 2006). The under-cage sediment : farm waste ratios were 1.7 for Cu and 1.4 for Zn, whereas under-cage sediment : net material ratios were 0.008 for Cu and 0.210 for Zn. Given that the partition coefficients for Cu and Zn between sediment and pore-water are 3.5 and 4.1, respectively (Allison and Allison 2005), this supports the hypothesis that the net material was the primary source of Cu, whereas faeces and waste feed were contributing a significant proportion of the Zn load. This is in accordance with the findings from the marine environment (Uotila 1991), and explains why the concentration of Cu was significantly higher beneath the cage edge than beneath the centre of the cage (Fig. 5); more net material overlies the edge, thus more of the abraded particles or algae would settle at the edge of the cage. The concentration of Zn was nearly the same beneath the cage centre and at the edge of the cage, supporting the idea that feed and faeces are the major source of Zn. Therefore, it is worth monitoring Zn, even at farms without treated nets, and Cu should certainly be monitored where treated nets are used.

Overall, the sediment and pore-water impacts of the L375 farm dissipated between 5 and 10 m from the edge of the cage. Previous studies conducted in freshwater lakes also reported that the impact of farming was spatially localised, although reported impact areas vary from ~20 to 50 m (e.g. Enell and Lof 1983; Doughty and McPhail 1995; Guo and Li 2003). This difference could be due to variability in loading rates at different farms or in the mobility of the many different chemical variables measured by different researchers (e.g. wastes bound to the sediment *v.* those dissolved in the water column). There is evidence from the marine environment that variation in farm footprint size could be attributed to differences in water depth (Giles 2008). Our results support this concept, because the distance beyond the cage farm to which benthic impacts were detected was smaller than that in other reports, whereas the magnitude of the impacts was often greater.

The spread of impacts on pore water (ammonia and pH) exceeded that on sediment chemistry (nutrients and heavy metals). This is likely because dissolved substances, such as ammonia, are more mobile than substances adsorbed to sediment particles (Lick 1987). They can be transported by diffusion and low-velocity water movements, whereas contaminants adsorbed to particles require sediment transport and thus higher-velocity water movements. Unfortunately, we could find no other study that compared the spatial extent of pore-water and sediment impacts below modern freshwater cage farms. Studies at marine farms generally find agreement in the spatial extent of pore-water (elevated sulfide or depressed pH) and sediment impacts (accumulation of metals and nutrients). For example, Carroll *et al.* (2003) noted that a variety of sediment variables all agreed that the affected area surrounding five marine cage farms along the western coast of Norway was limited to within ~10 m of the cages. The samples in the study of Carroll *et al.* (2003) were all taken at 30-m depth, so the slightly larger impact area could be attributed to greater waste dispersal than in L375 where the sampling depth was 15 m; however, this does not explain

why Carroll *et al.* (2003) found no difference in the areal extent of pore-water and sediment impacts. Giles (2008) observed that pore-water sulfide and sediment TP alike were detected up to ~40 m beyond cages at farms in water ranging from 15 to 30 m deep. It is likely that higher flow rates in the marine environment result in sediment transport, and thus the greater mobility of labile substances at low water velocities becomes unimportant.

If there is directional flow within a lake, the footprint of the impact of a farm will extend further downstream than upstream; thus, monitoring should be conducted in the downstream direction. Lake 375 does not appear to have a significant directional flow, because measures of the depth of accumulating farm waste made in 2006 and 2007 found that it was distributed fairly uniformly among each cardinal direction (C. L. Podemski, unpubl. data) and scuba divers noted that the pile of farm waste on the sediment beneath the cage was symmetrical. Even lakes that have currents, however, often lack strict upstream–downstream, wind-driven currents. In the absence of current data from a site, sampling should occur around the farm rather than in a single direction.

Monitoring tools

Our third objective was to review a wide range of sediment and pore-water chemistry variables, including those most often recommended as indicators in the marine literature, and assess their utility as indicators of the impact of cage farming in freshwater. Chemical variables with strong potential as indicators of farm-waste impacts in freshwater should respond rapidly, reliably and strongly (detectably) to farm-waste inputs. Changes in their values should occur early on, so that greater degradation can be anticipated and prevented. They should respond with similar sensitivity to farm-waste inputs in all types of lakes, and the changes should be of a magnitude that is easily detected using standard sampling and measurement. The selected variables should also be indicative of the risk to aquatic fauna, i.e. they should be biologically relevant.

An ordination of all sediment and pore-water data identified two distinct groups of variables along the only significant ordination axis. The first group (Group 1) included elements whose concentrations increased with proximity to the cage, and the second group (Group 2) included those that decreased with proximity to the cage. Most of the Group 1 variables were at higher concentrations in the farm waste and feed than in the sediment collected at 45 m from the farm, whereas those in Group 2 had lower concentrations in the farm waste and feed than in the sediment at 45 m from the farm (Table 2). Group 1 variables increased in concentration with proximity to the cage because farm-waste additions acted to spike their concentration in the subsample. Conversely, Group 2 variables decreased as their concentration in lake sediment was diluted by an increasing proportion of farm waste. Group 1 variables have indicator potential, because they will respond similarly to all regions receiving aquaculture waste, whereas variables belonging to Group 2 may differ among aquaculture sites on the basis of the composition of natural lake sediment.

Although all Group 1 variables had similar eigenvector scores, pore-water ammonia has the best potential as an early indicator of sediment impacts. Although pore-water sulfide is used and recognised as a sensitive indicator of sediment impacts in marine systems, its utility in freshwater appears limited,

especially compared with pore-water ammonia. Pore-water ammonia responded quickly, was detectably elevated further from the cage than any other chemical measured except pH and appears to have a larger response range because it had not reached a plateau after two production cycles, as the sediment nutrient concentrations did.

Pore-water ammonia is also of direct biological significance to sediment biota. To assess the potential toxicity to aquatic life, pH and temperature should also be monitored. Many authors who have reported total ammonia concentrations in aquaculture studies measured ammonia in the water column (e.g. Cornel and Whoriskey 1993; Veenstra *et al.* 2003) rather than in pore water, presumably because of the potential health risk to cultured fish. The concentration of ammonia in overlying water will always be less than in pore water (Phillips *et al.* 1997), and pore-water concentration will most directly affect benthic invertebrates because of their close association with sediments (Whiteman *et al.* 1996). Thus, it is pore-water ammonia and pH that should be monitored rather than pelagic levels.

The other two variables we recommend for inclusion in monitoring program are sediment Cu and Zn, which accumulated to potentially toxic levels in the sediment beneath the farm, in part because of the use of a treated farm net. The toxicity of these metals cannot be determined without assessing their bioavailability, which will depend on the pH and organic matter content of the sediment. Work in the marine environment, however, has shown promise at inferring Cu and Zn toxicity from the ratio of their concentration to the concentration of free sulfide (Brooks and Mahnken 2003), and perhaps a similar ratio of metals to organic content could be applied in freshwater.

Conclusions

The temporal development of sediment and pore-water impacts along a transect of increasing distance from a freshwater cage farm is documented here for the first time. The development of effects was rapid, particularly with pore-water ammonia. A definable edge existed to the area affected by the farm. Benthic conditions had returned to reference levels within 10 m of the cage; however, the distance beyond the cage to which effects were detectable had not stabilised by the end of the second production cycle, and continued expansion of effects is predicted. In deeper lakes, the area of impact is predicted to be larger and the intensity of impact to be less. This is the consequence of greater waste dispersal in deeper waters and where the flow rates are higher.

Recommendations are made regarding which of the measured variables has greatest potential as a monitoring tool. Because of its rapid rate of response, sensitivity, range of response and biological significance to benthic organisms, pore-water ammonia has the greatest utility as a monitoring tool. To infer its toxicity, pH and temperature should be measured simultaneously. Where the use of treated nets is known or suspected, measurement of sediment Zn and Cu are also recommended, although their toxicity will depend on the pH and the amount of organic matter in the sediment.

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