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Discrete water quality sampling at open-water aquaculture sites: limitations and strategies

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ABSTRACT: While environmental performance of cage-based aquaculture is most often monitored through benthic conditions, there may also be requirements that necessitate discrete, pelagic sampling. In the pelagic realm, adequately capturing the spatial and temporal dynamics of interest and attributing causality to aquaculture processes can be extremely challenging. Conditions are seldom ideal, and data adequacy concerns of discrete samples collected at open-water aquaculture sites are not uncommon. Further exploration of these challenges is needed. Herein, we aim to explore considerations for study design, analysis, and data interpretation of discrete pelagic sampling. As examples, we present 2 case studies where limited sampling occurred under conditions of complex pelagic dynamics. A Norwegian case study quantified particle abundance around salmon farms, and aimed to highlight the effects of spatial–temporal variation on sampling design, the need for inclusion of companion parameters, and the benefits of *a priori* and *a posteriori* data interpretation strategies. A Canadian case study collected discrete samples to measure ammonium concentrations with continuous current measurements at an Integrated Multi-Trophic Aquaculture (IMTA) farm, to explore issues of complex hydrodynamics, reference site suitability, sampling resolution, data pooling, and post hoc power tests. We further discuss lessons learned and the implications of study design, ambient conditions, physical processes, farm management, statistical analysis, companion parameters, and the potential for confounding effects. Pragmatic consideration of these aspects will ultimately serve to better frame the costs and benefits of discrete pelagic sampling at open-water aquaculture sites.

KEY WORDS: Sampling design · Pelagic · IMTA · Nutrients · Cage aquaculture · Farm-scale

INTRODUCTION

Increased environmental concerns about the release of effluents from open-water finfish culture have prompted monitoring and modelling to assess the potential negative impacts (Holmer et al. 2008) and approaches to utilize waste nutrients, such as Integrated Multi-Trophic Aquaculture (IMTA; Chopin et al. 2012). In most major fish-producing jurisdictions, cage-based aquaculture is primarily monitored through benthic environmental conditions (Wilson et al. 2009), with a few exceptions such as Malta (Holmer et al. 2008) and the Canadian Great Lakes (Boyd et al. 2001). Likewise, the majority of aquaculture environmental impact studies focus on the deposition of organic aquaculture wastes and the associated effects to benthic faunal and sediment biochemical processes (e.g. Karakassis et al. 2000, Kutti et al. 2007, Holmer et al. 2008, Borja et al. 2009, Chopin et al. 2012, Valdemarsen et al. 2012). Pelagic (horizontal) dispersion of organic particles and inorganic nutrients at fish culture operations is less well under-

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stood and studied (Sará 2007a, Handå et al. 2012a, Husa et al. 2014). Nevertheless, there can be various requirements that necessitate pelagic sampling of nutrients at fish cages. Farmers may require details on nutrient plumes for species placement in IMTA, regulators may want to know the potential for horizontal dispersion, and models require data validation.

Soluble metabolic by-products of fish culture that can be measured in the water column include ammonium (NH_4^+) and orthophosphate (PO_4^{3-}) (Sará 2007b). Respired carbon dioxide (CO_2) is not normally measured at fish cages, but trends are sometimes inferred through other water-quality proxies (Reid et al. 2006). Typically, nitrogen is the limiting nutrient in marine systems (Howarth & Marino 2006), as is phosphorus in freshwater (Dillon & Rigler 1974). These limiting nutrients are still commonly measured through discrete water sample collection followed by wet chemistry techniques, notwithstanding recent encouraging developments of *in situ* nutrient analysers (Arai et al. 2011, Wild-Allen & Rayner 2014). Though soluble nitrogen forms and phosphate are measurable with *in situ* analysers, total phosphorus (TP) is not at present, and this is the recommended nutrient form for aquaculture monitoring in freshwater due to the potential for rapid cycling of soluble phosphorus (Hudson et al. 1999, Boyd et al. 2001). There are several practical and logistical issues involved in measuring pelagic nutrients at open-water aquaculture sites (Brooks et al. 2003, Macleod et al. 2004, Gao et al. 2005). The process of collecting a discrete pelagic water sample is intuitively simple. However, collecting an appropriate amount of discrete samples in time and space to adequately capture the spatial and temporal scale of interest, or to attribute cause and effect relationships to aquaculture, evidently sets limits to such investigations. Ecosystem, farm type, hydrodynamics, site characteristics, and husbandry practices represent complex sources of variation in the quantification of aquaculture influences on water-column properties (Sará 2007b). Consequently, the sampling regimen necessary to acquire meaningful results may need to be intensive, prolonged, or impractical.

It is common for the discrete data collected in this complex environment to be influenced by confounding effects and undersampling, leading to difficulties in applying inferential statistics and drawing conclusions. With this paper, we aim to emphasize some of the challenges encountered in pelagic data collection by exploring the sampling requirements necessary to achieve meaningful results and by discussing several approaches to maximize information from limited data sets. Several of the issues addressed here have been debated previously in the international literature, but typically not in the context of open-water aquaculture. We suggest that a practical discussion on the challenges of discrete pelagic sampling is therefore warranted to infer the cause and effect relationships of aquaculture. In this paper we first present Norwegian and Canadian case studies as examples of the complex dynamics of pelagic sampling and possible strategies for analysing and interpreting less-than-ideal data sets. In the second part of the paper we explore lessons learned, through a review of sampling design, sampling strategies, data analysis, and data interpretation. We thereby aim to guide farm operators and researchers in the appropriate use of discrete sampling methods to assess water quality at open-water aquaculture sites based on practical considerations.

TWO FARM-SCALE CASE STUDIES

Challenges related to sampling of fine particulates at salmon farms in Norwegian stratified fjord systems

Study objectives

It is relevant to quantify and understand the dynamics of fine particulates at open-water fish farms in the context of IMTA development when bivalve filter-feeders are integrated with finfish (Brager et al. 2016). This case study presents a short-term sampling program that defines the abundance of fine particles in the water column. We thereby aim to highlight considerations of spatial-temporal variation on sampling design, the need for inclusion of companion parameters, and the benefits of *a priori* and *a posteriori* strategies for data interpretation.

Sampling design and data analysis

Field measurements were conducted at 2 large-scale Atlantic salmon farms in central Norway. The study was carried out in September 2012, 2 mo after the farms were stocked with juvenile Atlantic salmon *Salmo salar* of ~100 g. Site I (Flåtegrunnen), consisted of 7 circular net pens situated in a row parallel to the dominant current direction, and at Site II (Skrestedvika) 2 rows of 5 cages were situated perpendicular to the major current (Fig. 1). Samples were collected close to the fish net pens on the lee-

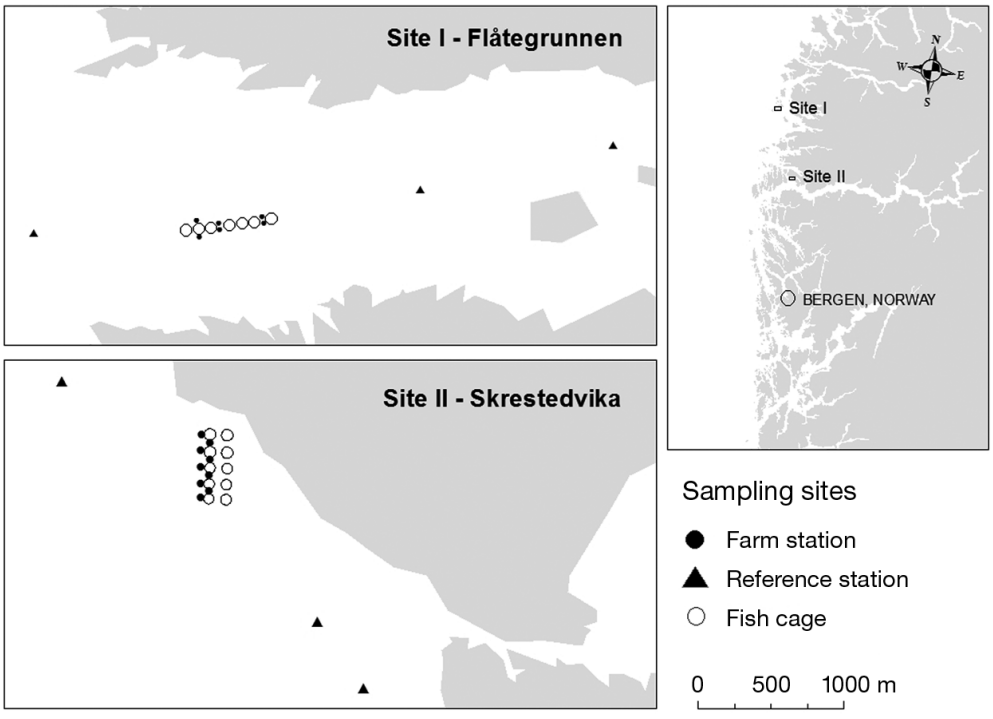

Fig. 1. Sampling locations for both study sites in Norway. An area overview is displayed in the right panel; the squares indicate the specific study areas (left panels): Site I Flåtegrunnen (61° 34.586′ N, 4° 48.942′ E) and Site II Skrestedvika (61° 9.417′ N, 5° 8.015′ E)

ward side (<10 m from the net pens; $n_{Site\ I}$ = 6 and $n_{Site\ II}$ = 9) and at reference stations 1–2 km east and west of the farm (n = 3 for both sites). Sampling was performed after the turning of the tide, in anticipation of unidirectional current flow, and all samples were collected within a timeframe of 3 h (Fig. 2). A vertical point water sampler was used to collect discrete samples. Water samples were collected at 1, 5, 10, 15, and 20 m depth, and continuous depth profiles (0–20 m) for temperature, fluorescence, and salinity were simultaneously obtained by running a STD/CTD 204 (SAIV A/S). Samples from a 'single-drop' were analysed in triplicate for particle abundance by using a Pamas particle analyser (Model S4031GO) with an overall size range set to 1–200 µm. Fixed current meters (SD6000 Sensor Data AS) were installed adjacent to the fish cages at 5 m depth over a period of 24 h to define hydrodynamic conditions. Independent *t*-tests were used to test the difference in particle abundance between farming and reference stations for each specific depth interval ($p < 0.05$) using SAS 9.3 and applying Satterthwaite degrees of freedom, because assumptions for equal sample size and equal sample variances were not met (Table 1).

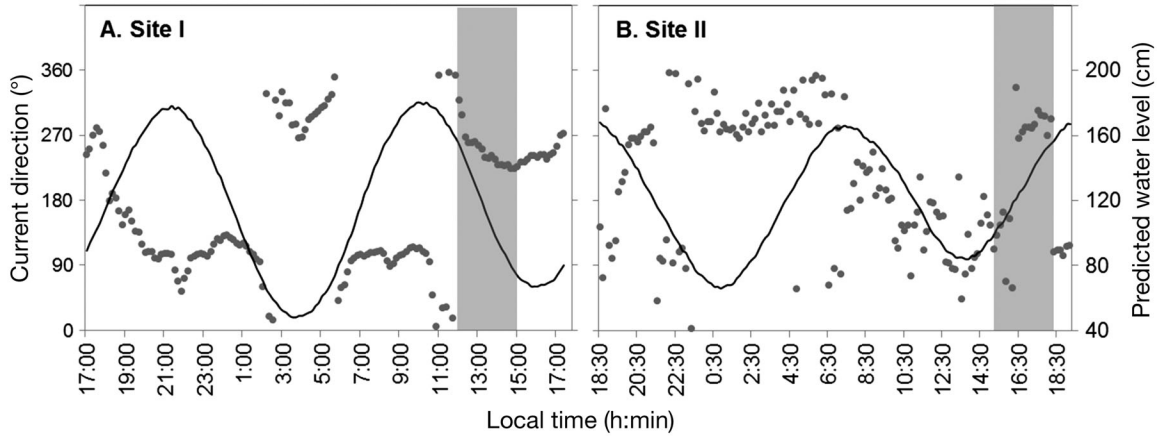

Fig. 2. Sampling time relative to hydrographical information at the 2 study sites in the Norwegian case study. Grey boxes indicate the time period of discrete water sampling. Circles: current direction; solid line: tidal cycle (data obtained from Norwegian Mapping Authority, Hydrographic Service)

Table 1. Paired comparisons (*t*-test) of particle concentrations between farm and reference (Ref) sites for the Norwegian case study. Statistical output includes both results for assumptions of unequal variances (Satterthwaite degrees of freedom) and equal variances (pooled degrees of freedom). *Equality of variances according to folded *F*-statistics

Depth (m)	Sample	Particle conc. (mean ± SD)	*t*-test assumption for sample variance	df	*t*	p
Site I: Flategrunnen						
1	Farm	11501 ± 2401	Satterthwaite*	6.71	1.94	0.0950
	Ref	9700 ± 704	Pooled	7	1.44	0.1924
5	Farm	9435 ±1913	Satterthwaite*	6.07	4.61	0.0036
	Ref	5623 ± 473	Pooled	7	3.30	0.0132
10	Farm	4871 ± 1476	Satterthwaite*	6.62	2.41	0.0486
	Ref	3279 ± 483	Pooled	7	1.78	0.1186
15	Farm	3247 ± 1800	Satterthwaite	5.44	1.13	0.3056
	Ref	2397 ± 274	Pooled*	7	0.79	0.4575
20	Farm	3167 ± 922	Satterthwaite*	5.51	2.96	0.0280
	Ref	2024 ± 150	Pooled	7	2.06	0.0778
Site II: Skrestedvika						
1	Farm	7675 ± 341	Satterthwaite*	2.42	0.18	0.8717
	Ref	7609 ± 614	Pooled	10	0.24	0.8123
5	Farm	7721 ± 659	Satterthwaite*	8.78	3.73	0.0049
	Ref	6706 ± 279	Pooled	10	2.53	0.0301
10	Farm	7585 ±1593	Satterthwaite*	8.78	6.24	0.0002
	Ref	3418 ±621	Pooled	9	4.29	0.002
15	Farm	5110 ± 1220	Satterthwaite*	9.47	6.85	<0.0001
	Ref	2162 ± 242	Pooled	10	4.03	0.0024
20	Farm	2918 ± 546	Satterthwaite*	6.57	4.28	0.0042
	Ref	1828 ± 308	Pooled	10	3.22	0.0091

Sampling execution and data interpretation

Even with the low fish biomass (0.72 kg m^{-3}) present at the start of the production cycle, enhanced particle abundance was observed close to the salmon sea cages (Fig. 3A,D). At Site I differences in particle concentrations between the farm and reference stations were most profound at 5 m depth (Fig. 3A), and at Site II enhancement of particle concentrations at the farm stations was significant for all depth intervals except the surface (Fig. 3D). Furthermore, distinct stratification of the water column was observed in the upper 10–15 m (Fig. 3B,C,E,F), which is common in Norwegian fjord systems during summer and early autumn (Sætre 2007). At Site I, vertical profiles of companion parameters, such as temperature and salinity, showed similarity between the farming and reference stations (Fig. 3B,C). This suggests that the water body close to the farm was representative of the surrounding environment, and differences in particle abundance can be attributed to waste release from the farms. At Site II, however, dissimilar profiles were observed (Fig. 3E,F) with the stratification occurring at 10 m depth at the reference station, while at the farming stations stratification was observed at 15 m. This suggests that enhanced particle abundance may also be related to factors other than waste release from the farm only. Explanations for this might relate to (1) local hydrodynamic conditions around fish cages (spatial), (2) tidal influences during the sampling execution (temporal), or (3) the appropriateness of reference sites.

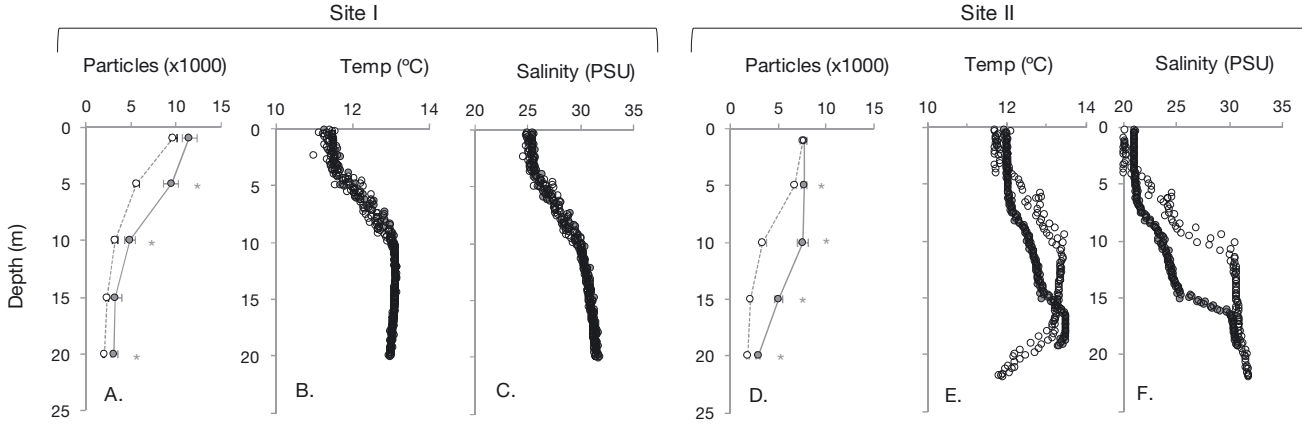

Fig. 3. Depth profiles of particle concentrations (counts between 1 and 200 µm ml^{-1}), temperature (°C), and salinity (PSU) obtained by discrete water sampling at Site I (A, B, C) and Site II (D, E, F) in Norway, respectively. Open circles: reference stations (1–2 km east and west of the farm; n = 3); solid circles: farming stations (<10 m from the net pens ; $n_{Site\ I} = 6$ and $n_{Site\ II} = 9$). Particle data are presented as means (±SE), and significant differences for a specific depth interval are indicated by asterisks under the assumption of unequal samples variances (see also Table 1)

Stocked fish cages can change adjacent current patterns (Harendza et al. 2008, Gansel et al. 2011, 2012), which may have affected the local vertical water column profiles at the Site II farm stations. Swimming fish can create vortices (Gansel et al. 2011), where water can be drawn upwards or downwards, exiting at the depth of maximum fish biomass. The vertical distribution of fish biomass at the time of sampling was unknown, but 15 m could possibly be the depth of maximum biomass according to the farm manager. Were this the case, cooler, less saline, particle-rich surface water might be drawn down through the cage, exiting at lower depths. Higher farm particle abundances at 10 and 15 m might thus either originate from fish waste or from shallower waters drawn down by the vortex. These 2 potential sources could not be differentiated within the present study design. Resuspension was not a likely factor of influence, as both sites were situated in deep waters (100–200 m).

The duration of sample collection might also have influenced the outcomes of the study. Although the sampling plan was based on *a priori* knowledge of hydrodynamic conditions, which were known to be driven by tidal exchanges (hence, sampling started 2 h after tidal shift), a change in current direction was observed at Site II during sampling (Fig. 2B). As reference stations were sampled prior to farming stations (non-randomized sampling program), 'timing' as a consequence of different tidally induced barotrophic forces may have interfered with the comparison between stations. Effects of such confounding factors highlight the importance of minimizing sampling time, and suggest the option of multiple-vessel sampling to reduce collection time.

Finally, there is a possibility that the reference sites were not appropriate, particularly for Site II. This can be ruled out as *a priori* information from a baseline study demonstrated that environmental conditions were similar for reference and farming stations at both sites prior to stocking based on CTD data (authors' unpubl. data). Furthermore, results among the 3 spatially separated reference stations were consistent for both sites, indicating a homogeneous water mass in the study area. This suggests that the observed differences are a result of spatial/temporal hydrodynamic effects rather than induced by geographical variances between reference and farming stations.

Conclusions and lessons learned

Results of this case study demonstrated that detection of enhanced particle abundance around fish cages may not be solely a function of salmon farm waste, but potentially also of local influences (e.g. the effect of stocked cages on hydrodynamic patterns) and sampling effects (e.g. the effect of tidal cycles). Besides careful planning and execution of sampling programs we thereby stress the need for appropriate documentation of hydrodynamic conditions and the importance of collection of companion water-quality parameters (e.g. high resolution depth profiles of temperature and salinity) simultaneously with the target variables, such as particle concentrations, to ensure valid conclusions. With the current sampling design we were not able to differentiate between farm- and non-farm-related factors, but *a priori* (e.g. historical data and baseline studies) and *a posteriori* (e.g. analysis of companion parameters) data interpretation strategies helped to place the right level of confidence in the obtained results.

It should be noted that the results included here were above all a tool to highlight challenges in the design of sampling programs and data interpretation, and should not be used to form conclusions on the (absolute) effect of particle enhancement by salmon farming, as temporal coverage was limited (see also the section 'Align sampling regimen with study objectives' below). Furthermore, aspects highlighted here are not only relevant for quantification of particle abundances but do apply for most pelagic variables, including organic and inorganic nutrients in the water column.

Challenges related to sampling ammonium along a transect at an IMTA site in Canada

Study objectives

This study aimed to identify an ammonium 'signal' or concentration gradient, along a multi-depth transect, leading away from a mussel circle located at an IMTA farm in southwestern New Brunswick, Canada (Fig. 4).

Sampling design and data analysis

Specific species production data were unavailable, but the site consisted of eight 100 m circumference, 12 m depth, stocked Atlantic salmon *Salmo salar* polar circles, and one mussel circle. The mussel circle was constructed from a 70 m polar circle enclosing 4 concentric polar circles, each of decreasing circumference, where continuous socks of blue mussels *Mytilus*

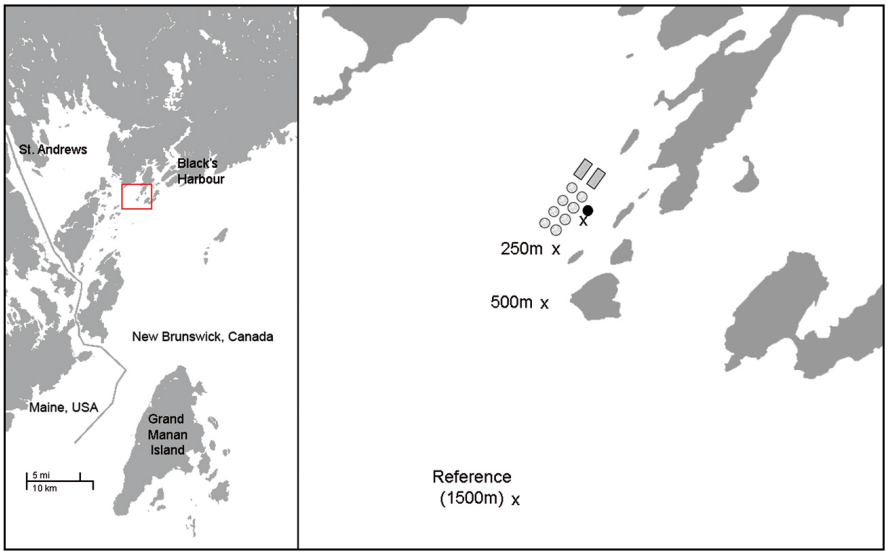

Fig. 4. Sampling locations at an integrated multi-trophic aquaculture farm in Canada. An area overview is displayed in the left panel; the square indicates the specific study area at high tide (right panel). Dark circle: a blue mussel 'polar circle' (70 m circumference); rectangles: kelp rafts; grey circles: Atlantic salmon cages. Sampling locations are denoted with an 'X', with liner distances indicated from 0 m. Latitude and longitude at 0 m are 66.865829°N and 45.029544°E, respectively

edulis were hung to a depth of 7 m. Mussels were approximately 40–50 mm at the time of sampling and were hung from the 4 inner circles. The mussel circle was located on the southeast side of a small salmon farm, and samples were collected along a linear transect at distances of 0, 250, and 500 m, with a reference location at 1500 m (Fig. 4). Sampling was in part opportunistic, based on vessel availability, weather, and the availability of analytical personnel. Sub-samples were collected for quality assurance of analytical analysis. An observation replicate was defined as the mean of 2 sub-samples drawn from 1 sampler 'drop'. Each station and depth (2, 5, and 10 m) was observed 3 times, replicated on 3 different days within the same week (11, 12, and 15 August 2011), with some exceptions due to technical difficulties. On Day 1 at the reference site, only 5 m depth samples were collected, and only 2 samples were collected at 5 and 10 m depth for the 250 m location on Day 2. Total sampling time on a specific day, took approximately 1 h, aiming to collect samples during the same tidal cycle, ebb or flood (see Fig. 6). Ammonium concentrations were analysed manually using a spectrophotometric technique by Holmes et al. (1999). Current was measured continuously during the 5 d deployment with Acoustic Doppler Velocimeters (ADV, Sontek Argonaut) at 5 m depths at the 0, 250, and 500 m locations, with speed and direction recorded as 5 min averages.

Ammonium results were statistically analysed by 1-way ANOVAs to determine differences between locations for each specific depth, and were followed by 1-tailed *t*-tests for comparisons of interest (Palisade 2014). Where *F*-tests identified unequal variances, *t*-tests for unequal variances were applied to compare means. To determine location means over the range of conditions during the sampling period, location concentrations were averaged across days (see Fig. 5). Post hoc statistical power tests for 1-way ANOVA and 1-tailed *t*-tests for independent means were estimated with G*power software (Faul et al. 2014). The best theoretical distribution was fitted to velocity data distributions using a parameter estimation approach, with best fits ranked by Akaike's information criterion using @RISK software (Palisade 2014). Current velocity data were non-normal, and normality was not achieved after transformation, so non-parametric Mann-Whitney tests were used to identify significant differences ($p < 0.05$) between locations.

Data interpretation

For sampling results on individual days, significant differences in ammonium concentrations were identified at each depth, except at 10 m on Days 1 and 2, for which there was insufficient statistical power for a credible test (Fig. 5). The largest concentration differences were observed on Day 3 at 5 m depth, when the concentration at the mussel circle was over a third higher than at other locations. Significant concentration differences at locations averaged over

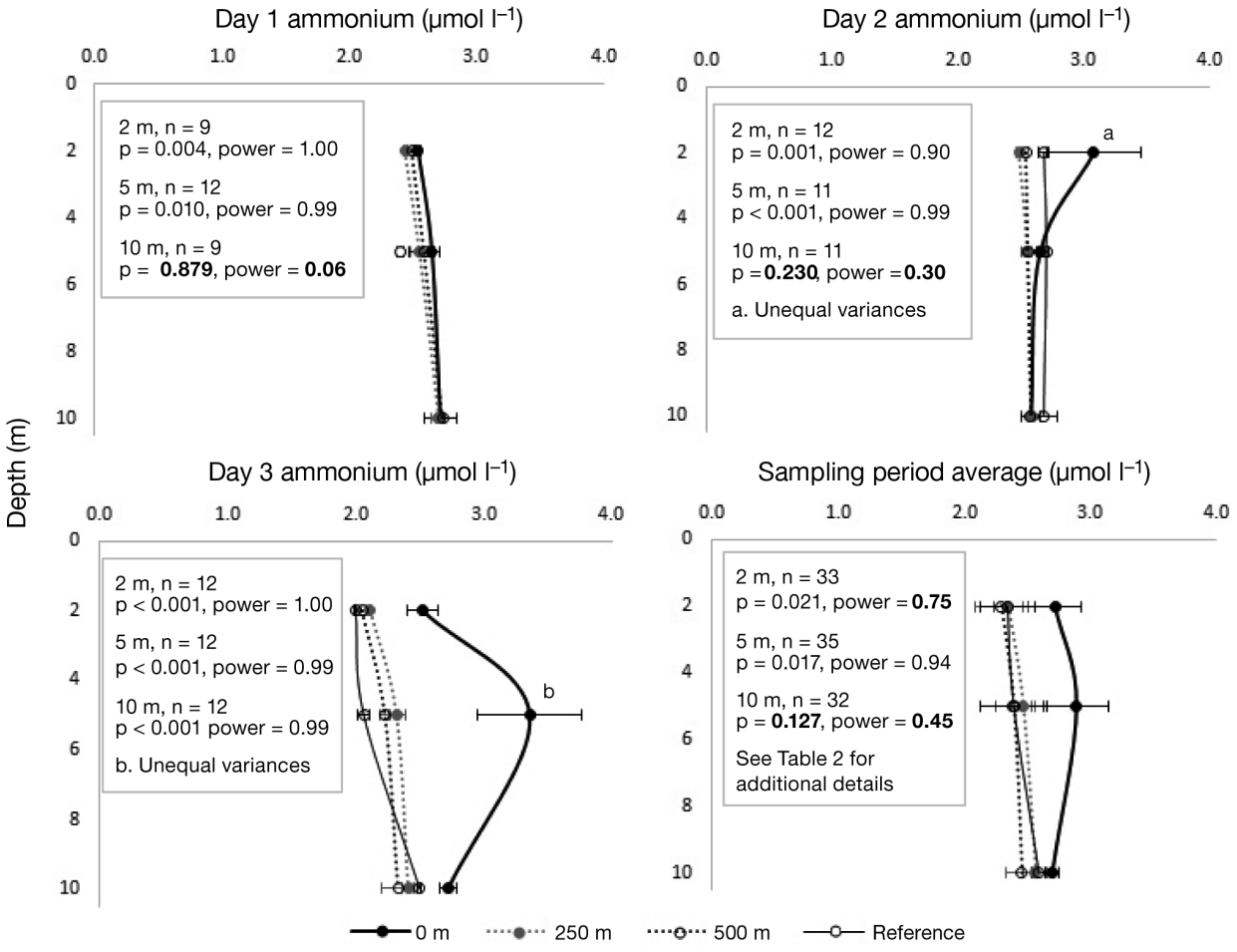

Fig. 5. Ammonium depth profiles measured various distances from a mussel circle at an IMTA site in Canada. Samples were collected on 3 different days over a 5 d summer period (11, 12, and 15 August). Lower right panel: location means averaged over the study period. Error bars are confidence intervals ($\alpha = 0.05$). Results of 1-way ANOVAs across depths and post hoc statistical power achieved (*F*-test family) are detailed within respective panels. Statistical insignificance ($p > 0.05$) and insufficient statistical power ($\beta - 1 < 0.80$) are indicated in **bold**. Where the ANOVA assumption of homogeneous variances was violated (a, b), *t*-tests for unequal variances were used to compare the mean farm (0 m) concentration. a: the difference between the farm and the closest concentration value, the reference site, was insignificant ($p = 0.90$), with insufficient test power (1-tailed = 0.63, 2-tailed = 0.45); the difference between the farm and 250 m, the largest concentration difference, was significant ($p = 0.03$) with sufficient power as a 1-tailed test (1-tailed = 0.90, 2-tailed 0.74); b: the difference between the farm concentration and 250 m, the closed concentration value, was significant ($p = 0.019$) with sufficient power (1-tailed = 0.99, 2-tailed = 0.95)

the study period (Fig. 5) were identified at 2 and 5 m depth, but not at 10 m (Table 2). However, only the ANOVA at 5 m depth achieved sufficient statistical power. The power of the 2 m depth ANOVA was 0.75. Upon closer examination, a *t*-test at 2 m depth between the farm concentration and that at 500 m (the location with the smallest concentration difference from the farm) was also significant and achieved sufficient power (0.84) as a 1-tailed test (Table 2), but insufficient power (0.71) as a 2-tailed test. This suggests slightly different outcomes depending on the test, in which data are partitioned for analysis, and greater power is achieved if a 1-tailed test can be used.

Ammonium concentrations at the reference site were variable, and reference site measures did not always have the lowest ammonium concentrations either. On Day 2, for example, reference site concentrations were significantly higher than those at the 500 and 250 m locations ($p < 0.001$). If it is assumed that the elevated ammonium was from the mussels and this was generally consistent during and between sampling days, the observed concentration differences would largely be a function of current flow and tidal cycle during sampling. Current direction at the mussel circle (0 m) and at 250 m locations were highly variable throughout the entire deployment (Fig. 6). However, samples collected during periods associated

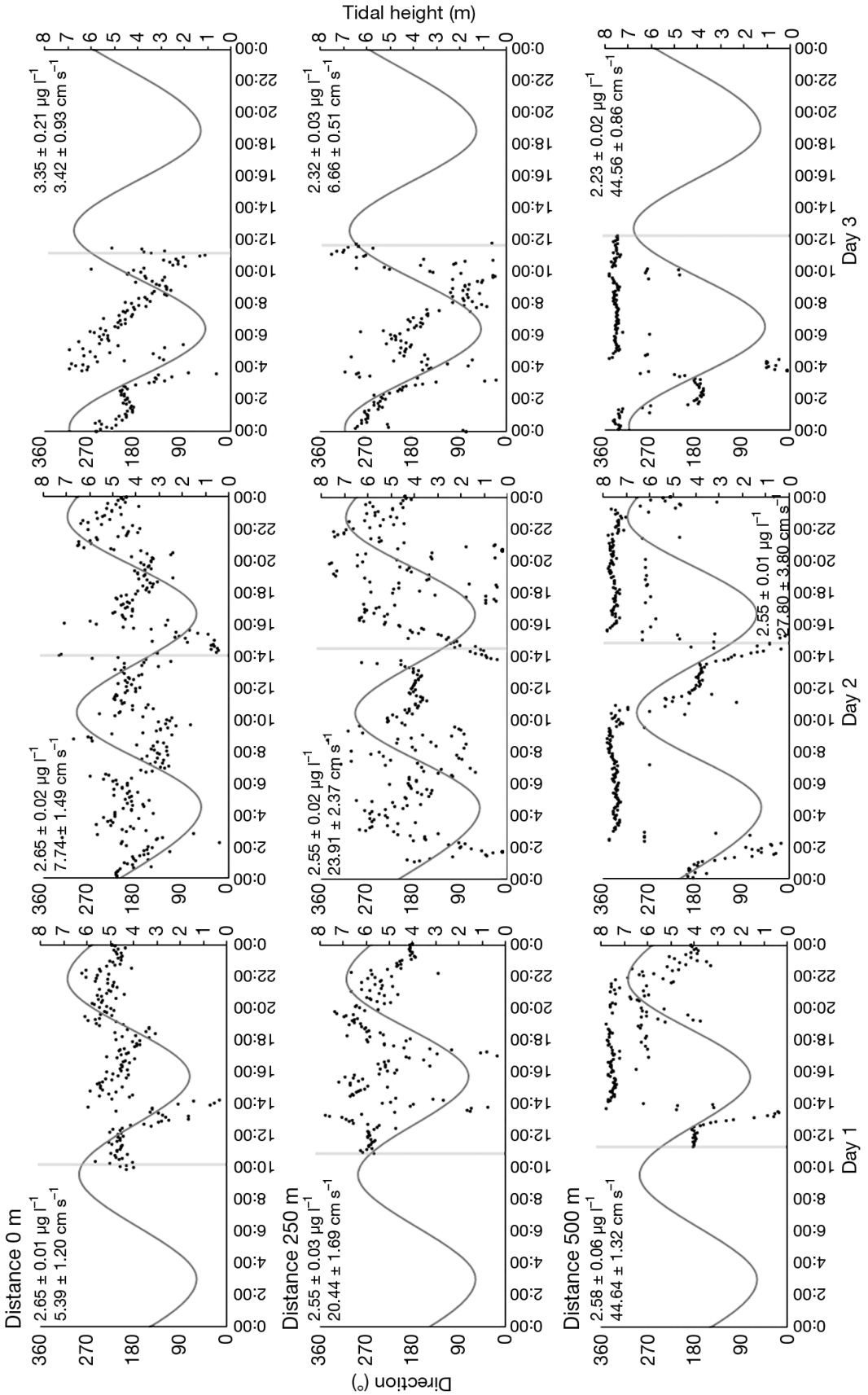

Fig. 6. Ammonium sampling times relative to current direction (dots, left *y*-axis) and tidal cycle (line, tidal height on the right *y*-axis; recorded from Back Bay, 2.6 km away) for the case study in Canada. Sampling times are indicated by the vertical line. Ammonium concentrations (n = 3) and mean current magnitude (n = 6) at 5 m depth during sample collection time are indicated in their respective panels (±SE). Internal ADV compass measures were corrected for magnetic declination, using Natural Resources Canada's magnetic declination calculator (http://geomag.nrcan.gc.ca/calc/mdcal-eng.php) for data collection dates

Table 2. Paired comparisons between averaged farm concentrations and the location with the smallest difference for the Canadian case study. One-way ANOVAs applied across days at each depth, excluding farm means (250 m, 500 m, and the reference site), showed no significant differences between other location means (2 m depth, p = 0.99; 5 m depth, p = 0.72; 10 m depth, p = 0.87) during the sampling period. *t*-tests were therefore used to confirm differences only between the farm mean and the closest concentration value at depth, as listed in the table. The *t*-tests and post hoc power tests are 1-tailed. Insufficient power in **bold.** Coefficient of variation (CV; SD/mean)

Paired comparison	n	Ammonium (mean; µmol l^{-1})	SD	CV (%)	Difference from farm	*t*-test, p-value	Power achieved
Depth 2 m							
0 m (farm)	9	2.72	0.32	12.0			
500 m	9	2.36	0.23	9.9	13.1%	0.030	0.84
Depth 5 m							
0 m (farm)	9	2.89	0.40	13.7			
250 m	9	2.46	0.13	5.3	14.6%	0.007	0.90
Depth 10 m							
0 m (farm)	9	2.70	0.08	2.9			
Reference	6	2.59	0.12	4.5	3.9%	0.028	**0.62**

with directional change, such as Days 2 and 3, could be subject to influence from other farm sources such as salmon. The high variability of flow direction, even within a particular stage of the tidal cycle, suggests an absence of a stable near-field 'nutrient plume', making it difficult to draw conclusions from sampling programs based on single sample mean. The same distribution shapes of current velocity for the 0 and 250 m stations (Fig. 7) suggest similar hydrodynamic influences at both locations; this seems consistent with similarities of near-shore locations and the close proximity to farm structures. The current velocity, direction (Fig. 6), and distribution shape (Fig. 7) of the 500 m sample suggest an entirely different exposure and current regimen which was more consistent during stages of the tidal cycles. This is not surprising given the bathymetry of the area, but demonstrates the difficulty of selecting appropriate reference sites for this location.

The slowest average current speed measured over the 5 d sampling period occurred at the mussel circle, along with the highest mean ammonium concentration in the study (Day 3, 5 m depth). This particular outcome is consistent with expectations of volumetric loading, dilution, and advection with distance, where minimal flushing manifests increased nutrient concentrations; however, this was not always the case. Day 1 and 2 concentrations for this station were similar, despite faster current flow on Day 2. Single concentration means measured at the mussel circle could not consistently be explained by changes in current flow. This aspect, in combination with the apparent unreliability of reference concentrations, make it difficult to determine the proportionate contribution of ammonium from aquaculture sources to individual measures. Averaged current velocities, however, do show a progressive increase ($p < 0.05$) in mean velocity, moving away from the potential influence of farm structures (Fig. 7).

Conclusions and lessons learned

A logistically practical sampling design of samples collected on 3 separate days, at 4 different locations, and at 3 depths was capable of identifying a definitive ammonium signal at an IMTA site. Elevated concentrations at the farm could, in part, be explained by proximity to a mussel circle and current flow dynamics. This supports the use of companion parameters, such as current speed and direction in this study, to help interpret results of discrete water-quality samples. While higher concentrations were measured at the farm site, a well-defined gradient measured across sampling locations was not readily identifiably. This suggests sampling transects should occur closer to the farm (<250 m), to improve chances of quantifying a nutrient gradient. Ammonium concentrations, in general, were variable but relatively modest, with the maximum farm concentration approximately a third that of other locations. Variation of natural sources is not surprising in light of historical August variation in the embayment—presumably a function of tidal cycle, other anthropogenic loading sources, and marine life. All ammonium concentrations measured in this study were well within the range of historical values (Martin et al. 2006), suggesting that, under the study conditions, ammonium was not accumulating in any substantial quantities and near-field pelagic impact from the farm was limited.

In the absence of good information on natural variation (*a priori* data) to guide sampling design, post hoc power tests were a useful tool to determine if sufficient statistical power was achieved. Insufficient statistical power occurred when differences between means were small or the coefficient of variation (CV; standard deviation/mean) increased, which is consis-

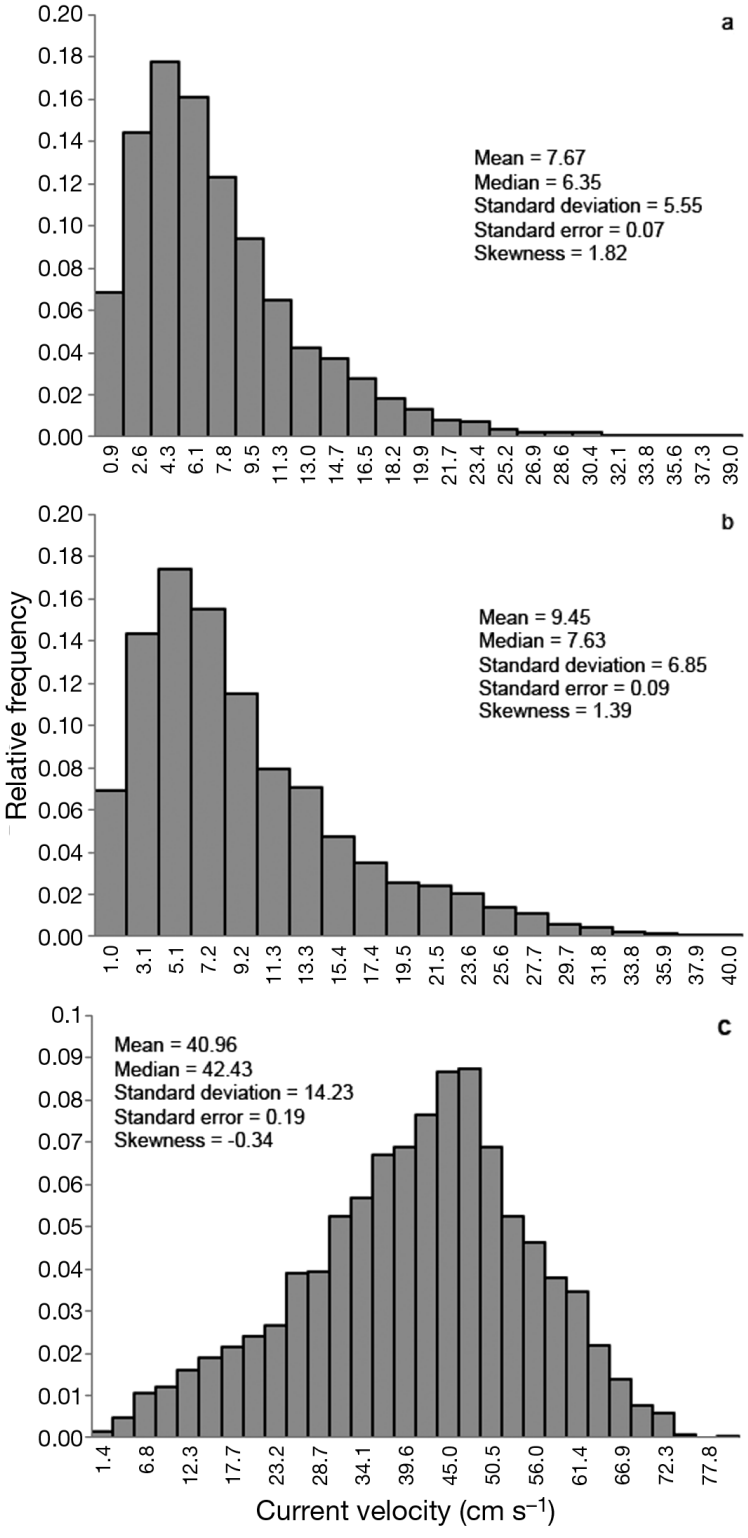

Fig. 7. Current velocity data distributions at 5 m depths, measured over 4 d. (a) Mussel circles: (a) 0 m, (b) 250 m, and (c) 500 m. All distributions are non-normal, and significantly different from each other ($p < 0.001$). Best theoretical distribution fits for 0 and 250 m locations are the Pearson 6 distribution, and a general beta distribution, for 500 m location

tent with statistical principles of power analysis (Berndtson 1991). From a scientific perspective, insufficient power due to small differences between means was less problematic, since these concentration differences were ≤0.15 µmol l^{-1}, and arguably negligible, in a biological sense. Away from the farm influence, the CV was small for individual samples means (i.e. collected in 1 d), suggesting that sampling duration (time to collect 3 samples at depth) occurred well within the timeframe for changes of natural cycles (e.g. tidal). These outcomes may provide a cost–benefit rational for reducing sample requirements at locations where less variation is anticipated (e.g. reference sites), assuming due consideration is given to statistical comparisons with unbalanced data.

CONSIDERATIONS FOR SAMPLING DESIGN, EXECUTION, AND DATA INTERPRETATION

The case studies emphasized several unique challenges related to quantification of pelagic influences from open-water aquaculture farms. In the following section we further explore sampling requirements that should be considered to achieve robust results and to discuss methods to improve the cost–benefit of sampling regimens. We thereby aim to provide an integral overview (Table 3), including lessons learned from the case studies, as well as additional information drawn from the literature.

Align sampling regimen with study objectives

Defining study objectives is the first step in designing a sampling scheme. While this seems rudimentary, different objectives may manifest very different approaches with regard to the parameters measured, sampling frequency, and spatial or temporal scaling. If for example, the objective is to quantify the maximal nutrient or particle signal at a northern temperate water fish farm, sampling should occur at peak temperatures (usually early fall) during second-year production (Atlantic salmon have an 18–24 mo production cycle) to ensure biomass, growth rates, feed intake, and, consequently, nutrient loading are at a maximum (Reid et al. 2013). If the study objective is to accurately quantify the effect of annual nutrient loads, sampling must occur across

Table 3. Overview of considerations for sampling design, sampling execution, and data interpretation of discrete water-quality sampling around aquaculture facilities

Sampling design
- Ensure sampling design can meet study objectives, with due consideration for statistical sample requirements, analytical capacity, and logistical practicalities
- Obtain *a priori* knowledge of abiotic and biotic conditions, if available, to help quantify hydrodynamics and 'patchiness' of ambient water-quality parameters to guide sampling design
- Exploit high-resolution autonomous or automated sampling of companion water-quality parameters to assist interpretation of discrete water samples
- Sample in a horizontal transect, orthogonal transect, or grid surveys where possible, to identify a gradient of effects if reference sites are inadequate
- Selection of multiple reference sites will help identify other sources of spatial variation that could confound data interpretation
- Define vertical sampling to account for seasonal stratification and depth-dependent data
- Consider requirements for minimal detectable difference between sample means as this will largely dictate the sample numbers required to achieve acceptable statistical power—the smaller the difference to be detected, the larger the sample size required

Sampling execution
- Ideally, obtain (planned) information on production, farm management, and husbandry (i.e. feeding regimens, disease treatment, feed delivery, or biofouling removal) during the timeframe of sampling to assist with determining causality of potential effects
- Be aware of vertical water sampler drift to ensure sampling occurs at the intended location
- Consider short-duration sample collection (use of multiple vessels could be considered) to reduce confounding effects of fluctuating tides and currents
- A single 'drop' of deployment and sample retrieval from a vertical point sampler should be considered a sample. Multiple water draws from a container should be considered sub-samples and not replicate samples

Data analysis & interpretation
- Skewed data distributions of water quality at aquaculture sites are not uncommon. Given enough samples, parametric and non-parametric data metrics are apt to present similar information on data spread (e.g. variation, range). Non-parametric analysis is unlikely to reduce the number of samples required for valid statistical comparison
- Averaging location means across a timeframe of interest (e.g. days, weeks) will help to capture additional sources of variation
- Irrespective of careful study design and sample collection, conditions at small scales may still vary due to inherent patchiness. *A posteriori* interpretation of target-variable data from discrete sampling, together with companion parameter data, will facilitate data interpretation to improve robustness of conclusions drawn from limited datasets

the range of seasons and biomass values present over a full production cycle. Additional parameters would be required if the objective were to identify the best placement for co-cultured IMTA species to intercept transient nutrients. Not only are nutrient concentrations, current direction, and frequency of change required, speed data would also be required, as the rate at which organic particles are delivered is just as important as the amount (Cranford et al. 2014). The achievement of ideal study objectives, however, is often tempered by logistic and analytical realities. To achieve meaningful results, it should be considered whether the maximum practical sampling effort is sufficient to achieve the minimally acceptable study objectives.

Ambient environmental conditions

Evaluation of the variability of the ambient background conditions is an important consideration for assessing aquaculture effects (Fernandes et al. 2001). Prior (*a priori*) historical knowledge of abiotic and biotic conditions at the study site is highly beneficial for defining sampling requirements. Coastal environments are usually characterized by large natural spatial variability. The natural variability of ammonium concentrations in the Canadian case study made it difficult to infer the contribution of farm nutrients to sample means. 'Baseline analysis' is also a useful strategy to identify starting points for evaluation. For fish farming this would be represented by the conditions prior to farm establishment or when no fish are present in pens. This method is sometimes described as the BACI (Before-After-Control-Impact) design (Stewart-Oaten et al. 1986, Underwood 1991) and has been used in some studies of ecology and aquaculture (Rodríguez-Gallego et al. 2008). Baseline data can also be used to validate reference stations, as in the Norwegian case study. Reference site(s) provide insight into ambient conditions and variability, if selected appropriately. Selection of reference sites is non-trivial. Ideally they should be close enough to the farm to reflect local conditions, but distant enough so as not to be influenced by the farm itself. This is not just an aquaculture research problem; choosing reference sites is an ongoing challenge for all ecological studies (Stewart-Oaten et al. 1986, Smith et al. 1993).

An additional concern with the assessment of pelagic aquaculture nutrients is that most coastal systems are affected by multiple anthropogenic sources, with the potential to be superimposed over natural

variability (Fernandes et al. 2001). For cage aquaculture studies in developed coastal regions, it may be extremely difficult to find reference sites, remote enough from other aquaculture operations or other anthropogenic sources, while still reflective of farm-exposed hydrodynamics (Troell et al. 2003). Where baseline data are unavailable, sampling of multiple reference sites can be a powerful assessment tool to help ensure ambient spatial variability is captured (Fernandes et al. 2001, Merceron et al. 2002, Reid et al. 2006, Yucel-Gier et al. 2007, Rodríguez-Gallego et al. 2008). However, many studies have only a single reference location, presumably due to practical constraints. Sampling along transects may circumvent some of the issues concerning single reference sites, as such sampling can potentially identify concentration gradients as a means of inferring causality (discussed in the next section).

The amplitude of ambient nutrient concentrations may require special procedural considerations. Typically, the limiting nutrient is of most interest in evaluating farm-induced effects, i.e. nitrogen in marine systems (Howarth & Marino 2006) and phosphorus in freshwater (Dillon & Rigler 1974). Both require different sampling considerations. Collection of larger sample volumes is, for example, advised for ammonium measurements in low concentrations (Holmes et al. 1999). Most total phosphorus (TP) techniques involve acid digestion of non-filtered samples (Reid et al. 2006), which means that the presence of particulates may result in higher sample variability or 'spikes'. Sub-samples may then be necessary to identify sources of variation.

Physical processes

Both case studies indicated that hydrographic conditions and water exchange mechanisms are keys to understanding the distribution of aquaculture discharges. The speed at which current passes through fish cages dictates the volumetric exchange (flushing) and thereby dilution potential (Merceron et al. 2002, Reid & Moccia 2007, Middleton & Doubell 2014). Sampling regimens are often based on site hydrodynamics, and benefit from previous knowledge of current flows. As shallows or islands can influence local current patterns, impacting current speed and nutrient concentrations (Sanderson et al. 2008, Groeskamp & Maas 2012), it is also important to consider the bathymetry and hydrography along the chosen transect.

For discrete sampling regimens in coastal ecosystems a horizontal transect is often adopted, with stations established at a certain distance (e.g. up to 1000 m from the cages) down-current from the aquaculture site (Mantzavrakos et al. 2007, Navarro et al. 2008, Neofitou & Klaoudatos 2008, the present Canadian case study). Identification of a horizontal gradient — with the appropriate resolution — helps to minimize the confounding effects of variable hydrodynamics and multiple anthropogenic sources. Other spatial study designs, for application to complex flow conditions, include orthogonal transects or a grid survey (Fernandes et al. 2001, Sanderson et al. 2008). Spatial gradients obviously provide a greater ability to assess spatial resolution of nutrient spread than simple comparisons between farm and reference sites. Furthermore, Sanderson et al. (2008) and Petrell et al. (1993) have reported that inorganic nitrogen transit from fish cages is commonly non-linear; this may be reflected in the occasionally higher concentrations measured further from the farm compared to measurements directly beside net pens. The authors suggest this relates to complex near-field hydrodynamics in the proximity of fish cages. As detailed in the Canadian case study, a range of factors may affect flow patterns, resulting in variable and fluctuating current directions (Petrell et al. 1993, Huang et al. 2008, Reid et al. 2010), consequently leading to complex nutrient plume dynamics in the near-field or close to structures (Reid & Moccia 2006, 2007). In addition to hydrodynamic influences from cages (Løland 1993, Helsley & Kim 2005, Lader et al. 2007, Le Bris et al. 2007), near-cage currents can be affected by the swimming behavior of fish (Gansel et al. 2011, 2014) as postulated in the Norwegian case study. The potential influence to currents and depth of fish excretion and egestion may be important considerations, not only for horizontal vectors, but also for vertical. Waste release is expected to occur at the depth of maximum fish biomass, which, in turn, is a function of environmental influences such as temperature and oxygen, as well as feed location and perceived threats (Oppedal et al. 2011), which may vary hourly, diurnally, and seasonally. Sampling programs that aim to quantify morphology of a 'nutrient plume' need to target both vertical and horizontal profiles. At sites with well-mixed water columns, background values will be similar across depths (e.g. Fig. 5, Canadian case study), whereas in areas with stratified water columns, depth profiles may vary for several variables (e.g. Fig. 2, Norwegian case study). As the latter water bodies can be partitioned by sharp halo-, thermo-, or pycnoclines, values may change within just a few meters depth. Under such conditions, sampling depth may strongly affect environmental parameters.

Sample collection mechanics (sampling execution) should therefore be considered, especially in relation to the effects of flow velocity and depth. Current and drag forces can induce swing of the deployment line holding the water sampler, with swing magnitude potentially increasing with depth. This may result in sampling bias, with the greatest potential for deviation along the horizontal axis. The authors have routinely observed drag inducing a swing of between 5° and 15°. If for example, a sample is collected at a 10 m depth with a drag-induced swing of 15° on the deployment line, the Pythagorean theorem indicates a horizontal displacement of 2.6 m and a vertical deviation of 0.35 m. While this may not seem much, some near-field data requirements such as the placement of co-cultured species in IMTA may necessitate spatial data on the scale of several meters. Displacement becomes more problematic if such accuracy is required at greater depths. If the depth is increased to 30 m, the same angle of swing causes a horizontal displacement of 7.8 m and a vertical deviation of 1 m.

Apart from the effects of physical processes on horizontal and vertical spatial variability, temporal aspects should not be neglected given that, in tidally driven areas, water column properties can change within hours. If a study aims to quantify water-quality data under relatively consistent environmental conditions, sampling should occur within the duration of a specific tidal phase, to improve the chances of capturing such conditions. This presents a very short sampling window. However, sampling within a specific tidal phase is no guarantee of consistent predictable flow either (see Norwegian case study), and unpredictable and unstable flow directions might be observed near and around farm structures (see Canadian case study). Appropriate data integration across time, at each location, will help to quantify temporal variation and should ultimately reveal the magnitude of effects spatially (see Canadian case study). The resultant location mean will be a combined function of the effect magnitude and occurrence frequency. When comparing between seasons, or sampling during a stormy season, one should be aware of the resuspension of bottom material or breaking down of pycnoclines. Resuspension of material requires a certain threshold level of energy; once such thresholds are reached by extreme events, nutrient conditions can change and affect water-column properties significantly, especially for shallow sites (Wallin & Håkanson 1991, Jones et al. 2012).

Farm management and husbandry

Waste released from commercial farms is not a continuous process but varies on temporal scales and, in addition to environmental factors, can be influenced by farm husbandry practices. Short-term (diurnal and daily) variation in waste release may be influenced by feeding regimens (Lander et al. 2013) and variable ammonia release with post-prandial excretion peaks (Brett & Zala 1975), while phosphorus can leach immediately from aquafeeds upon submersion in water (Reid & Moccia 2006). Occasional disease and treatment may result in a reduction or cessation of feeding (Ashley 2007). Furthermore, the cleaning of nets to remove biofouling adds a recurring waste flux from farms. Sampling should be avoided during such episodic events. Long-term variation in nutrient loading is also a function of the farm production cycle. Fish biomass increases until harvest, and, in the case of Atlantic salmon in temperate waters, almost 4 times more nutrients will be released in the second year of production (Reid et al. 2013). The stage of the production cycle therefore strongly influences the amount of feed entering the water, consumption, and nutrient loading accordingly. Variation in farm husbandry practices, such as changes in feeding regimen, fish harvesting, off-feed events (e.g. veterinary treatment), and site fallowing may influence pelagic sampling strategies and the interpretation of results. It is therefore beneficial to obtain knowledge about the farm site, husbandry practices, and production details, if available, before devising an appropriate sampling strategy.

Statistical considerations

The data analysis approach is primarily a function of study objectives, and there are many good resources detailing experimental design and analysis (e.g. Quinn & Keough 2002). We therefore only highlight some of the most relevant considerations to the statistical analysis of discrete water samples.

The 'patchiness' and variability of nutrient concentrations around fish cages may result in skewed data distributions. This is often a result of a few elevated measures in combination with a fixed minimum value, such as the background concentration, which may result in a non-normal distribution (Reid et al. 2006). As there will be no measures below the background concentration, the lower end of the data distribution may be abrupt, and the asymptotic tail which occurs in a normal distribution, absent. It may there-

Table 4. Replicates needed per treatment group for studies of 80% power at $p < 0.05$, for 2-tailed tests with 2-treatment experiments (modified from Berndtson 1991). For experiments with a 1-tailed test, the replication shown would provide an experiment of 90% power at $p < 0.025$. Coefficient of variation (CV; SD/mean)

CV (%)	Difference from reference to be detected (%)									
	5	10	15	20	25	30	35	40	45	50
1	3	2								
2	4	3	2							
3	7	3	3	2						
4	12	4	3	3	2					
5	17	6	4	3	3	2				
6	24	7	4	3	3	3	2			
7	32	9	5	4	3	3	3	2		
8	42	12	6	4	3	3	3	3	2	
9	52	14	7	5	4	3	3	3	3	2
10	63	17	9	6	4	4	3	3	3	3
12	91	24	12	7	5	4	4	3	3	3
14	124	32	15	9	7	5	4	4	3	3
16	161	42	19	12	8	6	5	4	4	3
18	204	52	24	14	10	7	6	5	4	4
20	252	63	29	17	12	9	7	6	5	4
25	393	99	45	26	17	12	10	8	6	6
30	566	142	63	37	24	17	13	10	9	7
35	770	193	86	50	32	23	17	14	11	9
40	1005	252	112	63	42	29	22	17	14	12
45	1272	318	142	80	52	37	27	21	17	14
50	1571	393	175	99	63	45	34	26	21	17

fore be tempting to consider non-parametric statistical approaches in these circumstances. However, non-parametric statistics are not as good at detecting differences among means (Steel et al. 1997) and may therefore require more samples in order to achieve sufficient power for detecting differences compared to parametric approaches. This suggests that the use of non-parametric statistical tests does not resolve issues of large sample requirements. As the number of sample 'replicates' increases, error measures of means (parametric) and medians (non-parametric) from the same samples will eventually overlap and communicate similar information (Reid et al. 2006).

The collection of true replicates for discrete water sampling is an important consideration. Multiple water draws from a single sampler 'drop' are, in essence, sub-samples and are likely to reflect the analytical technique rather than the variation in time and space at the sampling location. Treating these as true replicates is apt to underestimate the actual variation. This means that collection of statistical replicates necessitates multiple sampler 'drops'. However, as a result of dynamic water movement around aquaculture facilities, deploying multiple 'drops' actually leads to sampling of different pockets of water. Therefore, this approach does not necessarily result in the collection of true sample replicates either, but samples can be considered spatial replicates during the time of collection.

The number of sample replicates required to achieve the desired statistical power is an important consideration for experimental design or the post hoc assessment of the power achieved. For discrete concentrations in the Canadian case study, it was the probability of correctly rejecting a null hypothesis of ammonium concentrations being equal, when they are not. Table 4 shows the replicates per treatment group for *a priori* power testing, for the 2-tailed tests ($p < 0.05$) needed to achieve a power of 80%, which is the minimum statistical power recommended for parametric tests (Berndtson 1991). As the percent difference that is detected between samples decreases or the treatment coefficient of variation (CV) increases, the replicates needed increase exponentially. Therefore, careful consideration of study objectives, such as the minimum effect size requiring detection, is needed, as this could substantially affect the number of replicates required. In the absence of detailed *a priori* variation data, post hoc power tests are a useful tool in determining whether sufficient statistical power has been achieved. Results from the Canadian case study suggest how increasing spatial and temporal sample coverage can affect sample numbers. Under a similar CV and detectable difference between means (>0.15 µmol l^{-1}), a sample size (n) of 9 was just sufficient to achieve acceptable statistical power for 1-tailed tests of independent means (see also Table 2 and Fig. 5). Under similar conditions of 9 replicates per location, a modest sampling regimen of 4 locations at 3 different depths necessitates 108 samples. This suggests that a thorough spatial coverage of aquaculture sites with discrete sample collection, necessitating wet chemistry techniques, can quickly become logistically and analytically expensive. Large sample numbers will also require longer collection times, increasing the chance that environmental conditions may change during collection. In the Norwegian case study, current direction changed from 60° to 160° within 30 min, emphasizing the need for rapid sampling to document instantaneous conditions (i.e. 'snap-shot').

Companion parameters

Given the limitations to discrete sampling and, therefore, the limited number of samples, companion parameter data can help to interpret discrete sample results or can help to design a robust sampling pro-

gram. Ideally, good current flow data obtained during the sampling campaign can indicate whether water flowing from cages is being directed to near-field sampling locations. Fine-scale hydrodynamic modelling of plume dynamics can provide the context for fieldwork site selection and sampling design. But there are also valuable alternative approaches. One option is the application of discrete sampling in combination with other continuously measured indicators, such as dissolved oxygen, temperature, pH, salinity, and turbidity. Continuous sampling using *in situ* sensors enables greater sampling frequency and, therefore, better measures of variation. Continuous CTD measures in the Norwegian case study (Fig. 2) showed that water column characteristics were different between farming and reference stations, suggesting that higher particle abundance was not necessarily a function of the farm and that results from discrete samples should be interpreted with care. Reid et al. (2006) demonstrated that a strong inverse relationship between total phosphorus and dissolved oxygen occurred directly in trout cages, a lesser relationship down-current, and no relationship up-current from the cages. The magnitude of the variation and the strength of the relationship (r^2) reflected current direction and dilution with distance, relative to fish location. The oxygen–phosphorus relationship suggests that continuously measured companion parameters collected *in situ* at fish cages could be used as proxies for nutrient concentration trends.

The development of *in situ* sensors in aquatic sciences is in its infancy but is evolving rapidly, for companion parameters as well as for variables of interest (Bende-Michl & Hairsine 2010, Mukhopadhyay & Mason 2013, Wild-Allen & Rayner 2014). At present, *in situ* electronic monitoring technology can enable either sufficient temporal coverage or good spatial coverage, but often not both simultaneously. Multiprobes or CTDs (i.e. conductivity, temperature, and dissolved oxygen) work well for temporal assessment, but when deployed autonomously they are typically left in 1 location over days or weeks. Continuously monitoring submersible instruments that are boat towed (Brager et al. 2015) or autonomous remote operating vehicles, can enable good spatial coverage, but these are typically limited to collection durations on the order of hours. Ironically, there may also be challenges with large amounts of data. Even short sampling episodes using sensors can produce large amounts of data compared to discrete sample results. Such large data volumes may require various tools for post-processing, and issues like auto-correlation may become relevant.

Another useful proxy for nutrient dispersal is the use of bioindicators. In aquaculture studies, biomarkers such as stable isotopes, fatty acid profiles, and pigments are progressively being used to trace fish wastes in seaweeds (Garcia-Sanz et al. 2010) and shellfish (Mazzola & Sara 2001, Both et al. 2012, Graydon et al. 2012, Handå et al. 2012b, Jiang et al. 2013). In order to identify a signal from biomarkers, the needed timeframe of applicability may be on the order of months to years. An alternative time-integrated approach suited to shorter timeframes, such as days to months, is the use of biocollectors or sediment traps. Biocollectors are substrates that can be used to sample commonly occurring fouling organisms and are similar in design to those used for monitoring aquatic invasive species (Sephton et al. 2011). With the rationale being that, with the provision of appropriate habitat and all things being of equal density, fouling would increase as a function of nutrient availability. The merits of this approach are under investigation in an aquaculture context (Cooper 2013). Sediment traps can also be deployed to collect settling material (Findlay & Watling 1997), although this is typically done to assess benthic impacts. Slow-settling particulate wastes are often diluted to an extent that limits detection in discrete water samples; sedimentation over a timeframe of days to weeks may provide greater discriminative power when comparing locations (authors' unpubl. data).

Considerations of biological activity, far-field, and cumulative effects

Upon consideration of pelagic sampling design, it should be acknowledged that dispersion of farm wastes is not solely a function of hydrodynamics, as concentrations of dissolved particulate and inorganic nutrients are also a function of fish nutrient load, background concentrations, as well as ambient biological activity. The uptake and transformation of farm waste products can be very rapid, especially in nutrient-limited systems, and may become manifest as reduced farm nutrient concentrations in the water column. Overall environmental impact should therefore be evaluated according to both the living and non-living suspended fractions in the water column (Sará 2007c).

Discrete sampling programs often target the localized footprint of a farm, usually as a function of study objectives, but intensive fish farming may also affect regional impacts on marine ecosystems. Hence, envi-

ronmentally sustainable fin-fish farming necessitates an understanding of the farming impact potential beyond the immediate production area (Husa et al. 2014). There is, however, a knowledge gap on waste spread and persistence over large areas, as well as on the cumulative effects of multiple farms in a region (Price et al. 2015). Far-field or regional effects generally require different tools, such as biomarkers (see also 'Companion parameters' above) or modelling (Skogen et al. 2009). Near-field nutrient plumes can be modelled assuming an appropriate distance from cages, choosing a reasonable timeframe for sample integration (e.g. a daily average), and making the appropriate simplifying assumptions (Reid & Moccia 2007, Middleton & Doubell 2014). As an extension of this approach, models with volumetric loading and spatial components can subsequently be scaled up to determine the assimilative capacity of a region (Strain & Hargrave 2005, Skogen et al. 2009; see also ECASA toolbox at http://www.ecasatoolbox.org.uk/the-toolbox/informative/matrix-files/fin-fish-farming-environmental-impact-assessment). In regions where there are multiple potential loading sources within the same body of water, the possibility of synergistic effects could also be considered through modelling (Fernandes et al. 2001). Models do not negate the need for data collection, but require data for model development and validation, and, consequently, appropriately designed sample regimens.

CONCLUSIONS

With this paper we demonstrated that discrete pelagic sample collection within a dynamic system like commercial-scale aquaculture sites presents a unique set of challenges. Adequately capturing the spatial and temporal dynamics often requires large sample numbers, which may lead to practical and logistical limitations in discrete sampling regimens. Consideration of the balance between the information needed and the effort expended to acquire it is, therefore, non-trivial. Assuming study limitations have been properly identified and applying the strategies discussed in this paper (summarized in Table 3), the amount of information from discrete sample data sets can be maximized. When the need does arise for discrete sample collection of pelagic nutrients, farm operators and researchers need not be discouraged once armed with the appropriate tools.

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