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Aquaculture organic enrichment of marine sediments: assimilative capacity, geochemical indicators, variability, and impact classification

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ABSTRACT: Benthic organic enrichment at 2 high-flow Atlantic salmon *Salmo salar* farms and at a low-flow mussel *Mytilus edulis* farm was studied to assess the capacity of local physical and biological processes to assimilate organic waste inputs. Geochemical metrics served as proxies for detecting potential biological effects. High-flow sites are generally predicted to assimilate organic enrichment by flow- and wave-induced waste dispersion and metabolic processes. However, a decrease in porewater dissolved oxygen was detected out to 100 m at the salmon farm with cohesive sediments and to approximately 1000 m outside the farm with permeable sediment. Sediment oxygen consumption was responsive to the vertical flux of organic matter, resulting in hypoxic conditions. An increase in total free sulfides ($H_2S + HS^- + S^{2-}$) in porewater was restricted to the immediate vicinity of both salmon farms. Despite exhibiting a high degree of small-scale patchiness, benthic effects were greatest at the fish farms during the pre-harvest period, regardless of season. Natural organic enrichment at the mussel farm constrained the assimilative capacity for biodeposition, resulting in substantial free sulfide accumulation. Sediment free sulfide analysis at a wide array of fish and shellfish farms showed that the ion-selective electrode method that is widely prescribed for regulatory aquaculture monitoring gave biased readings relative to methylene blue colorimetry and direct UV spectrophotometry. The ecological quality status classification system was extended to include quantitative relationships between a wide range of geochemical and biological variables employed worldwide to monitor and regulate the effects of benthic organic enrichment.

KEY WORDS: Organic enrichment · Assimilative capacity · Free sulfide · Hypoxia · Benthic macrofauna · Monitoring · Ecological quality status

1. INTRODUCTION

The effects of aquaculture organic wastes on marine benthic geochemistry and micro- and macrofauna communities are well known and follow the general pattern of responses to an organic enrichment gradient described by Pearson & Rosenberg (1978), Nilsson et al. (1991), Diaz & Rosenberg (1995), Nilsson & Rosenberg (2000), Pearson & Black (2001), Gray et al. (2002), Brooks & Mahnken (2003), Diaz et al. (2004), Hargrave et al. (2008), Keeley et al. (2012), and Cranford et al. (2020). The effects of organic enrichment on the benthos are largely attributed to a combination of oxygen stress and the toxic effects of

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free sulfide accumulation in surficial sediments (reviewed by Hargrave et al. 2008). Organic loading can increase the biological oxygen demand of sediments to the point where natural processes that resupply oxygen into the sediment are unable to prevent the development of hypoxic to anoxic conditions. In the absence of oxygen, microbes continue to decompose the excess organic matter (OM) through several anaerobic respiration processes that occur in a characteristic sequence. Quantitatively, the most important of these processes in marine systems is sulfate reduction (Fenchel 1987) in which sulfate is reduced to sulfide gases (H_2S, SH^- and S^{2-}) that dissolve in sediment porewaters. These total free sulfides (collectively referred to as S^{2-}) are highly toxic to most invertebrate species (Bagarinao 1992, Grieshaber & Völkel 1998, Wang & Chapman 1999, Gray et al. 2002, Hargrave et al. 2008, Cranford et al. 2020), and the adverse effects are enhanced by the hypoxic and anoxic conditions (Vaquer-Sunyer & Duarte 2010).

Credible environmental management systems are required to minimize, mitigate, or eliminate potential adverse habitat and biological effects associated with OM deposition at open-water aquaculture farms. Numerical modelling is an integral part of such a system through the ability to simulate the major physical processes that control solid waste deposition and redistribution. However, prediction of the magnitude and spatial scale of benthic habitat and community effects can be confounded by numerous additional factors that can act as natural buffers against the development of adverse benthic effects. These site-specific factors include (1) physical and geological conditions influencing the rate of oxygen re-supply into the sediment, (2) geochemical conditions limiting S^{2-} precipitation and/or re-oxidation, and (3) biologically mediated degradation processes. Biogeographic variations in the natural ability of these and other processes to absorb detrimental effects from organic deposition (i.e. the assimilative capacity) appears to explain why carbon deposition rates reported to cause significant benthic habitat and community effects are known to vary widely across different studies (Giles 2008, Keeley et al. 2013, Bravo & Grant 2018). Bravo & Grant (2018) reported the results of a numerical model designed to assess the capacity of surface sediments to assimilate organic enrichment through biological processes. These authors concluded that the capacity of the environment to assimilate sediment organic enrichment should not be exceeded in high-flow environments, which they defined as having mean tidal currents >9.5 cm s^{-1}. The effect threshold used in that study was 1500 µM S^{2-} in surficial sediments, which indicates approximately a 50% reduction in benthic infauna taxa (Hargrave et al. 2008). The present study investigated the effects of aquaculture organic enrichment at high-flow fish farms located over cohesive and permeable sediments, and at a relatively low-flow suspended shellfish farm containing cohesive sediments, to empirically assess the capacity of local physical and biological processes to assimilate organic wastes from aquaculture.

Owing to uncertainty in model predictions of the aquaculture zone of benthic effect, monitoring programs are an essential part of environmental management systems. Geochemical indicators of organic enrichment, including S^{2-}, dissolved oxygen (DO), redox-potential (Eh), and pH, can serve as practical alternatives to the costly, labour-intensive and time-consuming taxonomic approaches used to detect minor to severe disturbances to benthic macroinvertebrate communities (Wildish et al. 2001, Schaaning & Hansen 2005, Hargrave 2010, Cranford et al. 2017, 2020). S^{2-} measurements in particular are widely applied for aquaculture regulatory monitoring (e.g. in Canada, USA, New Zealand, and Australia) and farm certification (e.g. Aquaculture Stewardship Council) programs. Hargrave et al. (2008) presented a comprehensive scale describing the effects of S^{2-} stress on benthic macroinfaunal communities that has served as the basis for classifying organic enrichment zones at aquaculture sites. Cranford et al. (2020) proposed that the S^{2-} concentration limits that separate each of the 5 'oxic status' zones defined by Hargrave et al. (2008) be substantially reduced owing to the large contribution of non-toxic particulate sulfides (iron sulfides and pyrite) known to contaminate S^{2-} measurements obtained using the standard ion-selective electrode (ISE) protocol (see Brown et al. 2011, Cranford et al. 2017, Brodecka-Goluch et al. 2019). The development of a practical field analysis protocol for measuring S^{2-} (direct UV spectrophotometry; Cranford et al. 2017) resolved the multiple biases inherent with the ISE method and showed that the toxicity of S^{2-} to marine macrofauna is considerably greater than previously indicated by ISE analysis (Cranford et al. 2020). Altering a standard regulatory protocol and the related impact classification system raises questions about the potential consequences to the current state of knowledge on benthic macroinvertebrate effects from organic enrichment and the impact on regulatory decisions that depend on S^{2-} monitoring. These consequences were addressed in the present study by comparing site classifications established using 3 different S^{2-} analytical methods.

Additional objectives addressed in the present study include (1) determining temporal (husbandry cycle) and spatial (m to km) scales of variability in S^{2-} measurements to aid in improving monitoring program designs, (2) conducting further comparisons of S^{2-} results obtained using 3 analytical protocols across a variety of aquaculture types and habitats towards recommending accurate, precise, and practical methodologies for use in monitoring programs, (3) determining the effect of organic enrichment on DO concentrations in surficial sediments, and (4) expanding the benthic ecological quality status (EQS) classification system based on S^{2-} analysis (Cranford et al. 2020) to include a wider range of impact indicators. Defining quantitative inter-relations between multiple organic enrichment metrics is meant to increase awareness of the abiotic and biotic effects defined within different sediment classifications and facilitate comparison of effects measured across a wider range of studies.

2. MATERIALS AND METHODS

2.1. Locations and sampling

Seasonal and spatial variations in benthic organic enrichment were investigated at 2 Atlantic salmon *Salmo salar* farms in eastern Canada that exhibit high-flow conditions and contrasting sediment characteristics. Farm A overlayed cohesive clay–silt sediments in Passamaquoddy Bay, New Brunswick (see Table 1), where the water depth and tidal range are approximately 23 and 7 m, respectively. The farm contained 15 circular net-pens in 2 parallel rows. Farm B was situated over a permeable sand seabed in Jordan Bay, Nova Scotia (see Table 1), which has a water depth of 17 m and tidal range of 1.5 m. Farm B contained 2 parallel rows of 10 circular net-pens. The dimensions of both farms were similar at approximately 500 m long and 200 m wide. Upward-looking acoustic Doppler current profilers previously moored on the seabed 100 m from these 2 farms showed that both sites exhibited a similar range of current velocities (2–22 cm s^{-1}; Law & Hill 2019), with average velocities exceeding 9.5 cm s^{-1}. Farm B was located on the open Atlantic coast, where it was exposed to higher wind waves and longer period swells than the relatively sheltered Farm A. Both farms were stocked with juvenile salmon in April and May 2015. Salmon stocking levels are not reported by industry in Canada, but each salmon cage is known to contain 30 000–50 000 fish, giving a minimum of 450 000 fish under culture at Farm A and 650 000 fish at Farm B. The typical grow-out period in open-water cages for Atlantic salmon in eastern Canada ranges between 14 and 24 mo. Salmon were harvested in October 2016 at Farm A and in April 2017 at Farm B, after which the farms were fallowed before restocking. Sediment sampling was conducted during different stages of the grow-out and fallow periods, including prior to harvesting when peak biomass, feed input, and faeces deposition is expected to be greatest.

A third site (Farm C) was selected to investigate the spatial scale of benthic organic enrichment under suspended long-line mussel *Mytilus edulis* culture activities. Farm C was located near the mouth of Tracadie Bay, Prince Edward Island (see Table 1), which is one of the most extensively leased aquaculture embayments in Canada. The sampling location contained cohesive silt sediments (see Table 1) with a water depth of 3 m and tidal range of 0.6 m. Mean (±SD) current speeds reported near this location indicate low-flow conditions (1.2 ± 1.0 cm s^{-1}; Grant et al. 2005).

Data reported in Law & Hill (2019) on the magnitude and directional distribution of tidal currents were employed in the development of sampling designs for Farms A and B. Sediment was collected at multiple locations 0–1000 m from the edge of the net pens along transects situated in the predominant current direction as well as in the opposite direction. Two additional transects, located orthogonal to the predominant current flow, were sampled on the first visit to Farm A. Sampling at Farm C, which contained harvest-size mussels, was conducted along a transect that ran from 30 m outside the farm edge to 37 m inside the farm. The transect ran in parallel with the mussel longlines, and single core samples were collected at approximately 3 m intervals along the transect. Sediment geochemical analysis at Farm C was more limited than at Farms A or B, and only included physical properties, organic content (%), and S^{2-} measured by UV spectrophotometry as described below.

Seabed samples were obtained at the salmon farms using a specialized 50 kg coring device (Mini Slocorer; Law & Hill 2019) that provided 10.8 cm internal diameter core samples (92 cm^2 surface area) with an undisturbed and clearly visible sediment–water interface. A minimum of 3 replicate core samples were collected at each sampling station, with 12 replicates collected at stations immediately beside the net-pens (0 m distance) to provide data on small-scale geochemical variability. Coring at Farm C was conducted with a hand-operated gravity corer containing a 5 cm internal diameter core tube. The transparent plastic core tubes used in both samplers contained holes at 1 cm intervals along the side that were covered with clear duct tape to provide access for porewater extraction devices to be inserted at different sediment depths, as described in Section 2.2 (Fig. 1).

Additional sediment sampling was conducted at multiple fish (salmon and trout) and shellfish (mussel) aquaculture sites in eastern and western Canada to investigate potential geographic biases in S^{2-} measurements performed using 3 different analytical protocols. See Table 2 for a summary of site and sampling details for these farms. Surficial sediment samples were collected at these locations using a variety of coring (Slo-core and SCUBA diving) and grab (Van Veen and Ponar) methods (see Table 2).

The vertical flux of particulate OM at Farms A and B was measured during the period when each farm contained 2nd year salmon of harvestable size (September–October 2016). Paired 3.8 l sediment traps containing steering fins (Model 28.100; KC-Denmark) were moored 1 m above the seabed at 50, 200, and 500 m distances from the farms in the direction of the main currents. An additional time-series trap was moored on the seabed at 0 m distance from the net-pens during this same period; these data have previously been reported (Law & Hill 2019). Each trap deployment lasted approximately 4 d. Upon retrieval, the contents of the 80 mm diameter tubes were analysed for total particulate matter (mass of material retained on GF/C filters after drying at 80°C to constant weight) and OM content (%OM: percentage weight loss after combusting at 450°C for 6 h), and these data were used to calculate average daily fluxes (g m^{-2} d^{-1}).

2.2. Sediment processing and analysis

S^{2-} and DO sampling and analysis was immediately conducted onboard the sampling vessel as described in Cranford et al. (2017, 2020). In brief, porewater in core samples was extracted at 1 and 2 cm depths below the sediment–water interface by inserting RhizoCera porewater extractors through the tape and holes in the side of the core tube (Rhizosphere Research Products). Porewater in grab samples was collected by inserting the extractors at a 45° angle into the upper 2 cm of sediment. Porewater was collected under low vacuum for approximately 2 min using a syringe (Fig. 1). The DO concentration was then determined for the core samples by inserting a fiber optic sensor (NTH-PSt7 needle-type microsensor; PreSens Precision Sensing) inside the RhizoCera while still inserted in the core (Fig. 1). A PreSens Micbox 4 fiber optic oxygen meter provided compensation of salinity, temperature, and pressure. The sensor was calibrated several times a day and found to remain stable over several days. After each DO reading stabilized and was recorded, a 100 µl subsample of porewater was removed from the RhizoCera with a gas-tight syringe, and the S^{2-} concentration was immediately analysed onboard the boat

Fig. 1. Left: core sample processing at sea using RhizoCera porewater extractors attached to syringes (right core) followed by dissolved oxygen analysis of porewater using a needle-type fiber-optic microsensor (left core). Right: eddy correlation system being lowered to the seabed

by UV spectrophotometry (S^{2-}_{UV}). The instrument (Implen C40 Mobile Nanophotometer) was calibrated prior to each sampling trip with 5 serial dilutions of an ISO-certified $Na_2S{\cdot}9H_2O$ standard (Sulfide Whole Volume; Sigma-Aldrich). A separate 100 µl subsample of porewater from the RhizoCera was then preserved in 1 ml zinc acetate:EDTA:NaOH (1:1:0.8% weight/volume) for later analysis onshore by methylene blue (MB) colorimetry (S^{2-}_{MB}) using the bulk microplate method described in Cranford et al. (2020).

Cores collected at Farms A and B were also analysed for S^{2-} using the ISE protocol (S^{2-}_{ISE}) prescribed in Canada, and elsewhere, for aquaculture regulatory purposes. After processing each core as stated above, the seawater overlying the sediment was drained by removing the tape from above the sediment surface. A 5 ml subsample of sediment was then collected for S^{2-} analysis by inserting a cut-off 5 ml plastic disposable syringe into the upper 2 cm of sediment. The syringes were capped and stored at 4°C for S^{2-}_{ISE} analysis within 12 h of collection according to Wildish et al. (2001). Both the S^{2-}_{MB} and S^{2-}_{ISE} analysis instruments were calibrated using standards prepared from $Na_2S{\cdot}9H_2O$ crystals after testing the stock standard for purity by titration. The remaining upper 1 cm of sediment in the core was retained for analysis of %OM, water content (% water), and dry bulk density. Water content was determined from sediment mass before and after drying at 80°C. The %OM in sediment was determined from the weight loss on ignition at 450°C for 6 h (Kristensen 1990). Dry bulk density (in g cm^{-3}) was determined by subsampling a known volume of homogenized sediment, drying and weighing the sediment, and then dividing by the initial volume. Sediment grain size was determined using Coulter Counter and Coulter laser methods (cf. Law & Hill 2019).

S^{2-} and DO data reported for each analytical method represent the average across all within- and between-core measurements taken at each sampling station. This included the 1 and 2 cm depth measurements for DO, S^{2-}_{UV}, and S^{2-}_{MB} measurements.

2.3. Sediment oxygen consumption

Sediment oxygen consumption (SOC) flux was measured using the non-invasive aquatic eddy correlation technique (Berg et al. 2003, Lorrai et al. 2011). The *in situ* sensing system consisted of a Nortek Vector acoustic Doppler velocimeter (ADV) attached to a MicroSquid modular sensor package (Rockland Scientific) that included a fast response micro-oxygen probe (AMT galvanic sensor) and an FP07 micro-thermistor. The sensors were calibrated prior to each sampling trip according to the manufacturer's specifications. The MicroSquid was powered by the ADV, and the 2 analog signals were synchronized and logged with the 3D current measurements. The system components were attached to a benthic lander frame (Fig. 1) such that the microsensors were positioned precisely at the edge of the velocity sensing volume, which was adjusted to be located 15 cm above the sediment surface (i.e. the frame base). An upward-looking light sensor (Hobo Pendant; Onset Computer Corporation) was attached to the lander during each deployment to measure ambient light levels (Long et al. 2012).

The eddy correlation system was deployed on the seabed during daytime hours at multiple stations between 0 and 1000 m away from the net-pens at Farm A (1–5 October 2016) and Farm B (5–10 September 2016) in the main current flow direction. Each day, the oxygen electrode was allowed to polarize for at least 2 h prior to data collection, and the system was placed sequentially at several stations for a minimum of 2 h with data continuously collected at 32 Hz. This minimum flux data collection period exceeds the minimum 1 h record recommended for research on the spatial and temporal scales of oxygen fluctuations in aquatic systems (Lorrai et al. 2010). Each sampling period produced a high volume of data that was initially post-processed using MicroSquid and Nortek software to provide the appropriate data and format needed for input to SOHFEA v.2.0 software (http://sohfea.dfmcginnis.com), which was used for the oxygen eddy correlation analysis. The data processing sequence followed that described in Lorrai et al. (2010) and included despiking and filtering the oxygen and current signals to remove noise, applying a tilt correction, calculation of turbulent fluctuations through running mean detrending, applying time-shifting to correct for the DO sensor time-lag relative to the current measurements, and calculation of vertical O_2 fluxes (mmol m^{-2} d^{-1}) averaged over 1 min sampling intervals.

3. RESULTS

3.1. Sediment organic enrichment

The seabed at Farm A in Passamaquoddy Bay consisted of cohesive silt sediments (Table 1) with an average %OM of approximately 5.8% at sam-

Table 1. Sampling details and sediment characteristics at Atlantic salmon and bivalve farms used to study spatial variations in benthic organic enrichment effects.

Farm location and species	Sampling dates	*T* (°C)	No. of transects, distances, and cores	Median ± SD grain size (µm)	Mean ± SD density (g cm^{-3})	Mean ± SD organic matter (%)	Mean ± SD porosity (%)
Farm A	October 2016: 2nd year fish	13.5	4, 22, 81	9.8 ± 0.8	0.41 ± 0.04	6.4 ± 0.9	66.7 ± 2.5
Passamaquoddy Bay	April 2017: Pre-harvest	2.9	2, 10, 44	9.2 ± 1.0	0.39 ± 0.07	9.5 ± 5.3	68.4 ± 4.5
45.032° N, 67.006° W	July 2017: Fallow	12.3	2, 14, 54	9.8 ± 0.9	0.41 ± 0.06	7.6 ± 1.1	66.8 ± 3.7
Salmo salar	September 2017: Fallow	15.0	2, 12, 52	10.3 ± 1.0	0.43 ± 0.04	6.6 ± 0.4	65.6 ± 2.5
	February 2018: Fallow	5.0	2, 6, 28	9.7 ± 0.5	0.42 ± 0.03	6.9 ± 0.3	65.9 ± 1.6
Farm B	September 2016: Pre-harvest	14.5	2, 14, 46	111.8 ± 5.3	1.26 ± 0.09	0.6 ± 0.2	28.4 ± 3.5
Jordan Bay	May 2017: Fallow	7.5	2, 5, 16	107.5 ± 1.5	1.40 ± 0.02	0.4 ± 0.1	22.4 ± 0.9
45.700° N, 65.194° W	October 2017: 1st year fish	12.9	2, 6, 25	108.6 ± 1.8	1.40 ± 0.03	0.3 ± 0.1	20.9 ± 0.3
Salmo salar							
Farm C	September 2017:	17.5	2, 21, 21	25.1 ± 6.2	0.32 ± 0.08	9.9 ± 1.8	72.8 ± 5.4
Tracadie Bay	2nd and 3rd year mussels						
46.409° N, 62.996° W							
Mytilus edulis							

pling stations >200 m from the net-pens (Fig. 2). In October 2016, when the salmon were in their second year of production, %OM was enhanced by up to 2% within 100 m distance from the net-pens and in multiple directions from the farm. During pre-harvest sampling in April 2017, mean %OM increased to between 11.8 and 13.4% at stations within 10 m of the net-pens and declined rapidly to near-baseline levels at 100 m distance (Fig. 2). Fallowing of Farm A after the fish harvest resulted in a gradual decline in %OM, and background levels were largely achieved at all sampling stations within 6 mo after the harvest. The relationship between sediment %OM and porosity showed a progression of changes in sediment properties over the fish production cycle, with maximum changes observed prior to harvesting at stations located within 50 m distance from the net-pens (Fig. 3). Seasonal sampling indicated that the maximum degree of sediment organic enrichment at Farm A occurred during the pre-harvest period when water temperature was 2.9°C, and this farm effect was localized to within approximately 10 m of the net-pens.

The Farm B site in Jordan Bay contained highly sorted fine-sand sediments (Table 1) with a natural %OM content of approximately 0.3% as indicated by measurements collected in May 2017 during the farm fallow period (Fig. 2). %OM was highest beside the net-pens in all directions from the farm during pre-harvest sampling in September 2016. Mean %OM was slightly elevated in the main directions out to 1025 m (mean 0.6%) relative to levels measured in May and October 2017 after the farm was harvested and fallowed (Fig. 2). The porosity data also supports this indication of widespread sediment organic enrichment at Farm B prior to the fish harvest in September 2016. Average sediment porosity was near maximum at all sampling distances from the net pens during the pre-harvest sampling period but declined to relatively low levels after the farm was fallowed over winter and after it was restocked with first-year salmon (Fig. 3).

A 1-tailed independent *t*-test was employed to assess the hypothesis of enhanced organic enrichment of surficial sediments located within Farm C. Mean (±SE) %OM of 18 sediment samples collected inside the farm boundary (10.49 ± 0.10%) compared with the 24 samples from the reference area (9.50 ± 0.23%) demonstrated significantly greater organic enrichment inside the farm (t = 1.765, p = 0.043).

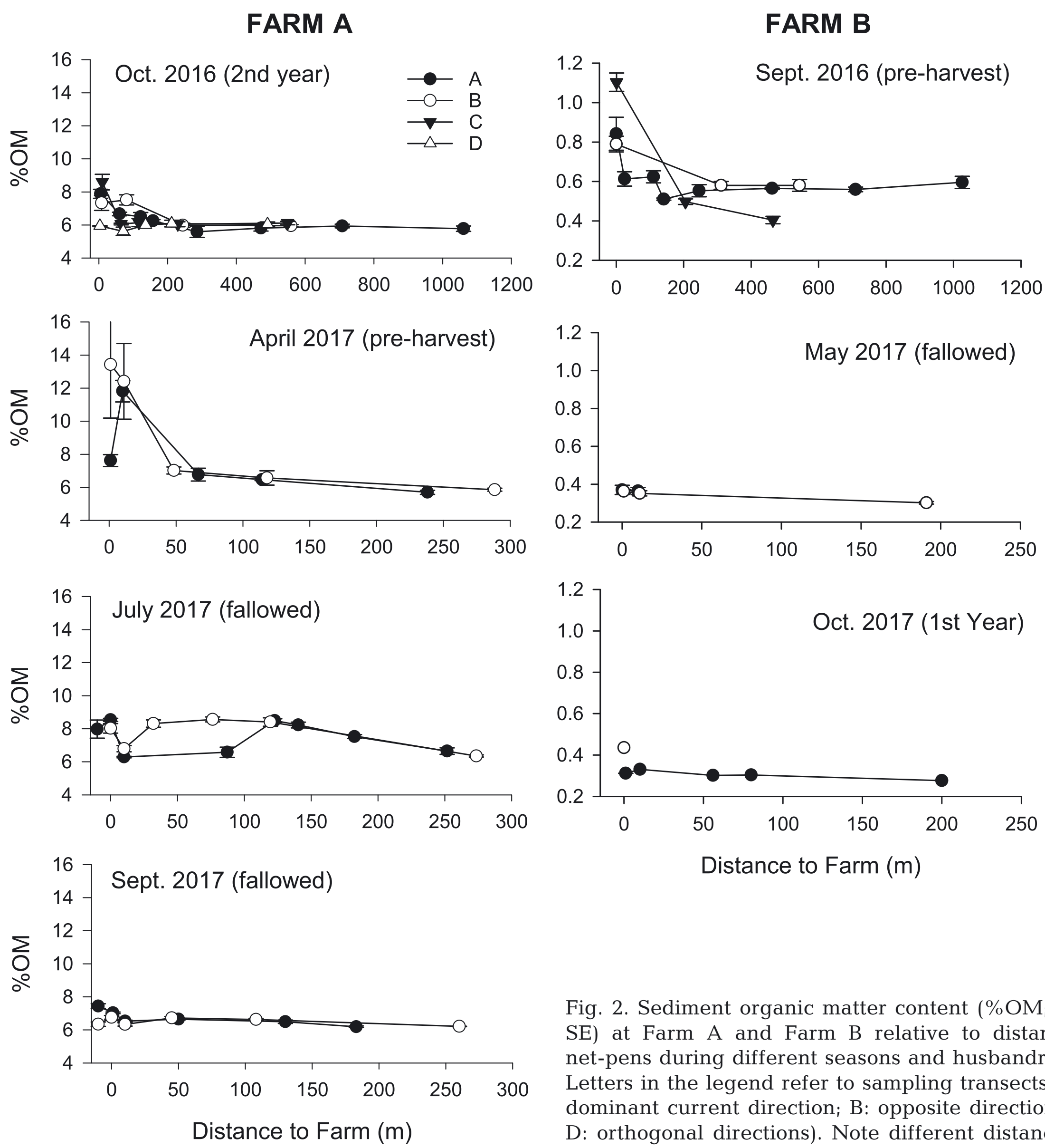

Fig. 2. Sediment organic matter content (%OM; mean ± SE) at Farm A and Farm B relative to distance from net-pens during different seasons and husbandry stages. Letters in the legend refer to sampling transects (A: predominant current direction; B: opposite direction; C and D: orthogonal directions). Note different distance scales

3.2. DO and S^{2-} in sediment porewater

Mean DO concentrations in the silt sediments at Farm A were low at all sampling sites throughout this study (<1 mg l^{-1}), but the highest values were measured in April 2017 when water temperature was low (2.9°C; Fig. 4). Porewater within the sand substrate at Farm B generally contained higher DO levels than observed at Farm A, with the highest DO concentrations observed at sampling stations located farthest from the net-pens (Fig. 4). Hypoxic sediment conditions (DO < 3 mg l^{-1}) were observed out to at least 70 m during the first year of fish stocking (October 2017). Sampling conducted immediately prior to fish harvesting in September 2016 detected hypoxic conditions in surface sediments out to 700 m. Porewater concentrations in sediments located within 10 m of the net-pens showed a return to oxic conditions during the fallow period, albeit with some indication of a patchy spatial distribution of hypoxic and oxic conditions close to the farm (Fig. 4).

S^{2-} concentrations in sediment porewater during the latter stages of salmon production at Farm A followed a similar pattern with distance as %OM (Figs. 2 & 5). The highest S^{2-} concentrations were measured in sediments within 10 m of the net-pens during the second year of grow-out and during pre-harvest sampling (Fig. 6). Replicate core samples re-

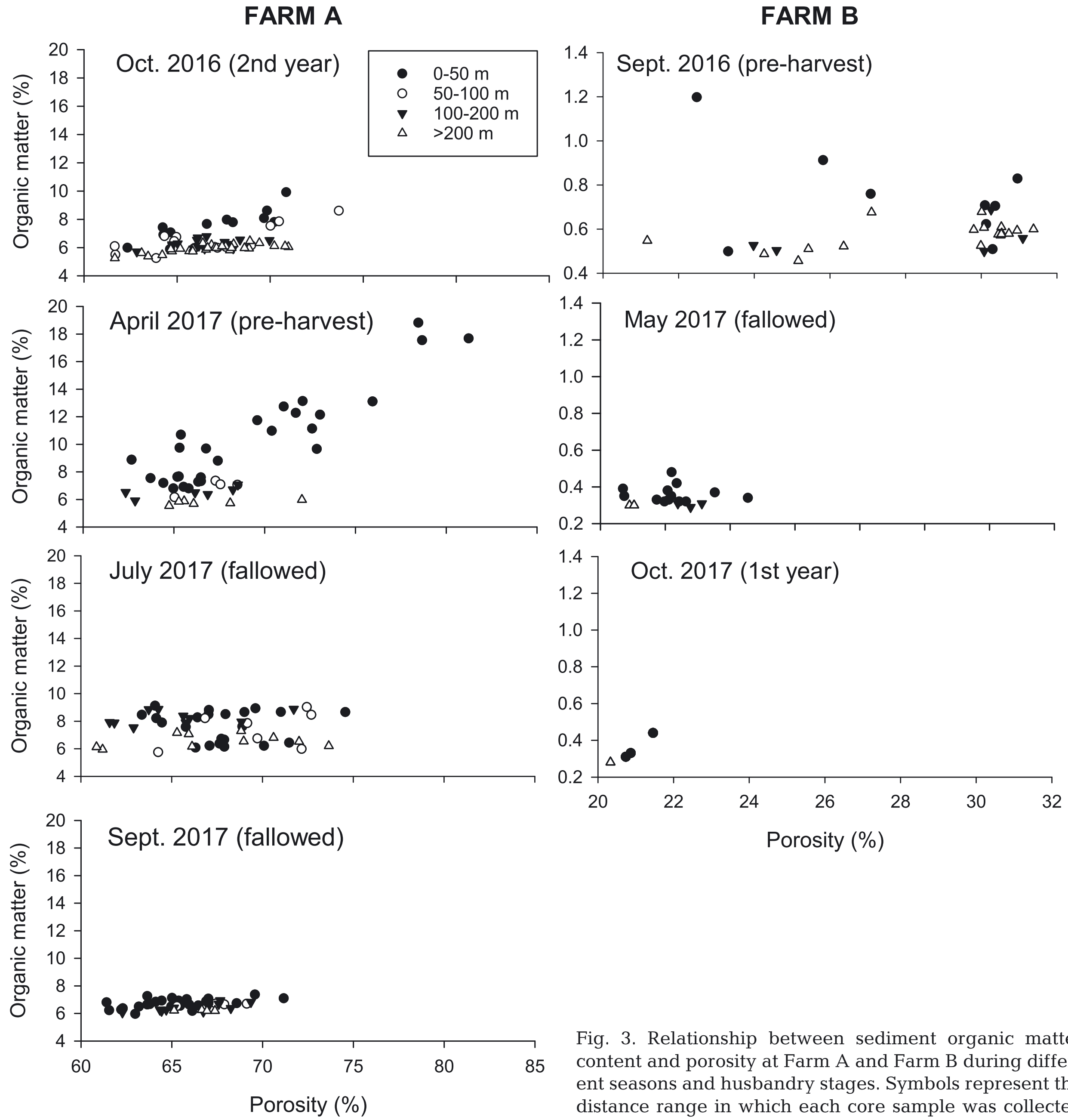

Fig. 3. Relationship between sediment organic matter content and porosity at Farm A and Farm B during different seasons and husbandry stages. Symbols represent the distance range in which each core sample was collected

vealed a high degree of S^{2-} patchiness in sediments collected close to the farm in April 2017 (Fig. 5). Although local S^{2-} levels declined during the fallow period, average concentrations at the cage edge and under the fish pens (0 and −10 m distances) remained above 500 µM for 5 mo after the fish were harvested (Fig. 5). S^{2-} concentrations measured at all stations outside 10 m at Farm A were low (<250 µM) during all seasons. Farm B sediments contained S^{2-} concentrations below 500 µM at all sampling stations and dates, except for a single core sample collected in May 2017 which greatly skewed the average towards the high value shown (Fig. 5; 1450 µM). Exclusion of this core resulted in a mean (±SE) concentration of 435 ± 170 µM.

S^{2-} concentrations at Farm C varied substantially along the sampling transect (Fig. 5), with concentrations averaging 2707 ± 569 µM for sediments collected beside the mussel long-line inside the farm boundary compared with 653 ± 94 µM measured in sediments outside the farm. This farm was located close to an extensive eel grass *Zostra marina* bed,

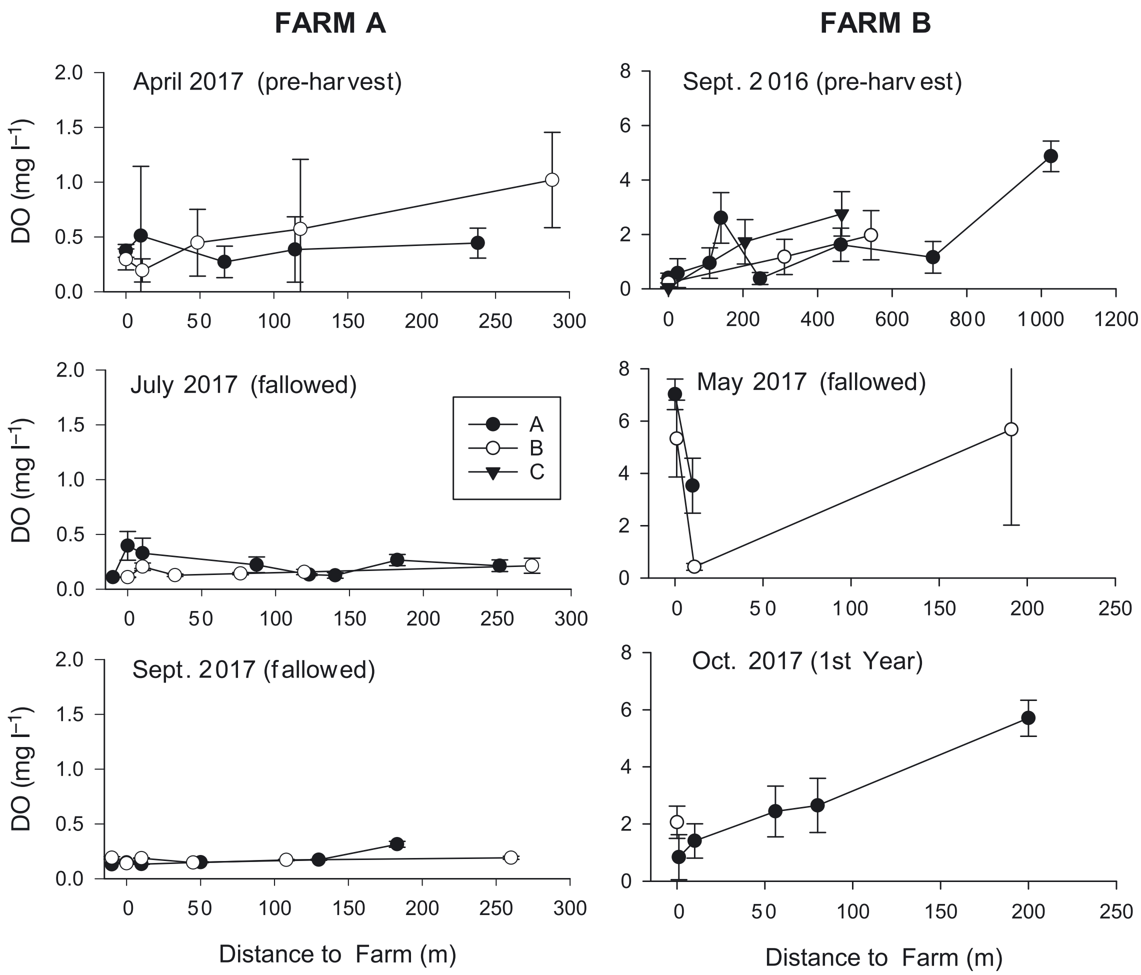

Fig. 4. Sediment dissolved oxygen (DO; mean ± SE) concentrations at Farm A and Farm B relative to distance from net-pens during different seasons and husbandry stages. Letters in the legend refer to sampling transects (A: predominant current direction; B: opposite direction; C: orthogonal direction). Note different distance scales

and all sediment samples contained decomposing grass detritus.

As noted above, the mean S^{2-} concentration determined for stations located close to the edge of Farm A was heavily influenced by small-scale patchiness. A total of 18 grab samples were collected beside Farm A (0 m distance in all 4 orthogonal directions) in April 2017. S^{2-}_{UV} concentrations ranged from 117–6768 µM with an arithmetic mean of 1537 ± 524 µM. These data were positively skewed by several high S^{2-}_{UV} values and followed a lognormal distribution. The geometric mean ± SE concentrations (542 ± 1.0 µM) provided more appropriate descriptors of central tendency and precision in these data. The collection of 18 replicate core samples at this location provided an opportunity to estimate the sample size (n) required to maximize S^{2-}_{UV} measurement precision while minimizing sampling costs. This retrospective analysis of measurement precision was conducted using the arithmetic SE because aquaculture management decisions are typically informed by the arithmetic mean. SE was calculated for n = 3–18 by repeatedly conducting randomized draws of S^{2-}_{UV} concentrations from the pool of 18 replicate stations. Up to 7 draws were performed for each n-value to aid in determining the sample size at which precision approached a steady state. The results are shown in Fig. 6 and indicate that 9 replicate seabed samples are sufficient to constrain S^{2-}_{UV} precision to less than 50% of the mean.

S^{2-} data presented in Fig. 5 only include results obtained using the UV spectrometry method. The same sediment samples were analysed using the MB and ISE methods. S^{2-}_{UV} concentrations from all sites listed in Tables 1 & 2 were strongly correlated with S^{2-}_{MB} data (Fig. 7, n = 927) regardless of the farm location.

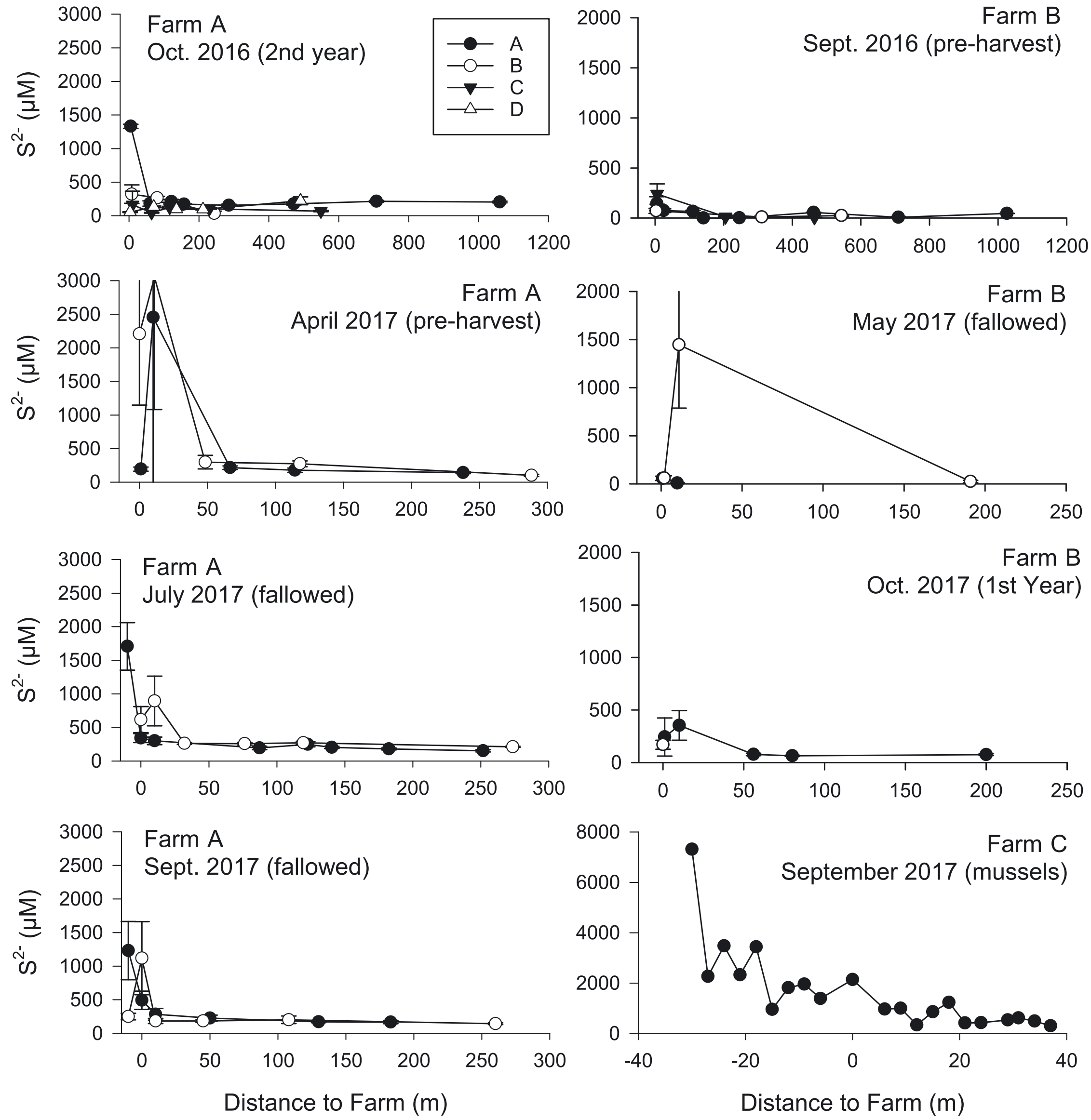

Fig. 5. Sediment total free sulfide (S^{2-}_{UV}; mean ± SE) concentrations at Farm A, Farm B, and Farm C relative to distance from farm during different seasons and husbandry stages. Letters in the legend refer to sampling transects (A: predominant current direction; B: opposite direction; C and D: orthogonal directions). Note different distance scales and negative distances for stations inside the farm

In contrast, comparisons of S^{2-}_{UV} and S^{2-}_{ISE} data showed much more variable results (Fig. 8), with large differences apparent both within and between aquaculture embayments. For all locations, S^{2-}_{ISE} measurement methods differed by up to an order of magnitude from either the S^{2-}_{UV} or S^{2-}_{MB} measurements made on the same samples. Although S^{2-}_{ISE} generally increased with increasing S^{2-}_{UV}, the relationship remained highly variable and inconsistent. For example, S^{2-}_{ISE} measurements tended to overestimate S^{2-} in Clayoquot Sound and St. Anns Bay while underestimating concentrations in Whycocomagh Bay. All 3 locations contained silt sediments of similar grain size (Table 2).

3.3. OM flux and SOC

The vertical flux of OM at 50 m distance from both salmon farms in September–October 2016 was elevated relative to fluxes measured at greater distance (Fig. 9). The average %OM of particulate matter col-

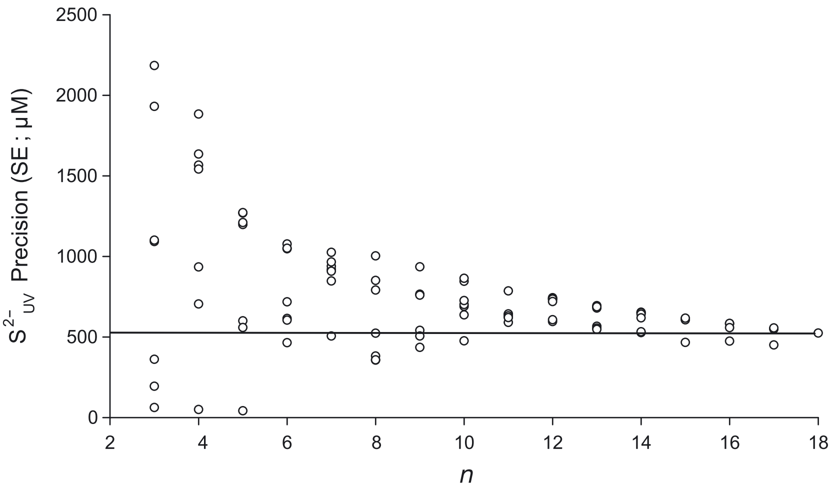

Fig. 6. Relationship between the precision (SE) of total free sulfide measurements (S^{2-}_{UV}), based on UV spectrophotometry, and the number of replicate sediment samples (n) collected at the edge of salmon net-pens at Farm A. The data points show SE values calculated from randomized draws of n size from a pool of 18 replicate S^{2-}_{UV} measurements. The horizontal line is the SE for all 18 replicate samples

lected in traps at the 50, 200, and 500 m stations also declined with distance from 11.7–8.8%. Simultaneous measurements of OM flux at the farm edge, reported in Law & Hill (2019), are included in Fig. 9 and indicate that OM flux was lowest at the farm edge. OM fluxes measured near Farm B in September 2016 were higher than observed at Farm A (Fig. 9). Although the average flux declined with distance from this farm, the %OM content of particulate matter collected in traps from all distances was similar, with mean ± SE 22.6 ± 0.4%. The previously reported average OM flux, measured adjacent to Farm B net-pens during the same period, was also lowest beside the farm.

Daily averaged SOC rates at the 2 salmon farms are shown in Fig. 9 along with the OM flux data. The SOC of cohesive silt sediments at Farm A was highest at the farm edge and decreased to stable low levels at and beyond the 200 m station. In contrast, peak SOC in the fine sand substrate at Farm B occurred at 97 m from the farm and then declined to what appears to be baseline levels at 500 m distance. The relatively low mean SOC measured within 50 m distance from the farm corresponded with the relatively low OM flux measured near this farm. The maximum SOC rate measured for the sand sediments at Farm B was considerably higher than for the silt sediments at Farm A (684 and 256 mmol m^{-2} d^{-1}, respectively), and this is consistent with differences in the mean OM flux at each farm. The cumulative 1 min SOC fluxes measured over each instrument deployment period increased linearly, and the stability of instrument responses resulted in low variability in the mean SOC measurements shown in Fig. 9. Light intensity near the seabed, measured during each eddy correlation system deployment, was less than 1% of surface measurements, and it is therefore assumed that photosynthesis had a negligible affect on oxygen flux during this study.

Table 2. Sampling details at fish and shellfish aquaculture farms used to study potential geographic biases in total free sulfide measurements. These sites are in addition to the 3 bays described in Table 1

Embayment and farm location	Farmed species	Sampling dates	Sampler	n	Median ± SD grain size (µm)
Clayoquot Sound					
1) 49.49° N 125.73° W	*Salmo salar*	Aug 17	Van Veen grab	21	–
2) 49.21° N 125.77° W	*Salmo salar*	Aug 17	Van Veen grab	20	–
3) 49.21° N 125.77° W	*Salmo salar*	October 2018	Van Veen grab	16	13.4 ± 7.7
Whycocomagh Bay					
45.95° N 61.13° W	*Oncorhynchus mykiss*	Sep 18	Ponar grab	35	8.4 ± 4.0
St. Anns Bay					
46.23° N 60.58° W	*Mytilus edulis*	Aug 18	SCUBA core	30	15.1 ± 8.8
Lime Kiln Bay					
1) 45.06° N 66.83° W	*Salmo salar*	February 2018	Slo-core	14	10.2 ± 0.6
2) 45.06° N 66.83° W	*Salmo salar*	Sep 18	Slo-core	14	8.3 ± 0.7
3) 45.04° N 66.82° W	*Salmo salar*	Sep 18	Slo-core	17	8.7 ± 0.7

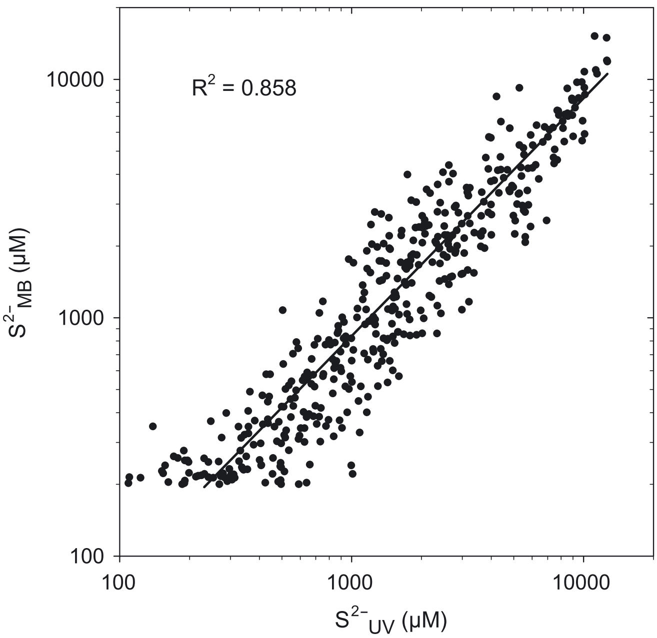

Fig. 7. Relationship between sediment total free sulfide concentrations analysed by UV spectrophotometry (S^{2-}_{UV}) and methylene blue (S^{2-}_{MB}) methods from all aquaculture sites and sampling depths included in this study (Tables 1 & 2, n = 927). The regression line and correlation coefficient are shown. S^{2-}_{MB} data below the 200 µM limit of detection are excluded

4. DISCUSSION

Aquaculture sites have been characterized as 'high-flow' or 'dispersive' if the average current velocity exceeds 9.5 cm s^{-1} (Keeley et al. 2013, Bravo & Grant 2018). Tidal currents above this critical velocity are believed to resuspend and disperse fish farm organic wastes (Cromey et al. 2002) and thereby prevent adverse effects on benthic macrofauna. Salmon Farms A and B are located in environments with maximum current velocities of approximately 20 cm s^{-1}, and sediment erosion studies at these 2 farms have shown that seabed shear stresses at Farm A in Passamaquoddy Bay are dominated by tidal currents, while those at Farm B in Jordan Bay are dominated by wave activity (Law & Hill 2019). Under these hydrodynamic conditions, accumulation of OM in surface sediments around fish cages is generally assumed by regulators to not occur. On the contrary, the %OM in the cohesive muddy sediments at Farm A was approximately doubled within 10 m distance from the cages in April 2017 prior to fish harvesting and appeared to be slightly elevated to a distance of at least 100 m. The permeable sands at Farm B also exhibited a doubling in %OM during the

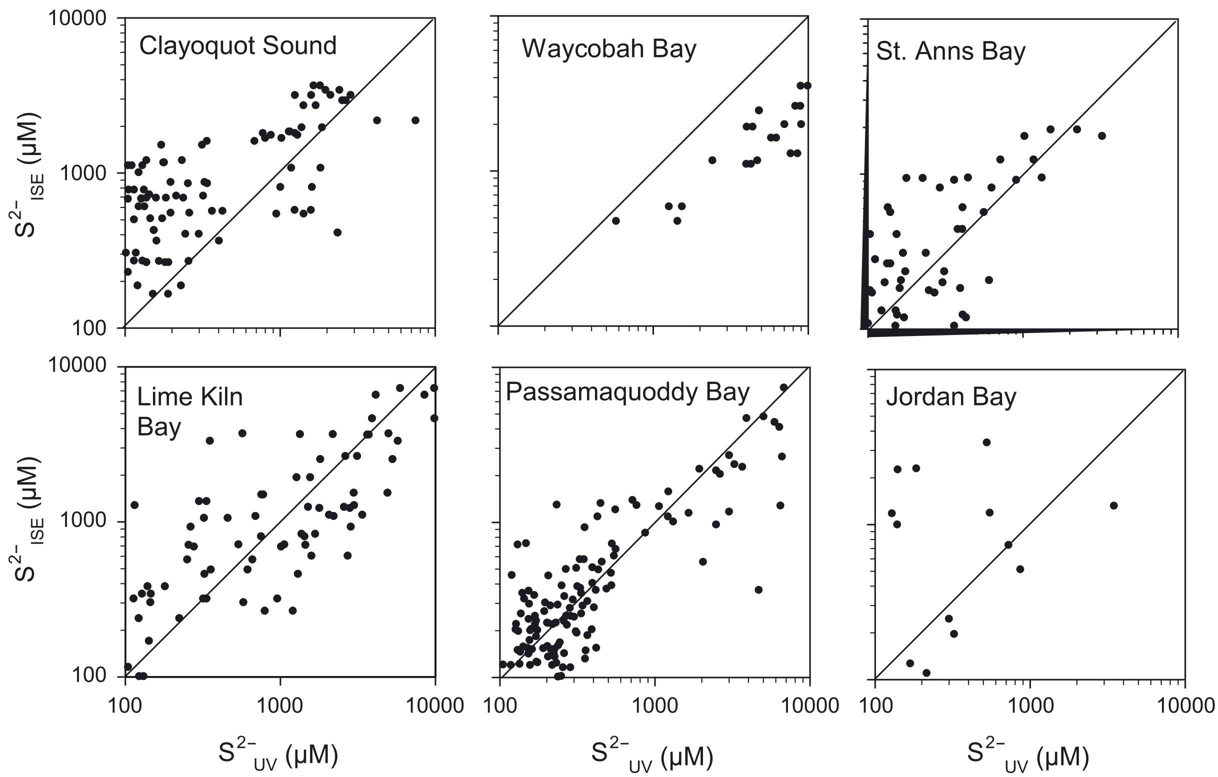

Fig. 8. Relationships between sediment total free sulfide concentrations analysed by UV spectrophotometry (S^{2-}_{UV}; mean for 1 and 2 cm depth samples) and ion-selective electrode (S^{2-}_{ISE}, 0 to 2 cm depth samples) methods for multiple aquaculture locations (Tables 1 & 2). The unity line (1:1 relationship) is shown in each plot

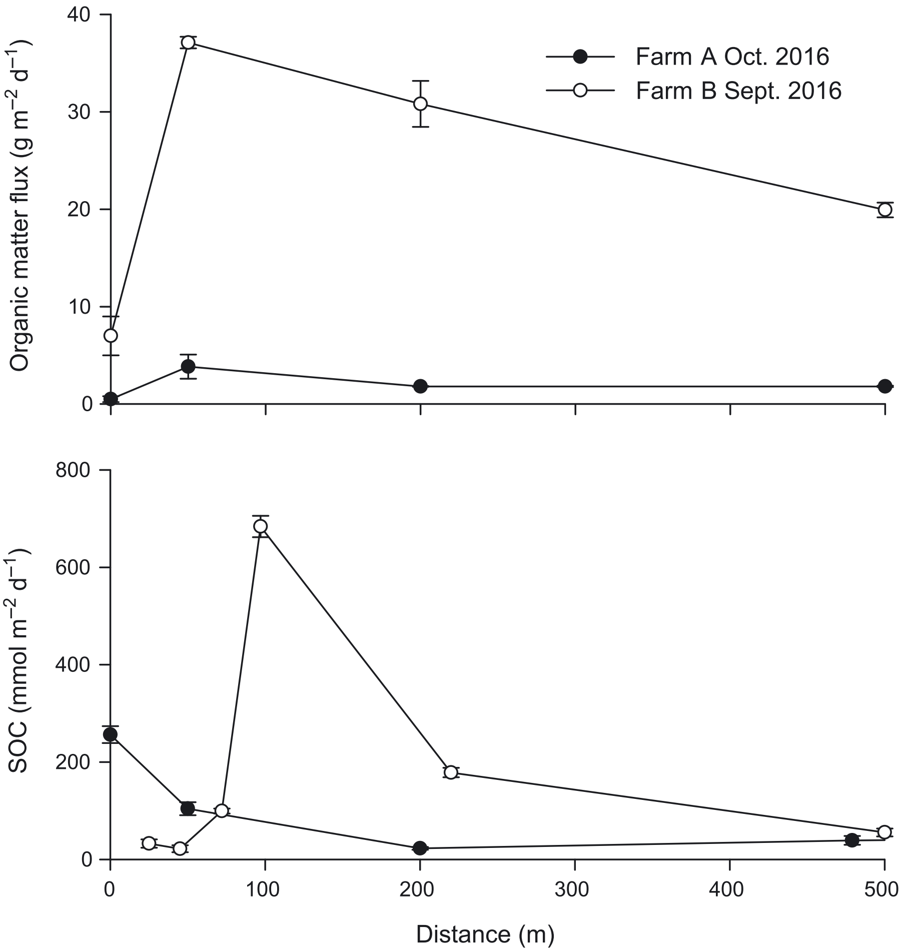

Fig. 9. Vertical fluxes (mean ± SE) of particulate organic matter and sediment oxygen consumption (SOC) measured at different distances from the edge of Atlantic salmon net-pens at Farms A and B. Measurements were made in the predominant current direction from each farm. Organic matter flux rates shown for the 0 m distance are from Law & Hill (2019) and were measured simultaneously with those shown at greater distances

pre-harvest period (September 2016), with evidence of organic enrichment out to 1000 m from the farm. Sediment organic enrichment was detected in multiple directions from both farms and corresponded with changes in sediment porosity.

The assumption that benthic impacts at open-water, high-flow fish farms are negligible can be contested based on the measured sediment geochemical changes associated with organic enrichment in cohesive sediments at Farm A. An examination of international aquaculture regulations for benthic organic enrichment at open-water marine fish farms indicates that severe effects on benthic communities are generally permitted within the farm footprint (the area immediately below the farm pens) and that moderate effects are generally not permitted beyond approximately 25–50 m from the farm. According to the Canadian Aquaculture Activities regulations (Government of Canada 2015), the 2 high-flow farms studied in eastern Canada cannot exceed 'Hypoxic A' status ($S^{2-}_{ISE} \leq 3000$ µM) at the farm boundary (the edge of the net-pens). This is equivalent to not exceeding a 'Moderate' EQS (Phillips et al. 2014, Borja et al. 2019) classification outside the net-pen boundary. Although the mean S^{2-}_{ISE} data indicated that Farm A did not exceed the local regulatory limit, both the mean S^{2-}_{UV} and S^{2-}_{MB} data indicated that the impact on the macrofaunal community beside this farm is more severe. Spatial variations in the macrofaunal community at Farm A have not been studied, but published relationships between S^{2-}_{UV} concentrations and benthic macrofauna community health metrics measured at Farm A (Cranford et al. 2020; species richness [*S*], proportion of first-order opportunists, polychaete/amphipod ratio, Shannon's diversity, AZTI's marine biotic index, and AZTI's multivariate marine biotic index [mAMBI]) indicate that sampling stations outside 10 m from this farm largely contained communities of 'Good' EQS (Cranford et al. 2020) while stations inside 10 m can be classed as being of 'Poor to Bad' status during all sampling periods, including fallowing. Multiple biases inherent with the standard S^{2-}_{ISE} protocol (see Section 1) can strongly influence benthic quality classifications and related regulatory decisions (Cranford et al. 2020). Additional research and monitoring at multiple marine farms are required to more thoroughly assess the impact of implementing alternative S^{2-} methodologies and benthic quality status thresholds for the purposes of aquaculture and habitat management.

The direct UV spectrophotometry method provided similar S^{2-} results at all study sites as obtained using the MB method. The latter is based on Standard Methods: 4500-S2-D, which was adapted for bulk sample analysis using a micro-plate reader (see Cranford et al. 2017). Both protocols require porewater extraction to eliminate particulate sulfide contamination and use equally small sample volumes (0.1 ml). These methods also prevent errors associated with the oxidation and volatilization of S^{2-} during sample collection and processing. The major advantages of the UV method are related to the inherent analytical robustness (capacity to produce unbiased results despite small changes in the method), the relative simplicity of direct optical methods, the high analytical precision and low limit of quantification (5 and 37 µM, respectively; Cranford et al. 2020), low per-sample cost, and the capacity for near real-time field analysis on small vessels. Both the UV and MB sulfide methods can be performed using a wide array of sediment core and grab methods. $S^{2-}{}_{MB}$ analysis requires preservation of the sample followed by multiple analytical steps and reagent preparations/additions in a laboratory. Unlike the UV method, a notable disadvantage of the MB method is the relatively high limit of detection (~200 µM), which makes it difficult to detect the biological effects of low free sulfide concentrations.

The highest $S^{2-}{}_{UV}$ concentrations at Farm A were measured prior to fish harvesting when water temperature was only 2.9°C. These data indicate that the timing of peak fish biomass, which can be assumed to occur prior to harvesting, is more important than temperature in determining when the peak benthic impact will occur. The timing of peak fish biomass consequently provides direction on when benthic monitoring is most appropriate.

The muddy sediments at Farm A have naturally occurring low oxygen levels owing to the constraints of molecular diffusion-dominated transport of dissolved materials across the sediment–water interface. The effects on macrofauna from organic enrichment in muddy sediments are therefore largely attributed to the accumulation of toxic metabolic products such as S^{2-} and ammonia, as opposed to oxygen depletion (Fenchel 1987, Gray et al. 2002). Permeable sand substrates are typically well-oxygenated owing to advection-dominated porewater flushing, preventing the accumulation of these reduced compounds in surface sediments. Despite the presence of permeable sand sediment, benthic organic enrichment at Farm B appears responsible for the widespread sub-oxic conditions. A decrease in sand permeability through the trapping of particulate matter between sand grains reduces porewater flushing and appears to have contributed to the ability of microbial aerobic mineralization processes to deplete oxygen levels. Oxygen depletion at this farm was limited to concentrations above the 0.5 mg l^{-1} threshold at which S^{2-} begins to rapidly accumulate (Gray et al. 2002).

Middelburg & Levin (2009) reviewed the effects of hypoxia on benthic macrofauna, which include stimulation of migration behaviour, mortality of some taxa, reduced density and biomass, changes in functional diversity, smaller community body size, and the shallowing of macrofauna distribution and activities (bioturbation and irrigation). These authors also noted that the duration of hypoxia is important for determining the severity of impacts. Although the sand sediments were largely reoxygenated during the farm fallow period, hypoxic conditions developed after the reintroduction of juvenile fish to the cages. Information on the macrofauna community at this farm has previously been reported for the October 2017 sampling period (Cranford et al. 2020), although not in relation to spatial variations. Further analysis of those published data using mAMBI as a proxy for macrofauna community health revealed a positive relationship with sediment oxygen concentration ($r^2 = 0.73$, n = 17), which indicates a negative biotic effect from increasing hypoxia. However, the impact on the benthic community was negligible, with 'Good' EQS indicated for sediments collected immediately adjacent to the fish cage (0.68 ± 0.06) and 'High' EQS status indicated by an average (±SD) mAMBI at 200 m (0.85 ± 0.07). Apparently, most resident taxa were able to obtain sufficient oxygen at the sediment–water interface to counteract the potential effects of sediment hypoxia.

Physical waste dispersal processes are important for maintaining natural benthic habitats and preventing the manifestation of benthic community impacts. However, the waste assimilative capacity of an aquaculture site also depends on inherent biotic processes, including the oxygenation of surface sediments through bioturbation and bioirrigation and the mineralization of excess OM. Biological and chemical waste assimilation processes appear to be important at both farm sites given that physical processes did not fully prevent OM enrichment in either the permeable or cohesive sediments. SOC rate measurements serve as an indicator of total benthic metabolism and chemical oxygen demand. SOC rates measured at both farms largely followed a similar spatial pattern as the vertical flux of OM. An unexpected observation was that both the SOC and OM fluxes were relatively low adjacent to Farm B compared with more distant locations. Differences in sediment trap designs (cylindrical tubes at distances ≥50 m [this study] and funnel-type at 0 m [reported in Law & Hill 2019]) are known to affect particle trapping efficiency, with funnel-type traps tending to under-collect particles in turbulent flow conditions (Butman 1986). This type of measurement bias may explain why the OM vertical flux appeared to be relatively low beside both farms. An alternate explanation stems from 3D hydrodynamic model predictions that show that fish cages can enhance the water velocity in the bottom layer beneath the cages for farms in relatively shallow waters (Wu et al. 2014). Farm B, which is located in shallower waters than Farm A, occupies a larger fraction of the water column, and faeces exiting the bottom of the cages may have been rapidly advected away from the farm prior to deposition on the seabed.

Although the muddy sediments in Passamaquoddy Bay contained considerably more natural OM than the sands in Jordan Bay, SOC rates were considerably higher at the sandy site. Martinez-Garcia et al. (2015) reported that sandy sediments have a greater metabolic capacity than muddy sediments to assimilate high levels of organic enrichment from aquaculture. Oxygen uptake by muddy sediments can be diminished by reducing conditions, which favor less efficient anaerobic decomposition pathways compared with aerobic ones in sand substrates (Kristensen & Holmer 2001). Rusch et al. (2006) similarly suggested that decomposition rates of OM in permeable sands can exceed those of fine-grained, organic-rich deposits. The maintenance of bioturbation and bioirrigation would help to offset the increased biological and chemical oxygen demand of enriched sediments. Although both farms were of similar size and had a similar range of tidal currents, a combination of physical (wave-induced resuspension and advection-dominated oxygen transport) and biological processes (high metabolic capacity and bioirrigation) can largely explain why the effects of organic enrichment on the macrofaunal community were greater in the muddy substrate than at the sand site.

Sediment oxygen flux at aquaculture facilities has been measured using a variety of *in situ*, shipboard, and laboratory incubation methods, but the eddy correlation technique employed in the present study is the only approach that retains biogeochemical and biological conditions in the sediment and preserves boundary layer currents and vertical exchanges across the sediment–water interface. Measurements also represent a relatively large area of the seabed (Berg et al. 2003, 2009, Chipman et al. 2012). Coring methods cause disturbances that can alter the availability of OM and oxygen to the microbial community, resulting in measurement biases (Mogg et al. 2017). Sandy sediments have been a particular challenge for measuring oxygen uptake because sediment enclosures exclude the advective circulation of porewater. The previous maximum oxygen flux rate previously measured at a salmon farm was 434.9 ± 139.7 mmol m^{-2} d^{-1} (Nickell et al. 2003), which is lower than the 684 ± 22 mmol m^{-2} d^{-1} value reported herein at 97 m outside Farm B. These oxygen flux rates are not strictly comparable, owing to differences in methodology and sediment grain size; however, previously documented spatial trends in SOC at marine fish farms are comparable with results reported here. For example, SOC has been shown to decline away from cages to about 50 m for muddy sediments (Hargrave et al. 1997, Papageorgiou et al. 2010) and to approximately 600 m for more coarse sediments (Keeley et al. 2019).

The waste assimilative capacity of any marine environment is not an absolute measurable property owing to the contribution of a wide variety of related abiotic and biotic processes. However, the point at which S^{2-} concentrations begin to rapidly accumulate in surface sediments provides an indication that the impact-buffering capacity of the system has been exceeded. Although very low S^{2-} concentrations can affect some infauna, the inflection point above which macroinfauna *S* rapidly declines with increasing organic deposition occurs at approximately 250 µM $S^{2-}{}_{UV}$ (Cranford et al. 2020). The extremely high S^{2-} levels measured inside the boundary of the mussel farm at Farm C illustrates how farming in an area where the assimilative capacity is already stressed by autochthonous organic enrichment (e.g. seagrass detritus) can profoundly affect the accumulation of S^{2-} concentrations inside the farmed area. Oxygen depletion near the seabed has previously been observed in Tracadie Bay, presumably the result of the high chemical oxygen demand of these highly reduced sediments (Cranford et al. 2006). Surficial sediments with naturally occurring S^{2-} levels exceeding

250 µM should raise concerns regarding the capacity of these sediments to effectively assimilate additional organic inputs. The natural organic enrichment status of surficial sediments is an important consideration for assessing the potential impacts of proposed aquaculture sites.

Environmental management frameworks include activities that identify, evaluate, and address predictions of environmental threats. The results of the present study show that environmental impact predictions at both high-flow open-water fish farms and low-flow suspended mussel culture farms are subject to some level of uncertainty. Monitoring programs remain essential for ensuring that actual effects do not exceed what is authorised. Owing to apparent gaps in knowledge on the complex array of physical, chemical, and biological processes that define the waste assimilative capacity of a particular area, the results of organic biodeposition modelling (i.e. allowable zone of effect predictions) should not presently be used in isolation for defining where monitoring stations are to be located.

Numerically characterizing the quality/health status of benthic communities with a single indicator is highly complex, and numerous studies designed to identify an 'ideal' index have been unsuccessful (Daly et al. 2018). A multitude of abiotic and biotic organic enrichment impact indicators have been developed and employed for research and regulatory purposes, but this diversity of metrics makes it difficult to compare results. The uniformity of geochemical and biotic responses along an organic enrichment gradient supported the development of a unified quantitative scale for classifying the status of benthic communities (Hargrave et al. 2008). This 'oxic status' scale was linked to a nomogram that describes inter-relationships between $S^{2-}{}_{ISE}$ concentrations and a range of variables used for aquaculture monitoring. Keeley et al. (2012) reported inconsistent sediment ecological classifications based on $S^{2-}{}_{ISE}$ and a wide range of biotic indices, with an apparent tendency for oxic status classifications to underestimate the magnitude of biotic effects. A revised benthic classification system was presented by Cranford et al. (2020) to rectify $S^{2-}{}_{ISE}$ methodological biases (see Section 1) and to link sediment $S^{2-}{}_{UV}$ concentrations with the standardized descriptions of normal to severely impacted macrofauna communities that underpin the EQS system. The elimination of site-specific S^{2-} methodological artefacts and the intercalibration with biotic organic enrichment metrics (Cranford et al. 2020) was meant to improve confidence in sediment ecological classifications. Published information was used herein to broaden this initiative by establishing numerical boundaries between the 5 EQS classifications (High, Good, Moderate, Poor, and Bad) for an expanded range of organic enrichment indicators. This quantitative scale of organic enrichment effects on abiotic and biotic variables is shown in Fig. 10. Normalized metrics that indicate benthic EQS at farm sites relative to values at reference sites were not included in this analysis because this approach alters the standard normative descriptions of community EQS, and the results are therefore specific to the farm site where they are employed. Fig. 10 is meant to show how geochemical and biotic changes in marine sediments are quantitatively linked and to provide a means of quantitatively describing and comparing organic enrichment responses across multiple marine aquaculture research and regulatory/certification monitoring programs, regardless of the intensity of culture operations or species farmed.

This study highlights several key issues relevant to the provision of a credible, efficient, and measurable performance-based environmental management system designed to minimize, mitigate, or eliminate negative impacts from seabed organic enrichment at marine aquaculture farms. First, the results of the present study provide further evidence beyond those in Brown et al. (2011), Cranford et al. (2017, 2020), and Brodecka-Goluch et al. (2019) that the ISE method typically provides inaccurate readings relative to the MB (Standard Methods: 4500-S2-D as described for bulk analysis in Cranford et al. 2017) and UV methods (Guenther et al. 2001, as adapted for a broad range of concentrations by Cranford et al. 2017), resulting in a high likelihood for erroneous benthic ecological quality classifications. Second, S^{2-} concentrations around fish farms tend to be highly patchy, and the results of replicate sediment sampling in Passamaquoddy Bay showed that maximizing measurement precision can require high sampling replication ($n \geq 9$), particularly at the farm edge. Third, near real-time shipboard analysis of S^{2-} by the UV method provides accurate results and is analytically robust and practical for routine monitoring purposes. Rapid field analysis by the UV method permits the immediate interpretation of preliminary monitoring data, and this property can inform decisions on the need for additional sampling. The capacity for rapid S^{2-} analysis supports the potential use of a tiered monitoring approach in which a rapid assessment of S^{2-} conditions at a farm can be used to trigger a more rigorous monitoring program that includes additional abiotic and biotic indicators and more widespread sampling.

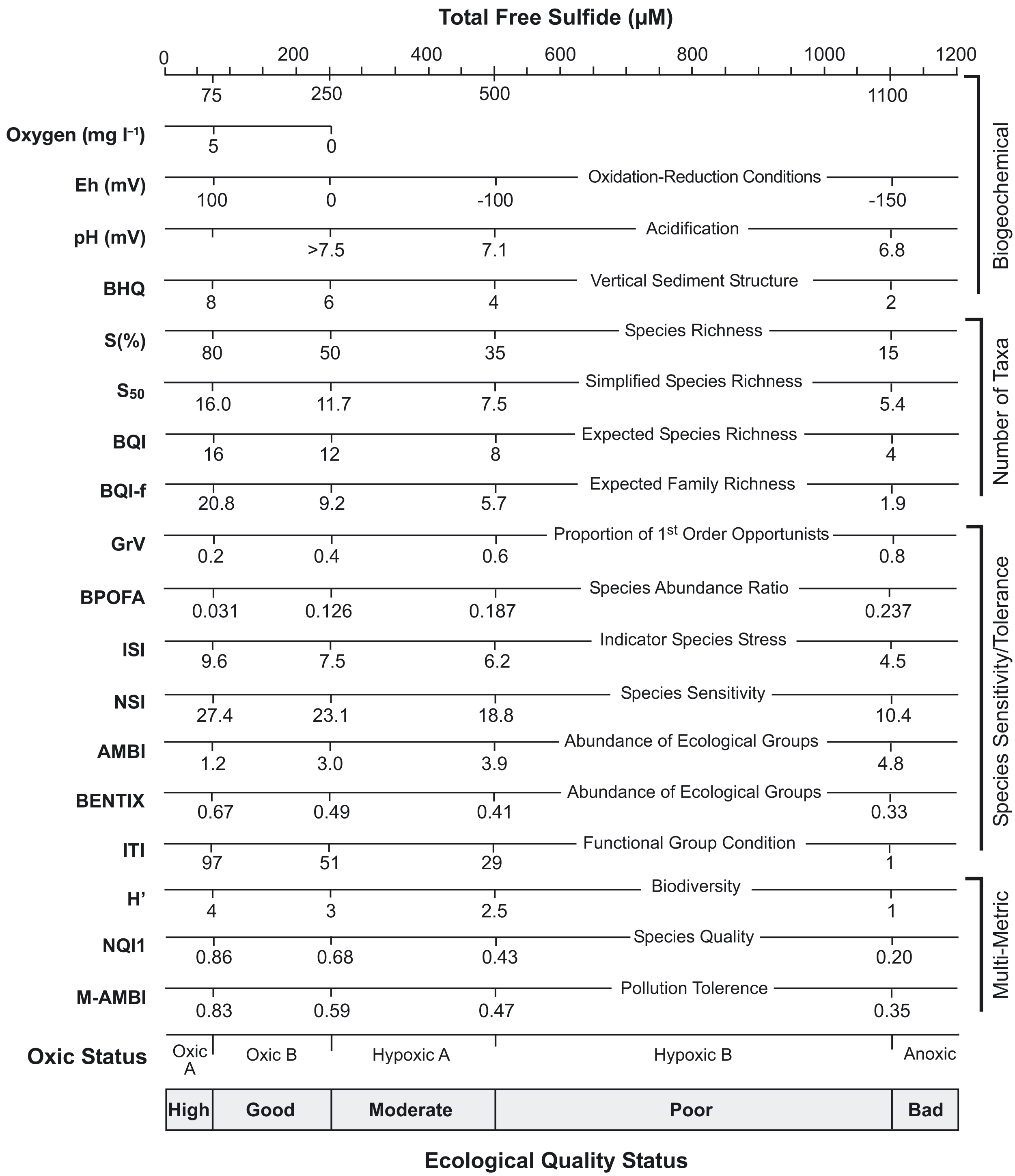

Fig. 10. Numerical ecological quality status (EQS) classes for benthic organic enrichment based on abiotic and biotic indicators. Equivalent EQS boundaries for total free sulfides, redox potential (Eh), Shannon-Wiener diversity (*H'*), species richness (*S*%), group V opportunistic species (GrV), and AZTI's Marine Biotic Indices (AMBI and M-AMBI) are from Cranford et al. (2020). Sediment oxygen EQS zones are from the present study. pH and benthic polychaete opportunistic families amphipod index (BPOFA) classes are from Schaaning & Hansen (2005) and Dauvin et al. (2016), respectively. The benthic habitat quality (BHQ) and number of species among 50 individuals (S_{50}) boundaries are derived from relationships in Hargrave (2010). Benthic quality index (BQI), benthic quality families index (BQI-f), infaunal trophic index (ITI), and BENTIX boundaries are from Rosenberg et al. (2004), Dimitriou et al. (2012), Ruellet & Dauvin (2007), and Simonini et al. (2009), respectively. The Norwegian quality (NQI1), sensitivity (NSI) and indicator species (ISI) index classes are from Husa et al. (2014) and Rygg & Norling (2013)

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LITERATURE CITED

Bagarinao T (1992) Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquat Toxicol 24:21–62

Berg P, Røy H, Janssen F, Meyer V, Jørgensen BB, Huettel M, de Beer D (2003) Oxygen uptake by aquatic sediments measured with a novel non-invasive eddy-correlation technique. Mar Ecol Prog Ser 261:75–83

Berg P, Glud RN, Hume A, Stahl H, Oguri K, Meyer V, Kitazato H (2009) Eddy correlation measurements of oxygen uptake in deep ocean sediments. Limnol Oceanogr Methods 7:576–584

Borja A, Chust G, Muxika I (2019) Forever young: the successful story of a marine biotic index. Adv Mar Biol 82: 93–127

Bravo F, Grant J (2018) Modelling sediment assimilative capacity and organic carbon degradation efficiency at marine fish farms. Aquacult Environ Interact 10: 309–328

Brodecka-Goluch A, Siudek P, Bolałek J (2019) Impact of sampling techniques on the concentration of ammonia and sulfide in pore water of marine sediments. Oceanol Hydrobiol Stud 48:184–195

Brooks KM, Mahnken CVW (2003) Interactions of Atlantic salmon in the Pacific Northwest environment II. Organic wastes. Fish Res 62:255–293

Brown KA, McGreer ER, Taekema B, Cullen JT (2011) Determination of total free sulphides in sediment porewater and artefacts related to the mobility of mineral sulphides. Aquat Geochem 17:821–839

Butman CA (1986) Sediment trap biases in turbulent flows: results from a laboratory flume study. J Mar Res 44: 645–693

Chipman L, Huettel M, Berg P, Meyer V, Klimant I, Glud R, Wenzhoefer F (2012) Oxygen optodes as fast sensors for eddy correlation measurements in aquatic systems. Limnol Oceanogr Methods 10:304–316

Cranford PJ, Anderson R, Archambault P, Balch T and others (2006) Indicators and thresholds for use in assessing shellfish aquaculture impacts on fish habitat. Can Sci Advis Sec Fish Aquat Sci 2006/034

Cranford PJ, Brager L, Wong D (2017) A dual indicator approach for monitoring benthic impacts from organic enrichment with test application near Atlantic salmon farms. Mar Pollut Bull 124:258–265

Cranford P, Brager L, Elvines D, Wong D, Law B (2020) A revised classification system describing the ecological quality status of organically enriched marine sediments based on total dissolved sulfides. Mar Pollut Bull 154: 111088

Cromey CJ, Nickell TD, Black KD (2002) DEPOMOD — modelling the deposition and biological effects of waste solids from marine cage farms. Aquaculture 214:211–239

Daly AJ, Baetens JM, De Baets B (2018) Ecological diversity: measuring the unmeasurable. Mathematics 6:119–147

Dauvin JC, Andrade H, de-la-Ossa-Carretero JA, Del-Pilar-Ruso Y, Riera R (2016) Polychaete/amphipod ratios: an approach to validating simple benthic indicators. Ecol Indic 63:89–99

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological effects and the behavioral responses of benthic macrofauna. Oceanogr Mar Biol Annu Rev 33: 245–303

Diaz RJ, Solan M, Valente RM (2004) A review of approaches for classifying benthic habitats and evaluating habitat quality. J Environ Manage 73:165–181

Dimitriou PD, Apostolaki ET, Papageorgioua N, Reizopoulouc S, Simbourac N, Arvanitidis C, Karakassis I (2012) Meta-analysis of a large data set with Water Framework Directive indicators and calibration of a benthic quality index at the family level. Ecol Indic 20: 101–107

Fenchel T (1987) Ecology — potentials and limitations. Excellence in Ecology, Book 1. International Ecology Institute, Oldendorf/Luhe

Giles H (2008) Using Bayesian networks to examine consistent trends in fish farm benthic impact studies. Aquaculture 274:181–195

Government of Canada (2015) Aquaculture activities regulations. SOR/2015-177. https://laws.justice.gc.ca/pdf/sor-2015-177.pdf

Grant J, Cranford P, Hargrave B, Carreau M and others (2005) A model of aquaculture biodeposition for multiple estuaries and field validation at blue mussel (*Mytilus edulis*) culture sites in eastern Canada. Can J Fish Aquat Sci 62:1271–1285

Gray JS, Wu RS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. Mar Ecol Prog Ser 238:249–279

Grieshaber MK, Völkel S (1998) Animal adaptations for tolerance and exploitation of poisonous sulfide. Annu Rev Physiol 60:33–53

Guenther EA, Johnson KS, Coale KH (2001) Direct ultraviolet spectrophotometric determination of total sulfide and iodine in natural waters. Anal Chem 73: 3481–3487

Hargrave BT (2010) Empirical relationships describing benthic impacts of salmon aquaculture. Aquacult Environ Interact 1:33–46

Hargrave BT, Phillips GA, Doucette LI, White MJ, Milligan TG, Wildish DJ, Cranston RE (1997) Assessing benthic impacts of organic enrichment from marine aquaculture. Water Air Soil Pollut 99:641–650

Hargrave BT, Holmer M, Newcombe CP (2008) Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Mar Pollut Bull 56: 810–824

Husa V, Kutti T, Ervik A, Sjøtun K, Hansen PK, Aure J (2014) Regional impact from fin-fish farming in an intensive production area (Hardangerfjord, Norway). Mar Biol Res 10:241–252

Keeley N, MacLeod C, Forrest B (2012) Combining best professional judgement and quantile regression splines to improve characterisation of macrofaunal responses to enrichment. Ecol Indic 12:154–166

Keeley NB, Cromey CJ, Goodwin EO, Gibbs MT, Macleod CM (2013) Predictive depositional modelling (DEPOMOD) of the interactive effect of current flow and resuspension on ecological impacts beneath salmon farms. Aquacult Environ Interact 3:275–291

Keeley N, Valdemarsen T, Woodcock S, Holmer M, Husa V, Bannister R (2019) Resilience of dynamic coastal benthic ecosystems in response to large-scale finfish farming. Aquacult Environ Interact 11:161–179
Kristensen E (1990) Characterization of biogenic organic matter by stepwise thermogravimetry (STG). Biogeochemistry 9:135–159
Kristensen E, Holmer M (2001) Decomposition of plant materials in marine sediment exposed to different electron acceptors (O_2, NO_3^-, and SO_4^{2-}), with emphasis on substrate origin, degradation kinetics, and the role of bioturbation. Geochim Cosmochim Acta 65:419–433
Law BA, Hill PS (2019) Spatial and temporal variation in cumulative mass eroded and organic matter percentage in surface sediments near areas of active salmon aquaculture. Aquacult Environ Interact 11:305–320
Long MH, Rheuban JE, Berg P, Zieman JC (2012) A comparison and correction of light intensity loggers to photosynthetically active radiation sensors. Limnol Oceanogr Methods 10:416–424
Lorrai C, McGinnis DF, Berg P, Brand A, Wüest A (2010) Application of oxygen eddy correlation in aquatic systems. J Atmos Ocean Technol 27:1533–1546
Martinez-Garcia E, Carlsson MS, Sanchez-Jerez P, Sanchez-Lizaso JL, Sanz-Lazaro C, Holmer M (2015) Effect of grain size and bioturbation on decomposition of organic matter from aquaculture. Biogeochemistry 125:133–148
Middelburg JJ, Levin LA (2009) Coastal hypoxia and sediment biogeochemistry. Biogeosciences 6:1273–1293
Mogg AOM, Attard KM, Stahl H, Brand T, Turnewitsch R, Sayer MDJ (2017) The influence of coring method on the preservation of sedimentary and biogeochemical features when sampling soft-bottom, shallow coastal environments. Limnol Oceanogr Methods 15:905–915
Nickell LA, Black KD, Hughes DJ, Overnell J and others (2003) Bioturbation, sediment fluxes and benthic community structure around a salmon cage farm in Loch Creran, Scotland. J Exp Mar Biol Ecol 285–286:221–223
Nilsson HC, Rosenberg R (2000) Succession in marine benthic habitats and fauna in response to oxygen deficiency: analyzed by sediment profile-imaging and by grab samples. Mar Ecol Prog Ser 197:139–149
Nilsson HC, Jönsson B, Lindström Swanberg I, Sundbäck K (1991) Response of a marine shallow-water sediment system to an increased load of inorganic nutrients. Mar Ecol Prog Ser 71:275–290
Papageorgiou N, Kalantzi I, Karkassis I (2010) Effects of fish farming on the biological and geochemical properties of muddy and sandy sediments in the Mediterranean Sea. Mar Environ Res 69:326–336
Pearson TH, Black KD (2001) The environmental impacts of marine fish cage culture. In: Black KD (ed) Environmental impacts of aquaculture. Sheffield Academic Press, Sheffield, p 1–31
Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr Mar Biol Annu Rev 16: 229–311
Phillips GR, Anwar A, Brooks L, Martina LJ, Miles AC, Prior A (2014) Infaunal quality index: Water Framework Directive classification scheme for marine benthic invertebrates. UK Environment Agency Report SC080016. Environment Agency, Bristol
Rosenberg R, Blomqvist M, Nilsson HC, Cederwall H, Dimming A (2004) Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Mar Pollut Bull 49:728–739
Ruellet T, Dauvin JC (2007) Benthic indicators: analysis of the threshold values of ecological quality classifications for transitional waters. Mar Pollut Bull 54:1707–1714
Rusch A, Huetel M, Wild C, Reimers CE (2006) Benthic oxygen consumption and organic matter turnover in organic-poor, permeable shelf sands. Aquat Geochem 12:1–19
Rygg B, Norling K (2013) Norwegian Sensitivity Index (NSI) for marine macroinvertebrates, and an update of Indicator Species Index (ISI). Report SNO 6475-2013. Norwegian Institute for Water Research, Oslo
Schaaning MT, Hansen PK (2005) The suitability of electrode measurements for assessment of benthic organic impact and their use in a management system for marine fish farms. In: Hargrave B (ed) The handbook of environmental chemistry, Vol 5: water pollution, Part M. Springer-Verlag, Berlin, p 381–408
Simonini R, Grandi V, Massamba-N'Siala G, Iotti M, Montanari G, Prevedelli D (2009) Assessing the ecological status of the north-western Adriatic Sea within the European Water Framework Directive: a comparison of Bentix, AMBI and M-AMBI methods. Mar Ecol 30: 241–254
Vaquer-Sunyer R, Duarte CM (2010) Sulfide exposure accelerates hypoxia-driven mortality. Limnol Oceanogr 55: 1075–1082
Wang FY, Chapman PM (1999) Biological implications of sulfide in sediment—a review focusing on sediment toxicity. Environ Toxicol Chem 18:2526–2532
Wildish DJ, Hargrave BTH, Pohle G (2001) Cost-effective monitoring of organic enrichment resulting from salmon mariculture. ICES J Mar Sci 58:469–476
Wu Y, Chaffey J, Law B, Greenberg DA, Drozdowski A, Page F, Haigh S (2014) A three-dimensional hydrodynamic model for aquaculture: a case study in the Bay of Fundy. Aquacult Environ Interact 5:235–248

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