Biogeochemistry **32:** 15–39, 1996.

Seasonality of sulfate reduction and pore water solutes in a marine fish farm sediment: the importance of temperature and sedimentary organic matter

MARIANNE HOLMER & ERIK KRISTENSEN
Institute of Biology, Odense University, Campusvej 55, DK-5230 Odense M.

Received 25 May 1995; accepted 12 September 1995

Key words: fish farm sediment, carbon mineralization, sulfate reduction, acetate

Abstract. Sulfate reduction and pore water solutes related to sulfur cycling and anaerobic processes (short chain fatty acids (SCFA), SO_4^{2-}, TCO_2, NH_4^+, dissolved sulfides ($\sum H_2S$) and CH_4) were examined during one year at a marine fish farm. Mineralization of fish farm waste products was rapid in this non-bioturbated, organic rich sediment. Stimulation of sulfate reduction rates (SRR) occurred primarily in the surface layers where the organic matter was deposited. Acetate was the most important (>99%) of the measured SCFA attaining high concentrations during summer months (up to 4.7 mM). The acetate profiles exhibited distinct seasonal cycles, where periods with high concentrations in the pore waters were found coincident with a high pool of particulate organic matter in the surface sediments and a low activity of the sulfate reducing bacteria (early spring and late summer). Periods with low acetate pools occurred when sulfate reduction rates were high in early summer and in winter were pools of particulate organic matter were decreasing. Methane production was observed concurrent with sulfate reduction in the microbial active surface layers in late summer. Subsurface peaks of SO_4^{2-}, TCO_2, NH_4^+ and $\sum H_2S$ were evident in July and August due to rapid mineralization in these surface layers. With decreasing autumn water temperatures mineralization rates declined and subsurface peaks of these solutes disappeared. A strong relationship was found between pore water TCO_2 and NH_4^+. Ratios between TCO_2 and NH_4^+ were low compared to a control site, attaining minimum values in mid-summer. This indicated rapid nitrogen mineralization of nitrogen rich labile substrates in the fish farm sediment during the entire season.

Introduction

Cycling of organic matter in coastal environments often exhibits pronounced seasonal variations. Fluctuations in rates of benthic metabolism, nutrient regeneration and sediment-water interactions are usually considered a response to the annual variation in water temperatures (Jørgensen & Sørensen 1985; Klump & Martens 1989; Kristensen 1993). Benthic metabolism is generally proportional to carbon input (Westrich & Berner 1984), but not necessarily with organic content of sediments (Jørgensen 1978; Burdige 1991). However, in sediments receiving large amounts of organic matter, e.g. due to eutrophication, sewage deposit or fish farming, organic matter input may bias a strict temperature control (Sampou & Oviatt 1991; Holmer & Kristensen 1992). Diagenetic analysis of newly deposited sediments have

shown that the quality as well as the quantity of sedimentary organic carbon controls microbial processes (Westrich & Berner 1984; Burdige 1991; Kristensen & Hansen 1995). However, the combined effect of organic loading and temperature on benthic mineralization has not been widely studied, especially in eutrophic sediments (Oenema 1990; Sampou and Oviatt 1991; Hargrave et al. 1993).

Fish farm sediments generally receive large amounts of high quality organic matter which leads to a concurrent increase in benthic metabolism primarily by stimulating anaerobic processes like sulfate reduction and methanogenesis (Hall et al. 1990; Holmer & Kristensen 1992; Hansen et al. 1993). Accordingly, the seasonal variation in sulfate reduction rates can only partly be explained by variations in water temperature. As the terminal mineralization of organic carbon in these sediments occurs mainly through sulfate reduction, the seasonal variation in dissolved substrates (e.g. short chain fatty acids) for the sulfate reducing bacteria (SRB) is an important controlling factor in the overall benthic metabolism. Sulfate reducing bacteria do use a wide range of electron donors (Widdel 1988; Parkes et al. 1989; Hansen & Blackburn 1995), but short chain fatty acids (SCFA) have been found to be the most important, with acetate as the primary substrate (Parkes et al. 1989). SCFA are produced by anaerobic decay of a wide variety of compounds, e.g. during hydrolysis and fermentation of proteins (amino acids), lipids and carbohydrates (McInerney 1988). The turnover rates of SCFA are usually very high and only small pools are found in the pore waters (Sansone & Martens 1982; Christensen 1984; Alperin et al. 1994).

The purpose of this study was to determine factors controlling sulfate reduction in an organic rich fish farm sediment from observations of seasonality in measured sulfate reduction rates, inorganic pore water solutes including gasses (SO_4^{2-}, TCO_2, NH_4^+, $\sum H_2S$, CH_4) and substrates (SCFA) for the SRB. The study is an extension of previous work on benthic metabolism and sediment-water interactions in the same fish farm sediments (Holmer & Kristensen 1992).

Materials and methods

Sample collection

Sediment was sampled in 1992 at a marine fish farm situated in Kolding Fjord, Denmark (Fig. 1). A detailed description of the study site is given by Holmer & Kristensen (1992). The farm contained 14 circular net cages with a diameter of 12.7–15.9 m. Two stations were examined: Sta. 1 underneath one of the net cages, and Sta. 2 at a control site 30 m away from the farming

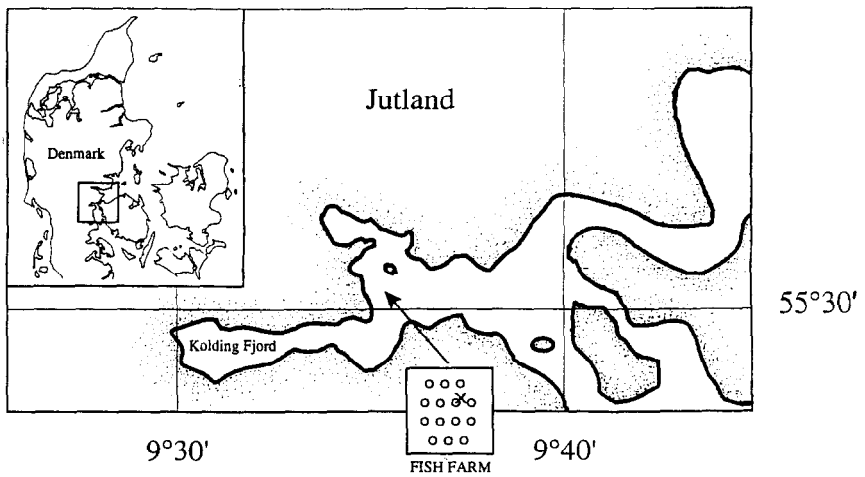

Fig. 1. Location of the fish farm in 1992 (indicated by arow). Insert display outline of the net cages in the fish farm, where the position of Station 1 is shown (x).

area. Fish farming was initiated in April and terminated in November. The production of rainbow trout, *Oncorhynchus mykiss*, in the cage overlying Sta. 1 was 11.0 tons, which is approximately 2 times higher than during a former study in 1989 (Holmer & Kristensen 1992). The food conversion coefficient (the ratio between food input(ww) and net fish production(ww)) was 1.52, which is 6% higher than in 1989. The trout were fed with dry pellets (Aller Diamant 4300; Aller Mølle), consisting of ~50 %dw organic carbon and ~7 %dw organic nitrogen.

Sediment cores for solid phase analysis, pore water extractions (5.0 cm i.d.) and cores for sulfate reduction assays (2.6 cm i.d.) were collected at monthly intervals from March to November 1992 by scuba diving using plexiglass core liners.

Sulfate reduction

Sulfate reduction rates were determined in 1-cm intervals to 10 cm depth in 3 replicate cores from the 2 stations. A volume of 2 μl carrier-free $^{35}S\text{-}SO_4^{2-}$ solution (~70 kBq) was injected through silicone ports at each depth. Cores from Sta. 1 were incubated for 3–6 h and from Sta. 2 for 6 h in darkness at *in situ* temperature (Fig. 2). After incubation and sectioning, each sediment slice (5.3 cm^3) was fixed in 5 ml 1 M zink acetate, and subsequently frozen. One additional core from each station was fixed similarly, but without previous incubation, and tracer was injected after fixation to determine a sediment blank value (Fossing pers. comm.). Separation of reduced sulfur compounds was

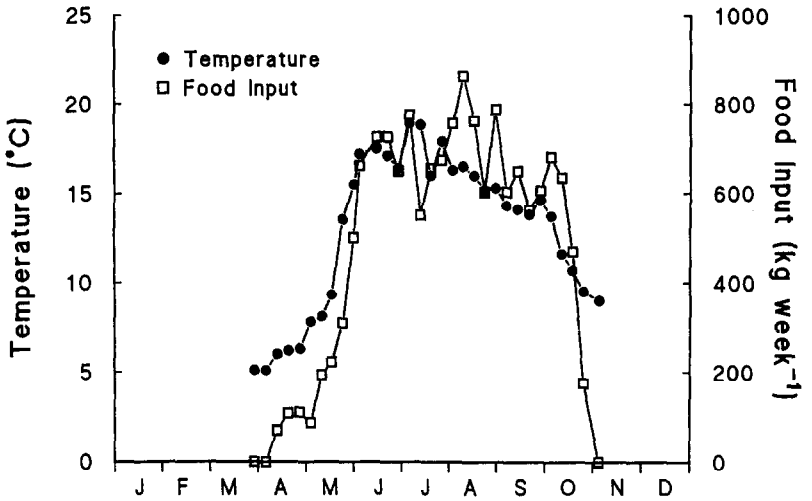

Fig. 2. Seasonal changes in water temperature measured weekly at the fish farm and food input to the net cage overlying Sta. 1 given in kg food pellets pr. week (data provided from fish farmer).

performed by the 1 step (TRIS, total reducible inorganic sulfur) distillation procedure of Fossing & Jørgensen (1989). The sediment pellet was transferred directly to a reaction flask, and after degassing for 10–15 min the sediment was distilled by boiling for 40 min using 0.5 M Cr^{2+} in 3 M HCl (34 ml). TRIS was trapped as H_2S in 10 ml 250 mM buffered zink acetate. Subsamples of traps were mixed with Ultima Gold scintillation liquid and the radioactivity was counted on a Packard Tri-Carb 2200 Liquid Scintillation Analyzer.

Pore water

Pore water was extracted from 3 replicate cores within 4–6 h of sampling by squeezing through combusted (300 °C) GF/C filters according to Reeburgh (1968). The cores were sectioned in 1-cm segments down to 6 cm, and in 2-cm segments down to 16 cm. The first ml of the obtained pore water was discarded. The remainder (5–10 ml) was analyzed for short chain fatty acids (SCFA, C_1-C_4), SO_4^{2-}, TCO_2, NH_4^+ and dissolved sulfides ($\sum H_2S$). A test of the squeezing method showed constant concentrations of all components until ~25% of the pore water was left in the sediment pellet.

Samples for SCFA were stored frozen until analysis, and were measured by ion exclusion HPLC as described by Bøtte & Jørgensen (1992) modified for seawater analysis. The HPLC was equipped with a conductivity detector, an ion-exclusion column (Ion-Pac, Waters) with 1.00 mM H_2SO_4 as eluent, and an Anion MicroMembrane Suppressor (AMMS-ICE, Dionex corp.) with

5.00 mM TBaOH as regenerant. The precision was better than 5%, and the detection limit was 2–5 μM for chain lengths of C_1-C_4.

Samples for sulfate were preserved in HCl (pH = 2) and stored at 5 °C until analysis by HPLC anion chromatography. TCO_2 was determined within 24 hours by flow injection analysis according to Hall & Aller (1992) with 30 mM HCl as carrier and 10 mM NaOH as receiver. Interfering sulfides were precipitated with $ZnCl_2$. Samples for NH_4^+ were stored frozen until analysis using the salicylate-hypochlorite method (Bower & Holm-Hansen 1980) on a strip reader (Microwell, EL301). Dissolved sulfides ($\sum H_2S$) were determined by the method of Cline (1969) on samples precipitated with 0.5 M zink acetate. Measurements of pH were done on intact sediment by inserting a pH electrode (Radiometer) directly into the sediment and allowing the reading to stabilize for 2–3 min.

Sediment characteristics

Sediment characteristics (density, water content and loss-on-ignition (LOI)) were determined on duplicate cores in 1-cm intervals to 6 cm depth and in 2-cm down to 16 cm depth. Density was obtained from the wet weight of a known volume. Water content and LOI were obtained after drying for 6 hours at 105 °C and 520 °C, respectively. Total pools of particulate organic carbon (POC) and nitrogen (PON) were measured in duplicates for each interval in the upper 0–4 cm and only single samples in the lower 4–12 cm as described by Kristensen & Andersen (1987) using a Carlo Erba Elemental Analyzer 1100A.

Results

Visual observations

The sediment at the two stations appeared quite similar in March, one month before fish farming was initiated. The only difference being the presence of a few macrofauna (>1 mm) at Sta. 2, while no macrofauna were found at Sta. 1 throughout the study period. During April-May a massive spring bloom of the algae *Chrysochromulina sp.* occurred, and phytoplanktonic detritus was evident at the control site as a ~0.5 cm flocculent layer on top of the sediment. During the rest of the study period the sediment at Sta. 2 had a 1–2 cm brown oxidized layer, overlying a 4–6 cm black zone. Below 6 cm the sediment was a grey/green silty clay.

At Sta. 1 the upper sediment layer was still oxidized in April, but fish farm waste products, faeces and food started to appear as scattered pellets

on the surface. By May the oxidized zone was absent and a layer of waste products, intermixed with white spots of *Beggiatoa sp.*, covered the sediment surface. Meiofauna (unidentified nematodes) were abundant at the sediment-water interface in June whereas in July and August the sediment was free of fauna and completely covered by a white layer of *Beggiatoa sp.*. Small bubbles of methane (detected by GC analysis) were observed in the upper 1–4 cm of the sediment. The bacterial mat degenerated in September and only small amounts of waste products were visible at the surface. Finally, the sediment surface regained its oxidized appearence in October and November. Deeper sediment layers (>2 cm) remained black during the entire sampling period.

Fish food input

Due to the bloom of algae, which were toxic to the farmed fish, food input to the farm was lower than usual until the beginning of June. This was one month later than in normal years. Trout were placed in the net cage at Sta. 1 at the end of March, but food addition was fairly low until the end of May (Fig. 2). Food input was terminated at the end of October, and the fish were harvested in the beginning of November.

Particulate organic carbon and nitrogen

Elevated organic content was found in the upper layers (0–2 cm) at Sta. 1 from April to September (Fig. 3). POC and PON at Sta. 1 peaked in August (20 and 2 mmol g dw^{-1}, respectively). There was a strong correlation between the accumulated food input and the organic carbon content in the upper layers at Sta. 1 until September (Fig. 4). Later, POC decreased despite continued high input rates of food. At Sta. 2, the organic content (especially nitrogen) was slightly elevated during the spring bloom sedimentation, and decreased gradually during the rest of the sampling period. No significant difference between the 2 stations was evident below 3 cm depth in the sediment, where the POC content was 5.8-7.5 mmol g dw^{-1} and the PON content was 0.5–0.6 mmol N g dw^{-1}.

Short chain fatty acids (SCFA)

Of the four analyzed SCFA (formate, acetate, propionate and butyrate) only formate and acetate were found above detection limit (2–5 μM). Acetate attained very high concentrations (2.4–4.7 mM) in the upper 1–4 cm of the sediment at Sta. 1 during intense fish farming in July and August (Fig. 5). Except for April, acetate concentration was much lower during rest of the

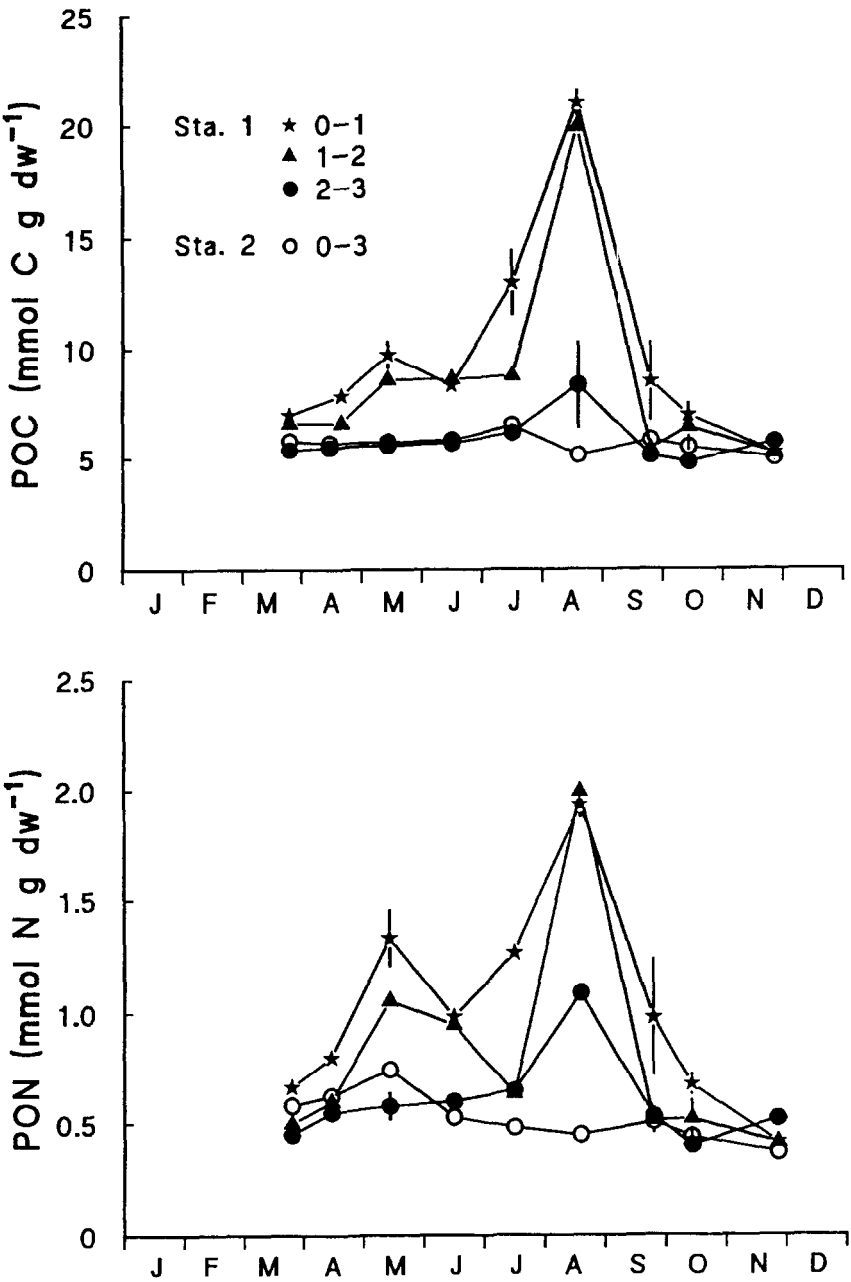

Fig. 3. Pools of particulate organic carbon (upper) and nitrogen (lower) in the surface layers at Sta. 1 (0–1, 1–2, 2–3 cm) and Sta. 2 (0–3 cm). Each point represents the mean ±range of duplicate cores at Sta. 1, and the average of the upper 3 cm (measured in 1-cm intervals on duplicate cores) at Sta. 2.

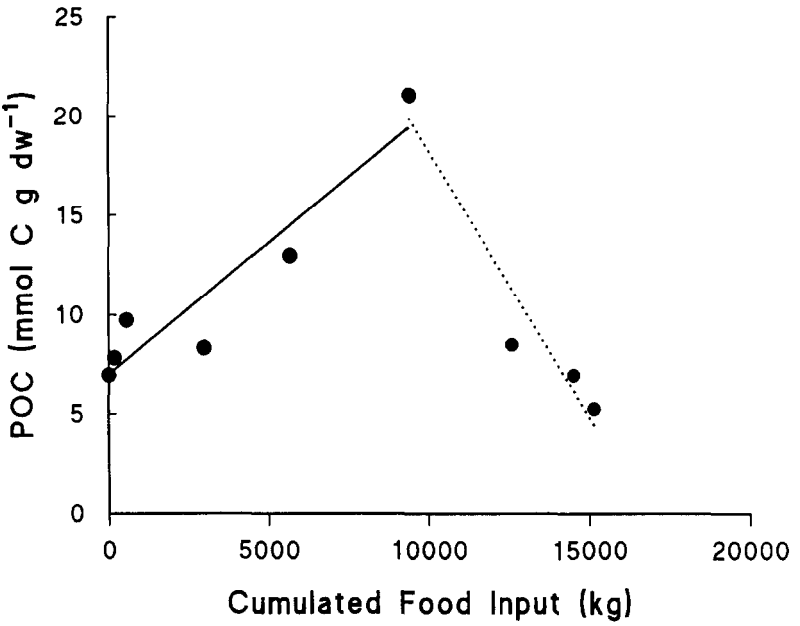

Fig. 4. The relationship between cumulated food input to the net cage overlying Sta. 1 and the particulate organic carbon content (POC) in surface sediment (0–1 cm).

study period (<100 μM). Maximum concentrations were always observed in the surface layers (0–2 cm), and decreased rapidly with depth and approached detection limit at about 6 cm depth, except for July and August where acetate was detected down to 12 cm depth. At Sta. 2, acetate was generally found close to detection limit, with maximum values of 20–50 μM at 3–5 cm in April and May (data not shown).

Formate was only present in the surface layers at Sta. 1 in April, July and August where concentrations reached 5–125 μM (data not shown). At Sta. 2 no SCFA other than acetate were measured.

Sulfate reduction rates

The sulfate reduction rates (SRR) at Sta. 1 were very high during the farming season (Fig. 6). The rates were generally highest close to the sediment surface, with notably higher SRR at Sta. 1 than at Sta. 2 down to depths of 6–8 cm. At Sta. 1 the rates in the upper layers increased from <158 nmol cm^{-3} d^{-1} in early spring to a maximum of ~3.8 μmol cm^{-3} d^{-1} in June. In July and August the core-injection technique was inappropriate due to low sulfate concentrations in the surface sediment and were estimated from the pore water profiles of sulfate (see below). Maximum SRR declined through the fall reaching 541 nmol cm^{-3} d^{-1} in November.

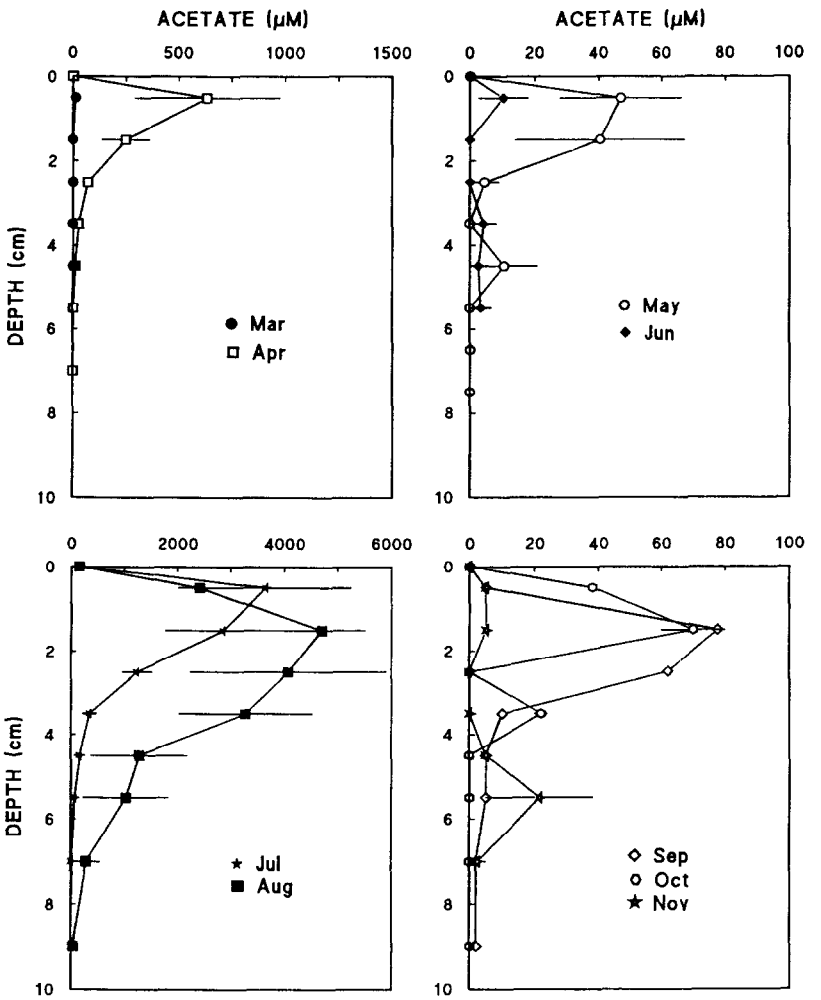

Fig. 5. Depth profiles of acetate at Sta. 1 at each sampling date. Values are given as mean ±SE of triplicate cores. Note the difference in concentration scales.

Sulfate reduction rates were highest in the surface layers and decreasing with depth at Sta. 2. A seasonal variation was primarily seen as increasing rates throughout the examined depth interval. In April, September and October, however, a subsurface maximum was seen in the 1–2 cm depth interval. Surface rates increase from 166 nmol cm^{-3} d^{-1} in March to a maximum of 721 nmol cm^{-3} d^{-1} in June.

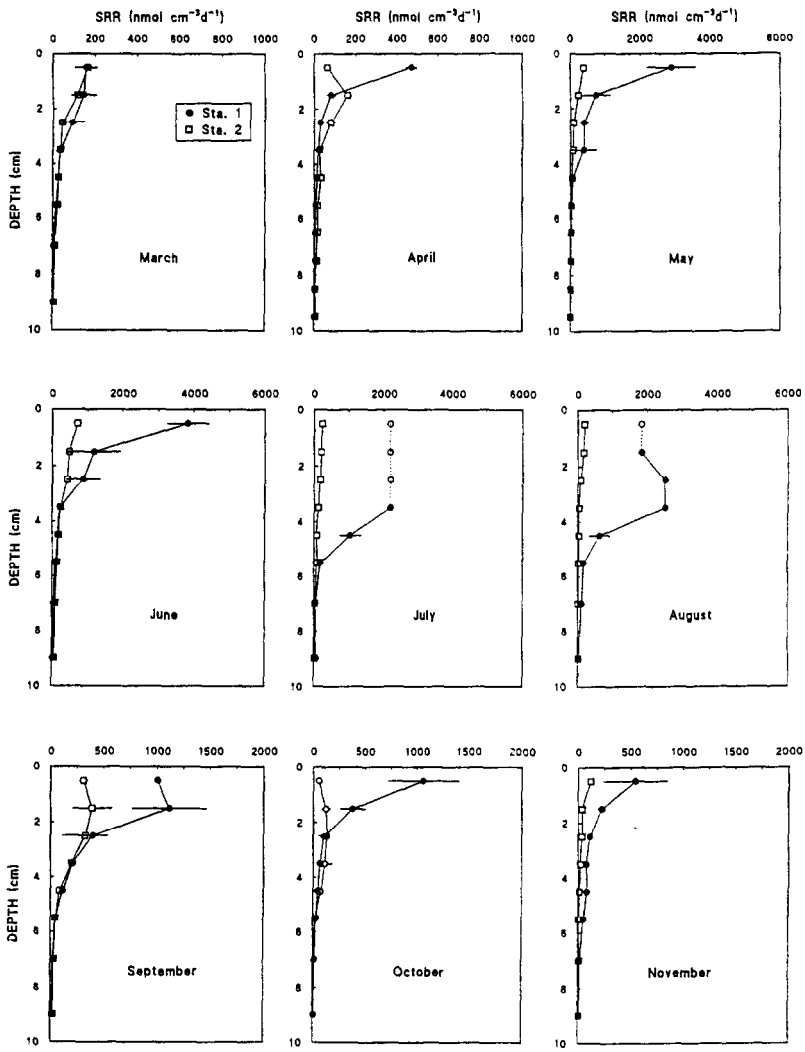

Fig. 6. Depth profiles of sulfate reduction rates (SRR) at Sta. 1 (closed circles) and Sta. 2 (open squares) at each sampling date. Values represent mean ±SE of triplicate cores. Note difference in scales.

Pore water sulfur

The pore water sulfate concentrations at Sta. 1 changed dramatically with time and depth. Most notably was the distinct subsurface minimum at 0–8 cm depth in July and August (Fig. 7). Before and after this period the sulfate concentrations gradually decreased and increased respectively in the surface layers and before April and after October the profiles were similar to those

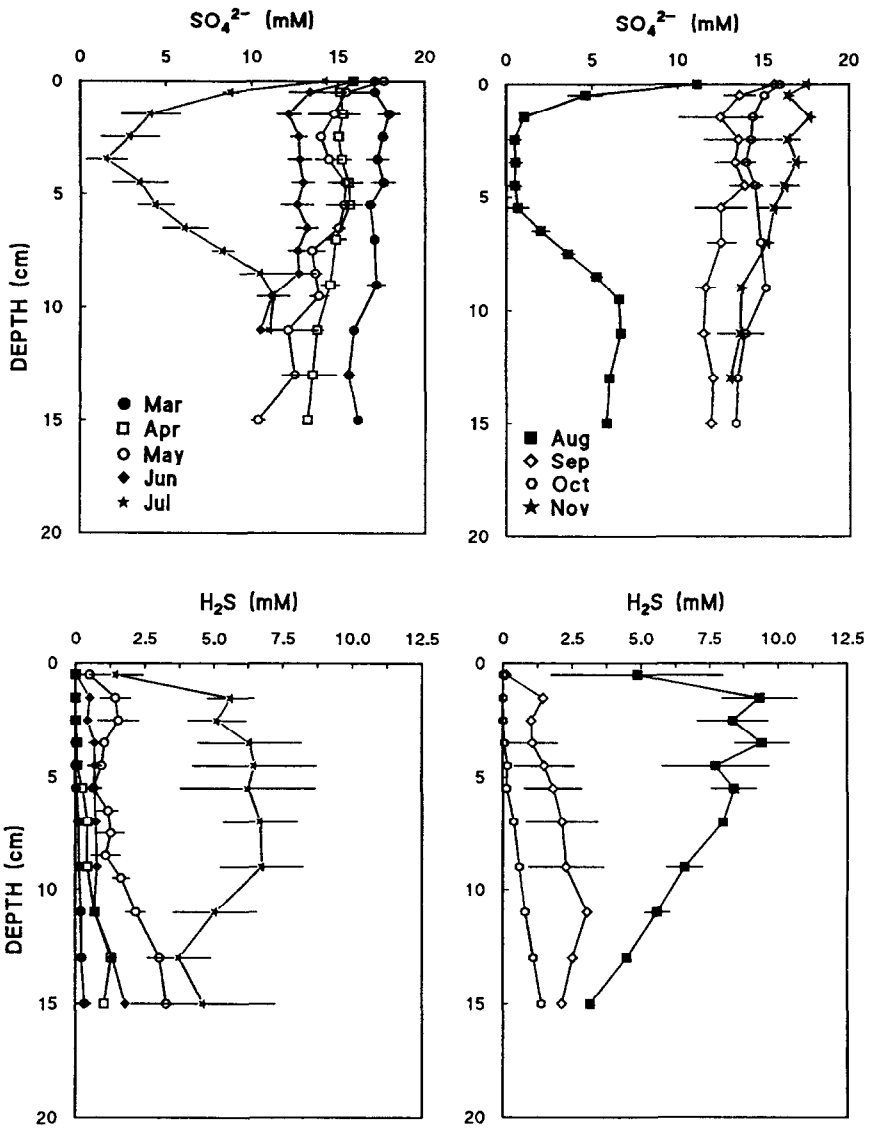

Fig. 7. Depth profiles of sulfate (SO_4^{2-}) (upper) and dissolved sulfides (H_2S) (lower) at Sta. 1. Values represent mean ±SE of triplicate cores.

at the control site, showing a slight decrease with depth (Fig. 8). In July and August methane bubbles were observed in the layers with low sulfate concentration (0.5 mM sulfate in 1–4 cm's depth).

During July and August the SRR in the upper layers at Sta. 1 were estimated based on fluxes calculated from pore water sulfate profiles (Fig. 7). According

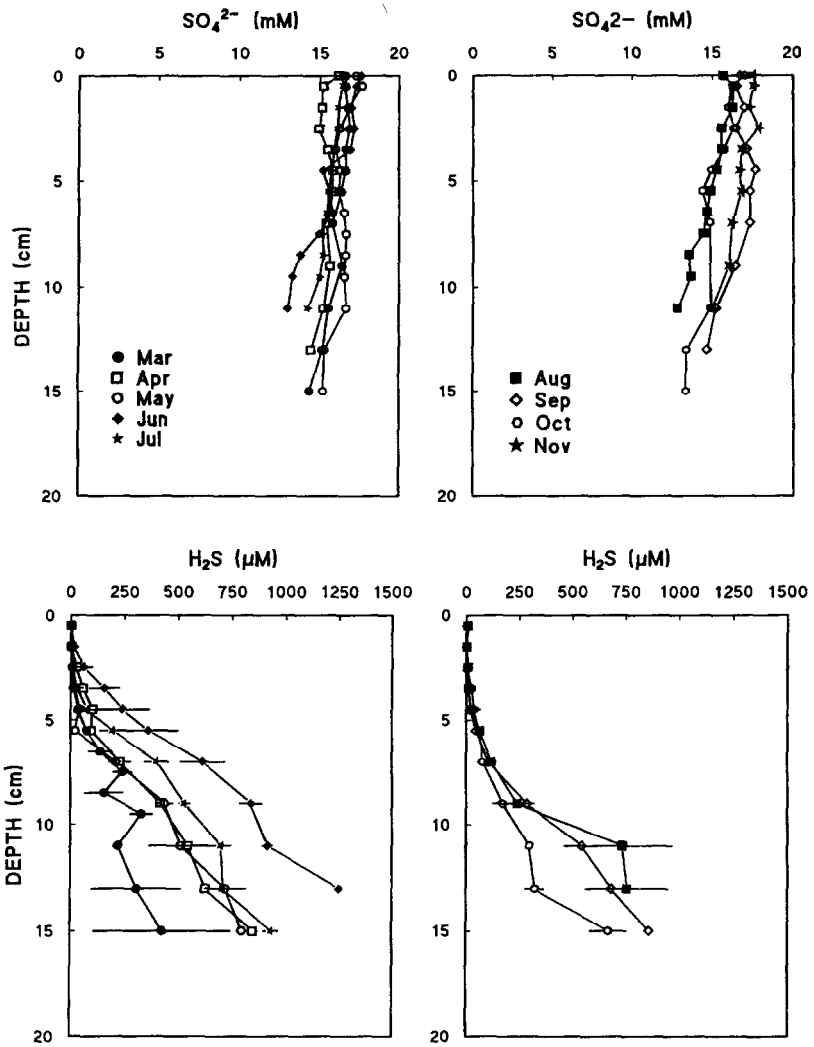

Fig. 8. As in Fig. 7 at Sta. 2.

to Ficks 1. law of diffusion, the flux can be estimated as: $J = -\phi * D_s * dC/dx$. J is the flux in mmol m^{-2} d^{-1} and D_s is the sediment diffusion coefficient of SO_4^{2-} in seawater ($D_0 = 5.00 * 10^{-6}$ cm^{-2} s^{-1}) corrected for temperature, according to Li & Gregory (1974), and porosity, according to Ullman & Aller (1984) for high porosity muds ($D_s = \phi^2 * D_0$). By calculating J for the concentration gradients (1-cm depth intervals) above and below the subsurface sulfate peak, an average SRR for the depth intervals examined was estimated. These rates were 2100 and 1900 nmol cm^{-3} d^{-1} in July and August, respectively (Fig.

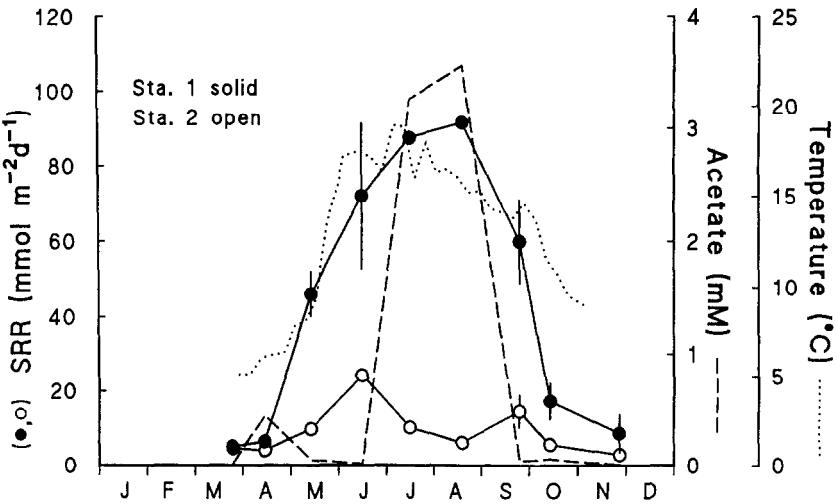

Fig. 9. The seasonal variation of depth integrated sulfate reduction rates (SRR) at Sta. 1 (closed circles), Sta. 2 (open circles), and the concentration of acetate in the surface layer at Sta. 1 (0–2 cm) (dashed line). The seasonal changes in water temperature is shown (dotted line).

6 open symbols). The method may underestimate the true sulfate reduction rate, as processes like reoxidation and mixing during handling (e.g. methane gas bubbling) were not considered.

Pore water concentrations of dissolved sulfides ($\sum H_2S$) attained high values during July and August at Sta. 1 (Fig. 7), when SRR were high. Subsurface peaks were present in both cases, with maximum values of 6.5 and 9.0 mM in July and August, respectively. In May, June and September, on the other hand, concentrations increased gradually from ~100 μM in the upper layers to ~2500 μM with depth. In April and in October-November $\sum H_2S$ was low until a depth of 5 cm, reaching a concentration of ~750 μM at 15 cm depth which was similar to profiles from Sta. 2 (Fig. 8). The seasonal variation in $\sum H_2S$ concentration at the latter station (500–1250 μM at 15 cm) was evident as a steeper increase with depth in June and July.

The seasonal variations in the depth integrated (0–10 cm) sulfate reduction rates ($\sum$SRR) and acetate concentrations in the surface layer (0–2 cm) are shown for the two stations in Fig. 9. SRR were 2–10 times higher during the farming season at Sta. 1 compared to Sta. 2, with maximum rates in July and August (87.4–91.5 mmol m^{-2} d^{-1}). Acetate concentrations in the surface layers showed two seasonal maxima, one minor in April (0.6 mM) and one major in July and August (2.4–4.7 mM), whereas the concentration was low (<100 μM) during rest of the season. At Sta. 2, SRR generally followed the

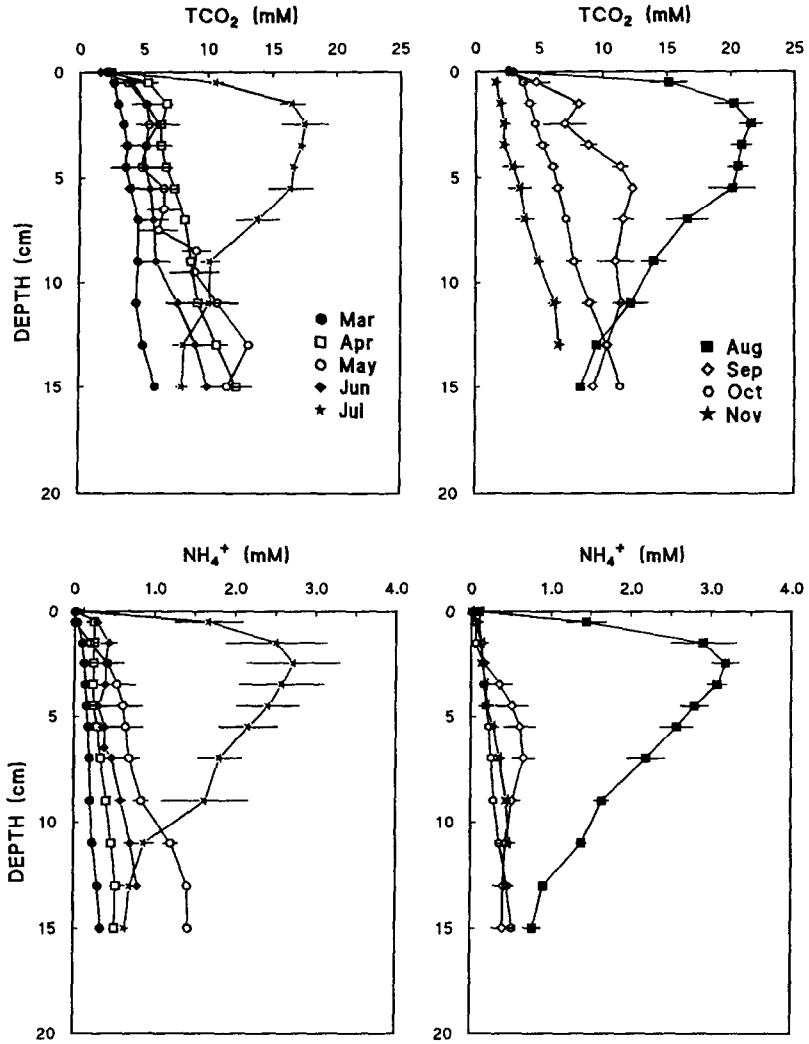

Fig. 10. Depth profiles of TCO_2 (upper) and ammonium (NH_4^+) (lower) at Sta. 1. Values are given as the mean ±SE of triplicate cores.

temporal variation in water temperature (2.7–24.2 mmol m^{-2} d^{-1}), except for a fall maximum in September despite decreasing temperature (14.5 mmol m^{-2} d^{-1}).

TCO_2 and NH_4^+

The depth profiles of TCO_2 and NH_4^+ in pore water at Sta. 1 showed rapid changes during the sampling period (Fig. 10). Both compounds attained

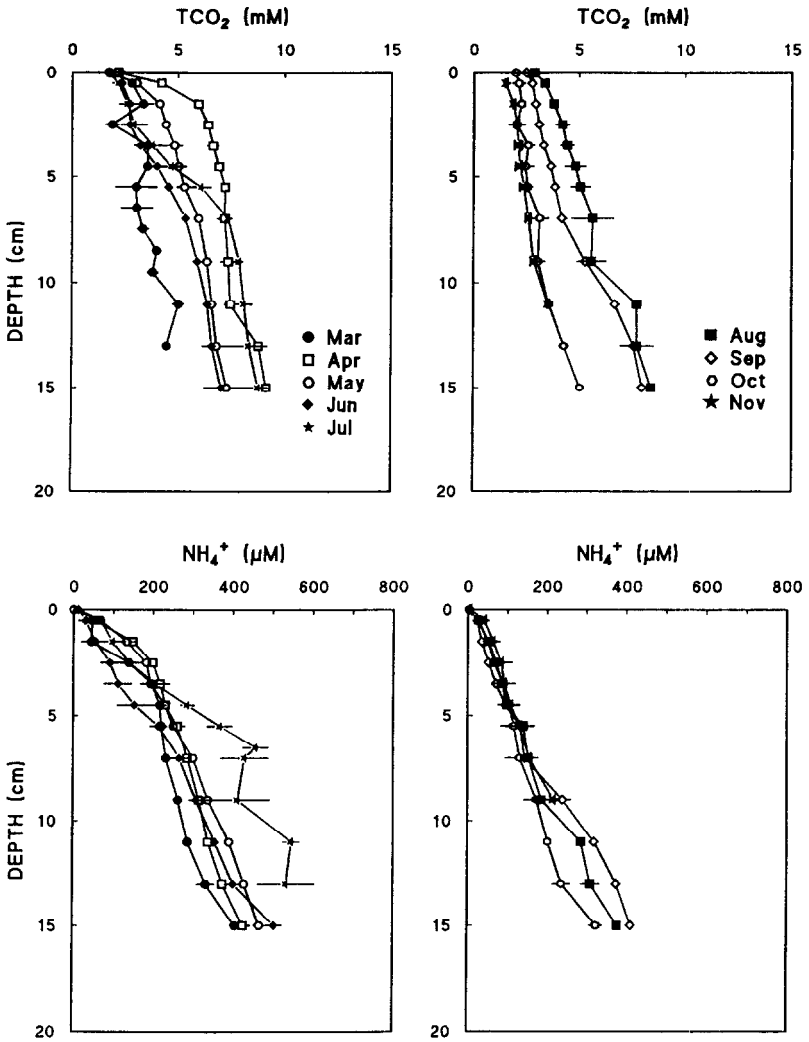

Fig. 11. As in Fig. 10 at Sta. 2.

higher concentrations (2–5 times) at this station compared to Sta. 2 (Fig. 11) during the entire study period. Subsurface maxima, similar to those for acetate and SRR, were found in July and August with values of 16–20 mM TCO_2 and 2.8–3.1 mM NH_4^+ in the 1–5 cm depth interval. During rest of the period both compounds attained lowest concentrations in the surface layer and steeply increasing to highest concentrations in the deep layers. Exceptions from these concentration gradients were seen in April, where elevated concentrations were measured in the 1–2 cm layer. High surface concentra-

tions were also found in June and September. At Sta. 2 the concentrations were increasing with depth, where changes in the profiles largely followed the seasonal variation in water temperatures with maximum values during June and July of 8 mM TCO_2 and 0.5 mM NH_4^+ in the 12–16 cm interval.

Sediment pH

The pH was generally lower at Sta. 1 (6.7–7.0) than at Sta. 2 (7.4–7.8) with a minimum in the upper 0–1 cm especially during July and August (data not shown). During October and November pH was between 7.4 and 7.7 similar to Sta. 2.

Discussion

The input of organic rich particulate waste products from fish farming to the underlying sediments strongly stimulates anaerobic metabolism, and affects the seasonal variation of major biogeochemical cycles (Hall et al. 1990; Hall et al. 1992; Hargrave et al. 1993). Seasonal variations in depth distributions of dissolved organic matter (DOM) reflect changes in rates of particulate organic matter (POM) hydrolysis, fermentation and DOM consumption while seasonal variations in sulfate, total CO_2 and sulfides reflect changes in terminal metabolism (Alperin et al. 1994).

Pore water pools of acetate varied considerably during the season with concentrations in summer several orders of magnitude higher than usually found in coastal sediments (Sansone & Martens 1982; Christensen 1984; Michelson et al. 1989; Alperin et al. 1994; Hines et al. 1994). The initial conditions of the studied farming season were atypical due to a bloom of toxic phytoplankton in April and May. The fish suffered and did not eat efficiently, which caused an extraordinarily high loss of undigested food pellets to the sediments, and the spring peak in pore water acetate may therefore be exceptional. Sulfate reducing bacteria (SRB) are considered substrate limited under normal conditions (Westrich & Berner 1984; Burdige 1991), given that the pool of SCFA in most sediments is usually low due to its higher consumption than production (Christensen 1984; Parkes et al. 1989; Parkes et al. 1993). Measurements of acetate oxidation have shown rapid turnover rates, often higher than accounted for by the sulfate reduction (Christensen 1984; Gibson et al. 1989; Kristensen et al. 1994), and this discrepency is probably caused by an overestimation of the bioavailable acetate pool (Novelli et al. 1988; Michelson et al. 1989; Hines et al. 1994). In a previous study at the present fish farm using same techniques as here, sulfate reduction and acetate oxidation rates were similar and indicated that

measured acetate in the pore water is all bioavailable (Holmer & Kristensen 1994a).

Vertical profiles of acetate suggested a maximum production rate at 0–2 cm depth in early spring and late summer. Studies have demonstrated elevated concentrations of short chain fatty acids due to rapid hydrolysis at high summer temperatures (Sansone & Martens 1981, 1982). However, during periods of high production, the rate of consumption may also be reduced if the sulfate reduction declines due to sulfate limitation (Alperin et al. 1994). The dynamics of the SCFA in the present fish farm sediment can be revealed by considering both the production and consumption of acetate. Acetate production rates (APR) are here estimated by extrapolating measured rates (experimental) (Holmer & Kristensen 1994b) to seasonal changes in the sediment pool of reactive particulate carbon (POC) and water temperature. The reactive POC pool is calculated by substraction of the carbon pool in the fish farm sediment from the control site at the date of sampling. The reactive POC used experimentially was food pellets, whereas the POC at the field site is a mixture of food pellets, faeces and planktonic detritus. This possibly leads to an overestimation of the acetate production rates, except for April and May where a large sedimentation of food pellets occurred. Acetate consumption rates are based on measured sulfate reduction assuming acetate as the sole electron donor. Net acetate production is estimated as the difference between the production and consumption. From these calculations it is possible to get an impression of the carbon metabolism at the date of sampling.

The estimated APR increased rapidly during the initial farming period and attained highest rates in August (Table 1) followed by a decline in autumn. This seasonal variation was similar to the changes in SRR, although the up to 5 times higher APR in July and August indicated a positive net production. This corresponded well with the high accumulation of pore water acetate during this period.

The production of acetate in April was not immediately followed by an increase in sulfate reduction. Acetate production increased rapidly in response to the massive input of POM at the start of farming while SRB appeared less stimulated. Thamdrup et al. (1994) also found that the response of sulfate reduction rates to POM buried in the anoxic sediment during a spring bloom was delayed until summer. The pool of reactive POM is usually high in spring due to low microbial decay during winter (Sansone & Martens 1982; Klump & Martens 1989; Alperin et al. 1994), deposition of a spring bloom (Jensen et al. 1990; Thamdrup et al. 1994), and/or sulfate reduction remaining low and temperature controlled. The response found at the fish farm indicated that hydrolysis and fermentation processes are more dependent on both organic matter deposition and temperature than terminal carbon mineralization.

Table 1. Seasonal acetate production rates (APR), sulfate reduction rates (SRR) and net production (Net Prod) of acetate in the surface layers (0–1 cm and 1–2 cm) at Station 1. The net production is calculated as the difference between APR (estimated from the enrichment of sediment (POC) and acetate production rates obtained in Holmer & Kristensen, 1994b) and SRR. The production is positive (+) when the acetate production exceeds the sulfate reduction (stoichiometry 1:1 of acetate consumed and sulfate reduced) and negative (−) when the sulfate reduction rates exceed acetate production rates. Rates are given in nmol cm^{-3} d^{-1}

	0–1 cm		Net Prod	1–2 cm		Net Prod
Month	APR	SRR	+/−	APR	SRR	+/−
April	927	471	+	461	80	+
May	927	2902	−	461	740	−
June	2967	3819	−	760	1176	−
July	7380	2100	+	2795	2100	+
August	11323	1830	+	13124	2500	+
September	3062	1000	+	89	1113	−
October	1674	1059	+	903	380	+
November	16	540	−	359	224	+

Alperin et al. (1994) also found a positive relationship between temperature and hydrolysis and fermentation, but the hydrolysis was also strongly dependent on the POM inventory.

The negative net production during early summer coinciding with a high POM inventory suggested that temperature at this time was important for the disappearence of acetate. The water temperature increased rapidly during this period (~10 °C), and sulfate reduction in the surface layers attained maximum rates of the season. Sulfate reduction rates as high as these have been found at this farm previously (Holmer & Kristensen 1992), but are high compared to rates in organic enriched sediments e.g. surrounding mussel farms (Oenema 1990) as well as in coastal sediments with high sedimentation rates (Skyring et al. 1987; Thamdrup et al. 1994). The very high sulfate reduction rates developed when the SRB had ideal conditions with rapid and continuous supply of reactive POM and sulfate from the overlying water. The depletion of acetate from the pore waters indicates that hydrolysis and fermentation processes did not respond as fast as sulfate reduction to temperature changes.

The positive net production of acetate during late summer suggests that temperature alone does not explain the seasonal variation. There was a potential for a large acetate production as the water temperature and especially the POM inventory both were high. The layer affected by the fish farm was to a depth of 3 cm compared to 1–2 cm in early summer. The consump-

tion of acetate was also extended as SRR were stimulated down to a depth of 5 cm. The depth integrated SRR accordingly attained maximum values of the season during late summer, although the rates in the upper layers were not as high as in early summer. Sulfate reduction rates may have been underestimated, as they were calculated from sulfate concentrations in the pore waters and/or rates may be reduced due to sulfate limitation. High rates of sulfate reduction in the fish farm sediment demanded a large supply of sulfate, and even relatively high concentrations in the pore waters (>0.5 mM) may limit sulfate reduction (Boudreau & Westrich 1984). The sulfate minimum zone was supersaturated with methane indicating a high methane production although sulfate reduction still occurred. Coexistence of SRB and methanogens has previously been found in this sediment (Holmer & Kristensen 1994a). Methanogens also utilize short chain fatty acids as electron donors, but they are usually inferior in competition with the SRB when acetate is limiting. In the present study acetate was not depleted from the pore waters during coexistence, suggesting that the acetate production was so high that it could support both metabolisms. Alternatively methanogens may use non-competitive substrates as methyl amines (Oremland & Polcin 1982).

During fall net production was still slightly positive, although acetate was slowly being depleted from the pore waters. In contrast to findings of Alperin et al. (1994), where the POM inventory was depleted in autumn, the POM content remained high in the fish farm sediment and inventory was not depleted until early winter, when fish farming was terminated. Water temperature was also high (>10 °C) in these months (September and October) and allowed a rapid hydrolysis as well as terminal metabolism, although the activity was only 10-30% of the summer rates. The farming activities appeared to prolong the normal summer situation in coastal sediments with continued high POM input and rapid SCFA production and consumption resulting in only minor accumulations of SCFA in the pore waters.

Acetate was hardly detectable in winter, and sulfate reduction rates were relatively low. The similarity in POM content under and away from the farm site indicated that the POM inventory was depleted or removed. After net cages were brought on shore, the location became less sheltered leading to resuspension of the upper sediment layers during storms. Although temperature may control the microbial activity, sulfate reduction rates were still higher than in spring at similar water temperatures. Alperin et al. (1994) also found a negative net production of dissolved organic matter in autumn and they suggested that decreasing water temperature as well as depletion of the reactive POM pool are the major controlling parameters.

Seasonality of anaerobic metabolism

Sedimentation of particulate organic matter at the farming site was very high, as indicated by the almost 3 times higher organic pool during summer than in winter. The difference in pool size from March to August was 88.5 mol C m^{-2} and 10.8 mol N m^{-2}, which is approximately 2.5 times higher than the annual primary production in the Kolding fjord area (Vejle Amt 1993), and corresponds to approximately 4% and 0.5% of the total food input as carbon and nitrogen, respectively. This pool is highly dynamic due to the extensive microbial activities and due to fish and mobile benthic macrofauna (crabs) feeding on the organic-rich surface layer, and the pool is possibly minimum estimate of the sedimentation. As the area affected by fish farming was restricted to ~7500 m^{-2}, the loss of organic matter in particulate form from the farming process was responsible for an accumulation in spring and summer of ~8 tonnes of organic carbon and ~1 ton of organic nitrogen in the sediments.

The correlation between microbial activity and seasonal changes in POM pools and water temperature is reflected in the depth integrated sulfate reduction rates at both stations. A regression analysis, using log transformed $\sum$SRR at the farm site resulted in a correlation with temperature and POC content which could explain 87% and 42 % respectively of the seasonal variation. By combining both parameters in a multiple regression analysis 89% of the variation was explained.

At the control site, 68% of the seasonal variation in $\sum$SRR was explained by temperature, but by including the POC pool the correlation was improved to 84%.

Although the organic matter input is high at the farming site, changes in sediment pool sizes correlated poorly to changes in sulfate reduction rates. A previous study at this farm during 1989 actually showed a strong correlation (r^2 = 0.975) between the total anaerobic metabolism (TCO_2 production) and the input of food pellets to the farm (indicator of reactive POM), whereas only 40% of the changes at that time was explained by temperature (Holmer & Kristensen 1992). One reason for the poor correlation could be an underestimation of the sulfate reduction rates in July and August, but maximizing these rates did not improve the correlation considerably. Also methane production was not considered in the anaerobic metabolism. Increased resuspension of sediments or feeding efficiency compared to the previous study may influence the POC pool and weaken the correlation. Factors controlling the SRR may have differed between the two studies. In the present study (1992) the POM pool increased rapidly during spring, and reached a level in summer which was significantly higher than in the 1989 season. In long periods of 1992 the pool of POM appeared not to limit the microbial mineralization as indicated

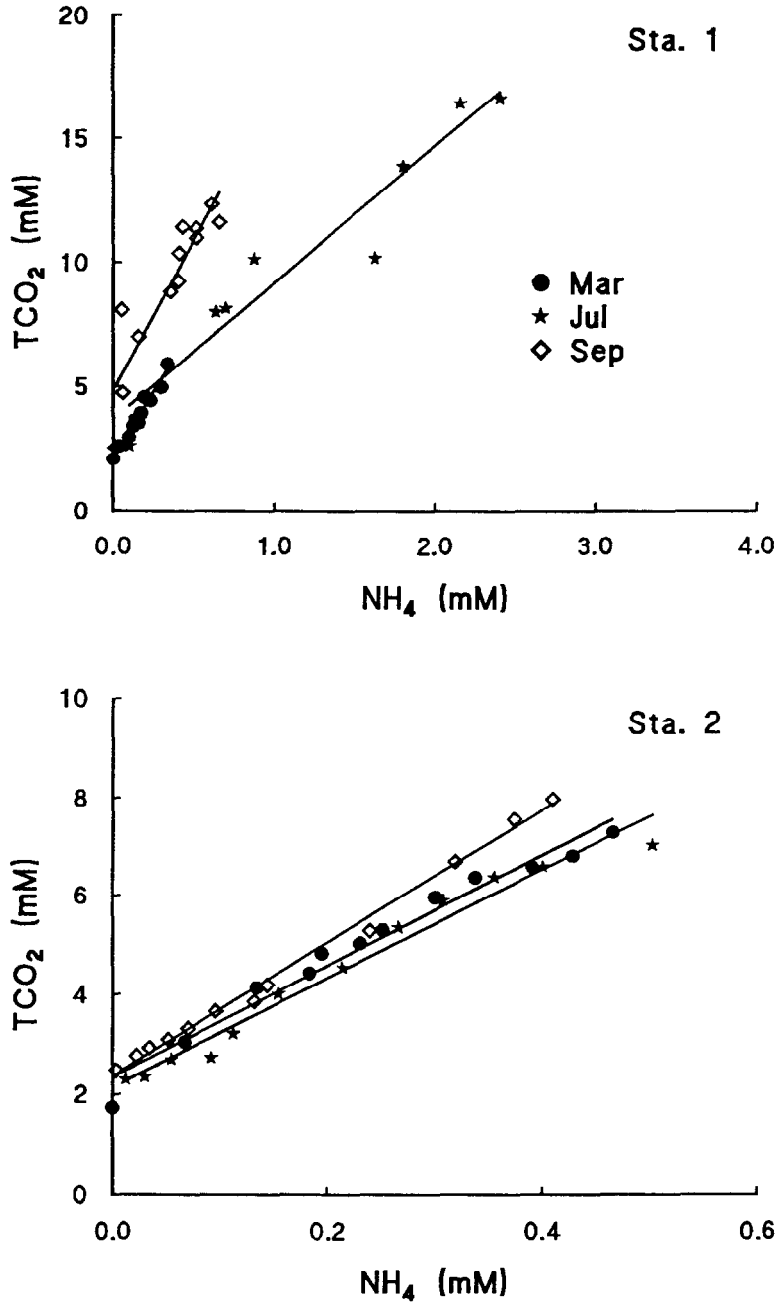

Fig. 12. Relationship between pore water TCO_2 and ammonium (NH_4^+) at Sta. 1 (upper) and Sta. 2 (lower) in March, July and September. Lines represent best fit by linear regression at the sampling date.

from the high acetate concentrations. In 1989, however, relatively moderate changes in the reactive POM pool controlled the microbial activity to a greater extent. Even the very small changes in the POM content at the control site were important for the seasonal variation in SRR. A similar dependence of SRR on deposited organic matter has been found in sediments enriched with phytoplankton deposits (Sampou & Oviatt 1991). When the input of labile POM to sediments is moderate compared to the indigenous pool, substrate availability may limit microbial activity during summer and fall. However, when the POM input is massive as seen at the farm site in 1992, other factors, such as temperature and in the worst cases the availability of electron acceptors (sulfate) are more important.

Stoichiometry of terminal mineralization

High microbial activity in the surface layers in the fish farm sediment during summer was reflected in pore water profiles of the mineralization products TCO_2 and NH_4^+. The actual C:N stoichiometry of mineralization can be examined by analyzing the ratios between TCO_2 and NH_4^+ in the pore waters (Aller & Yingst 1978; Andersen & Kristensen 1988). According to Kristensen & Hansen (1995) the average stoichiometry of the organic matter under decomposition can be approximated when DOC is low and TCO_2 and dissolved NH_4^+ are linearly related in the examined sediment layer. Highly significant linear relationships between pore water TCO_2 and NH_4^+ were found at the fish farm (excluding depths with high DOC) as well as at the control site throughout the year (r^2 = 0.82–0.99) (Fig. 12). The C:N stoichiometry at the fish farm was lower (2.3–7.6) than in the control sediment (4.3–9.0). The lowest values coincided with the highest organic matter loading suggesting that the decomposition of organic nitrogen from fish farm waste products was higher than the decomposition of organic nitrogen in the control sediment. A similar range and seasonal variation with low C:N ratios during spring and summer has been found in an organic poor coastal lagoon, supported by detritus from benthic microalgae (Kristensen 1993). Previous studies on decomposition of fish farm waste products (food pellets) have shown a fast mineralization of nitrogen (Hansen et al. 1993). The POM pool at the farm site had a low C:N ratio (7–8) and a labile nitrogen pool.

Acknowledgement

We thank the staff at the Fiskegruppen Lillebælt fish farm for patience and kind assistance during sampling and for providing data on water temperature and fish production. MH was supported by a grant from Odense University.

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