C- and N-mineralization in the sediments of earthen marine fishponds

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ABSTRACT· The rates of mineralization of organic-C and -N were measured in the sediments from 2 earthen fishponds in Eilat, Israel. The flux of the mineralized products (NH_4^+ and CO_2) across the sediment-water interface was also measured. It was concluded that the diffusional flux accounted for approximately 70 % of the flux; fish bioturbation was responsible for the remainder. The sediments were highly anaerobic and rates of SO_4^{2-} reduction had a mean value of 70 mmol m^{-2} d^{-1}. Nitrification was absent, denitrification was insignificant and much of the sedimenting organic detritus was mineralized. This amounted to 140 mmol C and 18 mmol N m^{-2} d^{-1}, equivalent to a minimum input of > 600 g C m^{-2} yr^{-1}.

INTRODUCTION

A study of the biogeochemical processes occurring in earthen marine fishponds is important both in the control of commercial systems and for an understanding of processes beneath a hypertrophic marine system. Previous studies of the fishpond complex in Eilat have concentrated on the processes occurring in the water column (Motzkin et al. 1982, Krom et al. 1985a, b). However, it was recognized particularly in those studies of nutrient budgets (Krom et al. 1985a) that microbial processes in the sediment were an unknown but potentially important factor in the water chemistry of these ponds. In fact, Krom et al. (1985c) found a correlation between mass mortality of fish and the state of the sediment within the pond. One of the main purposes of this study was to understand and quantify the microbial processes in the sediment and to measure the flux of metabolites between the sediment and the overlying water. This parallels previous studies which have examined various aspects of the sedimentary microbiology in freshwater fishponds (Ram et al. 1982, Rimon & Shilo 1982, Jana & Patel 1984).

These fishponds are also an example of a hypertrophic marine system. The ponds are supplied with high concentrations of nutrients from the inflow water (Krom 1986) and from fish food (Krom et al. 1985a). The annual water temperature is 18 to 33 °C (Krom et al. 1985b) and on most days there is little or no cloud cover. The resulting high phytoplankton growth rates (26 g O_2 m^{-2} d^{-1}) and biomass (150 µg chlorophyll a l^{-1}), are comparable to those found in freshwater fishponds (Shilo & Rimon 1982), sewage treatment ponds (Sreenivasan 1979) and highly polluted lakes (Barica & Mur 1979). Marine systems of comparable productivity are wastewater-algal treatment systems (Goldman & Ryther 1975). An unknown fraction of this primary productivity, together with fish faeces and uneaten food, reaches the sediment as particulate organic matter, where it is available for microbial degradation. This study sets out to examine the interrelationship between the various microbial processes in the sediment (CO_2 production, sulfate reduction, organic-N mineralization, nitrification, denitrification etc.) which were expected to be important in such a system (Blackburn 1986). Furthermore, it was known that the principal fish in these ponds bioturbate the sediment of the pond (Krom et al. 1986). Many studies have examined the importance of infaunal bioturbation in increasing the flux of dissolved nutrients from sediments to the overlying water (Aller 1980, Kristensen & Blackburn 1987). Very few studies, however, have considered the possible effects of fish bioturbation, although Tenore et al. (1982) suggested that fish bioturbation might explain the discrepancy between the rate of metabolite production and the flux of these metabolites from the system. In this study a similar procedure is used to calculate the nutrient flux from the sediment to the overlying water, caused by the action of fish.

MATERIALS AND METHODS

Sediment samples were taken from 2 earthen sea-water fishponds in Eilat, Israel on 4 occasions in spring 1986. The ponds have been previously described (Motzkin et al. 1982, Krom et al. 1985a, b). The pond complex is situated adjacent to the H. Steinitz Marine Laboratory on the Gulf of Eilat (Akaba) and was constructed in 1975. Each pond is approximately 10 m wide and 30 m long with a maximum depth of 1 m and with gradually sloping sides. The ponds were excavated from local beach sand, and lined with silt which was then covered with 10 to 20 cm of beach sand. Over the years of operation, the sand and silt have mixed together and with additional windblown particles. This has resulted in sediments containing a high proportion of fine-grained material. The 2 ponds involved in this study were dried for 4 wk in August 1985, and were then filled with water. Pond #6 was then stocked with 800 gilthead seabream *Sparus aurata* and 150 grey mullet (Mugilidea) of average weight 2 g. At the time of this study, the average weight of the fish was 40 g. Pond #2 was stocked with the same number and type of fish, but with larger individuals, such that by spring 1986, the average weight of the fish was 250 g. The fish were fed daily with 550 g (Pond #6) or 1800 g (Pond #7) food containing 53.3 % C, 6.9 % N and 1.25 % P wt/wt (Krom et al. 1985a). The pond was operated with a continuous flow of water from a nearby seawater well, with a dilution rate of 0.41 pond volumes d^{-1}.

Samples were removed manually in plexiglass sampling tubes from Pond #6 on 30 March and 1 April, from a position 4 m from the outflow in 1 m water depth, after wading into the pond. Samples were similarly removed from Pond #7 on 31 March, 4 m from the outflow in <1 m water depth, and on 2 April, 2 m from the outflow in 1 m water depth. All samples were taken at approximately 11:00 h. The same suite of experiments was performed on all samples, with the exception of the 1 April sample, in which a restricted suite was used, as the samples were from the same position in the pond as 31 March samples.

Pool sizes and general sediment characteristics were measured in sediment from 4 cores (4.6 cm diam., 12 cm long), which were fractionated in 2.0 cm slices; slices from the same strata were pooled and thoroughly blended together. Pore water was obtained by centrifugation at $17\,500 \times g$ and was assayed for NH_4^+, $NO_3^- + NO_2^-$, and SO_4^{2-}. Extracts of 20 g blended sediment were made at 20 °C for 0.5 h with 10.0 ml 1.0 M KCl, containing 0.5 % $ZnCl_2$ as a bacteriocidal agent and to remove H_2S. The extract was assayed for NH_4^+ after centrifugation at $3000 \times g$ for 10 min. Replicate samples ($10\,cm^3$) of blended sediment were weighed for specific gravity determination and were heated at 80 °C for 8 h to measure water loss. Portions of the dried sediment were ignited at 550 °C to determine organic loss. Other portions of the dried sediment were analysed for C and N content (Carlo Erba 1500 CNS analyser).

Rates of NH_4^+ production were measured by fractionating 2 sediment cores (4.6 cm diam., 12 cm long) in 2 cm slices; slices from the same strata were blended together anaerobically under a stream of N_2 and $^{15}NH_4Cl$ was added to give 10 % enrichment (Blackburn 1979). Portions of the enriched sediment (20 g) were transferred anaerobically to a series of stoppered glass scintillation vials. The vials were incubated at pond temperature and were extracted with 10 ml 1.0 N KCl containing 0.5 % $ZnCl_2$ at 0, 1, 2 and 3 d intervals. Extraction of the sediment was made at 20 °C for 0.5 h. The extracts were assayed for NH_4^+ after centrifugation at $3000 \times g$ for 10 min and were later assayed for $^{15}NH_4^+$ content by emission spectrometry (Blackburn 1979).

Sulfate reduction rates were measured in 3 cores (2.6 cm diam., 12 cm long) in plexiglass coring tubes with silicone-rubber ports; injections with $^{35}SO_4^{2-}$ were made at 1.0 cm intervals (Jørgensen 1977). After incubation at pond temperature for 4.0 h the cores were sectioned into 4 cm slices to 30 ml screw-top containers containing 4.0 ml 20 % $ZnCl_2$. The sediment was blended with the $ZnCl_2$ solution to ensure that all S^{2-} was trapped. The blended sediment was frozen until analysed for acid-volatile S^{2-} and $^{35}S^{2-}$.

Denitrification was measured in 4 sediment cores (3.6 cm diam., 8 cm long) in plexiglass tubes with multiple silicone rubber-filled ports, through which acetylene-saturated distilled water at 0 °C was injected to inhibit the reduction of N_2O to N_2 (Sørensen 1978, Andersen et al. 1984). The sediment cores were overlaid with water from the inlet to the fish ponds, which had been 10 % saturated with acetylene. The tubes were sealed top and bottom with rubber stoppers; there was no head space and bubbles were avoided. The overlying water was agitated by a magnet suspended from the top stopper with nylon fishing line. This magnet was itself moved by a larger magnet, rotating at 60 rpm, and situated centrally between the plexiglass tubes. The cores were incubated in the dark at pond temperature. The accumulation of N_2O in the overlying water and in the sediment was measured and the rate of denitrification was calculated (Andersen et al. 1984).

Exchange of solutes (O_2, CO_2, NH_4^+, $NO_3^- + NO_2^-$ and PO_4^{3-}) between the sediment and the overlying water was measured in 5 sediment cores (3.6 cm diam., 8 cm long) in plexiglass coring tubes. The cores were treated in exactly the same way as the cores for denitrification, with the exception that the coring tubes did not have injection ports and no acetylene was added. After 4 h incubation samples were removed from the overlying

water and analysed for O_2, CO_2, NH_4^+, $NO_3^- + NO_2^-$ and PO_4^{3-}. The rate of movement of these solutes across the sediment/water interface was calculated from a comparison with the initial concentrations in the overlying water.

O_2 was measured in duplicate 5.0 ml water samples by Winkler titration. Dissolved nutrients were measured in samples which had been filtered through 0.4 μm Millipore filters, and then stored in iodized plastic bottles at 4 °C for a maximum of 3 d before analysis. $NO_3^- + NO_2^-$ and PO_4^{3-} were measured using an automated procedure (Glibert & Loder 1977), while NH_4^+ was determined using the automated method of Krom et al. (1985d). Changes in ΣCO_2 were calculated from pH and total alkalinity measurements. pH was measured with a Radiometer M84 pH-meter using a combined pH electrode (Radiometer GK 2401C). The alkalinity was measured using an autobiuret (Radiometer ABU 80) and a Gran-type titration according to Sass

& Ben-Yaacov (1977). Potential nitrification rates were measured by incubating surface sediment with saturating NH_4^+ and O_2 (Hansen et al. 1981).

RESULTS

A summary of the physical and biological properties of the sediments is shown in Fig. 1. It had been assumed that the 2 samples taken from Pond #6 were identical; the 2 samples from Pond #7 were from 2 different sites and were clearly different from each other. The upper 2 cm of sediment in Pond #6 was flocculent, had a high porosity, a high organic content and a high organic-N content. The C:N ratio was ca 8 at all depths. The porosity of the 31 March sample from Pond #7 decreased linearly from the surface downward, as did organic content and organic-N content. The C:N ratio was variable and had a high value of
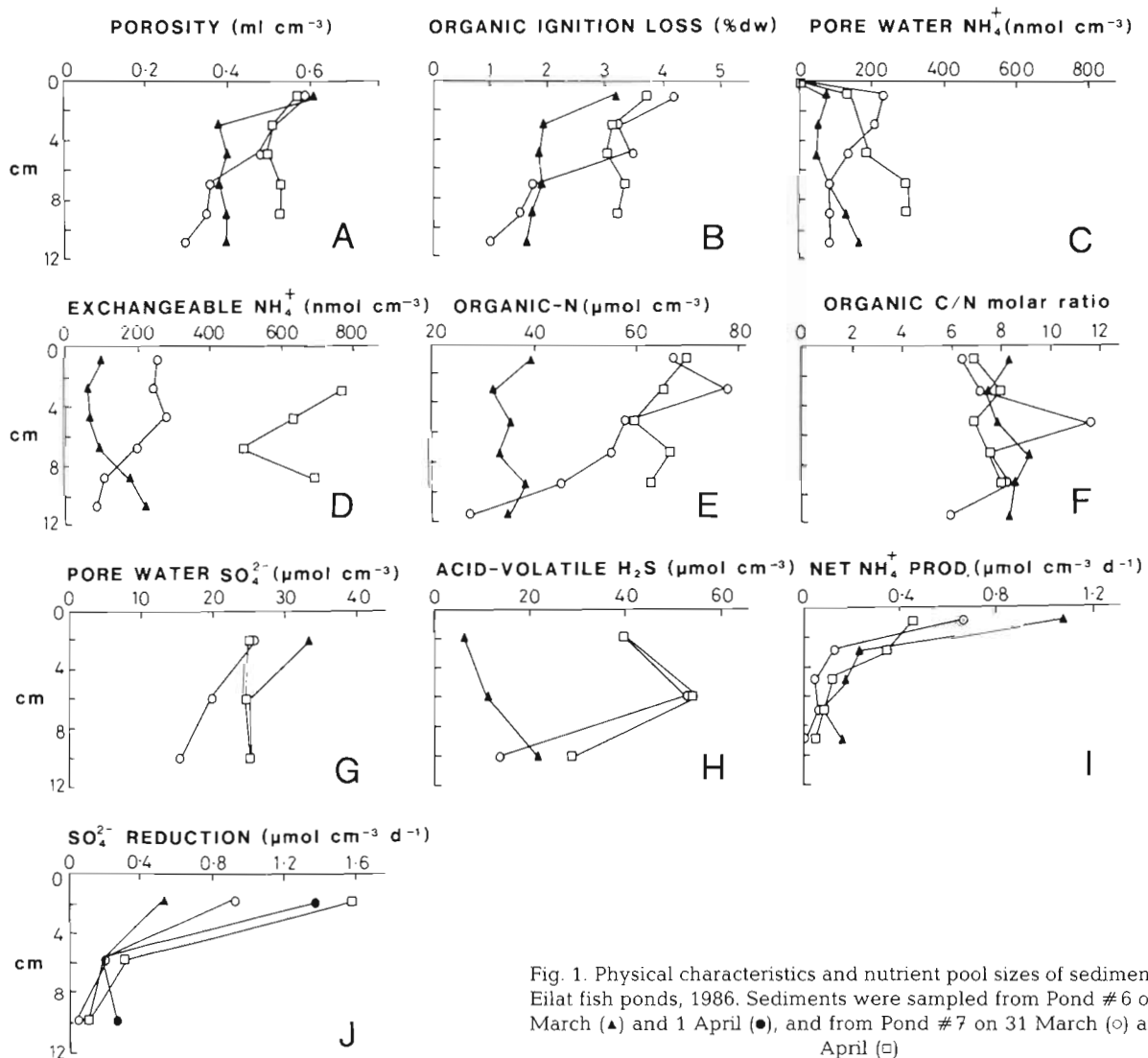

Fig. 1. Physical characteristics and nutrient pool sizes of sediments in Eilat fish ponds, 1986. Sediments were sampled from Pond #6 on 30 March (▲) and 1 April (●), and from Pond #7 on 31 March (○) and 2 April (□)

Table 1 Nitrate reduction and denitrification. All rates and standard deviations (SD) are in mmol m^{-2} d^{-1}. A negative sign indicates flux from water to sediment

Process	Pond # 6				Pond # 7			
	30 Mar		4 Apr		31 Mar		2 Apr	
	Rate	SD	Rate	SD	Rate	SD	Rate	SD
$NO_3^- + NO_2^-$ flux with acetylene inhibition	−5.8	0.8	−6.2	1.7	−8.1	0.7	−10.6	1.2
$NO_3^- + NO_2^-$ flux with no acetylene inhitibion	−5.1	0.4	−6.7	2.3	−7.2	1.3	−8.5	1.8
Denitrification	1.8	0.2	1.6	0.2	1.2	0.2	1.0	0.1

almost 12 in the 4 to 6 cm stratum. The 2 April sample from Pond #7, near the outflow in deeper water, changed little in porosity, organic content, organic-N content or C:N ratio with increasing sediment depth. The pore-water NH_4^+ concentrations (Fig. 1C) paralleled the organic-N concentrations, as did the exchangeable NH_4^+, with the exception of the 2 April samples from Pond #7. It is probable that these values are incorrect, due to poor refrigeration of the KCl extracts; the KCl extracts in the $^{15}NH_4^+$ experiment gave values between 200 and 300 nmol cm^{-3}. The ratio [NH_4^+ in pore water : exchangeable NH_4^+] was ca 1.0.

The concentration of SO_4^{2-} was very high, and generally decreased slightly with depth (Fig. 1G). The acid-volatile S^{2-} in Pond #6 increased from the surface downward in both samples (values for 1 April are not shown, but closely resembled those for 30 March). Both samples from Pond #7 showed a peak at 4 to 6 cm depth.

The net rates of NH_4^+ production (Fig. 1I) decreased almost exponentially from the surface downward. Pond #6 contained some worms in the upper 2 cm; it is likely that their death and mineralization resulted in exaggerated rates.

The rates of SO_4^{2-} reduction also decreased from the surface downward (Fig. 1J). The rates in the 0 to 4 cm stratum were very high and indicated that much organic-C was degraded via SO_4^{2-} reduction.

Rates of nitrate reduction are given in Table 1. There was a high flux of $NO_3^- + NO_2^-$ into the sediment from the overlying water in each of the 4 experiments. Acetylene had no effect on the rate of disappearance, and the rate was much higher than the measured rate of denitrification. It is presumed that NO_3^- was reduced to NH_4^+, resulting in an increased flux of NH_4^+ from the sediment. Measurements of potential nitrification rates indicated a total absence of this activity in the surface sediments of both ponds.

Table 2. Diffusion- and bioturbation-mediated exchange rates between sediment and water. All rates and standard deviations (SD) are in mmol m^{-2} d^{-1}. A negative sign indicates flux from water to sediment

Process	Pond # 6				Pond # 7			
	30 Mar		1 Apr		31 Mar		2 Apr	
	Rate	SD	Rate	SD	Rate	SD	Rate	SD
1. N-mineralization	34.2	2.2	34.2	2.2	18.3	1.8	21.1	6.5
2. N-mineralization	13.4		18.8		14.6		23.5	
3. NH_4^+ flux (diff.)	11.0	1.7	4.3	3.4	10.5	1.3	14.2	8.2
4. C-mineralization	106.6	25.2	149.2	38.0	115.8	69.6	186.6	64.0
5. CO_2 flux (diff.)	104.2	30.9	89.1	20.4	94.4	20.0	75.0	19.5
6. O_2 flux (diff.)	−42.0	6.0	−60.0	6.0	−42.0	5.0	−50.0	4.0
7. NH_4^+ flux (bioturb.)	2.4		14.5		4.1		9.3	
8. CO_2 flux (bioturb.)	2.4		60.1		21.4		111.6	
9. O_2 flux (bioturb.)	64.6		89.2		73.8		136.6	

[1] Measured rate of net NH_4^+ production
[2] Calculated rate of net NH_4^+ production = C-mineralization/7.93
[3] Measured diffusional flux of NH_4^+ from sediment to water
[4] Organic-C mineralization to CO_2 = twice the rate of SO_4^{2-} reduction
[5] Measured diffusional flux of CO_2 from sediment to water
[6] Measured diffusional flux of O_2 from water to sediment
[7] Calculated flux due to bioturbation = N-mineralization (calculated) minus NH_4^+-flux due to diffusion
[8] Calculated flux due to bioturbation = C-mineralization minus CO_2-flux due to diffusion
[9] Calculated flux due to bioturbation = twice SO_4^{2-} reduction rate minus O_2-flux due to diffusion

The rates of exchange of other solutes (CO_2, O_2 and NH_4^+) are summarized in Table 2, and various calculations are made using these rates. The implications of these calculations are discussed later.

DISCUSSION

One of the main purposes in performing these experiments was to establish the role of the sediments in the overall cycling of nutrients in the ponds. This discussion will concentrate on the interconnection between the various rate measurements in the sediments, particularly those concerning the reduction of oxidants (O_2, SO_4^{2-} and NO_3^-) and the liberation of mineralization products (NH_4^+ and CO_2), and how these rates were effected by fish bioturbation.

Under normal conditions, i.e. when there was a substantial bloom of phytoplankton in the pond, the bottom water contained very low NO_3^- concentrations (<1 μM) even though the inlet water contained >40 μM NO_3^-. The potential for denitrification was, therefore, minimal since nitrification was almost zero in these sediments and potential nitrification acivity is generally low in the water column of fishponds (Shilo & Rimon 1982). Even if denitrification potential is high (Jana & Kalyani 1985), it is unlikely that either process was of significance in this situation.

These fishponds are subject to periodic (approximately monthly) phytoplankton crashes (Motzkin et al. 1982). During such crashes, for a few days the uptake of nutrients is drastically reduced and dissolved nutrients, including NO_3^-, accumulate in the water column (Krom et al. unpubl.). At such times it would be expected that the rates of denitrification would approach the values measured in this study (range 1.0 to 1.8 mmol N m^{-2} d^{-1}; Table 1), where inlet water was used as the overlying water in the experiment. Krom et al. (unpubl.) estimated a sediment denitrification rate of 4.0 to 4.9 mmol N m^{-2} d^{-1} for spring 1985 and of 6.0 mmol N m^{-2} d^{-1} at the time of the present study. This is similar to the present rates of NO_3^- reduction (range 5.1 to 8.5 N m^{-2} d^{-1}; Table 1). It is possible that denitrification, as measured by acetylene blockage, has been underestimated due to the presence of sulfide which can interfere with the acetylene inhibition (Sørensen et al. 1980), and that the rates of NO_3^- reduction give a better estimation of denitrification rates.

The methods for the measurement of the rates of organic-N mineralization did not yield very satisfactory results. The rate of $^{15}NH_4^+$ dilution was too low to be measured in the incubation time employed; the results are not presented. The net rate of NH_4^+ production is thought to be valid only for Pond # 7 samples. Pond # 6 had worms in the 0 to 2 cm stratum, and these were

disintegrated in the blending procedure, giving erroneously high rates of organic-N mineralization (Fig. 1I). The integrated value of these rates are presented on an areal basis in Table 2, Row 1. The net rate of organic-N mineralization (Table 2, Row 2) was, therefore, calculated from the rate of organic-C oxidation, assuming that C- and N-mineralization were in proportion to the C:N ratio in the detritus. This assumption was based on the relatively constant C:N ratio observed at different sediment depths (Fig. 1F; mean C:N ratio was 7.93). These calculated rates of N-mineralization agreed well with the measured rates for Pond # 7.

Mineralized NH_4^+ diffused out of the sediment, but the measured diffusional flux of NH_4^+ to the overlying water (Table 2, Row 3), probably contained some part of the NO_3^- that had disappeared, presumably being reduced to NH_4^+ (Table 1). The diffusional flux (Table 2, Row 3), measured under these conditions of high NO_3^- concentration in the overlying water, may have been too high, but was less than the net rate of NH_4^+ production (Table 2, Row 1 and 2). If all the NH_4^+ that was produced in the sediment (area 200 m^2) was transferred to the overlying water, the contribution would be ca 3000 and 4000 mmol N $pond^{-1}$ d^{-1} for Ponds # 6 and # 7 respectively. The other major sources of NH_4^+ to the pond at this time were from the inlet water (60 mmol N $pond^{-1}$ d^{-1}) and fish excretion, which in Pond # 6 was 630 mmol N $pond^{-1}$ d^{-1}, and in Pond # 7 was 2050 mmol N $pond^{-1}$ d^{-1} (Krom et al. unpubl.). The sediments are, therefore, very significant sources of N to the overlying water, and it must be inferred that most organic-N mineralization occurs in the sediments, rather than in the overlying water. The net mineralization of organic-N in the sediment may be compared with the input of N to the ponds as fish-food: 2700 and 8900 mmol N $pond^{-1}$ d^{-1} for Pond # 6 and Pond # 7 respectively.

The rate of SO_4^{2-} reduction in all experiments was much higher than the rate of O_2 uptake. Reducing conditions extended up to the surface of the sediment, as indicated by the high acid-volatile S^{2-} that was observed in the 0 to 4 cm surface layer (Fig. 1J). It was, therefore, assumed that no O_2 or NO_3^- was used for C-oxidation, and that all C-mineralization was due to SO_4^{2-} reduction (Table 2, Row 4). Some, but not all, of the CO_2 produced as a result of this mineralization diffused to the sediment surface, as measured in sediment cores (Table 2, Row 5). The rate of O_2 uptake was, as has been discussed, less than the rate of SO_4^{2-} reduction.

All the diffusional fluxes were less than might have been expected from the rates of detrital mineralization in the sediment. The main difference between the ponds and the cores in which the diffusional fluxes were measured was that fish were absent from the

latter. It is assumed that the difference between the rates of production and the diffusional fluxes was due to periodic fish-bioturbation, which resulted in the rapid exchange of solutes between the sediment and the overlying water. This assumption is probably correct for the NH_4^+ and CO_2 fluxes (Table 2, Rows 7 and 8), but may give too high a value for the O_2 flux (Table 2, Row 9). This is because it is assumed that all S^{2-} that is produced is almost immediately oxidized back to sulfate. This is only partially true; the mean turnover time for the S^{2-} pools was 42 d, indicating some accumulation of unoxidized S^{2-}. This relatively rapid reoxidation of S^{2-} due to fish bioturbation is quite different from the accumulation of S^{2-} that is observed in some marine sediments (Blackburn 1987). The overestimation of the O_2 rate is reflected in the higher proportion of the O_2 flux that must be attributed to fish bioturbation; the mean was 64 %, compared to 41 and 30 % for N- and C-fluxes respectively. The possibility that the N-flux is too large, due to NO_3^- interference, has been discussed above.

We conclude that ca 30 % of the flux of solutes across the sediment/water interface is due to sporadic bioturbation by fish. Krom et al. (1985a) showed that the amount of sediment resuspended into the water column was proportional to the size of the fish in the pond. For 60 g fish, approximately the size in this study, there was 40 ppm total particulate matter suspended into the water column. Assuming a depth of bioturbation of 2 cm, which is compatible with the data presented here, this corresponds to 0.05 to 0.10 % of the pond bottom being resuspended at any given moment. Because the diffusional flux of O_2 into the sediments is probably maximally 50 to 60 mmol m^{-2} d^{-1}, depending on porosity, the effect of fish bioturbation can be very important in causing the oxidation of S^{2-} in the sediment. S^{2-} is thus prevented from entering the water column and causing fish death.

The rates of sulfate reduction were very high (mean value ca 70 mmol m^{2-} d^{-1}) compared to normal marine sediments and are 3 times higher than at the 'Black Hole' in Long Island Sound, USA, a highly polluted location receiving local sewage effluent, and 30 times that from NWC, a normal station nearby (Westrich 1983). These high rates of sulfate reduction in the fishpond were due to the high in situ temperatures and to the high degradability of the sedimenting organic matter. The concentration of organic matter in these sediments is rather low (1.1 % C) compared to nearshore marine sediments. The rate of organic turnover is clearly much higher in the ponds, since the input estimated from sulfate reduction is in excess of 600 gC m^{-2} yr^{-1}. The 2 situations are obviously very different from each other also in terms of the age of the organic matter present. The fishpond sediments have not had time to accumulate resistant organic detritus, which is probably largely non-degradable bacterial cell residues (Blackburn 1987, 1988), both because the sediments have been in place for only 10 yr and because they are dried at the end of most seasons, allowing much organic matter to be oxidized in the air.

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