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Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines

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Abstract

Sedimentation and sediment metabolism was measured at eight active milkfish fish pens and at one abandoned site in the Bolinao area, Philippines in order to examine the interactions between sediment and water in this shallow coastal zone. The rates of sedimentation were high in the area due to siltation, but the activities in the fish pens also contributed to enhanced sedimentation as indicated by the difference between the abandoned and active sites. The sediment metabolism appeared to decrease with increasing rates of sedimentation indicating that the microbial activity reached a saturation level in the fish pen sediments. Anaerobic processes dominated the organic matter decomposition, and sulfate reduction rates are among the highest measured in fish farm sediments. The rates decreased with increasing organic loading despite high concentrations of sulfate (>10 mM) at all sites. Presence of methane bubbles in the sediments suggests that sulfate reduction and methanogenesis were coexisting. The sediment metabolism was significantly reduced at the abandoned site indicating that the stimulation of microbial activities is due to active fish production. The anaerobic activity remained high at the abandoned site indicating that the sediment biogeochemical conditions remain affected long time after fish production has ceased.

Keywords: Fish pen; Organic enrichment; Sediment metabolism; Sulfate reduction rates

1. Introduction

Marine fish farming ranks amongst the fastest growing industries in the world (FAO, 2002). The growth of marine fish farming is generating concern on the possible impacts for coastal ecosystems. One of the most important environmental impacts of marine fish farming is the release of particulate waste products, which increase the organic loading of the benthic environment (Hall et al., 1990; Holmer and Kristensen, 1992; Karakassis et al., 2000). Organic rich components are lost from fish farms as waste food and faeces, and increase the organic load of the sediments leading to a number of negative effects on sediment biogeochemistry and benthic communities (Holmer and Kristensen, 1996; Delgado et al., 1999; Karakassis and Hatziyanni, 2000). Decomposition of this organic matter may lead to water column hypoxia and higher rates of nutrient cycling (Hall et al., 1990; Holmer and Kristensen, 1992). The sediments remain oxidized as long as electron acceptors such as oxygen, manganese and iron are available, but when these are exhausted the sediments become highly reduced due to accumulation of sulfides in the sediment pore waters (Thamdrup, 2000). Such a benthic environment is unfavorable for growth and survival of benthic organisms, and fauna and seagrasses may eventually disappear (Terrados et al., 1999; Karakassis and Hatziyanni, 2000).

Changes in the dominant fauna and flora reduce the chemical and physical modifications of the surface sediments due to their activities, and the decomposition of organic matter is shifted to microbial processes (Holmer and Kristensen, 1996). Oxic processes such as nitrification are inhibited, and this may also limit denitrification

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due to low nitrate concentrations resulting in a high release of ammonium from fish farm sediments (Hargrave et al., 1993; Christensen et al., 1999). Enhanced release of ammonium in turn stimulates phytoplankton production during nitrogen limited periods (Pedersen and Borum, 1996). In addition, consumption of the oxidized iron pools, either by microbial iron reduction or by enhanced reoxidation of reduced compounds may result in a large release of phosphate from the sediments due the reduced capacity for binding of phosphates to oxidized iron (Jensen and Thamdrup, 1993) thereby further stimulating primary production (Heijs et al., 2000). Sulfate reduction is the most important mineralization process in marine anaerobic sediments and yields sulfides, which are toxic to organisms. If sulfate is depleted, methanogenesis becomes the dominant pathway subsequent decomposition. This may create gas bubbles in the sediments, and release of gas from the sediments is a clear sign of excess accumulation of organic matter and thus poor farming practice in marine fish farms (Ervik et al., 1997).

Marine fish farming is expanding rapidly in SE Asia, where it is a major source of environmental deterioration (Bell and Gervis, 1999; Adeel and Pomeroy, 2002; Sumalde et al., 2002; Terrados and Duarte, 2002). Rapid aquaculture expansion is occurring in the Bolinao area in the Philippines, where production of milkfish has flourished in the shallow waters of the coastal back reef areas since 1995 (Holmer et al., 2002). The back reef areas experience siltation due to discharges from the Alaminos River (Terrados et al., 1998), and the interactions between sediment and water are important due to the shallow water depth. The milkfish production takes place in enclosures made by fixing nets to the seafloor by bamboo sticks, or in floating cages. The various production structures reduce the water flow in the area. Previous analyses of the impacts of these fish pens on the sediments indicated increased oxygen consumption and nutrient release (Holmer et al., 2002). In this study we assess the influence of increased organic carbon inputs derived from fish pens, and the associated increased oxygen consumption and nutrient release, on sulfate reduction rates and sulfide pools in the sediments.

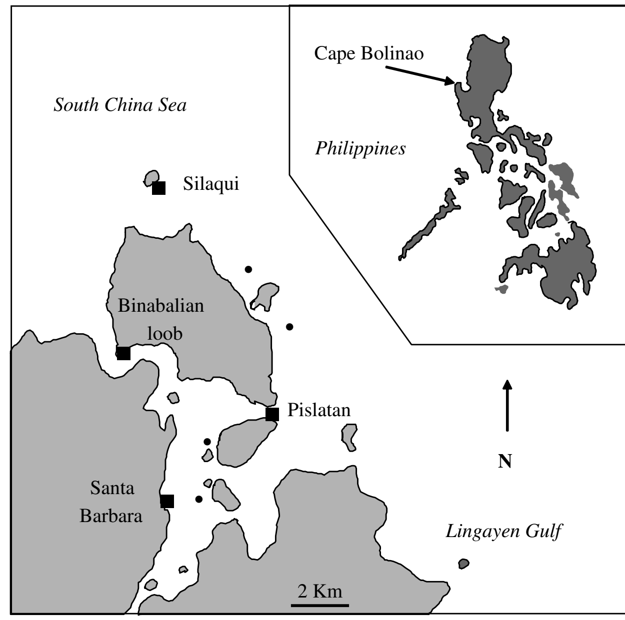

Fig. 1. Location of sampling sites (closed circles) in the Bolinao area, The Philippines. Two to four fish pens were investigated at each site.

2. Methods

The study was conducted in November 2001 in the Bolinao area (Pangasinan province, Luzon Island, The Philippines, Fig. 1), where mariculture operations were initiated in 1995. Eight net pens, two of which were also studied the year before, encompassing a range of fish biomass and feed inputs (Holmer et al., 2002) and one recently (~4 months) abandoned net pen were selected. All net pens consisted of nets fixed with bamboo sticks into the sediments and ranged in size from 300 to 3500 m^2 and had been in operation for up to six years (Table 1). Milkfish was produced in all net pens and the age of the fish ranged from 1.3 to 3 months. The abundance at harvest ranged from 4000 to 45,000 fish per pen (Table 1). All farms used dry pellets as feed and the feed type was either "Grower" or "Finisher" (Holmer et al., 2002) and the feeding load was between 50 and 250 kg pen^{-1}. Due to the large difference in size the aerial food input varied between 0.017 and 0.583 kg m^{-2} day^{-1} at the time of sampling. The water depth ranged from 0.5 to 3 m, the salinity was measured by refractometry at each net pen during sampling and was about 31 PSU. The water temperature was measured at the same time and was about 27 °C.

Sampling was performed by skin or SCUBA diving inside the fish pens away from the main feeding area in order to avoid a direct input of food pellets to the sediments. Feeding was done from one platform in all the fish pens. Three replicate sediment cores (i.d. = 8.0 cm) and three smaller cores (i.d. = 2.6 cm) were sampled, and afterwards sediment traps were placed inside the net pens and away from the feeding area to estimate organic inputs. The sediment traps were deployed at the sampling depths (0.5–3 m) for 24 h, and after transportation to the laboratory, the content of the each sediment trap was collected on a combusted, preweighed Whatman GF/F filter, and its dry weight was obtained after drying at 60 °C to constant weight. Sedimentation rates were estimated according to Blomqvist and Håkanson (1981) and Hargrave and Burns (1979) as described in detail in Holmer et al. (2002).

Table 1
Description of the fish pens studied during 2000 and 2001

Fish pen #	Size (m^2)	Production No. fish at harvest	Years of operation (year)	Fish age (month)	Food input ($kg\,pen^{-1}$)	Food input ($kg\,m^{-2}\,day^{-1}$)
2001						
FP3	1680	n.a.	6	2.0	n.a.	n.a.
FP4[a]	1404	–	–	–	–	–
FP5	2886	n.a.	2	2.0	n.a.	n.a.
FP10	300	5500–7000	0.9	2.5	175	0.583
FP11	3500	45000	0.8	2.2	50	0.017
FP12	400	4000	1.0	2.0	100	0.250
FP13	600	23000	0.6	1.3	175	0.292
FP14	3000	30000	1.9	3	250	0.083
FP15	250	5000	1.7	3	125	0.500
2000						
FP3	1680	15000[b]	5	3–4	75–100	0.052
FP4	1404	10000[b]	0.3	0.2	10	0.007
FP5	2886	20000[b]	1	1	25	0.008
FP6	2520	15000[b]	4	4 (some 9)	250	0.099

n.a. not available.
[a] Fish pen #4 was abandoned in 2001.
[b] Stocking density at production start is given.

The sediment cores were brought to the laboratory, where the large cores were submerged in an aquarium (50 l) aerated by an air pump and kept at in situ temperature. Each core was equipped with a magnet stir bar and stirred by a central magnet (50 rpm, see detailed description in Holmer et al., 2002). The cores were allowed to equilibrate for 3–4 h before flux measurements of oxygen, total inorganic carbon (TCO_2) and nutrients were initiated. After initial samples were taken the cores where closed with rubber stoppers and incubated for 1–3 h in darkness before a final sample was taken. Samples were analyzed for oxygen, TCO_2, ammonium, nitrate, nitrite and phosphate concentrations. Oxygen was determined by standard Winkler technique within 4 h of sampling. TCO_2 samples were fixed with $HgCl_2$ and analyzed within one month by the method described by Hall and Aller (1992). Samples for nitrate and nitrite were stored frozen and measured by standard methods in a flow injection autoanalyzer (Tecator FIA star 5012, Armstrong et al., 1967). Nitrite concentrations were low and are included in the nitrate pool (NO_3^-). Samples for ammonium (NH_4^+) were stored frozen and measured according to the method by Bower and Holm-Hansen (1980) and phosphate (DIP) was determined by the method of Koroleff (1983).

Once flux measurements were completed, the sediment cores were sliced into 1-cm intervals (to 4 cm) and 2-cm intervals (to 10 cm). Specific sediment density was obtained by weighing a known volume of sediment and water content was obtained by drying overnight at 105 °C. Porosity was calculated from sediment density and water content. The sediment particulate organic carbon (POC) and nitrogen (PON) content were measured on dry sediment with a Carlo Erba elemental analyzer 1100EA following Kristensen and Andersen (1987). Total P was obtained after boiling combusted sediment in 1 M HCl for 15 min followed by spectrophotometric determination of phosphate (Koroleff, 1983).

The small sediment cores were used for determining rates of sulfate reduction (SRR) and total pools of reduced sulfides (TRS). Sulfate reduction rates were determined by the core-injection technique (Jørgensen, 1978) where 2 μl of ^{35}S-sulfate (70 kBq) were injected into the cores at 1-cm intervals through predrilled silicone filled holes. The cores were subsequently incubated in darkness for 3 h. The incubation was terminated by sectioning the core into the same intervals as used above and fixing the sediment in 1 M zinc acetate (vol:vol). The samples were stored frozen until distillation according to the 1-step extraction scheme obtaining a total pool of reduced sulfides (Fossing and Jørgensen, 1989). The concentrations of reduced sulfides were determined by spectrophotometry according to Cline (1969).

3. Results

The fish pens yielded very high sedimentation rates, ranging from 124 to 331 g DW m^{-2} day^{-1} (Table 2), and the lowest rate was found in FP11, characterized by low feed supply rates (Table 1), where the sedimentation was similar to the abandoned fish pen (FP4). The maximum sedimentation rate was found at FP10, which was a relatively small fish pen with high fish biomass and a large food input per area compared to the other fish pens (Table 1). There was a strong positive linear correlation between sedimentation rates and the food input ($R^2 = 0.95$, $p < 0.001$) when excluding FP15 where a

Table 2
Rates of sedimentation and sediment characteristics (0–1 cm) at the fish pens studied in 2000 and 2001

Fish pen #	Rates of sedimentation ($g\,DW\,m^{-2}\,day^{-1}$)	Sediment organic carbon (%DW)	Sediment organic nitrogen (%DW)	Sediment total P ($\mu mol\,(g\,DW)^{-1}$)	C:N ratio (mol)
2001					
FP3	143 ± 10	11.7	0.70	40.9	19.6
FP4[a]	129 ± 41	10.0	0.52	22.2	22.4
FP5	254 ± 93	4.4	0.30	34.2	16.9
FP10	331 ± 17	10.9	0.49	37.7	25.7
FP11	124 ± 16	14.5	1.06	73.0	16.0
FP12	248 ± 17	9.2	0.84	48.6	12.7
FP13	268 ± 49	4.2	0.42	17.8	11.8
FP14	168 ± 82	2.4	0.14	49.7	20.3
FP15	146 ± 04	7.0	0.97	43.8	8.4
2000					
FP3	339 ± 46	5.4	0.37	36.9	17.1
FP4	292 ± 18	1.5	0.12	28.2	14.4
FP5	242 ± 19	6.3	0.35	24.5	20.9
FP6	493 ± 12	11.0	0.39	92.0	32.8

n.d. not determined.
The sedimentation rates are given as mean ± SD ($n = 2$).
[a] Fish pen #4 was abandoned in 2001.

high input was not reflected in the sedimentation. The sediment organic matter was high at all sites (POC 2.4–14.5 %DW, PON 0.14–1.06 %DW, Table 2), but there was no direct relation between sedimentation rates and the sediment organic matter content (linear regression, $p > 0.05$). The molar C:N ratio of the sediments was highly variable and ranged between 8.4 and 25.7 (Table 2) with no significant relationship to activities in the fish pens. The total P content in the sediments showed a similar lack of pattern, and was highest at FP11, the fish pen with lowest rates of sedimentation.

The sediment oxygen uptake was higher at the active fish pens than at the abandoned site (Table 3), with the highest sediment oxygen uptake, found at FP11, exceeding that at the abandoned fish pen by 6-fold. The sediment oxygen uptake showed an unexpected decrease with increasing organic input, as indicated by the negative relationship between sediment oxygen uptake and sedimentation rate (Fig. 2A, $R^2 = 0.44$, $p = 0.07$). On the other hand sediment oxygen uptake was found to increase with increasing total P content in the sediments (Fig. 2B, $R^2 = 0.80$, $p = 0.001$). The TCO_2 production

Table 3
Sediment metabolism (sediment oxygen uptake (SOU), TCO_2 production (TCO_2) and depth integrated sulphate reduction rates (0–10 cm, SRR)) and nutrient fluxes (phosphate (DIP), ammonium (NH_4) and nitrate + nitrite (NO_3)) at the fish pens sampled in 2000 and 2001

Fish pen #	SOU ($mmol\,m^{-2}\,day^{-1}$)	TCO_2 ($mmol\,m^{-2}\,day^{-1}$)	SRR ($mmol\,m^{-2}\,day^{-1}$)	DIP ($mmol\,m^{-2}\,day^{-1}$)	NH_4 ($mmol\,m^{-2}\,day^{-1}$)	NO_3 ($mmol\,m^{-2}\,day^{-1}$)
2001						
FP3	163.56 ± 9.86	312.37 ± 30.90	185.25 ± 8.61	0.52 ± 0.08	−0.29 ± 0.03	−0.65 ± 0.12
FP4	50.95 ± 9.35	150.03 ± 16.53	150.01 ± 16.97	−0.05 ± 0.01	−0.14 ± 0.05	−0.30 ± 0.04
FP5	154.89 ± 10.06	247.94 ± 34.04	114.04 ± 16.69	0.04 ± 0.08	−0.07 ± 0.28	−0.74 ± 0.16
FP10	166.62 ± 22.99	919.49 ± 25.25	91.39 ± 24.17	0.64 ± 0.25	2.23 ± 0.34	−0.56 ± 0.14
FP11	336.88 ± 19.85	844.29 ± 26.85	99.72 ± 16.01	0.34 ± 0.09	0.99 ± 0.37	−0.62 ± 0.11
FP12	206.58 ± 18.49	327.54 ± 28.71	140.83 ± 19.84	0.17 ± 0.19	2.38 ± 0.39	−0.66 ± 0.11
FP13	174.07 ± 22.12	245.06 ± 27.08	116.86 ± 22.24	0.31 ± 0.14	1.24 ± 0.36	−0.86 ± 0.19
FP14	261.20 ± 23.43	442.43 ± 30.23	51.56 ± 16.07	0.36 ± 0.23	−0.30 ± 0.06	−1.33 ± 0.03
FP15	243.40 ± 22.14	296.70 ± 29.53	97.81 ± 22.38	1.38 ± 0.11	1.90 ± 0.32	−0.78 ± 0.15
2000						
FP3	119.81 ± 17.89	141.09 ± 20.17	35.88 ± 7.82	0.32 ± 0.10	−1.72 ± 1.08	−4.34 ± 1.07
FP4	60.66 ± 6.05	140.76 ± 8.91	14.00 ± 1.07	−0.05 ± 0.20	0.71 ± 0.41	−1.69 ± 0.98
FP5	89.96 ± 6.25	88.26 ± 2.98	32.18 ± 7.57	−0.003 ± 0.05	1.35 ± 0.55	−1.33 ± 0.10
FP6	260.92 ± 19.43	640.84 ± 91.28	n.d.	4.81 ± 0.73	21.64 ± 5.92	−5.38 ± 0.32

Negative nutrient fluxes indicate a sediment uptake.
Values are given as mean ± SEM ($n = 3$).

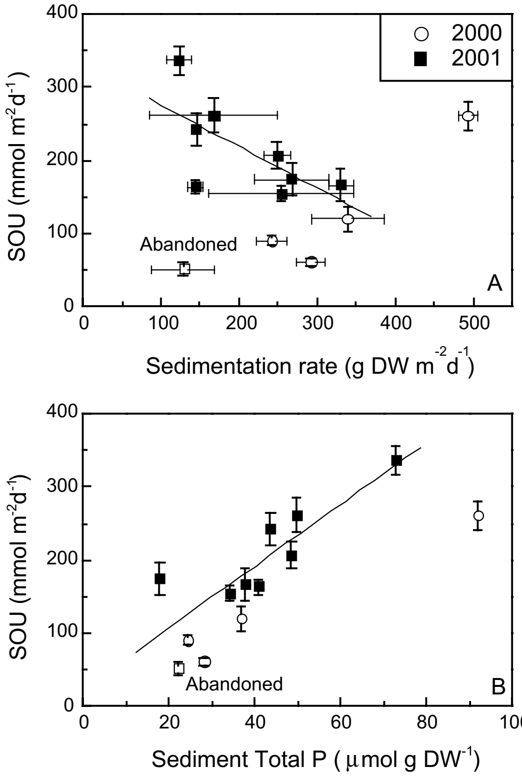

Fig. 2. Sediment oxygen uptake (SOU, $mmol\,m^{-2}\,day^{-1}$) with (A) increasing sedimentation ($g\,DW\,m^{-2}\,day^{-1}$) and (B) increasing sediment Total P content ($\mu mol\,g\,DW^{-1}$) at the fish pens sampled in 2001 (closed squares) and in 2000 (open circles). The abandoned site is indicated. Regression lines for 2001 are given (A: $R^2 = 0.44$, $p = 0.07$; B: $R^2 = 0.72$, $p = 0.003$). Symbols represent mean values (±SE, $n = 3$).

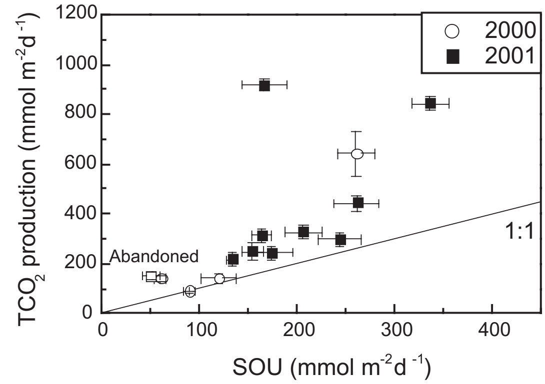

Fig. 3. Sediment oxygen uptake (SOU) versus TCO_2 production ($mmol\,m^{-2}\,day^{-1}$) at the fish pens sampled in 2001 (closed squares) and in 2000 (open circles). The line represents a ratio of 1:1 and symbols represent mean values (±SE, $n = 3$).

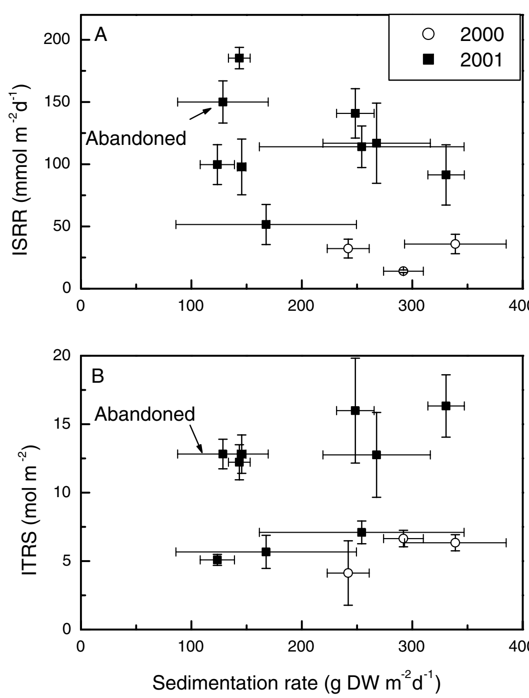

Fig. 4. Depth integrated sulfate reduction rates (A: ISRR ($mmol\,m^{-2}\,day^{-1}$)) and depth integrated pools of reduced sulfides (B: ITRS ($mol\,m^{-2}$)) with increasing rates of sedimentation ($g\,DW\,m^{-2}\,day^{-1}$) at the fish pens sampled in 2001 (closed squares) and in 2000 (open circles). The abandoned site is indicated. Symbols represent mean values (±SE, $n = 3$).

was also enhanced in the active fish pens compared to the abandoned pen and was up to six times higher at FP11 (Table 3). TCO_2 appeared to be produced in excess of the oxygen consumed in most fish pens, in particular at the two fish pens located on coral rubble (FP10 and FP11, Fig. 3).

The sulfate reduction rates were independent of the activity of the fish pens. Sulfate reduction rates were generally high, and the abandoned site attained one of the highest rates (Table 3). The sulfate reduction rates were extremely high in the surface layers (3–17 $\mu mol\,cm^{-3}\,day^{-1}$) and decreased with depth (35–655 $nmol\,cm^{-3}\,day^{-1}$, data not shown). There was no significant relationship between sulfate reduction rates and the rates of sedimentation (Fig. 4) or total P content in the sediments (data not shown). The fish pens could be grouped into two sets according to the pools of reduced sulfides in the sediments: a group of fish pens in the eastern part of the sampling area with an average of 5–7 $mol\,S\,m^{-2}$, and a group in the channel area with higher pools averaging 12–17 $mol\,S\,m^{-2}$ (Fig. 4). The pools of reduced sulfides tended to increase with increasing sulfate reduction rates although the correlation was not statistically significant (linear regression, $p > 0.05$).

The flux of phosphate across the sediment–water interface was clearly affected by the activity in the fish pens as all the active fish pens showed an efflux, whereas phosphate was taken up by the sediments at the abandoned site (Table 3). The flux varied by a factor of 35 among the fish pens, and the efflux showed an increasing trend, albeit not significant ($p > 0.05$), with increasing sediment oxygen uptake (Fig. 5). In contrast to phosphate, the sediments consumed ammonium at several of

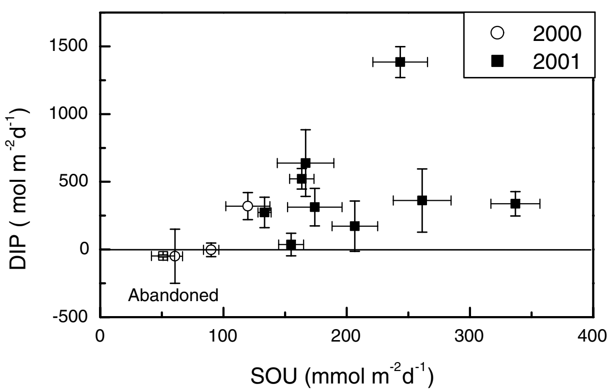

Fig. 5. Phosphate flux (DIP μmol m^{-2} day^{-1}) versus sediment oxygen uptake (SOU, mmol m^{-2} day^{-1}) at the fish pens sampled in 2001 (closed squares) and in 2000 (open circles). The abandoned site is indicated. Symbols represent mean values (±SE, $n = 3$).

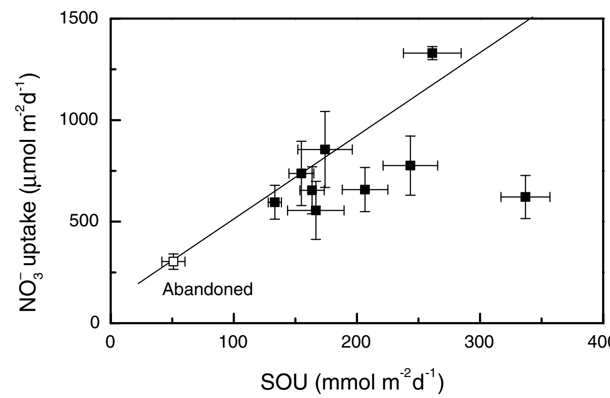

Fig. 7. Nitrate uptake (NO_3^-, μmol m^{-2} day^{-1}) versus sediment oxygen uptake (SOU, mmol m^{-2} day^{-1}) at the fish pens sampled in 2001. The abandoned site is indicated. Symbols represent mean values (±SE, $n = 3$).

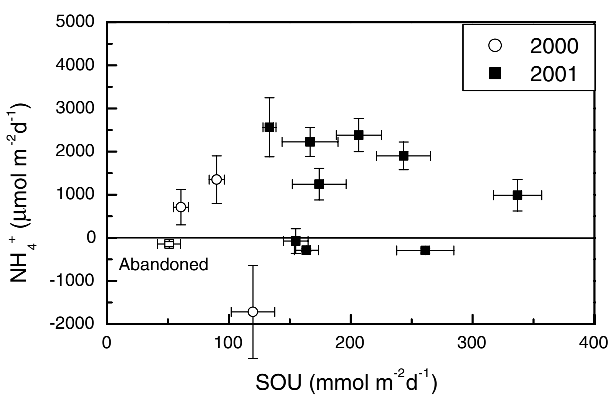

Fig. 6. Ammonium flux (NH_4^+ μmol m^{-2} day^{-1}) versus sediment oxygen uptake (SOU, mmol m^{-2} day^{-1}) at the fish pens sampled in 2001 (closed squares) and in 2000 (open circles). The abandoned site is indicated. Symbols represent mean values (±SE, $n = 3$).

the active fish pens (FP3, FP5 and FP14) and at the abandoned site (Table 3). There was no apparent relationship between the ammonium fluxes and the sediment oxygen uptake (Fig. 6). Nitrate was taken up in the sediments at all sites with lowest uptake at the abandoned fish pen (Table 3). The nitrate uptake increased with increasing sediment oxygen uptake (Fig. 7, $R^2 = 0.77$, $p \ll 0.001$).

4. Discussion

The fish pen sediments in this study were highly impacted by the aquaculture operations. The sediments were characterized by high contents of organic matter, as reported for fish cages in the past (Hall et al., 1990; Hargrave et al., 1993; Holmer and Kristensen, 1992, 1996) and in fish pens (Holmer et al., 2002). As a consequence of the high organic inputs the sediments were anoxic, as indicated by the high ratio between TCO_2 production and sediment oxygen uptake and the high rates of sulfate reduction. The unexpected negative trend between the rates of sedimentation and the sediment metabolism (e.g. sediment oxygen uptake) found in this study indicates that the mineralization of organic matter decreases with increasing loading rates. This is in contrast to findings in coastal temperate sediments, where an input of fresh organic matter often leads to an immediate stimulation of sediment processes such as oxygen uptake, nitrogen mineralization and sulfate reduction rates (Graf et al., 1982; Jensen et al., 1990; Holmer, 1999; Gray et al., 2002). The most likely explanation for this contrast is that the sedimentation rates are much lower in these coastal areas compared to inside the fish pens, and that the source of organic matter is of higher quality in the fish pens (Holmer et al., 2002). The decrease in sediment metabolism with increasing sediment rate suggests that the microbial activity in the sediments is not able to keep up with the organic matter loading. There are several possibilities for reduced microbial activity: (1) lack of supply of electron acceptors, e.g. oxygen; (2) limited mixing of microbial substrates due to lack of bioturbation and (3) accumulation of fermentation and mineralization products to levels which inhibit microbial activity (Holmer and Kristensen, 1994b). We did not measure oxygen penetration depth in the examined sediments, but applied silver sticks to detect the presence of free sulfide in the pore waters. In most cases free sulfide was detected at depths of <5 mm suggesting that the presence of oxygen must be restricted to the top few mm of the sediments at best.

The 2- to 5-fold higher TCO_2 production relative to sediment oxygen uptake also shows that aerobic decomposition was probably quite limited in the fish pen sediments (Thamdrup, 2000). Possible metabolic pathways may include denitrification, iron/manganese reduction, sulfate reduction or methanogenesis (Thamdrup and Canfield, 2000). The large uptake of nitrate suggests that denitrification may be an important process in these sediments, and the positive relation between rates of nitrate uptake and sediment oxygen uptake suggests that the role of denitrification was increasing with increasing organic matter oxidation. It is, however, unlikely that iron and manganese play significant roles in anaerobic decomposition, as the content of oxidized iron probably is low in these carbonate-rich fish pen sediments (Berner, 1984; Kamp-Nielsen et al., 2002) and manganese is generally expected to be low in carbonate-rich sediments (Thamdrup, 2000). In organic-rich coastal sediments, most anaerobic mineralization occurs through sulfate reduction (Jørgensen, 1982; Canfield, 1994; Holmer and Kristensen, 1996). The molar ratio between TCO_2 production and sulfate reduction was close to 2:1 (1 mol of sulfate reduced produces 2 mol of TCO_2) for most fish pens (Fig. 8) suggesting that sulfate reduction was an important process and accounted for most of the production of TCO_2. Most of the oxygen taken up by the sediments must be consumed, instead, in reoxidation processes similar to findings in marine fish farm sediments in temperate regions (Hall et al., 1990; Holmer and Kristensen, 1996). The sulfate reduction rates were particularly stimulated in the surface layers where the organic enrichment occurred, and the rates are the highest ever recorded in coastal sediments (Jørgensen and Richardson, 1996), and even higher than found under fish cages in temperate regions (Holmer and Kristensen, 1996). The sediments were highly reduced and often without a visual oxidized zone and with white spots of *Beggiatoa*, strongly suggesting that the oxygen penetration was very limited. The TCO_2 production was very high at FP10 and FP11 and much higher than the sediment oxygen uptake (2.5–5.5 times) and sulfate reduction

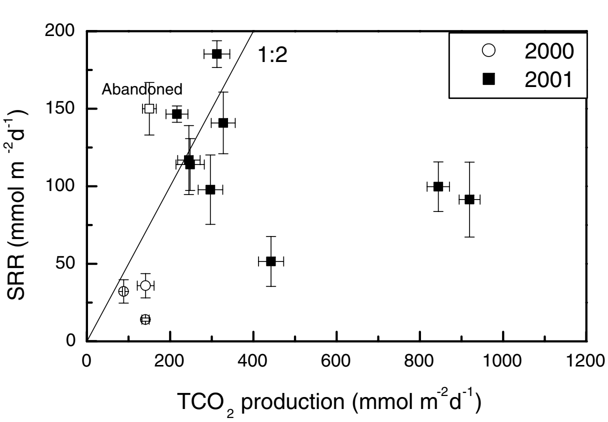

Fig. 8. Depth integrated sulphate reduction rates (ISRR, mmol m^{-2} day^{-1}) versus TCO_2 production (mmol m^{-2} day^{-1}) at the fish pens sampled in 2001 (closed squares) and in 2000 (open circles). The line represents a ratio of 1:2 and symbols represent mean values ($\pm$SE, $n = 3$).

rates (~10 times). Ku et al. (1999) found that dissolution of calcium carbonate in organic-rich shelf carbonate sediments is related to sulfate reduction rates, and as these two fish pens were located on coral rubble it is likely that carbonate dissolution was particularly high in these sediments.

Sulfate reduction appeared to decrease with increasing organic loading rates, although it was not statistically significant due to heterogeneity in the measurements. A negative relationship can be expected under several conditions, as sulfate reduction depends, in addition to the organic matter supply, upon the availability of sulfate. Whenever sulfate concentrations fall below 3 mM in the pore waters reduction rates becomes controlled by the sulfate supply (Boudreau and Westrich, 1984). There were no indications of limiting sulfate concentrations in the fish pen sediments, as the concentrations remained >10 mM. Sulfate reducing bacteria can also be inhibited if the pH decrease to low levels and sulfides at the same time accumulate (McCarthney and Olezkiewicz, 1991). A low pH increases the toxicity of sulfides and depressed sulfate reduction rates have been found under experimental conditions in fish farm sediments (Holmer and Kristensen, 1994b) and in active sludge (Choi and Rim, 1991). Unfortunately we did not measure pH in the fish pen sediments, but dissolved sulfide concentrations, which were measured in the four fish pens studied in 2000, ranged from 50–100 μM to 1–4 mM concentration at the most productive fish pen (Holmer, unpublished results). Such levels have been found to be inhibitory (Holmer and Kristensen, 1994b).

The decreasing importance of sulfate reduction suggests that methanogenesis may be a significant process at the high loading rates. Coexistence of sulfate reduction and methanogenesis has been found in fish farm sediments (Holmer and Kristensen, 1994a) and although we did not quantify methane production in this study, the observed release of gas bubbles from the sediments during collection and handling of sediment cores suggests that methanogenesis was an important mineralization pathway. Accumulation of gas bubbles in surface sediments only occurs at high rates of methane production (Holmer and Kristensen, 1996).

4.1. Nutrient regeneration in fish pen sediments

The regeneration of nutrients was rapid in the fish pen sediments, and most of the sites showed a significant release of phosphate to the water column, which may stimulate the benthic and pelagic primary production in the area (Marbá et al., in preparation). The concentration of nutrients in the water column has generally increased in the area since fish farming was initiated (San Diego-McGlone and Ranches, in press), and in particular the concentrations of phosphate and nitrate have shown major elevations. We found a positive trend between sediment oxygen uptake and release of phosphate from the sediments, suggesting that the release of phosphate is controlled by sediment mineralization. Fish farm wastes contain high amounts of phosphorus due to high lipid content in wasted feed as compared to natural sources of settling material in coastal areas (Henderson et al., 1997).

In addition to the composition of the organic matter the release of phosphate from coastal sediments is controlled by a number of factors such as binding capacity in the sediments and uptake by benthic microalgae (Jensen and Thamdrup, 1993; Sundback and Miles, 2002). A previous study in this area showed a large accumulation of phosphorus in the sediments, and it was suggested that phosphorus was either buried with organic matter or bound in carbonates (Holmer et al., 2002). Phosphorus concentrations were also elevated in the surface sediments in the examined fish pens. At the same time, the sediments were highly reduced with large pools of reduced sulfides as compared to low-organic carbonate sediments in general (Chambers et al., 2001). The binding capacity between phosphate and oxidized iron was probably very low due to low iron-availability in these sediments and further constrained by competition with reduced sulfides (Jensen and Thamdrup, 1993; Jensen et al., 1998). Hence increasing release of phosphate with increasing metabolic rate was likely caused by a combination of increased phosphate release through mineralization, reduced sediment oxidation and increased competition for iron oxides with sulfides. Phosphate only fluxed into the sediments at the abandoned fish pen. This site is shallow and light availability at the sediment surface is probably sufficiently high during calm periods with low resuspension and low dispersion of fine particles from the surrounding fish pens to allow growth and nutrient uptake by benthic microalgae. Also binding of phosphates to carbonates is likely, as the total phosphorus concentration was lower here compared to the active sites.

There is in contrast no clear relationship between the sediment oxygen uptake and the flux of ammonium in the fish pen sediments. Uptake of ammonium by the sediment is found both at low and high sediment metabolism, which may be due to the presence of benthic algae at the sediment surface or due to immobilization of nitrogen by the benthic bacteria during decomposition of carbon rich organic matter (Fenchel et al., 1998). It is not likely that ammonium is taken up for nitrification in these reduced sediments with high ammonium and sulfide concentrations, which inhibits nitrification (Christensen et al., 1999). Denitrification may also be inhibited by high sulfide concentrations, and the nitrate fluxing into the sediments may be reduced to ammonium through dissimilatory nitrate reduction (Christensen et al., 1999).

4.2. Ecological consequences

This study shows, that sediment processes are so impacted by fish farming in the Bolinao area, that this activity can be considered to have moved beyond the threshold of ecological sustainability. This is further supported by the major fish kill, which took place only two months after this study, where 110,000 mt milkfish were lost to an estimated value of $16 million (Primavera, 2002). Unusual weather conditions with two weeks of neap tides and low water levels combined with calm wind conditions, which limited the dilution of the nutrients released from the sediments and oxygenation of the waters, resulted in a major bloom of dinoflagellates and oxygen depletion in the water column. This combination resulted in major fish kills of farmed milkfish, which was proceeded by a massive die-off of wild populations of puffer fish. The oxygen depletion event may have further enhanced the release of ammonium and phosphate from the sediments due to elimination of nitrification (no oxygen) and release of phosphate from iron-bound pools.

The observed decrease in organic matter mineralization with increasing organic matter loading rates leads to enhanced burial of organic matter, and thus a general organic matter enrichment of the sediments. This suggests that the rate of recovery after fallowing may be slow and that organic matter and nutrients may be transported to the nearby seagrass beds and coral reefs during resuspension events. Organic loading and nutrient enrichments have been found to interfere with coral reef (Barber et al., 2001) and seagrass (Hemminga and Duarte, 2000) communities. Also reduced sulfides accumulate in the sediments, and the impoverished sediment conditions appear to have major impact on the benthic flora (Terrados et al., 1999) and fauna. We found declines in seagrass distribution and diversity (Marbá, in preparation) and a change in the benthic fauna to more pollutant tolerant species (Heilskov, in preparation). The snapper shrimp *Alpheus macellarius*, which is a major agent of bioturbation and sediment ventilation in the Bolinao area (Nacorda, submitted for publication) was absent from the fish pens and was reduced in numbers outside the farms compared to control sites (Heilskov, in preparation.). The area has also been invaded by the benthic medusa *Cassiopeia andromeda*, which appear to be particularly abundant on the organic-enriched sediment surfaces of abandoned fish pens.

In conclusion, the high rates of sedimentation at the fish pens examined resulted in high rates of metabolic activity in the sediments. Methane production was most likely coexisting with sulfate reduction, a phenomenon which has only been observed in organically enriched marine fish farms, where excess organic matter causes the two processes to occur at the same time (Holmer and Kristensen, 1994a). Despite this stimulation, organic matter mineralization could not keep up with the organic matter loading and the sediments were enriched in organic matter and may thus act as a nutrient reserve even after the fish pens have been abandoned. Indeed, the abandoned fish pen included in this study was still enriched in organic matter compared to unimpacted sites (Holmer et al., 2002), although the metabolic activity and nutrient release were significantly reduced. This indicates that removal of active fish farming improve the conditions through reduced oxygen consumption and nutrient release across the sediment–water interface. The anaerobic activity, however, remained high.

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References

Adeel, Z., Pomeroy, R., 2002. Assessment and management of mangrove ecosystems in developing countries. Trees—Struct. Funct. 16, 235–238.

Armstrong, F.A.J., Stearns, C.R., Strickland, J.D.H., 1967. The measurement of upwelling and subsequent biological processes by means of the technicon autoanalyser and associated equipment. Deep-Sea Res. 14, 381–389.

Barber, R.T., Hilting, A.K., Hayes, M.L., 2001. The changing health of coral reefs. Human Ecol. Risk Assess. 7, 1255–1270.

Bell, J.D., Gervis, M., 1999. New species for coastal aquaculture in the tropical Pacific—constraints, prospects and considerations. Aquacult. Int. 7, 207–223.

Berner, R.A., 1984. Sedimentary pyrite formation: An update. Geochim. Cosmochim. Acta 48, 605–615.

Blomqvist, S., Håkanson, L., 1981. A review on sediment traps in aquatic environments. Arch. Hydrobiol. 91, 101–132.

Boudreau, B.P., Westrich, J.T., 1984. The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments. Geochim. Cosmochim. Acta 48, 2503–2516.

Bower, C.E., Holm-Hansen, T., 1980. A salicylate-hypochlorite method for determining ammonia in seawater. Can. J. Fish Aquat. Sci. 37, 794–798.

Canfield, D.E., 1994. Factors influencing organic carbon preservation in marine sediments. Chem. Geol. 114, 315–329.

Chambers, R.M., Fourqurean, J.W., Macko, S.A., Hoppenot, R., 2001. Biogeochemical effects of iron availability on primary producers in a shallow marine carbonate environment. Limnol. Oceanogr. 46, 1278–1286.

Choi, E., Rim, J.M., 1991. Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment. Water Sci. Tech. 23, 1259–1264.

Christensen, P.B., Rysgaard, S., Sloth, N.P., Dalsgaard, T., Schwærter, S., 1999. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. Aquat. Microb. Ecol. 21, 73–84.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14, 454–459.

Delgado, O., Ruiz, J., Pérez, M., Romero, J., Ballesteros, E., 1999. Effects of fish farming on seagrass (*Posidonia oceanica*) in a Mediterranean bay: seagrass decline after organic loading cessation. Oceanol. Acta 22, 109–117.

Ervik, A., Hansen, P.K., Aure, J., Stigebrandt, J., Johannessen, P., Jahnsen, T., 1997. Regulating the local environmental impact of extensive marine fish farming: I. The concept of MOM (Modelling-Ongrowing fish farms-Monitoring). Aquaculture 158, 85–94.

FAO, 2002. The state of world aquacultures 2002. ISBN 92-5-104842-8.

Fenchel, T., King, G.M., Blackburn, T.H., 1998. Bacterial Biogeochemistry. The Ecophysiology of Mineral Cycling. Academic Press, San Diego, CA.

Fossing, H., Jørgensen, B.B., 1989. Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method. Biogeochem. 8, 205–222.

Graf, G., Bengtsson, W., Diesner, W., Schulz, R., Theede, H., 1982. Benthic response to sedimentation of a spring phytoplankton bloom: process and budget. Mar. Biol. 67, 201–208.

Gray, J.S., Wu, R.S.-S., Or, Y.Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Mar. Ecol. Prog. Ser. 238, 249–279.

Hall, P.O.J., Aller, R.C., 1992. Rapid, small-volume, flow injection analysis for $\sum CO_2$ and NH_4^+ in marine and fresh water. Limnol. Oceanogr. 37, 1113–1119.

Hall, P.O.J., Anderson, L.G., Holby, O., Kollberg, S., Samuelsson, M., 1990. Chemical fluxes and mass balances in a marine fish cage farm. I. Carbon. Mar. Ecol. Prog. Ser. 61, 61–73.

Hargrave, B.T., Burns, N.M., 1979. Assessment of sediment trap collection efficiency. Limnol. Oceanogr. 24, 1124–1136.

Hargrave, B.T., Duplisea, D.E., Pfeiffer, E., Wildish, D.J., 1993. Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. Mar. Ecol. Prog. Ser. 96, 249–257.

Heijs, S.K., Azzoni, R., Giordani, G., Jonkers, H.M., Nizzoli, D., Viaroli, P., van Gemerden, H., 2000. Sulfide-induced release of phosphate from sediments of coastal lagoons and the possible relation to the disappearance of *Ruppia* sp. Aquat. Microb. Ecol. 23, 85–95.

Hemminga, M.A., Duarte, C.M., 2000. Seagrass Ecology. Cambridge University Press, Cambridge.

Henderson, R.J., Forrest, D.A.M., Black, K.D., Park, M.T., 1997. The lipid composition of sealoch sediments underlying salmon cages. Aquaculture 158, 69–83.

Holmer, M., 1999. The effect of oxygen depletion on anaerobic organic matter degradation in marine sediments. Estuar. Coast. Shelf Sci. 48, 383–390.

Holmer, M., Kristensen, E., 1992. Impact of marine fish cage farming on sediment metabolism and sulfate reduction of underlying sediments. Mar. Ecol. Prog. Ser. 80, 191–201.

Holmer, M., Kristensen, E., 1994a. Coexistence of sulfate reduction and methane production in an organic-rich sediment. Mar. Ecol. Prog. Ser. 107, 177–184.

Holmer, M., Kristensen, E., 1994b. Organic matter mineralization in an organic-rich sediment: Experimental stimulation of sulfate reduction by fish food pellets. FEMS Microbiol. Ecol. 14, 33–44.

Holmer, M., Kristensen, E., 1996. Seasonality of sulfate reduction and pore water solutes in a marine fish farm sediment: the importance of temperature and sedimentary organic matter. Biogeochem. 32, 15–39.

Holmer, M., Marbá, N., Terrados, J., Duarte, C.M., Fortes, M.D., 2002. Impacts of milkfish (*Chanos chanos*) aquaculture on carbon and nutrient fluxes in the Bolinao area, Philippines. Mar. Poll. Bull. 44, 685–696.

Jensen, H.S., Thamdrup, B., 1993. Iron-bound phosphorus in marine sediments as measured by bicarbonate–dithionite extraction. Hydrobiologia 253, 47–59.

Jensen, H.S., McGlathery, K.J., Marino, R., Howarth, R.W., 1998. Forms and availability of sediment phosphorus in carbonate sand of Bermuda seagrass beds. Limnol. Oceanogr. 43, 700–810.

Jensen, M.H., Lomstein, E., Sørensen, J., 1990. Benthic NH_4^+ and NO_3^- flux following sedimentation of a spring phytoplankton bloom in Aarhus Bight, Denmark. Mar. Ecol. Prog. Ser. 61, 87–96.

Jørgensen, B.B., 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. Geomicrobiol. J. 1, 11–27.

Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed—the role of sulphate reduction. Nature 296, 643–645.

Jørgensen, B.B., Richardson, K. (Eds.), 1996. Eutrophication in Coastal Marine Ecosystems. American Geophysical Union, Washington, DC.

Kamp-Nielsen, L., Vermaat, J.E., Wesseling, I., Borum, J., Geertz-Hansen, O., 2002. Sediment properties along gradients of siltation in South-east Asia. Estuar. Coast. Shelf Sci. 54, 127–137.

Karakassis, I., Hatziyanni, E., 2000. Benthic disturbance due to fish farming analyzed under different levels of taxonomic resolution. Mar. Ecol. Prog. Ser. 203, 247–253.

Karakassis, I., Hatziyanni, E., Tsapakis, M., Plaiti, W., 2000. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES J. Mar. Sci. 57, 1462–1471.

Koroleff, F., 1983. Determination of nutrients. In: Grasshof, K., Ehrhardt, M., Kremling, K. (Eds.), Methods of Seawater Analysis. Verlag Chemie, Weinheim, New York, pp. 125–139.

Kristensen, E., Andersen, F.Ø., 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. J. Exp. Mar. Biol. Ecol. 109, 15–23.

Ku, T.C.W., Walter, L.M., Coleman, M.L., Blake, R.E., Martini, A.M., 1999. Coupling between sulfur recycling and syndepositional carbonate dissolution: Evidence from oxygen and sulfur isotope composition of pore water sulfate, South Florida Platform, USA. Geochim. Cosmochim. Acta 63, 2529–2546.

McCarthney, D.M., Olezkiewicz, J.A., 1991. Sulfide inhibition of anaerobic degradation of lactate and acetate. Water Res. 25, 203–209.

Nacorda, H., submitted for publication. Aboveground behavior and significance of *Alpheus macellarius* Chace, 1988, in a Philippine seagrass meadow. J. Exp. Mar. Biol. Ecol.

Pedersen, M.F., Borum, J., 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar. Ecol. Prog. Ser. 142, 261–272.

Primavera, J.H., 2002. CRM case studies in the Philippines: Seeds of hope. 3rd meeting of the Asia–Pacific cooperation for sustainable use of renewable natural resources in biosphere reserves (ASP-ACO) project, Okinawa, Japan, p. 10.

San Diego-McGlone, M.L., Ranches, G.D., 2003. Water quality assessment of Lingayen Gulf. Philipp. Sci.

Sumalde, Z.N., Francisco, K.L.A., Peñales, M.H., 2002. Aquaculture pollution in Bolinao: Who pays the price? In: Proceedings of the Coastal Zone Asia Pacific Conference "Improving the State of the Coastal Areas" Bangkok, Thailand. Available from <http://www.vims.edu/czap/poster/Sumalde_Zenaida.ppt>.

Sundback, K., Miles, A., 2002. Role of microphytobenthos and denitrification for nutrient turnover in embayments with floating macroalgal mats: a spring situation. Aquat. Microb. Ecol. 30, 91–101.

Terrados, J., Duarte, C.M., 2002. Southeast Asian seagrass ecosystems inder stress: have we improved? In: Proceedings of the Coastal Zone Asia Pacific Conference "Improving the State of the Coastal Areas" Bangkok, Thailand. Available from <http://www.vims.edu/czap/oral/terrados_jorge.ppt>.

Terrados, J., Duarte, C.M., Fortes, M.D., Borum, J., Agawin, N.S.R., Bach, S., Thampanya, U., Kamp-Nielsen, L., Kenworthy, W.J., Geertz-Hansen, O., Vermaat, J., 1998. Changes in community structure and biomass of seagrass communities along gradients of siltation in SE Asia. Estuar. Coast. Shelf Sci. 46, 757–768.

Terrados, J., Duarte, C.M., Kamp-Nielsen, L., Borum, J., Agawin, N.S.R., Fortes, M.D., Gacia, E., Lacap, D., Lubanski, M., Greve, T., 1999. Are seagrass growth and survival affected by reducing conditions in the sediment. Aquat. Bot. 65, 175–197.

Thamdrup, B., 2000. Bacterial manganese and iron reduction in aquatic sediments. Adv. Microb. Ecol. 16, 41–84.

Thamdrup, B., Canfield, D.E., 2000. Benthic respiration in aquatic sediments. In: Sala, O.E., Jackson, R.B., Mooney, H.A., Howarth, R.W. (Eds.), Methods in Ecosystem Science. Springer, New York, pp. 86–103.