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Estuarine nitrogen retention independently estimated by the denitrification rate and mass balance methods: a study of Norsminde Fjord, Denmark

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ABSTRACT: Nitrogen retention was studied in a small, shallow estuary (Norsminde Fjord, Denmark) by 2 independent methods: (1) measurement of denitrification rate using the isotope pairing technique; and (2) estimation of mass balances established on the basis of hydrodynamic numerical modelling. Denitrification rates were found to range from 100 to 1600 µmol N m^{-2} d^{-1}, and varied considerably from February to May. The seasonal and spatial variation in denitrification rate was positively related to the water column NO_3^- concentration. The average annual denitrification rate was 29 kg N ha^{-1} yr^{-1}, or approximately 6 t N yr^{-1} for the entire estuary. This compared well with the annual nitrogen retention of approximately 8 t N yr^{-1} calculated from the mass balances. Annual denitrification and nitrogen retention in the estuary accounted for approximately 2% and 3%, respectively, of total nitrogen input from the catchment area. These low values are explicable by the high water exchange rate in the estuary, freshwater retention time being 1.5 to 13 d, with minimum values during the winter. The present study therefore lends no support to the widely held assumption that estuarine denitrification generally amounts to 40 to 50% of the nitrogen input. The findings indicate that in addition to nitrogen input from the land, consideration must also be given to the water residence when estuarine nitrogen retention is being estimated.

KEY WORDS: Estuary · Eutrophic · Denitrification · Isotope pairing · Loading · Mass balance · Model · Nitrogen · Retention

INTRODUCTION

Estuaries are often characterized by high productivity caused by high nutrient input from terrestrial runoff and sewage effluent. Of primary importance in this respect is nitrogen, although phosphorus may also play a role — typically during the spring (Ryther & Dunstan 1971, Boynton et al. 1982, Sand-Jensen & Borum 1991, Sand-Jensen et al. 1994). Part of the nutrient input in eutrophic areas is temporarily retained as high primary producer biomass, i.e. phytoplankton and benthic micro- and macrophytes (Monbet 1992, Sfriso et al. 1992, Valilea et al. 1992, Cahoon et al. 1993). Estuarine nitrogen retention (N_{ret}) also takes place through immobilization in the sediment or as a result of denitrification.

Most estimates of estuarine N_{ret} are based on estimates of sediment N_2 fluxes and terrestrial input of inorganic nitrogen. On the basis of an analysis of data from the Baltic Sea and 6 estuaries in different parts of the world, Seitzinger (1988, 1990) concluded that 40 to 50% of estuarine nitrogen load is retained by denitrification, the figure being independent of the biological and hydrographic structure of the estuary or the technique used to measure denitrification. Whether or not this '50% rule' is generally applicable is uncertain; moreover, no clear explanation has yet been offered to account for it. The question is important since the '50% rule' is sometimes applied when estimating nitrogen transport from land to the sea during the compilation of total nitrogen budgets for open marine waters, e.g. Kattegat/Skagerrak (Ærtebjerg et al. 1991, North Sea Task Force 1993).

Another approach to studying estuarine N_{ret} is to determine the ecosystem mass balance. However, this approach has only rarely been applied to marine ecosystems (Wulff et al. 1990, Kamp-Nielsen 1992, Larsen et al. 1992), probably due to difficulties in estimating nutrient exchange with the open sea. To our knowledge, no parallel determination of estuarine N_{ret} using both methods has previously been reported.

The purpose of the present study was to determine annual N_{ret} in a small, eutrophic estuary using both the denitrification rate (isotope pairing technique) and mass balance (hydrodynamic numerical modelling) methods. The study was undertaken in Norsminde Fjord, Denmark, and was part of the aquatic monitoring programme jointly conducted by the Aarhus County Council and the National Marine Research Programme, HAV 90.

MATERIALS AND METHODS

Study area. The study was undertaken from February 1992 to February 1993 in Norsminde Fjord, a shallow estuary situated on the east coast of Jutland, Denmark (Fig. 1, Table 1). The surface area is 1.86 km^2

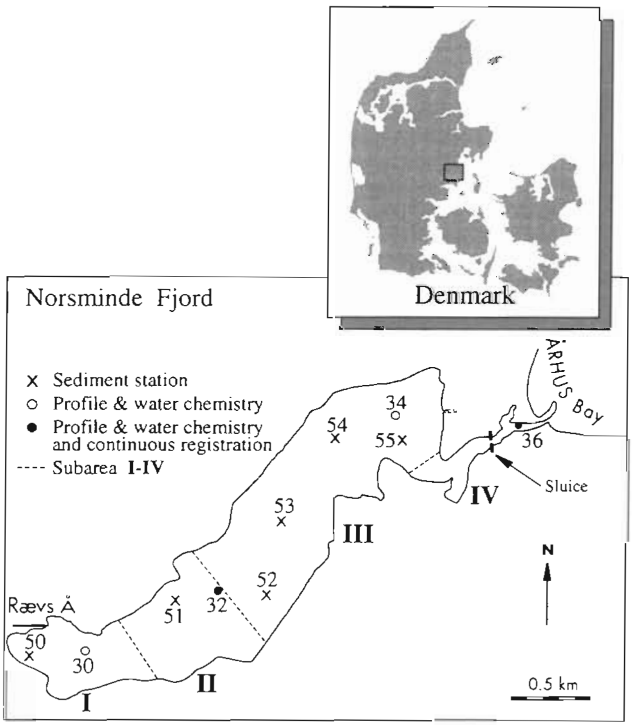

Fig. 1. Norsminde Fjord, Denmark, showing the main freshwater inlet, Rævs Å, the sluiced outlet to Aarhus Bay and the sampling stations for water chemistry, sediment and continuous registration of temperature and salinity. The 4 subareas used in the numerical hydrodynamic model are also indicated

Table 1. Characteristics of Norsminde Fjord

Catchment area	101 km^2
Length	5 km
Surface area	1.86 km^2
Mean depth	0.6 m
Maximum depth	2.0 m
Volume	1.1×10^6 m^3
Tide	0.2 m

and the mean depth only 0.6 m. The catchment area comprises 101 km^2 of intensively farmed agricultural land. The main freshwater input is Rævs Å, a small stream that drains 85% of the catchment area and enters the estuary at its innermost part. The outflow to Aarhus Bay is a narrow channel protected by a sluice which closes at sea water level +0.35 m (to prevent excessive influx of sea water).

The sediment ranges from soft mud with an organic matter content of about 10 to 15% (ignition loss) to fine/medium-sized sand with a low organic matter content (1% ignition loss). The organic matter content is generally highest in the relatively deeper, central part of the estuary (Aarhus County Council 1994.)

Loading and hydrography. The main freshwater input to the estuary was calculated on a daily basis from the water level using a calibrated water level/water flow relationship for Rævs Å. Additional input from the remainder of the catchment area was estimated on the basis of the input from the Rævs Å catchment area using the measured nitrogen input per km^2 from the latter. Chemical analysis of the stream water was performed 18 times during the study period. Nitrogen input was calculated from the product of daily water flow and nitrogen concentration using the *C/Q* method; knowing the daily water flow, the daily values of nitrogen concentration can be estimated from the empirical model of nitrogen concentration (*C*) and water flow (*Q*) (Walling & Webb 1981).

Salinity and temperature profiles were measured with a CTD probe every 2 to 3 wk at 4 permanent monitoring stations (Fig. 1). Surface water was analysed for total-nitrogen (dissolved and particulate), nitrate and ammonium every 2 to 3 wk at 3 stations. During stratification, bottom water was also analyzed. The water samples were stored at the *in situ* temperature until required, analysis being performed by conventional methods within 3 h (Greenberg 1992). Water level, salinity and temperature were recorded digitally every 15 min during most of the study period at 2 of the stations, 1 in the centre of the estuary and 1 at its mouth (Fig. 1).

The estuary was ice covered in January 1992, and temperature was at its maximum (20 to 25°C) during May to July. Salinity varied considerably, ranging from 0.1–19.4‰ in the inner part of the estuary to 3.7–27.3‰ in the outer part. Despite the shallow depth, the water column was stratified approximately 15% of the year (Aarhus County Council 1994).

Denitrification rate. The sediment at 6 stations distributed along the length of the estuary (Fig. 1) was sampled on 9 occasions at 2 to 7 wk intervals from February to December 1992. During each sampling, 2 sediment cores were collected in plexiglass tubes (10 cm^2, 3 cm sediment, 7 cm water column) mounted on a rod. The sediment cores were kept at the *in situ* temperature and subjected to both light and dark incubation within 1 to 5 h. Denitrification was measured using the isotope pairing technique whereby the denitrification rate is calculated from the accumulation of single- and double-labelled N_2 following addition of $^{15}NO_3^-$ to the water column (Nielsen 1992). A magnetic stirrer was placed in each core (60 rpm) and $^{15}NO_3^-$ from a 10 mM stock solution (99% $^{15}NO_3^-$) was added to the water column of each core to a final concentration of 50 to 100 µM $^{15}NO_3^-$. This concentration has previously been found to be optimal for measurements in similar estuarine sediments (Pelegri et al. 1994). The cores were stoppered and incubated for 0.5 to 3 h depending on the season. At the end of the incubation the whole core was mixed with a rod and a sample of the slurry taken for mass spectrometric analysis of accumulated $^{15}N_2$ ($^{14}N^{15}N$ and $^{15}N^{15}N$), as described by Nielsen (1992). The *in situ* denitrification of unlabelled NO_3^- (D_{14}) was calculated from the accumulation of the 2 $^{15}N_2$ species (Nielsen 1992):

$$D_{14} = \frac{^{14}N\,^{15}N}{2(^{15}N\,^{15}N)} \times [(^{14}N\,^{15}N) + 2(^{15}N\,^{15}N)] \quad (1)$$

The exact isotopic composition of NO_3^- in the water column during incubation was not measured, and the sources of NO_3^- for denitrification (diffusion from the overlying water or nitrification within the sediment) could not, therefore, be distinguished, except during periods when the water column lacked NO_3^-.

Numerical modelling. ***Model description:*** Numerical, hydrodynamic modelling of Norsminde Fjord was undertaken using a 2-dimensional, hydrodynamic model — the MIKE 21 model system — comprised of a hydrodynamic (HD) submodel and an advection-dispersion (AD) submodel (Abbott et al. 1981, Warren & Bach 1992, Danish Hydraulic Institute 1993). The HD submodel simulates the water level and the current velocity at each time step and in each grid point. It solves the depth-integrated hydrodynamic equations, i.e. the continuity equation and conservation of momentum in 2 horizontal dimensions (Ekebjærg & Justesen 1991). The momentum equation includes the effects of the wind shear stress on the water surface, as well as the effects of the Coriolis force and bed friction, the latter being described by a term including the Chezy number.

The MIKE 21 AD model simulates simultaneously with the HD calculations the transport and concentration of dissolved or suspended matter by solving the 2-dimensional advection-dispersion equation:

$$\underbrace{\frac{\partial c}{\partial t} + u\frac{\partial c}{\partial x} + v\frac{\partial c}{\partial y}}_{\text{advective transport}} = \underbrace{D_x\frac{\partial^2 c}{\partial x^2} + D_y\frac{\partial^2 c}{\partial y^2}}_{\text{dispersive transport}} \underbrace{- Fc}_{\text{linear decay}} \quad (2)$$

where c is concentration; t is time, Δt being the time step in the simulation; x and y are 2 horizontal directions (the x direction may be selected as any horizontal direction, and it will often be one of the main flow directions in the model area under consideration; the y direction is perpendicular to the x direction); u is the current velocity in the x direction; v is the current velocity in the y direction; D_x is the dispersion coefficient in the x direction; D_y is the dispersion coefficient in the y direction; F is the coefficient of linear decay, $F \geq 0$ (the term Fc can be considered as a sink term).

In the AD submodel, the dispersion coefficient is a measure of all longitudinal water exchange not taking place by advection with the depth-integrated mean flow in the calculation grid. Variations in bed topography, turbulence generated by bed or wind friction and the presence of longitudinal density (salinity) gradients may all influence the magnitude of the dispersion coefficient, which may therefore be subject to considerable temporal and spatial variation.

Model set-up: The MIKE 21 model of Norsminde Fjord was set up in a horizontal grid with the grid size fixed at 100 m in each direction. In the model, freshwater from the catchment area is set to discharge into the estuary at 3 locations, the major part being the innermost lateral inflow which drains 92% of the catchment area (i.e. Rævs Å and a few minor streams). Precipitation was included in the model as an additional freshwater source. All freshwater sources were based on daily average values (Water Consult 1993). The boundary condition at the coastal limit of Norsminde Fjord was the water level variation at Norsminde Sluice. This was digitized as 15 min values such that tidal movement of the water was included in the model.

In shallow waters like Norsminde Fjord, the wind action on the water surface may be of especially great significance for estuarine water flow. Such effects were included in the simulations as wind time series with 3-hourly values of wind speed and direction.

Model calibration: The hydrodynamic model of the estuary was calibrated on the basis of all the discharge, water level and salinity data. The main calibration parameter in the HD submodel was the size of the Chezy number (the bed resistance factor), while that in the AD submodel was the dispersion coefficient.

During calibration of the AD submodel it was found that the simulated salinity levels were extremely dependent on the dispersion coefficients employed, particularly in the inner part of the estuary. The study period of 1 full year was divided into 18 shorter periods ranging in duration from 5 to 30 d and averaged 20 d (Table 2). In addition, the estuary was divided into 4 subareas (Fig. 1) in which dispersion coefficients were specified separately, period by period.

Estimation of freshwater residence time: Freshwater residence time was mainly estimated from a few simulations with the calibrated MIKE 21 AD model involving the initial 'discharge' of a pulse of tracer into Rævs Å. The propagation of this pulse through the estuary to Aarhus Bay was followed by calculating mass transport at each time step through pre-selected cross sections, as well as total accumulated transport of mass through each section. Using this method the residence time of freshwater was calculated for 2 selected periods. The residence time for other periods was estimated on the basis of the residence times determined for the 2 selected periods, together with a knowledge of the variation in freshwater discharge and the estimated dispersion coefficients.

Mass balance determination: Nitrogen concentrations were simulated with the calibrated MIKE 21 AD model for all 18 time periods. The dominant nitrogen source was freshwater discharge from the catchment area, input in the form of precipitation being negligible. The simulations were carried out by discharging the freshwater with a varying concentration of nitrogen specified for each source. At the coastal boundary, the concentration time series was fixed on the basis of measurements in Aarhus Bay at intervals of 1 to 4 wk (Aarhus County Council unpubl. obs.).

Table 2. Calculated coefficients of dispersion ($m^2\ s^{-1}$) in the 4 subareas of Norsminde Fjord during the period February 1992 to February 1993

Period	Date	Coefficient of dispersion Subarea: I	II	III	IV
1	11 Feb 1992–11 Mar 1992	60	60	60	60
2	11 Mar 1992–18 Mar 1992	60	60	60	60
3	18 Mar 1992–23 Mar 1992	30	30	30	30
3b	23 Mar 1992–01 Apr 1992	5	20	30	30
4	01 Apr 1992–16 Apr 1992	30	30	30	30
4b	16 Apr 1992–23 Apr 1992	2	5	10	20
5	23 Apr 1992–16 Mai 1992	20	20	20	20
5b	16 May 1992–26 Mai 1992	2	3	5	20
6	26 May 1992–15 Jun 1992	2	3	10	20
7	15 Jun 1992–06 Jul 1992	20	20	20	20
8	06 Jul 1992–05 Aug 1992	3	5	10	20
9	05 Aug 1992–24 Aug 1992	3	5	10	20
10	24 Aug 1992–23 Sep 1992	2	3	10	20
11	23 Sep 1992–15 Oct 1992	3	3	3	3
12	15 Oct 1992–12 Nov 1992	10	10	10	20
13	12 Nov 1992–09 Dec 1992	2	20	40	60
14	09 Dec 1992–07 Jan 1993	2	20	40	60
15	07 Jan 1993–08 Feb 1993	10	20	20	40

Denitrification or immobilization in the sediment was included in these simulations by adjusting the decay coefficient (F) relating to the concentration of dissolved nitrogen. For each simulation period the decay coefficient was initially set to zero. If necessary, i.e. if simulated nitrogen concentrations were too high, the decay coefficient was adjusted until the simulated values fitted the nitrogen concentrations measured in the estuary. Release of nitrogen from the sediment to the water could be modelled by adding a source term.

The simulation providing the best fit was then used to calculate the mass balances. The latter provide information on nitrogen transport in and out of the estuary for each period, as well as storage, denitrification/sediment retention and sediment release.

RESULTS

Denitrification

Denitrification rates ranged from 100 µmol N m^{-2} d^{-1} in July to 1600 µmol N m^{-2} d^{-1} in April (Fig. 2). Considerable temporal variation was seen, especially during the period February to May, the denitrification rate thus decreasing from 1500 to 400 µmol N m^{-2} d^{-1} from February to March, then increasing again to 1600 µmol N m^{-2} d^{-1} from March to April. From May to July there was a gradual decline and the denitrification rate was almost constant (100 to 200 µmol N m^{-2} d^{-1}) during the summer. In December, the denitrification rate increased to 600 µmol N m^{-2} d^{-1}.

There was a significant correlation between seasonal variation in denitrification rate and NO_3^- concentration ($r^2 = 0.96$) (Fig. 3). That the denitrification rate exceeded 200 µmol N m^{-2} d^{-1} when NO_3^- was present in the water, but was only 100 to 200 µmol N m^{-2} d^{-1} when NO_3^- was absent (Fig. 3), indicates that the major source of NO_3^- for denitrification was NO_3^- in the water column rather than NO_3^- produced in the sediment by nitrification. The low NO_3^- concentrations during the summer period were attributable to the lower NO_3^- input via freshwater, as well as to the uptake of NO_3^- by macro- and microalgae. Annual mean denitrification rate decreased gradually from the inner to the outer part of the estuary, the rate being 3-fold greater at Stn 50 (810 µmol N m^{-2} d^{-1}) than at Stn 55 (270 µmol N m^{-2} d^{-1}) (Fig. 4). This is mainly due to the parallel decline in NO_3^- concentration in the

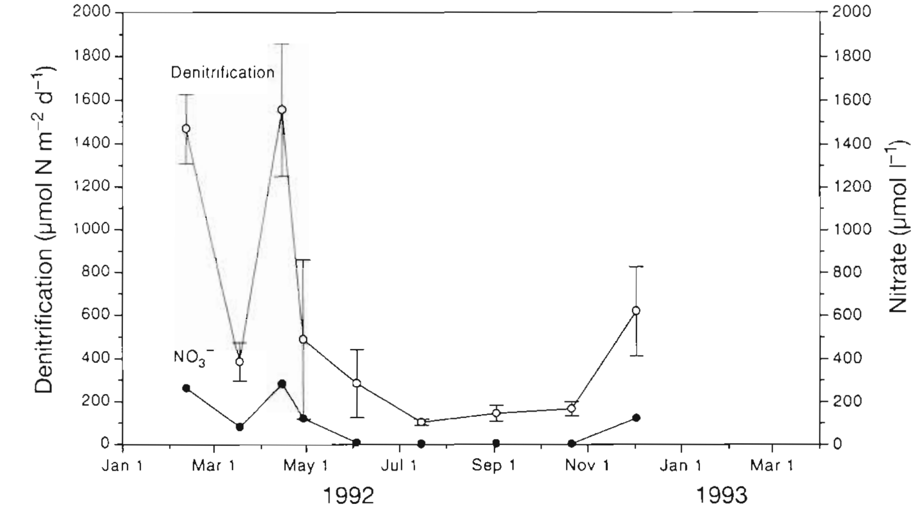

Fig. 2. Average denitrification rate and nitrate concentration in Norsminde Fjord during 1992 (average of 6 stations)

water due to gradual dilution of the inflowing NO_3-containing freshwater by saline water from Aarhus Bay.

Total annual N_{ret} in Norsminde Fjord due to denitrification was estimated to be 29 kg ha^{-1} yr^{-1}, or 5.4 t yr^{-1} for the entire estuary. $N_{ret\%}$ determined from denitrification therefore amounted to approximately 2% of total freshwater nitrogen input.

Dispersion coefficients and freshwater residence time

The estimated dispersion coefficients ranged from 2 to 60 m^2 s^{-1}, and were generally lowest in the inner part of the estuary (subareas I and II), reflecting the low rates of water mixing in these very shallow parts (Table 2). The great variation seen in the dispersion coefficients during 1992 is attributable to the weather conditions. The coefficients were generally lowest during the summer period.

The estimated freshwater residence time in Norsminde Fjord was less than 14 d throughout the year. During the winter period, when the freshwater input to the estuary was greatest, residence time was extremely low (1.5 to 5 d). During the summer period, freshwater residence time was typically 3 to 13 d. The fast water exchange reflects the relatively small volume of the estuary compared to the freshwater input (0.57 to 2.65×10^6 m^3 yr^{-1}) and the tidal exchange volume. In addition, wind-driven circulation in the shallow waters probably enhances the mixing processes.

Nitrogen mass balances

The estimated mass balance for the 18 separate periods revealed little or no nitrogen retention during most of the year, freshwater input being balanced by transport out of the estuary to Aarhus Bay. The exceptions were April to May (periods 4b, 5 and 5b), where N_{ret} ranged from 1800 to 6900 µmol N m^{-2} d^{-1}, and in August to October (periods 9, 10 and 11), where it ranged from 1400 to 3400 µmol N m^{-2} d^{-1} (Fig. 5).

The estimated monthly nitrogen input, output and retention are shown for Norsminde Fjord in Table 3. The annual nitrogen balance is illustrated in Fig. 6. Annual freshwater input of nitrogen to the estuary was estimated to be 307 t N yr^{-1} during the 1 yr study period (1 February 1992 to 31 January 1993). Annual atmospheric deposition of nitrogen was estimated to be

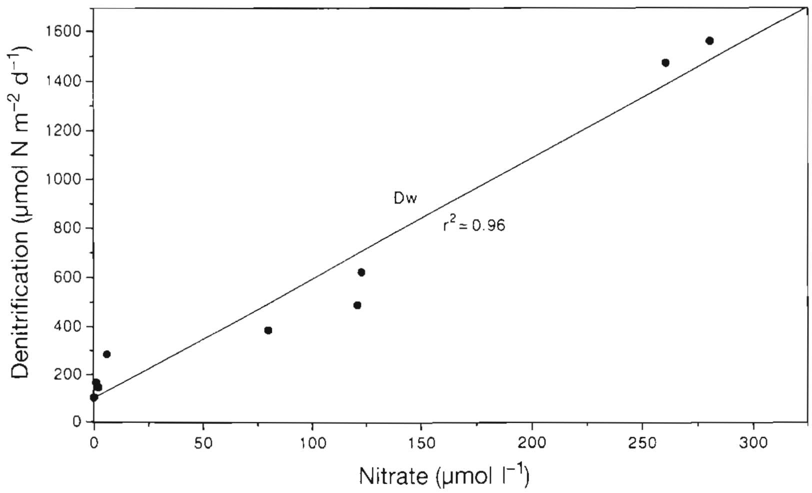

Fig. 3. Average denitrification rate in Norsminde Fjord as a function of the water column NO_3^- concentration (same data as Fig. 2)

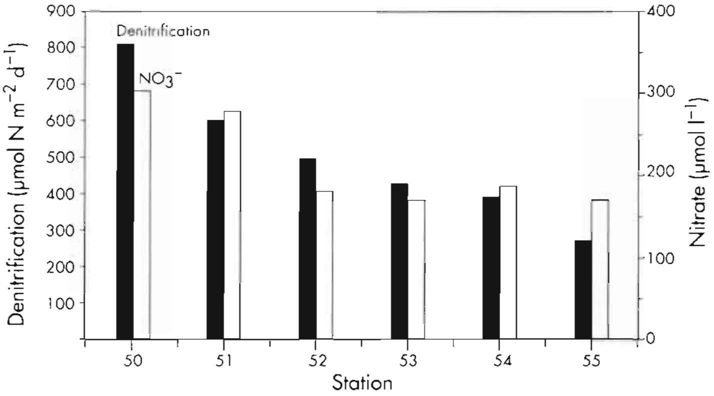

Fig. 4. Denitrification and nitrate concentration along a transect from the inlet to the outlet of Norsminde Fjord. Values are the yearly average

2 t N yr^{-1} based on the study of Hovmand et al. (1993). Net output of nitrogen to Aarhus Bay was 298 t yr^{-1}, annual N_{ret} in the estuary thus being approximately 8 t N yr^{-1}. $N_{ret\%}$ determined from the mass balance therefore amounted to approximately 3% of the total freshwater nitrogen input. The influx of nitrogen to the estuary from Aarhus Bay was estimated to be 37 t N yr^{-1}, or approximately 12% of net output from the estuary to the bay. The amount of nitrogen present in the estuary water column was approximately 3 t greater at the end of the 1 yr study period than at the beginning (Fig. 6).

DISCUSSION

The denitrification rate and mass balance methods are 2 very different and truly independent approaches to estimating estuarine retention of nitrogen. Thus the similar values for N_{ret} obtained with these 2 methods in the present study (6 and 8 t N yr^{-1}, respectively) support the validity and reliability of both approaches in ecosystems of this type.

Except for the month of April, when there was a peak in both measured denitrification rate and N_{ret} calculated from the mass balances, the seasonal variation in N_{ret} seen with the 2 methods did not compare well. While this is partly because the hydrodynamic model inadequately simulates short-term variation, temporal changes in the sediment nitrogen pool may also affect the estimates. Nitrogen accumulates in the sediment as a result of the deposition of particulate organic matter and the assimilation of nitrogen by benthic micro- and macrophytes. Loss from the sediment occurs through particle resuspension, mineralization and denitrification. As the seasonal pattern of these processes differs, they can only be expected to balance over a full annual cycle.

That considerable benthic nitrogen mineralization takes place in Norsminde Fjord is apparent from the high ammonia and urea effluxes measured over an annual cycle (1200 to 8000 μmol N m^{-2} d^{-1} and 100 to 1100 μmol N m^{-2} d^{-1}, respectively) (M. S. Therkildsen & B. Aa. Lomstein unpubl.). Benthic assimilation of NO_3^- in the estuary is reported to be 15000 μmol N m^{-2} d^{-1} (Risgaard-Petersen et al. 1994). Since the gross benthic fluxes of nitrogen therefore greatly exceed the net loss by denitrification (100 to 1600 μmol N m^{-2} d^{-1}), a general absence of synchrony between N_{ret} and denitrification is not surprising. The closely similar values for yearly N_{ret} obtained with the 2 methods suggests that burial of organic nitrogen in sediment is negligible in Norsminde Fjord, this being in contrast to the situation in some other estuaries (Yoon & Brenner 1992).

The linear correlation found between denitrification rate and water column NO_3^- concentration (Figs. 3 & 4) was surprising considering the expected variability of other known regulating factors, e.g. the penetration depth of oxygen in the sediment, effects of benthic microphytes and benthic fauna activity. In 2 other Danish estuaries, Kertinge Nor and Skive Fjord, it has been found that denitrification of NO_3^- from the water column is a function of both NO_3^- concentration and penetration depth of oxygen (Rysgaard et al. 1995, T. Dalsgaard et al. pers. comm.).

Benthic microphytes are usually abundant in shallow Norsminde Fjord, and numerous studies have shown

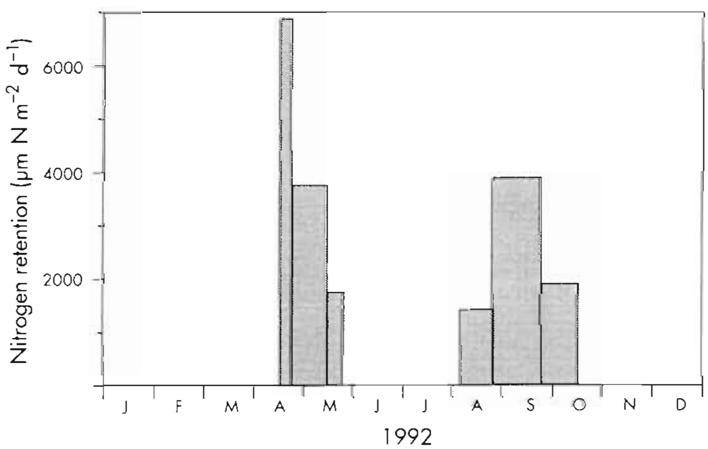

Fig. 5. Nitrogen retention in Norsminde Fjord calculated from the mass balance for each of the 18 periods in which the study period was divided (1 February 1992 to 31 January 1993). For all periods except those shown, retention was zero

Table 3. Monthly mass balance for nitrogen in Norsminde Fjord (1992 to 1993) calculated from the 2-dimensional hydrodynamic model, MIKE 21. The imbalance in individual months is attributable to storage in the estuary

Month	Freshwater input (t)	Atmospheric deposition (t)	Retention (t)	Net output to Aarhus Bay (t)
Feb	25.3	0.15	0	26.0
Mar	50.2	0.21	0	47.2
Apr	30.0	0.28	2.1	32.1
May	10.8	0.07	2.0	9.6
Jun	2.0	0.01	0	2.6
Jul	2.8	0.25	0	2.7
Aug	4.1	0.30	1.6	2.1
Sep	2.3	0.15	2.0	1.4
Oct	2.4	0.19	0.8	1.6
Nov	36.8	0.17	0	32.7
Dec	50.3	0.11	0	53.4
Jan	90.3	0.11	0	86.3
Total	307.3	2.00	8.5	297.7

that they can interfere with denitrification by assimilating inorganic nitrogen and altering both organic matter input and oxygen penetration depth (e.g. Christensen et al. 1990, Nielsen & Sloth 1994, Risgaard-Petersen et al. 1994). The most pronounced effect of benthic microphytes is the almost complete elimination of nitrification and denitrification when nitrogen availability from the water column is low (Nielsen & Sloth 1994, Nielsen et al. 1994). This may explain why the summer denitrification rates in Norsminde Fjord were low (100 to 200 µmol N m^{-2} d^{-1}) compared to those measured in the absence of microphytes at a depth of 15 m in Aarhus Bay (100 to 500 µmol N m^{-2} d^{-1}) (Nielsen et al. 1994).

Another factor of importance is the presence of bioturbating benthic invertebrates, which may stimulate denitrification by pumping water through the sediment. When the crustaceans *Corophium* spp. were added to sediment from Norsminde Fjord at a density of 20000 ind. m^{-2}, the denitrification rate increased 5-fold (Pelegri et al. 1994). At the time the present study was undertaken, *Corophium* spp. were abundant (up to 5000 ind. m^{-2}) in the inner part of Norsminde Fjord, although mean density in the estuary was only 650 ind. m^{-2} (Aarhus County Council 1994). Assuming a linear relationship between *Corophium* spp. density and stimulation of the denitrification rate, it can be calculated that the presence of this invertebrate explained approximately 13% of the total denitrification rate. Another bioturbating species common to the sediment of Norsminde Fjord and known to stimulate nitrification/denitrification is the polychaete *Nereis diversicolor* (Kristensen et al. 1991). Very high stimulation of denitrification [140% increase at a density of 1000 ind. m^{-2} (wet wt approximately 500 mg)] has recently been observed when worms of this species actively filter the water (M. P. Olsen & H. Blackburn pers. comm.). The mean *in situ* density of *N. diversicolor* in Norsminde Fjord was 460 ind. m^{-2}, but the individuals were very small (mean dry wt 3.5 mg) (Aarhus County Council 1994). Knowing that their dry weight is 17% of their wet weight, and that their pumping rate is proportional to their body weight (Riisgård 1991), it can be calculated that the presence of this species enhanced denitrification in the estuary by a maximum of 3%.

Denitrification rates are generally around 20 kg N ha^{-1} yr^{-1} in Danish coastal areas (Nielsen et al. 1994), but slightly higher in estuaries such as Norsminde Fjord (Rysgaard et al. 1995, Dalsgaard et al. pers. comm.). Thus although $N_{ret\%}$ was relatively low in Norsminde Fjord (2 to 3%), the annual denitrification rate was relatively high (29 kg N ha^{-1} yr^{-1}), this being due to the high water column nitrate concentration.

Denitrification in Norsminde Fjord has previously been estimated on the basis of measurements made at one of the permanent monitoring stations in the estuary (Jørgensen & Sørensen 1988, Binnerup et al. 1992). It was concluded that the denitrification rate was as high as 500 µmol N m^{-2} h^{-1}, i.e. that 25% of the freshwater input (about 50 t N yr^{-1}) was denitrified in the estuary. However, the station was located within 50 m

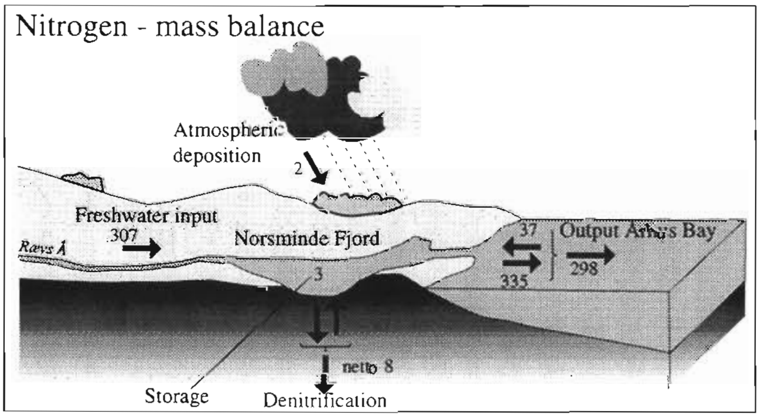

Fig. 6. Annual nitrogen balance for Norsminde Fjord over the period 1 February 1992 to 31 January 1993 showing main sources and sinks in t yr^{-1}. Nitrogen retention and net output to Aarhus Bay were calculated on the basis of the hydrodynamic mathematical model

of the Rævs Å outlet, where the nitrate concentration is high all year round (always exceeding 100 µmol N l^{-1}), and infaunal abundance and the precipitation of fresh organic material are high. The annual denitrification rate at that location (1.4 to 2.0 mol N m^{-2} yr^{-1}) therefore greatly exceeds the mean rate for the estuary as a whole. The previously reported $N_{ret\%}$ of 25 % for Norsminde Fjord is therefore an overestimate.

In marine ecosystems, the magnitude of $N_{ret\%}$ due to denitrification probably depends on the residence time. The relatively short residence time (1.5 to 13 d) in Norsminde Fjord implies that due to simple lack of physical contact with the sediment, only a minor part of the nitrogen in the water column will be denitrified. This effect is greatest during winter periods of high freshwater input since the water column nitrogen concentration is then high and the retention time low.

The low $N_{ret\%}$ found in the present study with the denitrification rate and mass balance methods (2 % and 3 %, respectively) lends no support to the concept that estuarine denitrification is generally 40 to 50 % (Seitzinger 1988, 1990). It is more likely the case that $N_{ret\%}$ is low in estuaries and coastal areas with a short residence time, and high (> 50 %) in large marine ecosystems with a long residence time, e.g. the Baltic Sea (Wulff et al. 1990).

Considerable amounts of nitrogen are transported to the Kattegat - Belt Sea area through estuaries with low residence times (Nielsen et al. 1993, Borum 1994). Thus if Seitzinger's '50 % rule' is applied when estimating nitrogen transport to open Danish seas (Ærtebjerg et al. 1991, North Sea Task Force 1993), nitrogen loading of this eutrophic area will probably be significantly underestimated.

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