Temporal trends in N & P concentrations and loads in relation to anthropogenic effects and discharge in Odense River 1964–2002

Morten Andreas Dahl Larsen, Henrik Soegaard and Klaus Hinsby

ABSTRACT

Using water quality data from 1964 to 2002, concentrations and loads of dissolved inorganic nitrogen (DIN) and phosphate-phosphorus (PO_4—P) in the Odense River were analyzed for trends in periods subsequent to and following the implementation of the Danish Action Plan I for reduction of nutrients in the environment (DAP) in 1987. In periods between 1964 and 1987 statistical significant increasing trends in DIN and PO_4—P concentrations and loads were detected. However, DIN generally showed stronger trends than PO_4—P and 19 of the 29 tested PO_4—P load tests showed non-significant results. In the subsequent periods between 1980 and 2002, significant nutrient reductions were detected at all sites and with the strongest trends for PO_4—P. Also, tests combining data from several sites showed equally strong trends. Significant positive correlations between DIN concentrations and discharge were found throughout the period using the non-parametric Spearman Rank Correlation, suggesting soil leaching to be the main nitrogen source. Conversely, correlations between PO_4—P and discharge changed from significantly negative to mostly significantly positive, a phenomenon most likely due to the change in phosphorus sources.

Key words | catchment, discharge, nitrogen, Odense River, trend test

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INTRODUCTION

Environmental effects caused by the increased use of nutrients in agriculture and industrial contamination during the second half of the 20th century have been reported in a number of countries (see e.g. Zakova *et al.* 1993; Böhlke & Denver 1995; Stow *et al.* 2001). During the last two decades many national governments have instituted regulations aimed at re-establishing the quality of water bodies and minimizing eutrophication. The increasing interest in protecting the environment from sources of pollution emphasized the need for a thorough knowledge of all elements in the water balance and their interrelation with the cycling of pollutants (Geological Survey of Denmark & Greenland 2003). Within Europe this interest led to the establishment of EU Water Framework Directive in December 2000, requiring all EU member states to monitor and control pollutant sources influencing the aquatic environment (EU 2000). The directive emphasizes the need for a thorough knowledge of the interaction between groundwater, surface water and the terrestrial environment to minimize point and non-point pollution, as well as improving drinking water quality. In Denmark, observations of eutrophication led to the implementation of the Danish Action Plan I and II for reduction of nutrients in the environment (DAP) in 1987 and 1998 respectively aiming at significant reductions in the total amount of N and P from agriculture and point sources emitted into streams (Kronvang *et al.* 1993, 2005; Mathiesen & Nielsen 2003; Fyns Amt 2004).

The various experimental studies concerning trends in river nutrients show different responses to input reductions.

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For example, Ulén (1998) reported that, despite efforts to substantially reduce nitrogen (N) and phosphorous (P) losses between 1987 and 1995 in Sweden, there has generally been no significant decrease of these pollutants in the water bodies studied since the implementation of pollution regulations. Löfgren *et al.* (1999) also found no consistent trend in nutrient loads from Sweden in the same period and Stålnacke *et al.* (1999) showed that there were no significant changes in the river nutrient loads discharged to the Baltic Sea for the period 1970–1993 despite drastic changes in land use and waste water treatment. Reductions in river nutrient concentrations have been found in most or some of the studied cases on Funen Island by Fyns Amt (2003*b*) and Kronvang *et al.* (2003), in Denmark in general (Larsen *et al.* 1999; Kronvang *et al.* 2005), in Colorado and New Mexico by Passell *et al.* (2005) and partly in Latvia by Stålnacke *et al.* (2003). In all of the studies showing decreases in P and N, the decrease in P concentrations was generally greater than for N concentrations.

In-stream nitrogen concentrations often show a positive correlation with discharge (Hinsby *et al.* 2006; Sigleo & Frick 2003; Vanderbilt *et al.* 2003), whereas phosphate concentrations tend to show both weak positive as well as negative correlations (Stålnacke *et al.* 2003). Because of this correlation between discharge and nutrients such as dissolved inorganic nitrogen (DIN) and phosphate-phosphorus (PO_4—P), the methods used to evaluate river nutrient concentration trends must be able to separate the influence of discharge trends from nutrient reductions caused by anthropogenic regulations or agricultural practices. This also makes it essential to use relatively high sampling frequencies to monitor both river nutrient concentrations as well as river discharge. Also, other factors than discharge have been shown to influence the stream nutrient loads, such as water temperature, suspended particulate matter and salinity (Hussain *et al.* 2004).

The Odense River catchment has been selected as a study catchment for several reasons: First and foremost, the authors were given access to a data set not previously used from the archives of the Odense Water Company from the period 1964–1989, which have not previously been thoroughly analysed. Together with a newer detailed data set collected by the County of Funen these data comprise important extensive monitoring data for periods both well before and after the first Danish Action plan on reduction of nutrients in 1987. This enables evaluation of the time trends in DIN and PO_4—P during the 1960–70s, prior to the implementation of regulations, as well as the possible decreasing trends after the implementation of the DAP I and II regulations. Secondly, the land use of the catchment represents a typical Danish landscape dominated by agriculture, but also including areas of a high population density. Thirdly, since the Odense River catchment is included in the EU Water Framework Directive as a Pilot River Basin (Fyns Amt 2003*a*), the need for investigations of long term nutrient trends and the relationship to hydrology is obvious. The reason for working with nutrient status in concentrations (mg/L) is the possibility to investigate the immediate relation between discharge and the DIN and PO_4—P concentrations, while still allowing calculations of nutrient loads, which are often important in governmental planning.

Trend tests were conducted to evaluate if statistically significant trends in DIN and PO_4—P concentrations and loads in Odense River could be detected in the examined period and to investigate the effect of the DAP I and II. To investigate if the change in nutrient sources has had an impact on the relationship between discharge and the DIN and PO_4—P concentrations, correlations were calculated throughout the period. Two equations were derived with DIN concentrations as a function of discharge using the measured values, to assess the accuracy of the considered variables as descriptors of DIN concentrations.

MATERIALS AND METHODS

Study area

Odense River catchment is located on Funen Island in Denmark (Figure 1). It consists of several tributaries and is dominated by agricultural land (68%) followed by city and roads (16%), forest (10%) and other natural areas (6%) (Fyns Amt 2003*a*; Kronvang *et al.* 2003). The catchment size for Ejby, the sampling site furthest downstream, is 535 km^2 and it is inhabited by approximately 200,000 people, most of whom live in the city of Odense (Fyns Amt 2003*a*, *b*). The altitude of the catchment is between 0 – 110 m above sea level (Fyns Amt 2003*a*). Odense River has undergone

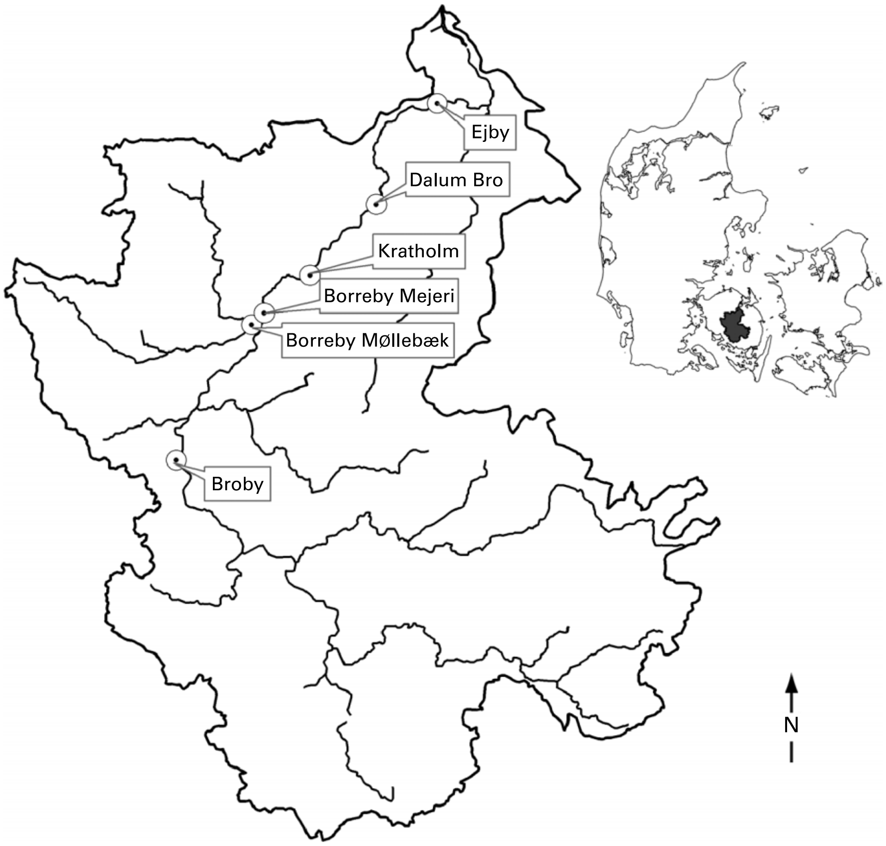

Figure 1 | The Odense River catchment showing the sampling sites and the placement of the Odense River catchment in Denmark.

substantial structural improvement, including straightening, draining, piping, damming etc. The soils consist mainly of loamy sand (49%) and sandy loam (40%). The annual mean 1964–2002 water balance consists of 821 mm of precipitation corrected to soil surface using the standards from The Danish Meteorological Institute (Allerup *et al.* 1998), 314 mm and 285 mm of runoff at Broby and Ejby respectively, and actual and potential evaporation of 493 mm and 580 mm respectively (1980–2002) (Fyns Amt, 2003*b*). The monthly mean water balance shows evaporation exceeding potential evaporation from end August to mid April and mean winter discharge values 5–6 times higher than summer values (Figure 2). In winter, discharge shows a fast response to precipitation both because precipitation exceeds evaporation, and because of extensive tile and ditch in the agricultural areas. Discharge in summer is mostly groundwater-fed and parts of the river network become ephemeral during the summer (Hinsby *et al.* 2006). In the 39-year period the mean discharges at Broby and Ejby were 3.0 m^3/s and 4.8 m^3/s respectively.

Data

The nutrient data evaluated in the present study were collected by both Odense Water Company and Fyns Amt (Funen County). These two sources of data divide the 39-year period under investigation into two periods: The years 1964–1980 include data from the sampling sites Borreby mejeri, Borreby Møllebæk (Holmehave stream), Dalum and Broby and the years 1980–2002 includes data from Broby, Ejby and Kratholm. Broby is the only sampling site used in both periods-1964–1980 and 1980–2002 - and therefore the only site applicable for calculations as a complete data series throughout the whole 1964–2002 period. The temporal variations in data availability between the different sites and the average number of yearly observations are shown in Table 1.

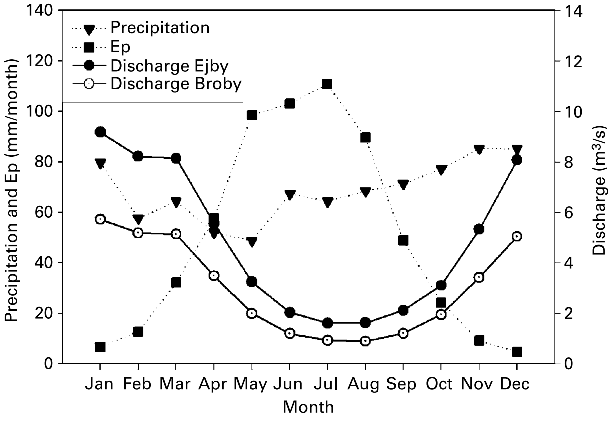

Figure 2 | The average water balance in the Odense River catchment. Precipitation data is surface corrected using (Allerup *et al.* 1998). Precipitation and discharge data is monthly means from 1964 to 2002 and potential evaporation is monthly means from 1980 to 2002.

Table 1 | The total and yearly number of observations at the different sites shown in periods related to data availability and data density – sampling sites change in 1980 and data density increases from 1987. N $*$ is the number of observations. The number of observations in total and pr. year is shown

Year	Site	Nutrient	N $*$	N $*$ /year
1964–80	Dalum	DIN	126	7
		PO_4—P	126	7
	Borreby mejeri	DIN	122	7
		PO_4—P	122	7
	Borreby mølle	DIN	127	7
		PO_4—P	127	7
	Broby	DIN	132	8
		PO_4—P	131	8
1980–86	Broby	DIN	56	8
		PO_4—P	70	10
	Ejby	DIN	70	10
		PO_4—P	70	10
	Kratholm	DIN	69	10
		PO_4—P	69	10
1987–2002	Broby	DIN	467	29
		PO_4—P	469	29
	Ejby	DIN	2898	181
		PO_4—P	3851	241
	Kratholm	DIN	4341	271
		PO_4—P	5161	323
1964–2002	Broby	DIN	655	17
		PO_4—P	670	17

A significant increase in the number of observations occurs from 1987 most significantly at Ejby and Kratholm.

The data include analysis for the total dissolved inorganic nitrogen (DIN) including the nitrogen contents of nitrate (NO_3—N), nitrite (NO_2—N), and ammonium (NH_4—N) as well as the phosphorous content of phosphate (PO_4—P) – all in mg/L. These can be seen as the main part of the dissolved inorganic N and P concentration (Allan 1995). The chemical analysis was carried out using the methods described in the Danish Standards numbered DS 223, DS 224 and DS 291 (Danish Standard 2004).

Data of daily discharge throughout the period were available from the two sites Broby and Ejby, the sites furthest up- and downstream respectively. Using the geographically nearest discharge dataset for the trend tests Borreby Møllebæk and Borreby mejeri have been carried out with data from Broby, and Kratholm and Dalum with data from Ejby. The river discharge data has been collected by Funen County using a Q/H relationship and water level measurements at a place of known cross sectional area. Temperature data from Odense Airport sampled in 3 hour intervals, 2 metres above ground level, were used as provided by the Danish Meteorological Institute.

Calculation procedure

Due to statistical non-normal distributions of both discharge and nutrient concentrations the non-parametric Mann Kendall test was used in the analysis of the nutrient state in Odense River involving both concentrations as well as total loads. For the trends in nutrient concentrations, two different forms of the Mann Kendall test were used. The first of these was based on yearly nutrient means and is referred to as the YMK-C test. The yearly means were derived summing the monthly means within each given year and using the previous observed sampling in months without data. This test was executed by methods described in Hirsch *et al.* (1982); Hirsch & Slack (1984); Kronvang *et al.* (2003) and US Geological Survey (2003). The second test used to assess the nutrient concentrations was the Seasonal and Partial Mann Kendall test, referred to as the SPMK-C test. As opposed to the YMK-C test, this test uses monthly means and was carried out removing the influence of both discharge and seasonality as a covariate. Further, the test is able to account for months of missing values (Hirsch *et al.* 1982; Hirsch & Slack 1984; Libiseller & Grimvall 2002; Stålnacke *et al.* 2003). In the evaluation of the monthly loads only the Seasonal and Partial Mann Kendall test was used, referred to as the SPMK-L test, and as for the SPMK-C test this test was carried out removing the covariates of discharge and seasonality.

For all seasonal Mann Kendall tests, the monthly values were obtained using linear interpolation, as described in Kronvang & Bruhn (1996). The trend tests were calculated with and without missing data. Tests omitting months of no observations were given the suffix M for missing – SPMK-CM and SPMK-LM.

The tests were carried out in both the 1964–1980 and 1980–2002 period for each sampling site and for both DIN and PO_4—P. The ability of the Seasonal and Partial Mann Kendall test to include several sites was utilized in a combined test of the four 1964–1980 period sites as well as the three 1980–2002 sites. Further, for the complete Broby 39-year DIN and PO_4—P data series calculations were done in periods previous to and following the year of the DAP I – 1980–1987 and 1988–2002. The yearly mean concentrations for Broby can be seen in (Figure 3).

Both types of the Mann Kendall tests (yearly and monthly) were carried out with a one-sided significance level of 5% which is typically used for trend detection in stream water quality (Fyns Amt 2003*b*; Kronvang *et al.* 2003; Stålnacke *et al.* 2003), the results are shown as the Mann Kendall statistics and the tests were calculated using descriptions and calculation procedures by Libiseller (2003).

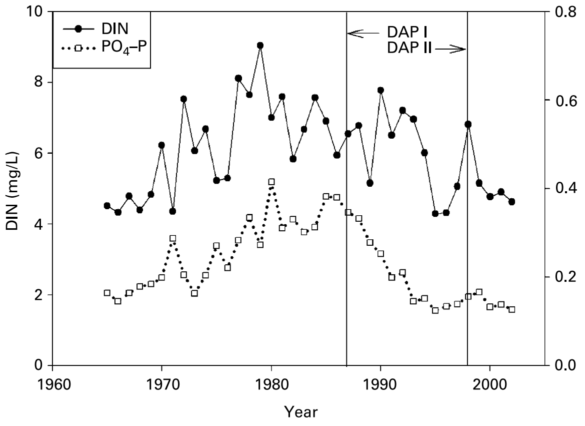

Figure 3 | The yearly mean DIN and PO_4—P concentrations at Broby.

Two equations using discharge as the descriptor of DIN concentrations were derived. Both include the physical relationship between DIN concentrations and discharge given by functions of power regression showing the highest possible r^2 correlation values. One is calculated with DIN-discharge correlations in the period before and after the DAP I and the other is calculated with separate expressions for each month in a year, as to investigate whether the equation could be improved by including seasonal differences. The equations are referred to as the simple and seasonal equation respectively. The general trend in the period before and after the DAP I was included in the equations being derived as the linear best fit in the two periods - as a variable expressed as the number of day in the 39-year period. The simple equation describing the DIN concentration (mg/L) at Broby in rounded numbers were calculated as: (1) $DIN = 3.34q^{0.578} + 0.00027D_1 - 1.20$ and (2) $DIN = 3.55q^{0.493} + 0.00044(D_2 - 8764) + 1.21$. Where q is the discharge in m^3/s and D_1 and D_2 is the number of day in the two periods respectively. (1) is to be used when $D_1 = (1;8764)$, and (2) when $D_2 = (8765;14245)$. The seasonal equation includes twelve expressions of power regression, $DIN = K_1q^{K_2}$, where K_1 and K_2 are the constants calculated with DIN-discharge correlation. The suffix including the number of day is the same as for the simple equation.

The data pairs used to derive the equations correspond with the density of data for Broby shown in Table 1. A plot of the DIN concentrations calculated using the equations and the measured DIN concentrations shows a fit of $r^2 = 0.65$ and $r^2 = 0.79$ for the simple and seasonal equation respectively (Figure 4). Calculated total loads based on these equations are referred to as simple modelled loads and seasonal modelled loads. When calculating measured loads, linear interpolation was used also for calculations of monthly means in months of no observations (Kronvang & Bruhn 1996).

Due to the non-normal data distributions the non-parametric Spearman Rank Correlation was used in calculating correlations between nutrient concentrations and discharge (Siegel 1956). The tests were performed using a two-sided 5% significance level. For each site all observations were used together with the discharge level

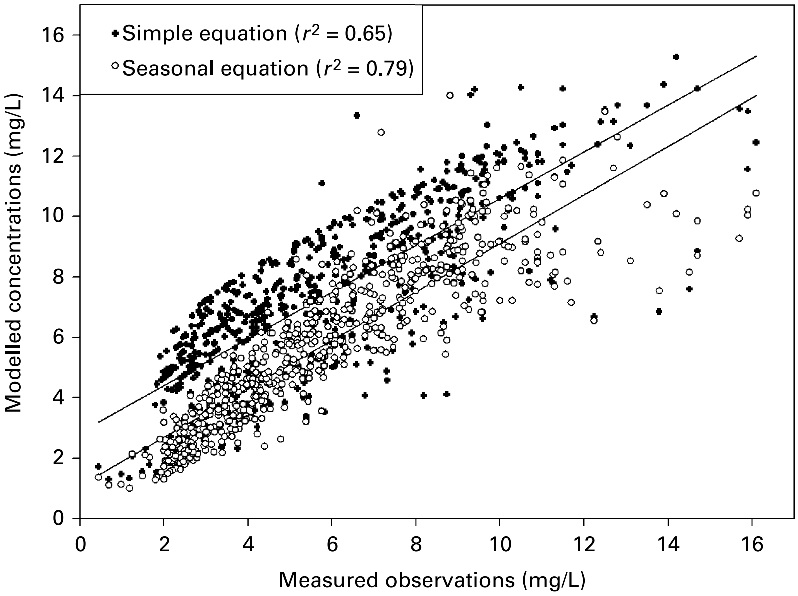

Figure 4 | The correlations between the measured DIN concentrations relative to the simple equation concentrations and the seasonal equation concentrations.

on the given day. The Spearman Rank Correlation indicates the direction of correlation by an R_s value between −1 and 1 and can thereby statistically assess the interaction between discharge and the DIN and PO_4—P concentration levels. Correlations were calculated in the periods 1964–1980, 1980–2002 before and after the DAP I and throughout the period in 4–5 year intervals for Broby and in 1–2 year intervals for Kratholm (1980–2002) having the highest density of data and in the periods previous to and following the DAP I.

The measured DIN and PO_4 concentrations from the individual days cannot be considered as independent samples as they will also depend on the concentrations on preceding days. Consequently the question of accuracy regarding the number of observations used to produce annual averages has been addressed by a Monte Carlo approach (Goulden *et al.* 1996) rather than Gaussian distribution statistics. For a selected year (1996) with a high measurement frequency (358 observations) 100 DIN concentration samples were drawn with sample sizes ranging from 1 to 120 using both an evenly distributed and a random sampling strategy. The sample average was compared to the average of 1996 calculating the standard error in percentages. This is illustrated in Figure 5 also showing the annual course in DIN concentrations both for the selected year of 1996 and for each day of the year

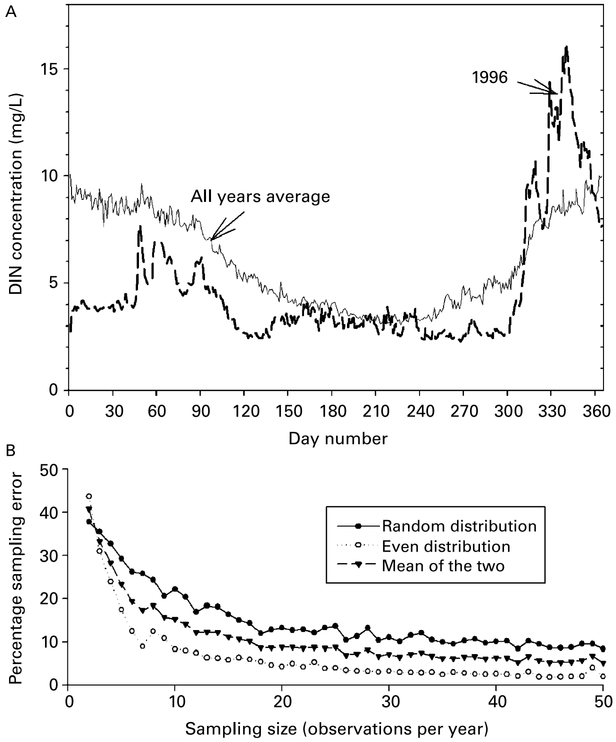

Figure 5 | (A) The annual DIN concentration course in the year 1996 as well as the daily average of all available observations at Kratholm. And (B), the effect of sampling density and distribution on the percentage sampling error throughout a year using Monte Carlo statistics drawing 100 samples both evenly and randomly in sample sizes between 1 and 120 (only sizes up to 50 shown) from the high frequency measurement year 1996.

calculated as a daily average of all available observations in the 23 year period of sampling at Kratholm.

RESULTS

Nutrient trends and loads

Table 2 displays the results from the YMK-C, SPMK-CM, SPMK-C, SPMK-LM and SPMK-L tests. In the periods of increasing nutrient levels, 4 out of 38 concentration trend tests and 17 out of 24 load trend tests show statistically significant trends for the individual as well as the combinations of sites. Of these, the yearly YMK-C test generally shows stronger trends than the monthly concentration and load tests. From 1964–1980, the DIN trends are stronger than the PO_4—P trends, whereas the opposite can be seen in the increasing periods for nutrient levels from 1964–1987. In the periods of decreasing nutrient levels, all individual and combined site tests show significant trends and PO_4—P generally show a stronger decrease than DIN.

In the periods of increase, the trend strength shows some variations but is approximately evenly distributed between tests with (SPMK-C and SPMK-L) and without (SPMK-CM and SPMK-LM) months of missing observations. These differences are almost diminished in the decrease-periods since data was available in nearly all months.

The DIN and PO_4—P measured loads and the DIN equation calculated loads at Broby are shown in Figure 6. All yearly loads show great variations throughout the investigated period with DIN values between approximately 200 and 1500 t/year and PO_4—P values between 6 and 45 t/year. The DIN modelled loads generally show larger loads than the DIN measured loads most pronounced in peak situations.

Discharge correlation

All Spearman Rank Correlations between DIN concentrations and discharge showed significant positive results in all of the tested periods with R_s correlation values between 0.63 and 0.97 (Table 3). For the Spearman Rank Correlations between PO_4—P and discharge the R_s results varied throughout the period. The 1964–1980 and 1964–1987 periods showed significant R_s correlation values for the different sites between -0.41 and -0.74 while the 1980–2002 and 1988–2002 periods showed 2 of 4 tests being significant. When calculating R_s correlations in 5 year intervals for Broby and in 1–2 year intervals at Kratholm, this pattern showed a somewhat systematic distribution throughout the investigated period: Broby showed significant R_s values up to -0.77 decreasing to -0.24 finally being positive with a R_s correlation of 0.31 and Kratholm showed the same tendency of correlation with values shifting from -0.70 to 0.47 in approximately the same years having a higher measurement frequency.

Sampling density

The results of the Monte Carlo analysis are shown in Figure 5B. As expected, the sampling error decreases with the number of yearly samples. 18 and 9 yearly samples are needed for 5% and 10% errors respectively using an evenly distributed sampling whereas the random sampling strategy requires 35 yearly samples to obtain a 10% error showing only slightly improved results by using additional samples.

DISCUSSION

The use of N and P in fertilizers and manure (data from Funen and Denmark respectively) and the waste water plant and industry emissions (point sources) into the sea from Funen Island in the period 1955–2002 are shown in (Figure 7). Temporal variations in nutrient inputs are clearly reflected in the trend tests showing significant trends in most cases (Table 2). The strength of each of the trend tests to a large extent follows the patterns of N and P use in fertilizers and manure, as well as the N and P from point sources: The significant increasing DIN concentration and load trends are consistent with the distinct increase in the use of N in fertilizers and manure. By contrast, P manure and fertilizer data show a less marked increase. As for the period of decrease, P apparently shows a stronger reduction than N especially for the relative decrease in point source emissions. As in other Danish investigations (Kronvang *et al.* 2003, 2005) this is equally detectable in the five trend tests used in this paper with stronger trends for PO_4—P than DIN.

Table 2 | The results of the YMK-C, SPMK-CM SPMK-C, SPMK-LM and SPMK-L trend tests at a 5% significance level (one-sided test) at the different sampling sites and as combined tests for all sites in each of the periods 1964–80 and 1980–2002 – shown as the Mann Kendall statistics. Statistical significant results are shown in bold and underlined typography

Period of increase			**Conc.**	**Conc.**	**Conc.**	**Load**	**Load**
Period	**Site**	**Resp. var.**	**YMK-C**	**SPMK-CM**	**SPMK-C**	**SPMK-LM**	**SPMK-L**
1964–80:	Dalum	DIN	**4.16**	**3.23**	**2.99**	**2.40**	**2.85**
		PO_4—P	**2.60**	1.23	**1.87**	0.75	1.19
	Borreby mejeri	DIN	**4.00**	**3.92**	**3.09**	**2.83**	**2.71**
		PO_4—P	**3.17**	**2.35**	**2.26**	1.50	1.41
	Borreby møllebæk	DIN	**4.57**	**3.93**	**3.96**	**2.74**	**3.23**
		PO_4—P	**2.84**	0.42	1.64	0.57	1.42
	Broby	DIN	**3.09**	**3.71**	**3.17**	**3.10**	**2.89**
		DIN-equation	0.12		**3.55**		
		PO_4—P	**3.09**	**2.60**	**2.78**	**2.61**	**2.12**
		q-Broby	1.44	−1.63	−1.63	−1.63	−1.63
	Ejby	q-Ejby	−0.95	−**2.21**	−**2.21**	−**2.21**	−**2.21**
1964–87:	Broby	DIN	**2.64**	**3.70**	**3.37**	**2.97**	**3.38**
		DIN-equation	**2.80**		**4.76**		
		PO_4—P	**4.81**	**4.16**	**4.48**	**3.54**	**3.92**
		q	−0.11	0.16	0.16	0.16	0.16
Period of decrease							
1980–2002:	Ejby	DIN	−**2.43**	−**3.21**	−**3.21**	−**2.67**	−**2.67**
		PO_4—P	−**3.27**	−**3.95**	−**3.92**	−**3.78**	−**3.78**
		q-Ejby	−0.21	−0.59	−0.59	−0.59	−0.59
	Kratholm	DIN	−**2.38**	−**3.17**	−**3.17**	−**2.34**	−**2.34**
		PO_4—P	−**4.38**	−**4.44**	−**4.44**	−**4.16**	−**4.16**
	Broby	DIN	−**2.64**	−**3.52**	−**3.52**	−**2.97**	−**3.02**
		DIN-equation	−**3.54**		−**4.23**		
		PO_4—P	−**4.65**	−**4.28**	−**4.27**	−**4.36**	−**4.27**
		q-Broby	−0.69	−0.83	−0.83	−0.83	−0.83
1988–2002:	Broby	DIN	−**2.18**	−**3.12**	−**3.12**	−**3.18**	−**3.18**
		DIN-equation	−**2.47**		−**3.56**		
		PO_4—P	−**3.17**	−**3.17**	−**3.17**	−**2.99**	−**2.99**
		q	0.30	0.75	0.75	0.75	0.75
Combined tests							
1964–80:	Dalum-Borreby mejeri-Borreby møllebæk-Broby	DIN		**3.86**	**3.41**	**2.86**	**3.06**
		PO_4—P		**1.93**	**2.54**	1.61	**1.75**
		q-Broby/Ejby		−**1.78**	−**1.78**	−**1.78**	−**1.78**
1980–2002:	Broby-Ejby-Kratholm	DIN		−**3.40**	−**3.49**	−**2.81**	−**2.83**
		PO_4—P		−**4.47**	−**4.46**	−**4.27**	−**4.30**
		q-Broby/Ejby		−0.68	−0.68	−0.68	−0.68

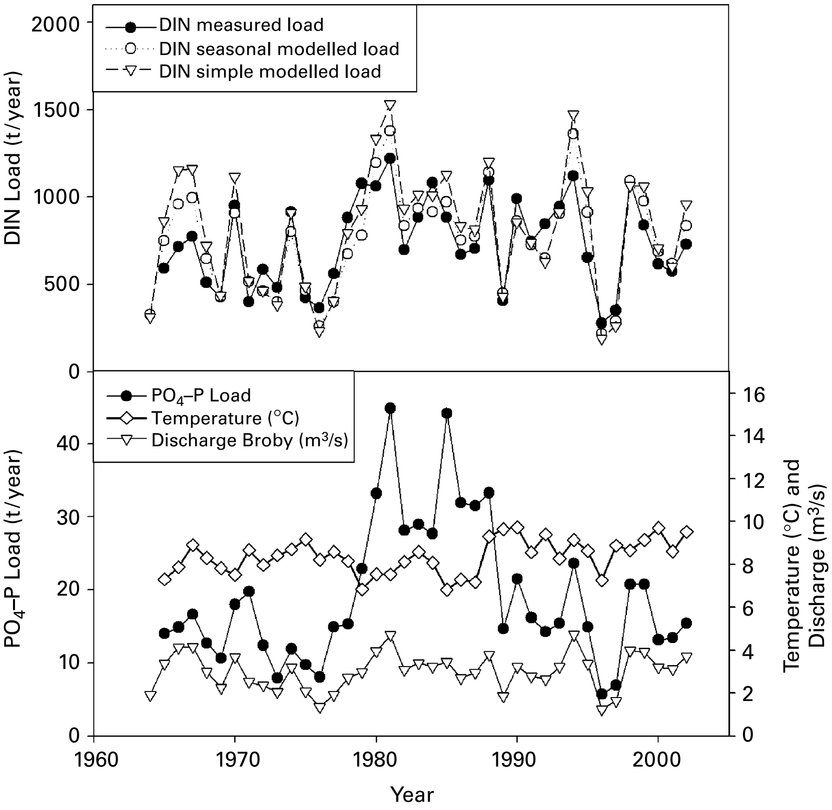

Figure 6 | The yearly DIN simple modelled loads, seasonal modelled loads, and measured loads as well as the yearly PO_4—P loads and discharge at Broby. Also shown are the yearly mean temperatures from Odense Airport.

Many factors affect the temporal variations between emission decreases and the actual measurable decrease in rivers, and this makes nutrient level investigations complex. The decreasing nutrient levels following the DAP I especially for PO_4—P in this study (Figure 3) is more apparent than in studies on emission decreases such as (Ulén 1998) and (Stålnacke *et al.* 2003). Also, significant decreasing trends are observed after the DAP I despite a nutrient surplus on Funen Island (Fyns Amt 2003*a*). This was caused by an excessive fertilizer use in the period under investigation which exceeded crop demands and this surplus is likely to have accumulated soil nutrient pools (Jensen & Reenberg 1986; Mathiesen & Nielsen 2003). Other factors mentioned in river nutrient studies as affecting the in-river nutrient trends include mineralization of organic matter (Jansson & Andersson 1988; Reynolds *et al.* 1992; Löfgren *et al.* 1999; Stålnacke *et al.* 2003), lake retention due to large residence times (Marion & Brient 1998; Stålnacke *et al.* 1999, 2003) and the in-river denitrification (Reynolds *et al.* 1992; Brady & Weil 2002; Stålnacke *et al.* 2003; Vanderbilt *et al.* 2003). The expected rate of nitrate denitrification is thought not to have a substantial effect on the long term in-stream nutrient levels (Christensen & Sørensen 1988) and the drainage area influencing the nitrogen state has been relatively constant in the investigated period (Olesen 2004). However, recent studies suggest that denitrification has been increasing as a result of increasing temperatures (Søndergaard *et al.* 2006). Yearly mean temperatures can be seen in Figure 6 showing a yearly mean increase of 0.029 °C from 1964 to 2002.

For dissolved P, sediment size is an important factor since sorption onto finer stream bottom sediments can affect the measurable concentration (Allan 1995; Ulén 1998; Löfgren *et al.* 1999; Stålnacke *et al.* 2003). Total P can however increase due to high flows bringing bottom material into resuspension and causing stream bank erosion (Svendsen *et al.* 1995). Equally important in the P cycle are the exchange between readily plant available compounds and compounds not accessible to plants (Brady & Weil 2002).

Table 3 | The results of the Spearman Rank Correlation test calculated at a 5% significance level (two-sided test). The periods 1964–80 and 1980–2002 and the periods before and after the DAP I are shown as well as 2-year divisions at Kratholm from 1980–2002 having the most observations and 5 year divisions at Broby having data from the entire 39-year period. Statistical significant results are shown in bold and underlined typography. N $*$ is the number of observation pairs and R_s is the Spearman Rank Correlation

		DIN		PO_4—P	
Period	**Site**	**N $*$**	**R_s**	**N $*$**	**R_s**
1964–80	Dalum	124	**0.78**	125	−**0.68**
	Borreby mejeri	122	**0.79**	122	−**0.64**
	Borreby møllebæk	124	**0.63**	124	−**0.74**
	Broby	127	**0.80**	126	−**0.70**
1980–02	Broby	529	**0.89**	545	0.04
	Ejby	2854	**0.84**	3806	−0.01
	Kratholm	3798	**0.88**	4647	**0.06**
1964–87	Broby	234	**0.86**	249	−**0.41**
1988–02		405	**0.87**	421	**0.10**
1980–81	Kratholm	25	**0.89**	25	−**0.70**
1982–83		21	**0.93**	21	−0.20
1984–85		24	**0.97**	24	−**0.57**
1986–87		220	**0.89**	188	−**0.24**
1988–89		494	**0.96**	498	−**0.42**
1990–91		617	**0.95**	624	−**0.25**
1992–93		710	**0.94**	712	**0.30**
1994–95		697	**0.91**	697	**0.12**
1996–97		726	**0.80**	726	−**0.13**
1998–99		357	**0.89**	712	**0.24**
2000–01		345	**0.91**	652	**0.17**
2002		184	**0.91**	361	**0.47**
1964–68	Broby	55	**0.93**	55	−**0.74**
1969–73		41	**0.93**	41	−**0.71**
1974–78		19	**0.86**	20	−**0.77**
1979–83		35	**0.90**	49	−**0.55**
1984–88		135	**0.94**	135	−**0.55**
1989–93		111	**0.94**	122	−**0.24**
1994–98		139	**0.89**	140	**0.31**
1999–02		104	**0.91**	108	0.17

This process tends to act as a balance, most often with P soil pool accumulation in areas of agriculture with fertilization. Following reductions in P emissions, a small part of these compounds can become more readily available to plants and leaching due to inadequate amounts for plants and other biological processes in the available P soil pool. As for N, the presence of lakes also adds to P retention as shown in Marion & Brient (1998) and Stålnacke *et al.* (2003).

The temporal patterns in nutrient loads between the different sites from 1980 to 2002 generally show the same variations although the total loads mostly vary relative to the catchment size (Figure 8). For DIN loads, Ejby and Kratholm are approximately similar whereas for PO_4—P significant variations occur from 1990 to 1995. The large PO_4—P values at Ejby could be due to proximity to a nearby upstream waste water plant with emissions in periods of extreme precipitation (Fyns Amt 2005).

The sampling strategy in the present study shows some evenness throughout the year although neither a totally even nor random distribution. For that reason the middle curve in Figure 5 showing the mean between errors from even and random sampling most likely shows the best resemblance with the sampling strategy. This curve of partly distributed sampling (the mean between random and even sampling) shown in Figure 5B reveals errors of approximately 17–18% for the 1964–1980 period with 7–8 observations (Table 1) and a constant error of approximately 7% from 1987. Almost no additional precision is obtained using more than 25 observations annually which is in line with the findings of Kronvang & Bruhn (1996). The proportionally high sampling errors occurring with 1–5 yearly observations are caused by the lack of capability of a few measurements to describe the seasonal variations in DIN concentrations. In other studies considering total river nutrient loads such as Rekolainen *et al.* (1995), Stålnacke *et al.* (2003), Hussain *et al.* (2004) and Passell *et al.* (2005) a yearly average of 6 to 34 yearly observations are used. Kronvang & Bruhn (1990, 1996) state a need for an increasing number of yearly observations with distributions of large runoffs in small parts of the year - between 12 and 26 annual observations - and Hussain *et al.* (2004) suggest that fewer observations are needed for larger rivers. The strength of the trend test results are considerable even when corrected for uncertainties regarding the number of observations since test results are

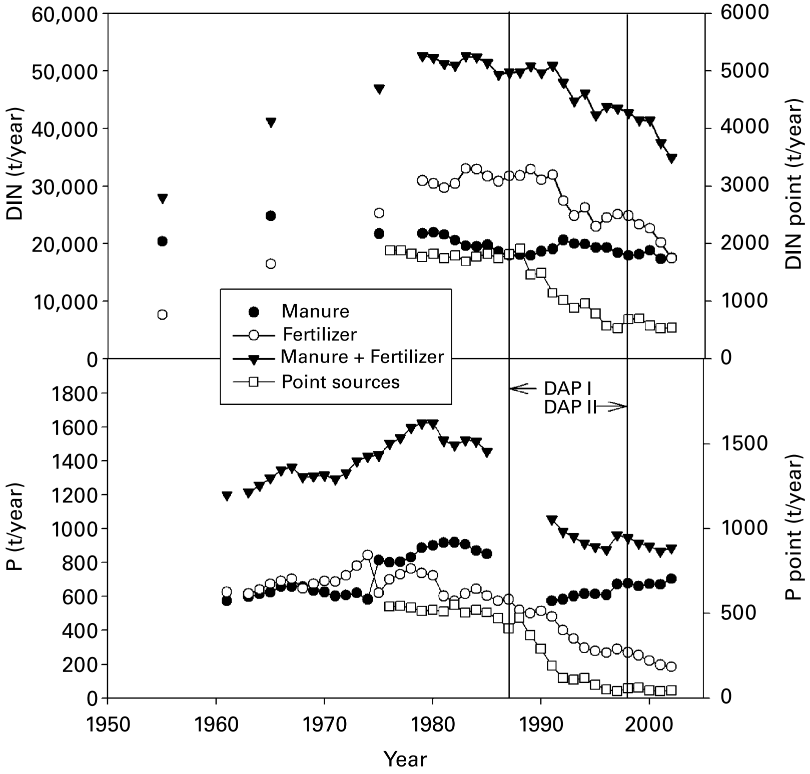

Figure 7 | The use of N and P in manure, fertilizers and in point source losses. The N fertilizer and manure use and the N and P point source loss are estimated totals from Funen. P fertilizer and manure use are the totals from Denmark downscaled relatively to the size of the Broby catchment since no data from Funen were obtainable. The latter changes estimation criteria in 1974/75 and is plotted with a 6 month delay because of different periods of yearly summation. Source: Danmarks Statistik (1960–2002).

not largely affected by missing data up to at least a level of 50% (Hirsch & Slack 1984) and only the years 1973–1979 have observations in less than 50% of the months (33.3%). The trend test values of the SPMK-CM and SPMK-LM tests somewhat corresponding to the results of the tests with no missing values, also reflect the ability of the Seasonal Mann Kendall test to account for missing values.

Figure 4 shows correlations between the measured concentrations relative to the simple equation concentrations and the seasonal equation concentrations of $r^2 = 0.65$ and $r^2 = 0.79$ respectively. The lowest correlation between data points for both equations can be seen for high concentration (and discharge) levels. The nature of the equations is closely related to the nitrogen cycle. In the summer months, a large amount of the readily available soil nitrogen is used by plant and biological uptake (Brady & Weil 2002) making the winter and spring months increasingly prone to diffuse leaching. The occurrence and amount of precipitation in relation to agricultural practices such as the time of tillage (causing aeration and thereby nitrate production), fertilization, plant root extent, combined with mineralization of plant residues in the fall are crucial in the correlation between discharge and stream DIN concentrations. Years or periods of drought as well as high precipitation levels will either empty or accumulate nitrogen in soils affecting the amount of nitrogen available for leaching (Vanderbilt *et al.* 2003; Bernal *et al.* 2005). The increased correlation for the seasonal equation relative to the simple equation confirms a varying pattern in the interaction between the water balance and DIN soil pools throughout a year. Lower overestimations relative to the measured loads can also be seen for the seasonal modelled loads relative to the simple modelled loads (Figure 6).

Hussain *et al.* (2004) mention temperature, suspended matter and salinity as possible covariates to stream DIN concentrations. A potential improvement of the equations is therefore possible with some of these variables included. Also, the measured concentration levels used for the equations are influenced by point source emissions not correlated with discharge reducing the accuracy of the

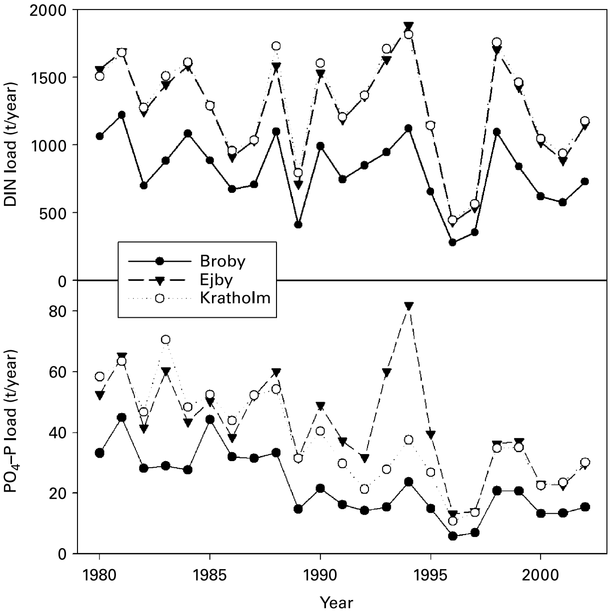

Figure 8 | The DIN and PO_4—P measured loads at the three sites Broby, Ejby and Kratholm from 1980 to 2002.

equation. Søndergaard *et al.* (2006) show that climate changes consisting of higher temperatures and stronger precipitation, will cause an increased diffuse leaching but also a longer growth season fixing more nitrogen in plants.

The uncertainties in empiric nutrient modelling due to extreme high or low flows have been addressed by Sigleo & Frick (2003) showing considerably improved discharge-concentration correlations for nitrate by removing values seen as representing periods of dry to wet transitions. This is addressed as the effects of nutrient build-up in soils in periods of drought. Among other studies investigating nutrient-discharge relations through equations or models Fyns Amt (2003*b*) obtained a correlation for total N of $r^2 = 0.80$ using the variables actual discharge, actual waste water discharge, rootzone NO_3—N, waste water N, discharge and waste water discharge in a previous year and total N load concentrations (in the previous year). And Stow *et al.* (2001) attained total P correlations between $r^2 = 0.63$ and 0.77 and N correlations between $r^2 = 0.46$ and 0.70 using discharge as the single variable.

The results from the correlation tests between discharge and both DIN and PO_4—P concentrations in the present study (Table 3) and the results of Stålnacke *et al.* (2003) and Vanderbilt *et al.* (2003) clearly emphasizes the need for removing the influence of discharge in trend tests. However, although significant decreases of both DIN and PO_4—P have been detected in this study, their correlations with discharge in the investigated period are dissimilar. No data on the P source distribution were available for 1964–1980 but the relative share of P emissions from waste water plants and industry (point sources) decreased from 70% (469 t/year) to 12% (25 t/year) in the period 1980/1988 (average) to 2002 (Fyns Amt 2003*b*) and the share from agriculture and housing with poor waste water treatment (diffuse sources) increased from 19% (126 t/year) to 49% (106 t/year). The results indicate that the shift from point sources to diffuse sources as the main P contributor in the investigated period is likely to have caused the fairly steady decrease in correlation between discharge and PO_4—P. This is supported by the non-existing or very low correlation between emitted point source P and precipitation amounts (Fyns Amt 2003*a*, *b*; Hinsby *et al.* 2006) and the expected positive correlation between P from diffuse sources and precipitation (and thereby discharge) accelerated by extensive drainage (Sharpley & Withers 1994) and also between nitrate from diffuse sources and discharge (Bernal *et al.* 2005) especially in non-growing seasons (Rekolainen *et al.* 1995). In the first part of the investigated period high discharge levels will therefore have a diluting effect decreasing PO_4—P concentrations, changing in the early 1980's to a higher diffuse share of P making correlations with discharge positive. The positive, significant and relatively uniform correlation between DIN concentrations and discharge throughout the investigated period match the distribution of N sources with diffuse sources as the main contributor – 63% (6100 t/year) in 1980/1988 (average) and 71% (5400 t/year) in 2002 (Fyns Amt 2003*b*). The tendency for DIN to be more correlated with discharge than PO_4—P after the DAP I due to the shift in nutrient sources can also be seen in Figure 3 where yearly fluctuations are stronger for DIN than PO_4—P. A similar result was found by Sigleo & Frick (2003) showing no diluting effect between stream nitrate concentrations and increased discharge.

CONCLUSION

By using the non-parametric Mann Kendall test it has been possible to prove statistical significant DIN and PO_4—P concentration and load trends in the periods of both increase and decrease, however with non-significant PO_4—P load trends from 1964 to 1980. PO_4—P Showed the strongest trends of decrease for the 1980/1987–2002 periods.

The Monte Carlo sampling error test showed substantial accuracy for the data densities used in the study with maximum errors for single observations of approximately 18% assuming the sampling distribution was comparable to the mean between errors from even and random sampling.

A general increase in DIN and PO_4—P loads was generally seen in the downstream direction although local factors such as nearby waste water plants possibly showed an increasing effect on PO_4—P loads. The DIN equation calculated loads were generally higher than the measured loads reflecting the tendency of both equations to overestimate concentrations at high discharges. Including the yearly variations the equation did however significantly increase its accuracy. The overestimation is likely to be due to percolating water not being able to transmit nutrients in the rate found by the discharge-concentration correlation based on a majority of observations under more typical discharge levels because of a limited N pool, being available for leaching.

The non-parametric Spearman Rank correlation values between discharge and both DIN and PO_4—P concentrations supported the distribution of nutrient sources: DIN showed significant positive correlations confirming agricultural field leaching to be the main source whereas the PO_4—P correlation change from negative to partly positive supported the fact that the main source changed from predominantly point sources to diffuse sources.

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