Science of the Total Environment 473–474 (2014) 262–274

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Responses of biological and chemical components in North East Atlantic coastal water to experimental nitrogen and phosphorus addition – A full scale ecosystem study and its relevance for management ☆

Yngvar Olsen [a,*], Helge Reinertsen [a], Ulrich Sommer [c], Olav Vadstein [b]

[a] Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway
[b] Department of Biotechnology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway
[c] GEOMAR Helmholtz Centre for Ocean Research Kiel, D-24148 Kiel, Germany

HIGHLIGHTS

- A full-scale 5 year experimental study of ecosystem responses to increased nutrients.
- Concentrations of DIN and DIP did not respond positively to increased nutrient input.
- Concentrations of PON and POP and phytoplankton biomass responded positively.
- PON is suggested as credible indicator for chemical and ecological state.
- A general scientific concept for managing nutrient input to coastal waters is presented.

ARTICLE INFO

Article history:
Received 14 July 2013
Received in revised form 20 October 2013
Accepted 6 December 2013
Available online xxxx

Keywords:
Experimental eutrophication
North East Atlantic
Biological and chemical responses
PON and POP
Indicators
Management concept

ABSTRACT

The objective of this study was to quantify chemical and biological responses to an experimentally increased nutrient input to an open coastal planktonic ecosystem and to contribute to a scientific concept and credible indicators for managing nutrient supply to coastal waters. Data were derived in a 5 year fertilisation experiment of a tidal driven coastal lagoon at the outer coast off Central Norway (*63°36' N, 9°33' E*), with a surface area of 275.000 m^2, volume of 5.5 mill m^3, mean depth of 22 m and a water exchange rate of 0.19 day^{-1}. The lagoon was fertilised in the summer season 1998 and 1999, while summer seasons 1996-97 and 2000 and inflowing water were used as unfertilised references. Most measured chemical and biological variables showed linear responses with an increasing loading rate of inorganic N and P (L_N and L_P, respectively). PON, POP and POC (< 200 μm) responded significantly ($P < 0.05$) as did chlorophyll *a* and phytoplankton C. DIN and DIP remained, however, constant and independent of L_N and L_P, respectively ($P > 0.05$) as did heterotrophic biomass ($P > 0.05$). We evaluate the response variables assuming a stepwise incorporation process of nutrients in the planktonic ecosystem and how that will interact with biological response times and water dilution rates. We suggest that PON is a credible indicator of both chemical and ecological states of the planktonic ecosystem and that natural background and upper critical concentrations are 46 and 88 mg PON m^{-3}, respectively. The study was supported by data from mesocosms. We discuss the scientific relevance of our suggestions, how results can be extrapolated to a broader geographical scale, and we propose a science-based concept for the management of nutrient emission to open coastal waters.

1. Introduction

Coastal eutrophication caused by human activities, or anthropogenic eutrophication, is primarily a problem in densely populated coastal regions. Reports describing causes and consequences of enhanced anthropogenic nutrient emission to the coastal zone are numerous (e.g., Schiewer, 1998; Capriulo et al., 2002; Colijn et al., 2002; Grizzetti et al., 2012). It has been well documented that enhanced nutrient inputs increase the primary production and phytoplankton biomass, but there is also evidence for an order-of-magnitude difference in biomass yield

* Corresponding author. Tel.: +47 97778249; fax: +47 73591597.
E-mail address: yngvar.olsen@ntnu.no (Y. Olsen).

0048-9697/$ – see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.12.028

per unit of nutrient input among systems (e.g., Nixon and Pilson, 1983; Borum, 1996; Cloern, 2001; Carstensen et al., 2011). This pronounced variability in the quantitative responses of increased nutrient input suggests that factors other than nutrient loading rate and nutrient concentration affect autotrophic biomass. The confusions and uncertainties associated with the lack of more uniform chemical and ecological responses (e.g., Capriulo et al., 2002) are among the reasons why there is still no general concept established for managing nutrient emission to coastal waters (Cloern, 2001).

The underlying reasons for system variability are probably complex, but the variable rate of water dilution driven by physical forcing and direct or indirect effects by zooplankton predation will obviously affect the structure and function of the planktonic food web and therefore also the autotrophic yields (Sommer and Stibor, 2002; Stibor et al., 2004a; Vadstein et al., 2004). Another factor is that phytoplankton biomass yields per unit of nutrients incorporated vary systematically by a factor >2 with the specific growth rate, or their nutritional state (Droop, 1983).

One practical way to reduce variability is to secure a standardised and representative data input for a geographic area instead of using more fragmented data for single days and sampling spots. European legislation requires that environmental management must be ecosystem-based, and the unit area of management – the ecosystem - must therefore have a certain geographical extension. It is also logical that the data for management must cover relevant time periods (Ferreira et al., 2011). For coastal eutrophication in moderately affected or pristine North-East (NE) Atlantic water, negative ecological effects of eutrophication in pelagic euphotic ecosystems will only become expressed in the summer season (Cloern, 2001), which is defined as the period from after the decline of the spring bloom until the onset of autumn turnover of water masses, i.e. June until the end of September in Norwegian temperate coastal waters. The use of input data that are temporarily and spatially representative will probably reduce variability in both data inputs and the outputs.

It is also important for management of coastal seas to establish science-based, credible and readily measurable indicators and procedures that can predict the ecological state of open coastal waters (e.g., Caruso et al., 2010; Andersen et al., 2011; Ferreira et al., 2011). We agree that there is no general concept established for a knowledge-based management of open coastal waters today (Cloern, 2001; Kitsiou and Karydis, 2011), in contrast to the simple principles used to freshwater eutrophication (Vollenweider, 1976). European environmental legislation has recently defined environmental objectives for a science-based management of surface waters in general (Tett, 2008; WFD-Water Framework Directive, 2000/60/EC; MSFD-Marine Strategy Framework Directive, 2008/56/EC), which would benefit from the establishment of a unified concept for assessing and managing coastal eutrophication. The WFD states that the concentrations of naturally occurring substances like nutrients should not be increased much above the natural background, whereas the structure and function of the ecosystem should be maintained in a Very Good or Good state category (Tett, 2008; Andersen et al., 2011). The principles of the WFD are attractive, but implementation is still a challenge because most present indicators (Caruso et al., 2010) are only determined empirically, showing a high variability among coastal regions (e.g., Nixon and Pilson, 1983; Borum, 1996; Cloern, 2001; Carstensen et al., 2011). The need to determine natural background values for the indicators is a particular challenge emphasised in the WFD (Tett, 2008), and comprehensive knowledge on background values are vital for their use as unified indicators.

The ultimate objective of the present study was to quantify chemical and biological responses of enhanced nutrient input to an open coastal planktonic ecosystem, and to contribute to the development of a scientific-based concept and credible indicators for managing anthropogenic nutrient supply to coastal waters. To address these questions, we carried out a full scale 5 year fertilisation experiment of a dynamic and tidal driven coastal lagoon situated at the outer coast off Central Norway in 1996-2000. The euphotic zone of the lagoon received a daily addition of inorganic nitrogen, phosphorus and silica (DIN and DIP, in Redfield proportions with half ration of Si) in the summer period in 1998 (low dose) and 1999 (high dose). The years 1996, 1997 and 2000 represented control years where no nutrients were added. In addition, regular sampling of the inflowing non-affected water also served as an internal control throughout the experiment. Sampling was performed weekly in the summer period, and involved many common physical, chemical and biological variables.

Our current interpretation of the results of the full scale fertilisation experiment is supported by a number of mesocosm experiments carried out in the course of and after the fertilisation experiment (e.g., Gismervik et al., 2002; Stibor et al., 2004a,b, 2006; Vadstein et al., 2004, 2012; Børsheim et al., 2005; Sommer et al., 2004, 2005; Olsen et al., 2006, 2007, 2011; Sundt-Hansen et al., 2006). The general knowledge gathered in these experiments is instrumental for the conclusions made in the present study.

2. Materials and methods

2.1. Description of the study area

The effects of experimental nutrient supply to coastal waters were studied in a tidal driven coastal lagoon named Hopavaagen (*63°36' N, 9°33' E*), situated in a sparsely populated area with a low human influence at the outer coast of Central Norway (Table 1). The coastal lagoon has a surface area of 275,000 m^2 and a total volume and a volume of euphotic waters (0–10 m) of 5.5 and 3.2×10^6 m^3, respectively. The depth of the main basin is 22-32 m, and the bottom is relatively flat with an average depth of 20 m. The mean water exchange rate of the mixed euphotic water layer is 0.19 day^{-1}, corresponding to a water renewal time of the mixed euphotic water layer of 5.2 ± 1.9 days. The inflowing water is drained from and considered representative of the Norwegian Coastal Current.

Due to the narrow inlet, the tidal range in Hopavaagen is smaller compared to that outside. The daily water exchange is variable and dependent primarily on the tidal cycle, but air pressure and wind speed and direction are also of importance. For prediction of the exchange rate of water, we used coastal astronomical tidal level changes for the area and meteorological data measured at Ørlandet airport close by.

Table 1
Characteristics of the tidal driven coastal lagoon Hopavaagen situated in Central Norway.

Characteristics of the coastal lagoon Hopavågen (SE)			Unit
Coordinates	N 63° 35.636' E 9° 32.809'		WGS 84
Surface area	275,000		m^2
Total volume	5.5		10^6 m^3
Mixed volume	3.2		10^6 m^3
Mean depth	20 (range 0-32)		m
Tidal range outside	1-2		m
Tidal range inside	0.3 – 1.0		m
Volume exchanges by tides	0.61 ± 0.22		10^6 m^3 day^{-1}
Replacement time of mixed volume	5.2 ± 1.9		day
Exchange rate of mixed volume	0.19 ± 0.07		day^{-1}
Exchange rate of total volume	0.11 ± 0.04		day^{-1}
Catchment area	1.9		km^2
Salinity and temperature in lagoon (0.5 - 10 m)	Temperature ± SD	Salinity ± SD	°C and ppt
1996	11.6 ± 1.6	32.6 ± 0.6	
1997	12.8 ± 2.3	30.2 ± 2.4	
1998	11.6 ± 1.8	31.3 ± 1.6	
1999	12.5 ± 1.5	31.4 ± 1.3	
2000	11.3 ± 1.0	31.1 ± 1.6	

2.2. Experimental conditions

The eutrophication experiment was run over a five-year period, where 1996, 1997 and 2000 provided data for an undisturbed, natural situation. The experimental eutrophication was initiated in late May 1998 and 1999, after termination of the spring bloom, and was terminated in early October in both years. The nutrients were supplied daily and automatically to the narrow inlet where water was exchanged with the coastal water outside. The addition started around 30 min after the start of water in-flow, when currents were strong, and the addition lasted for a period of 1 h on every rising tide. The proper distribution of nutrients was acquired by installing jet nozzles across the narrowest and most turbulent part of the inlet, and because inflowing water moved rapidly as a plume until it reached land on the opposite side, from where it was redistributed throughout the lagoon. The different nutrients were supplied through separate dosing channels to avoid chemical precipitation, and to control doses and nutrient composition. The operation was supervised by alarms.

The plan was to double the nutrient loading of the euphotic zone in summer 1998 by nutrient addition and then to double that addition in 1999. The nutrient input to the mixed euphotic waters in the natural situation were estimated from data for sedimentation rates of phosphorus (P) measured in 1996 and 1997, and assuming a quasi-steady-state of total nutrients in the mixed photic zone (0-10 m) and that the inputs of P (and other nutrients) were equal to the sedimentation of P over the complete summer season (Wassmann, 1990). This assumption was justified as there were no trends in the concentration of the main macro-nutrients in the photic zone during summer. The dose of added nutrients in 1998 was accordingly set to that of the average sedimentation rate of P during summer in 1996 and 1997, corresponding to 0.41 mg P m^{-3} day^{-1}. The addition of silicate and nitrogen was calculated assuming a molar ratio of 16:8:1 for N:Si:P. This corresponded to the optimal N:P ratio of phytoplankton and 50% of the optimal Si:P ratio of diatoms (e.g, Turner, 2002; Klausmeier et al, 2004). N was supplied as NH_4NO_3, P as H_3PO_4 and Si as water-glass ($Na_3Si_2O_7$), and all nutrients were in aqueous solutions. The system was designed for weekly maintenance and reservoir-supply, using 1000 litre containers as nutrient reservoirs. There was only small deviations between the planned and actual N:Si:P ratios.

2.3. Sampling and analytical procedures

The sampling frequency was once a week during the summer (June-September) and less frequent (around monthly) in other periods throughout the experimental period, with approximately 25 samplings per year. Depth profiles for temperature, salinity and *in vivo* florescence were recorded in the lagoon station each sampling day by a CTD. The depth of the euphotic zone, from which the mixed euphotic samples were taken, was estimated based on the profiles of salinity, temperature and *in vivo* fluorescence, and was almost always set to 10 m depth.

Inside the lagoon, a mixed integrated water sample was taken from the productive (euphotic) waters at the sampling station using a Ramberg sampler (2 m length tube sampler with opening and closing valves, V = 4.2 l). The integrated 0-10 m water samples were collected in 25 l light-protected containers. Meso-zooplankon samples were taken separately in the same way.

For in-flowing water, which served as a reference for all years, the mixed water samples were taken from the bridge over the channel by 5-8 casts with a stainless steel bucket. The first and last hour of the inflowing phase of the tidal cycle was avoided to ensure that samples were unaffected by the added nutrients and representative for the outside coastal water.

Sub-samples for the determination of phytoplankton and small heterotrophic plankton and chemical components were taken from the lagoon and in-flowing water samples. The zooplankton collected in separate samples was concentrated on a 35 μm net and preserved with acid Lugol's solution before further treatment. Methods of preservation, counting and the estimation of carbon biomass of the planktonic organisms assigned to the functional groups are summarised in Table 2.

Water used for the analysis of particulate C (POC), N (PON) and P (POP) and chlorophyll *a* (CHL *a*) was screened through a 200 μm nylon net by reverse filtration to remove larger organisms. The screened water samples were harvested on pre-combusted (450 °C, 4 h), acid washed (5% H_2SO_4) GF-F filters and frozen (-18 °C) for later analysis. POC and PON of filter samples were measured by a CHN analyser and POP was measured according to the method of Grasshoff et al. (1983). Chlorophyll *a* on filter samples was extracted for 24 h at 4 °C in 90% acetone, and quantified by fluorometry using a Turner Designs fluorometer (Strickland and Parsons, 1972). Inorganic nutrients were measured in water taken from the container after filtration through acid washed GF-F filters (25 mm, 5% H2SO4). Inorganic nutrients (nitrate, ammonium, phosphate) were measured according to Grasshoff et al. (1983).

2.4. Data treatment

Ordinary least square regression has been used to establish the relationship between variables. The r^2 and the p-value of the regression were used as indicators for the existence of a statistically significant relationship, and slope equal to zero as the null hypothesis for no significant effect of independent variables on dependent variables. Slopes were compared by t-tests. Due to deviations from normality, chemical measurements from Hopavaagen and Nord-Møre region were compared using the Mann-Whitney Rank Sum Test.

The concentrations of chemical and biological components in the inflowing water (IFW) varied slightly, but systematically, from one year to the other, and this variability was reflected in the respective concentrations in the lagoon (LAG). The lagoon data of particulate nutrients and biomass of different groups of organisms were therefore normalised as follows:

$$NLAG_{X,Y} = LAG_{X,Y} \times \text{Mean } IFW_Y \left(IFW_{X,Y} \right)^{-1} \quad (1)$$

where $NLAG_{X,Y}$ is the normalised value of component X for year Y in the lagoon, $LAG_{X,Y}$ is the measured value of component X for year Y in the lagoon, Mean IFW_X is the measured mean value of component X for all five years in in-flowing water, and $IFW_{X,Y}$ is the measured value of component X for year Y in in-flowing water. Inorganic nutrient concentrations were not normalised because their turn-over time is believed to be very fast, but the overall results and conclusions were independent of this. The mean normalisation factor (Mean IFW_Y $(IFW_{X,Y})^{-1}$) for all normalised variables X was 1.02 (range 0.72 – 1.4).

We have compared the lagoon data with results obtained in a mesocosm experiment. The methods used in the mesocosm experiment were described in Olsen et al. (2007), and sampling and analytical procedures were mostly similar to those described here.

3. Results

The mean temperature and salinity for the summer seasons from 1996 to 2000 (Table 1) showed relatively equal conditions for all years. The warmest summer was in 1997, but no statistical differences were found for water temperature and salinity among different years in the euphotic zone (Table 1).

The additions of P and N from natural sources and from experimental additions resulted in a 2.1 and 3.3 times increase above the natural supply rate of P to euphotic waters (0-10 m) in 1998 and 1999, respectively, and a 1.7 and 2.4 times increase for N (Fig. 1).

The mean background values of the measured variables in inflowing water over the summer seasons (June-September) during the 5 year period together with their global means for the period

Table 2
Principal methods for carbon biomass estimation for species assigned to different functional groups. BB: Biovolume-based carbon estimate; LB: Length-based carbon estimate; MS: obtained from microscope/Epifluorescence microscope analysis; IA: obtained by an image analysing system (Macintosh computer and an image processing program, IP Lab); fc: final concentration.

Functional group	Sedation, preserving, and staining of samples	Dominant taxonomic groups and method used for biomass determination
Pico-autotrophs	0.5 mmol EDTA l^{-1} (fc) Glutaraldehyde (1% fc)	Pico-cyanobacteria, <2 μm, BB-IA, 0.21 pg C μm^{-3} (Booth, 1993)
Nano-autotrophs	Acid Lugol (1% fc)	Diatoms <20 μm, autotrophic flagellates, small dinoflagellates <20 μm, BB-MS, group-specific regressions (Strathman, 1967)
Micro-autotrophs	Acid Lugol (1% fc)	Diatom colonies, dinoflagellates, autotrophic ciliates, BB-MS, group-specific regressions (Strathman, 1967)
Pico-heterotrophs	0.5 mmol EDTA l^{-1} (fc) Glutaraldehyde (1% fc) Staining by DAPI (Verity and Sieracki, 1993)	Bacteria (and Archaea), <1 μm, BB-IA, 0.16 pg C μm^{-3} (Vadstein and Olsen, 1989)
Nano-heterotrophs	0.5 mmol EDTA l^{-1} (fc) Glutaraldehyde (1% fc) Staining by DAPI (Verity and Sieracki, 1993)	Flagellates (HNF, 2-8 μm), bacterivorous ciliates, appendicularia, BB-IA, 0.22 pg C μm^{-3} (Børsheim and Bratbak, 1987; Gismervik et al., 2002)
Micro-heterotrophs	Acid Lugol (2% fc)	Ciliates, 20-50 μm, BB-MS, 0.20 pg C μm^{-3} (Putt and Stoecker, 1989).
Meso-heterotrophs	Acid Lugol (1% fc)	Calanoid and cyclopoid copepods, cladocera, LB-MS, group-specific regressions (Gismervik et al., 2002)

(n = 85 - 86) showed particularly high DIN concentration in 1996 and lower concentrations during later years (Table 3). Also the autotrophic and heterotrophic biomass peaked in 1996. There was considerable inter-annual variability in all variables, but no other major systematic variations among years than those mentioned above were apparent. The coefficients of variation (CV) for the five year period varied in the range 0.21-0.87 for the variables, with generally broader ranges for biological than for chemical variables. The standard error of the estimated global mean value (SE, Table 3) varied between 2.5% and 10%.

The mean concentrations (±SE) of different molecular species of nitrogen during the summer period was positively related to the total loading rate of N to euphotic waters or remained constant (L_N, natural plus added, see Fig. 1) (Fig. 2). The mean concentrations of inorganic nitrogen (DIN; $NO_3 + NH_4$) were variable among years, but were not positively related to the N loading rate of euphotic waters (P = 0.903, Table 4). Contrary to this, the concentration of PON, which is N incorporated in small organisms and in dead organic matter < 200 μm, was highly correlated with the N loading rate of the lagoon system through the summer periods (P < 0.001). The sum DIN + PON is a measure of the nitrogen incorporated in and readily available for the phytoplankton (Ptacnik et al., 2010), and was also correlated to the N loading rate, although not significantly (P > 0.05), mainly because of the high DIN concentration in 1996. Total nitrogen (TN < 200 μm) was not measured in 1996, and the concentration through 1997-2000 correlated poorly, although positively, to the loading rate of N (P = 0.15).

The mean concentrations of different molecular species of phosphorus showed a similar pattern of variation with variable loading rate of P to euphotic waters as for nitrogen (Fig. 3). The mean concentration of DIP was independent of the P loading rate (P = 0.300), whereas the concentration of P in organisms and dead matter (POP < 200 μm), the sum POP + DIP and the total P concentration (TP) were all positively and significantly correlated (P < 0.05, Table 3).

The mean seasonal concentrations of chlorophyll *a* (CHL *a*), phytoplankton carbon biomass (Phytoplankton C) and particulate organic carbon (POC, < 200 μm) were all significantly and positively related to the loading rate of N (P < 0.05, P < 0.001 and P < 0.05, respectively) (Table 4, Fig. 4). The concentration of all variables was about double from the lowest to the highest loading rates of nutrients. The total biomass of heterotrophs, including the functional groups of bacteria, heterotrophic nano-flagellates, ciliates, and copepods (Olsen et al., 2007), did not respond to the increased loading rate of nutrients (r^2 = 0.029, P = 0.784, Fig. 4C), and neither did the biomass of meso-zooplankton (r^2 = 0.152, P = 0.516), dominated by copepods. Both curves exhibited an apparent optimum, also observed in mesocosm studies (Olsen et al., 2007), but this is not further elaborated upon as the optimum value was not significantly different from the lowest extreme values (P > 0.05).

It is useful to compare the responses in selected variables in the open lagoon ecosystem with results obtained in a comprehensive mesocosm experiment carried out in the lagoon in August 1997 (Fig. 5) (Børsheim et al., 2005; Olsen et al., 2006, 2007, 2011; Vadstein et al., 2012). The responses in PON and POP (Fig. 5A, B) of euphotic waters of the lagoon system that was diluted at 0.19 day^{-1} were similar to the responses found in the stagnant mesocosm systems (mean values for 18 days, n = 11). The mean summer concentrations of the lagoon were generally slightly lower than those of the mesocosms, but the patterns were nevertheless similar (P < 0.001 for combined linear regression, Table 4). The slopes of the response curves for the lagoon only were not significantly different (P > 0.05) and were therefore combined.

The response patterns of Phytoplankton C and CHL *a* with increasing N loading rate showed lower responses (i.e., slopes, Table 4) in the lagoon than in the mesocosms, but the responses within both systems were positively and significantly related to the nutrient loading rate for both CHL *a* and Phytoplankton C (Fig. 5C, D, Table 4). For both

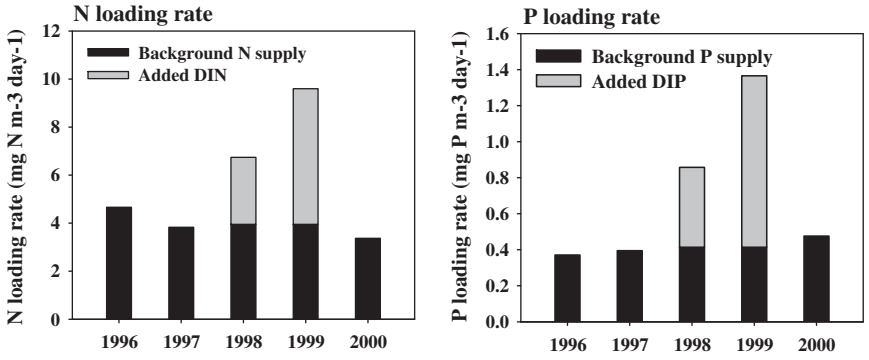

Fig. 1. Total loading rates of N and P to euphotic waters (0–10 m) in lagoon Hopavaagen (63°36' N, 9°33' E) through 1996-2000. Total loading rate is estimated as the sum of natural supply and experimentally added DIN and DIP.

Table 3
Mean background concentrations (mg m^{-3}) of key chemical and biological variables of water flowing into the lagoon during June – September in 1996-2000. Annual and global means and variability are given.

Variable, mg m^{-3}	1996 Mean ± SD (n)	1997 Mean ± SD (n)	1998 Mean ± SD (n)	1999 Mean ± SD (n)	2000 Mean ± SD (n)	Global Mean ± SD (n)	SE	CV
DIN	35.3 ± 13.9 (16)	9.81 ± 10.1 (18)	12.4 ± 10.6 (18)	6.6 ± 3.8 (18)	6.8 ± 6.8 (16)	13.9 ± 14.1 (86)	1.5	1.02
PON	43.1 ± 16.9 (16)	46.8 ± 13.9 (18)	42.8 ± 13.4 (18)	41.3 ± 16.2 (18)	55.5 ± 14.1 (16)	45.8 ± 15.4 (86)	1.7	0.34
DIN + PON	86.1 ± 17.4 (16)	60.3 ± 14.8 (18)	55.0 ± 13.8 (18)	47.9 ± 16.3 (18)	62.3 ± 14.6 (16)	62.4 ± 18.5 (86)	2.0	0.30
TN	-	146 ± 40.4 (18)	179 ± 21.1 (18)	127 ± 22.5 (18)	172 ± 26.8 (16)	155 ± 35.0 (86)	4.2	0.23
DIP	3.96 ± 3.40 (16)	4.25 ± 1.85 (18)	4.99 ± 1.17 (18)	4.73 ± 2.27 (18)	5.41 ± 2.18 (16	4.67 ± 2.22 (86)	0.25	0.47
POP	7.79 ± 4.18 (16)	7.55 ± 4.52 (17)	4.58 ± 1.93 (18)	5.62 ± 2.68 (18)	6.82 ± 1.70 (16)	6.42 ± 3.35 (85)	0.36	0.52
DIP + POP	11.9 ± 5.61 (16)	11.4 ± 5.29 (17)	9.56 ± 4.17 (18)	10.4 ± 4.04 (18)	12.2 ± 4.28 (16)	11.0 ± 3.67 (86)	0.40	0.33
TP	16.6 ± 4.54 (16)	17.5 ± 3.74 (17)	15.3 ± 4.22 (18)	14.0 ± 4.95 (18)	17.5 ± 4.18 (16)	16.1 ± 4.46 (86)	0.48	0.28
CHL *a*	2.18 ± 1.25 (16)	2.15 ± 1.24 (18)	1.74 ± 0.94 (18)	2.27 ± 0.73 (18)	2.54 ± 0.98 (16)	2.17 ± 1.05 (86)	0.11	0.49
Phyto C	75.8 ± 39.7 (16)	61.1 ± 42.7 (18)	48.9 ± 44.6 (18)	50.6 ± 42.6 (18)	69.4 ± 3.9 (16)	60.6 ± 37.9 (86)	4.1	0.63
Phyto C >2 μm	63.9 ± 42.8 (16)	54.0 ± 42.6 (18)	38.8 ± 44.6 (18)	41.8 ± 40.0 (18)	49.8 ± 289 (16)	49.3 ± 37.8 (86)	4.1	0.77
Heterotr C	101 ± 44.1 (16)	72.6 ± 41.2 (18)	60.7 ± 41.6 (18)	53.5 ± 44.2 (18)	70.5 ± 51.8 (16)	71.1 ± 41.5 (86)	4.5	0.59
MesoZoo C	61.1 ± 42.2 (16)	54.2 ± 39.2 (18)	42.8 ± 37.9 (18)	30.3 ± 37.7 (18)	43.4 ± 51.8 (16)	46.1 ± 39.4 (86)	4.3	0.86
POC	296 ± 154 (16)	302 ± 101 (18)	221 ± 85 (17)	269 ± 103 (18)	365 ± 105 (16)	288 ± 115 (85)	12.4	0.40

variables, the slopes obtained for the responses in the lagoon were less than half of the slope found in the mesocosms (42% for CHL *a*, 27% for Phytoplankton C).

The concentration of POC, a proxy for total living and non-living organic matter < 200 μm, showed a slightly different patterns of variation as the lagoon values were lower than those of the mesocosms at respective N loading rate while the slope of the response curves were statistically equal (Fig. 5E, Table 4).

The ratio of Phytoplankton C (including pico-cyanobacteria and eukaryotic algae) to total heterotrophic biomass (Heterotrophic C, including bacteria, protozoan zooplankton and meso-zooplankton) (Fig. 6A), the ratio between the biomass of eukaryotic algae (Phytoplankton C >2 μm) and meso-zooplankton (Fig. 6B), and the ratio between total chlorophyll *a* and meso-zooplankton (Fig. 6C) all showed positive relationships with the N loading rate, but only the ratio between biomass of eukaryotic alga and meso-zooplankton increased significantly ($P < 0.05$, Fig. 6B). Ratios of autotrophic and heterotrophic biomass have been suggested as an indicator for trophic state based on earlier mesocosm experiments (Olsen et al., 2006).

It is finally mentioned that the primary production during the summer season responded significantly to increased nutrient input ($P < 0.05$), but the sedimentation rate of C, N and P components did not respond positively and remained constant for all years ($P > 0.05$) (unpublished).

4. Discussion

There have been few, if any, relevant fertilisation experiments of complete marine planktonic ecosystems carried out to study how the ecosystem structure and function are influenced by sustained enhanced nutrient supply. Nixon and Buckley (2002) have excellently reviewed historical studies and summarised earlier efforts, which involve only one very early Scottish experiment studying the responses of fertilisation in higher trophic levels (Orr, 1947; Nutman, 1950; Raymont, 1950). We are not aware of later experiments, and the knowledge of ecosystem responses to increased nutrient input is almost entirely gathered from regional studies or reviews of regional data, combined with small scale experiments in mesocosms and modelling (e.g., Engqvist, 1996; Capriulo et al., 2002; Colijn et al., 2002; Andersen et al., 2011; Carstensen et al., 2011; Topcu et al., 2011; Grizzetti et al., 2012). Most of the efforts made to identify state indicators for ecosystem functioning (e.g., Ferreira et al., 2011) and to establish assessment methods and

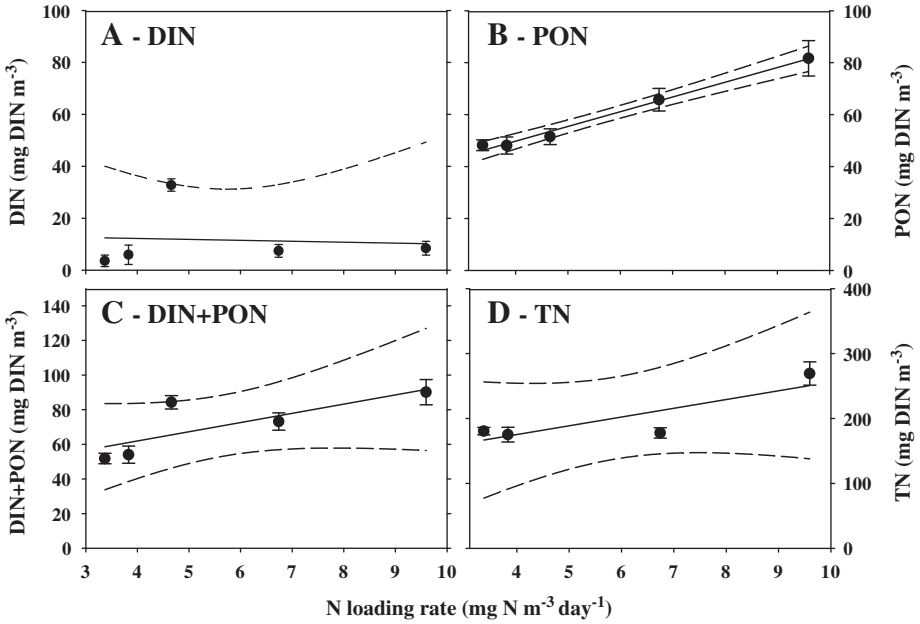

Fig. 2. Mean seasonal concentration of different N components (n = 16–18) as functions of the total loading rate of N to euphotic waters (0–10 m) in lagoon Hopavaagen through 1996 to 2000. A: inorganic N (DIN = NO_3 + NH_4), B: particulate organic N (PON); C: DIN + PON; and D: Total N (TN). Bars express 1SE and the dotted lines the 95% CL of regression curves.

Table 4
Regression coefficients for key chemical and biological variables *versus* loading rate of nitrogen and phosphorus for lagoon data with comparison to results derived in a mesocosm experiment (cf. Figs. 2, 3, 4 and 5).

	Intercept ± 1SE	Slope ± 1SE	r^2	P
Lagoon data				
DIN *versus* L_N	11.6 ± 13.4	−0.164 ± 2.69	0.006	0.903
PON *versus* L_N	27.1 ± 2.04	5.67 ± 0.34	0.990	<0.001
DIN + PON *versus* L_N	40.7 ± 14.8	5.31 ± 2.43	0.615	0.116
TN *versus* L_N	121 ± 37.6	13.5 ± 5.88	0.723	0.148
DIP *versus* L_P	3.70 ± 0.94	1.48 ± 1.19	0.342	0.300
POP *versus* L_P	3.69 ± 0.97	5.54 ± 1.23	0.827	<0.05
DIP + POP *versus* L_P	7.39 ± 0.453	0.174 ± 0.0371	0.879	<0.05
TP *versus* L_P	10.9 ± 1.34	7.98 ± 1.70	0.880	<0.05
Lagoon and mesocosm data				
CHL *a versus* L_N (lagoon)	1.04 ± 0.45	0.262 ± 0.074	0.806	<0.05
CHL *a versus* L_N (mesocosm)	0.777 ± 0.117	0.623 ± 0.020	0.997	<0.001
Phytoplankton C *versus* L_N (lagoon)	16.3 ± 4.71	9.98 ± 0.77	0.982	<0.001
Phytoplankton C *versus* L_N (mesocosm)	72.0 ± 20.2	36.7 ± 3.53	0.973	<0.01
POC *versus* L_N (lagoon)	148 ± 43.5	38.6 ± 7.16	0.958	<0.05
POC *versus* L_N (mesocosm)	220 ± 17.5	47.5 ± 3.05	0.988	<0.001
Combined lagoon and mesocosms				
PON *versus* L_N (mesocosms + lagoon)	32.2 ± 3.85	5.62 ± 0.65	0.903	<0.001
POP *versus* L_P (mesocosms + lagoon)	4.10 ± 0.55	4.54 ± 0.70	0.842	<0.001

management and monitoring schemes for nutrient emission to coastal waters over the last decade in Europe (e.g., Ferreira et al., 2007; Devlin et al., 2011; Andersen et al., 2011; Kitsiou and Karydis, 2011) have been motivated by the implementation of EU directives. These include the Water Framework Directive (WFD, 2000/60/EC) and more recently the Marine Strategy Framework Directive (MSFD, 2008/56/EC). Credible indicators for chemical and biological state of ecosystem functioning and their natural background levels (Tett, 2008; Ferreira et al., 2011; Topcu et al., 2011) are paramount for the implementation of environmental legislation for coastal waters. Our study can contribute with information needed to achieve this task.

Dissolved inorganic N and P (DIN and DIP) and total N and P (TN and TP) are the chemical indicators that have been most commonly used to assess trophic state of coastal waters both for the summer and the winter periods (e.g., OSPAR, 2005; HELCOM, 2009; Ferreira et al., 2011). Our experimental study has suggested that PON and POP are more credible indicators for the summer period (here June to September), at least in relatively pristine coastal waters. This can in fact also be deduced from general knowledge on the nutritional physiology of phytoplankton (Droop, 1983; Olsen, 1989), indicating that the uptake of nutrients is very fast and that phytoplankton biomass may vary by factors >2 relative to PON and POP because the cellular contents of the limiting nutrient, expressed in terms of cellular N:C and P:C ratios, are variable physiological characteristics. The quantitative information on background levels and the highly significant responses of both PON and POP and other variables to enhanced nutrient input to North-East Atlantic coastal waters in the summer period are the most important results of our study. DIN and DIP are not immediately taken up by phytoplankton in the winter period and are therefore remaining more constant with time. PON and POP will then be less relevant.

Our recommendation is to consider both N and P components in scientific studies on coastal eutrophication that aim to establish management methods of marine coastal systems. N and P in deep water is the major nutrient source of the plankton in relatively pristine coastal water, and the molar ratio of nitrate to phosphate in deep water is remarkably constant and close to 16 (Redfield ratio) in NE Atlantic coastal waters, and therefore in close balance relative to the requirements of phytoplankton (Falkowski, 2000). We found that N was the primary limiting nutrient for the phytoplankton community of the lagoon in 1997 (Børsheim et al., 2005; Olsen et al., 2006), and most likely in the following years, but P showed similar responses as N in all years. We suggest that the P supply was in fair balance with N supply. Many recent papers have performed in depth analyses of N *versus* P limitation in different ecosystems and situations, and our results and general understanding fit very well with these reports (Geider and La Roche, 2002; Arrigo, 2005; Howarth and Marino, 2006; Elser et al., 2007).

4.1. Chemical and biological responses

Our results demonstrated linear responses of most measured chemical and biological response variables with increasing nutrient loading rate in the range of 3–10 mg N m^{-3} day^{-1} (Table 4). The mesocosm experiment used as a reference, together with similar experiments

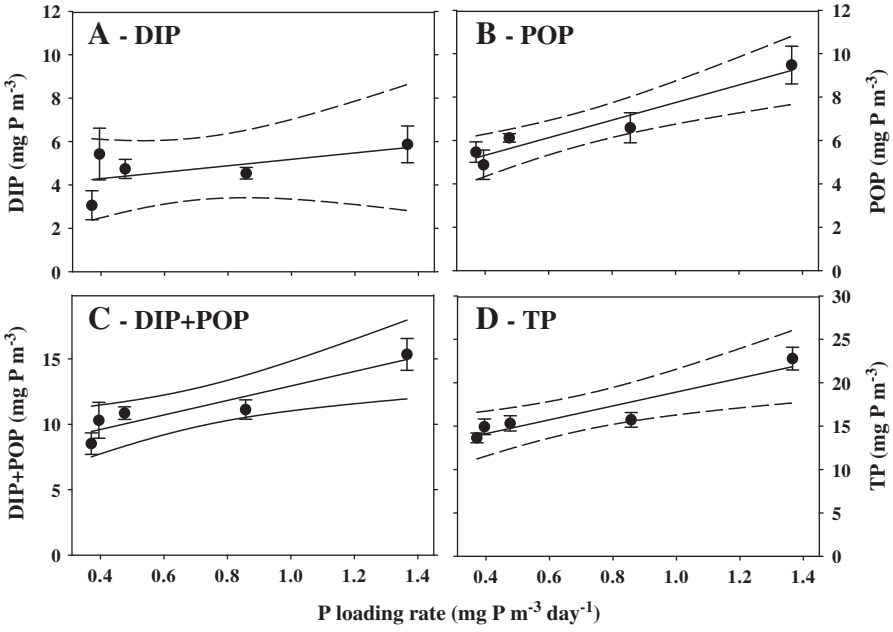

Fig. 3. Mean seasonal concentration of different P components (n = 16–18) as functions of the total loading rate of P to euphotic waters (0–10 m) in lagoon Hopavaagen through 1996 to 2000. A: inorganic P (DIP), B: particulate organic P (POP); C: DIP + POP; and D: Total P (TP). Bars express 1SE and the dotted lines the 95% CL of regression curves.

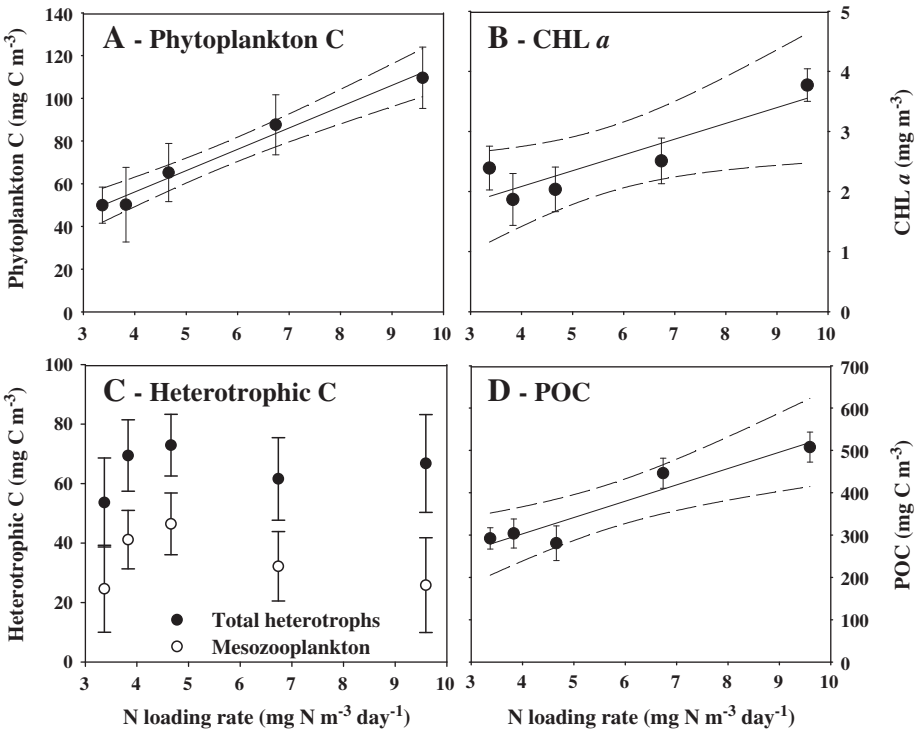

Fig. 4. Mean seasonal biomass of biological components and their proxies (n = 16–18) as functions of the total loading rate of N to euphotic waters (0–10 m) in lagoon Hopavaagen through 1996 to 2000. A: phytoplankton carbon (Phytoplankton C), B: Chlorophyll a (CHL *a*); C: Total biomass of heterotrophs (Heterotrophic C), and D: particulate organic carbon (POC). Bars express 1SE and the dotted lines the 95% CL of regression curves.

undertaken in the Baltic and Mediterranean Seas, all showed linear responses up to ~15 mg N m^{-3} day^{-1}, but responses generally levelled off for higher values in the Atlantic (Olsen et al., 2006).

Among the chemical variables, PON, which is mainly nitrogen incorporated in small organisms < 200 μm, showed the most significant response ($P < 0.001$) to enhanced nutrient supply. The increase in DIN + PON and TN was positive, although not significant ($P > 0.05$). The responses in the different P components were similar to those of the N components following enhanced input of P, and the responses in all variables except DIN and DIP were significant ($P < 0.05$). This result agrees with the general findings that the combined addition of N and P in bioassay experiments normally gives stronger growth responses of phytoplankton than the addition of a single element (Elser et al., 2007).

It was not surprising that neither DIN nor DIP concentrations responded positively to an increased loading rate of the respective inorganic nutrient. These nutrients are limiting, or close to limiting, and are therefore taken up rapidly and efficiently by the phytoplankton (and heterotrophic bacteria). There has been some confusion about the fate of nutrients released to open coastal waters, leading to an introduction of the term "Ghost nutrients" (Pitta et al., 2009). However, the disappearance of inorganic nutrients should be no surprise; they are rapidly assimilated by phytoplankton and are further transferred to heterotrophic zooplankton (Pitta et al., 2009).

Both the CHL *a* and Phytoplankton C, which are independent proxies for phytoplankton biomass, responded similarly and significantly (Table 4) to enhanced nutrient input, and both increased around 2 times in concentration from low natural to the highest rate of nutrient input. Contrary, the total biomass of heterotrophic organisms and meso-zooplankton biomass showed no significant positive response to enhanced nutrient input ($P > 0.05$), but perhaps an optimum response at intermediate loading rates?

Particulate organic carbon (POC) is a proxy for total organic matter < 200 μm, and the concentration of POC responded significantly ($P < 0.05$) to an enhanced rate of nutrient input, despite the fact that Phytoplankton C constituted only 16-22% of POC whereas Heterotrophic C constituted 13-21%. These living components therefore explained <43% of the increase in POC, and the remaining increase must have been caused by an increase in dead organic matter originating from an increased rate of zooplankton defecation and mortality. This is in agreement with other findings in mesocosm experiments (Olsen et al., 2007).

4.2. Phases of nutrient assimilation in the food web

It is important that increased nutrient input does not have an immediate effect on the growth of plankton and other organisms. The nutrient incorporation processes in different functional components of the planktonic food web involve different steps (Fig. 7). They all interact successively and have time-lags for their response, from the addition of DIN and DIP to the response in larger zooplankton.

Nutrient addition from point sources will immediately and without delay result in an increased ambient concentration in the surrounding water. Phytoplankton and heterotrophic bacteria will respond immediately and increase the uptake rate of nutrients that are limiting their growth rate. Nutrients that are not limiting, e.g. sulphate, are normally present in excess concentrations and the uptake is not affected by a further increase in the ambient concentration. The initial uptake rate of a limiting nutrient in phytoplankton is a very fast process with a time scale of minutes to a day (e.g., Olsen, 1989; Thingstad et al., 2005; McCarthy et al., 2007). This means that a limiting nutrient will be removed from the water very fast and become incorporated in the phytoplankton and bacterial cells.

Phytoplankton cells that are perturbed with and take up excess limiting nutrients will not immediately, but in due time, react by increasing their growth rate and biomass. It is very likely that phytoplankton and planktonic heterotrophs exhibit a lag-phase with a steady pre-perturbation growth rate before they react by a shift-up in the growth rate. This has been demonstrated for lagoon communities (Olsen et al., 2007, 2011), but the duration of these lag phases is variable and likely

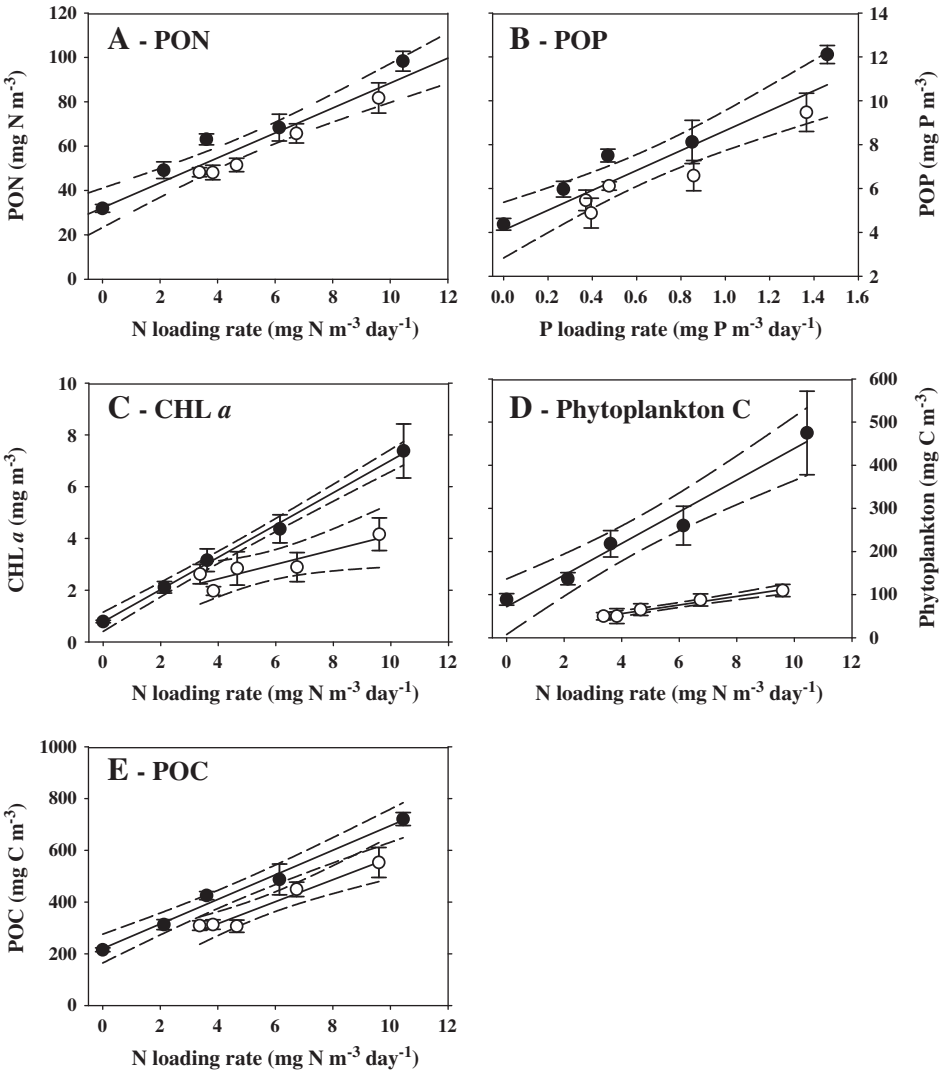

Fig. 5. Mean seasonal biomass of key chemical and biological components (n = 16–18) as functions of the total loading rate of N to euphotic waters (0–10 m) in lagoon Hopavaagen through 1996 to 2000 (open circles) and in a mesocosm experiment of 18 days duration (solid circles. n = 11; e.g., Olsen et al., 2007). A: particulate organic N (PON), B: particulate organic P (POP); C: Chlorophyll *a* (CHL *a*); D: phytoplankton carbon (Phytoplankton C) and E: particulate organic carbon (POC). Bars express 1SE and the dotted lines the 95% CL of the regression curves.

dependent on the initial nutritional state of the organisms. If the phytoplankton is initially only slightly nutrient limited, growing close to their maximum capabilities, nutrient addition will not affect the growth rate very much and the lag-phase will be difficult to detect. Contrary, if the phytoplankton is more severely limited, the lag phase will likely become clearly expressed.

Andersen et al. (2007) have reported small responses in chlorophyll *a* concentration after only one day of incubation in small-scale laboratory systems, whereas Elser et al. (2007), in a meta-analysis of published experiments, mention that the average incubation time of the bioassay experiments analysed was 7 days. Mesocosm experiments in the Hopavaagen lagoon have revealed lag-phases of several days for phytoplankton after a perturbation of the limiting nutrient (Olsen et al., 2007). The phytoplankton first accumulated the added nutrients which was observed through increased particulate N:C and P:C ratios, which express endogenous nutrients (Droop, 1983). A significant increase in primary production was thereafter found first after 4 - 5 days and the biomass of phytoplankton became significantly higher 5–6 days after the nutrient perturbation (Olsen et al., 2007). A lag phase in the growth response is important even if it is only 1-2 days (see below).

Herbivore heterotrophs depend on particulate carbon resources and will become stimulated to faster reproduction and growth first after a noticeable increase in phytoplankton biomass (i.e., food concentration, Fig. 7). This means that the response of heterotrophs, which also need time to adapt metabolically to a situation of increased food concentration, can normally be expected after the response in phytoplankton biomass. If an essential nutrient is limiting for the heterotroph, rather than carbon or energy, the growth response may be faster than for the phytoplankton. This was the case for ciliates in the mesocosm experiment conducted in Hopavaagen in 1997 as they appeared to be mineral P limited (Olsen et al., 2011).

4.3. Interaction of nutrient assimilation and water dilution

The lifetime of nutrient perturbations and responses of key variables will also depend on the dilution rates of the water after the nutrient perturbation. PON and POP, which are positively affected by nutrient addition and negatively by dilution, sedimentation and grazing, have lifetimes that are comparable to the lag in growth response of the phytoplankton and the water residence time of the lagoon (~5 days,

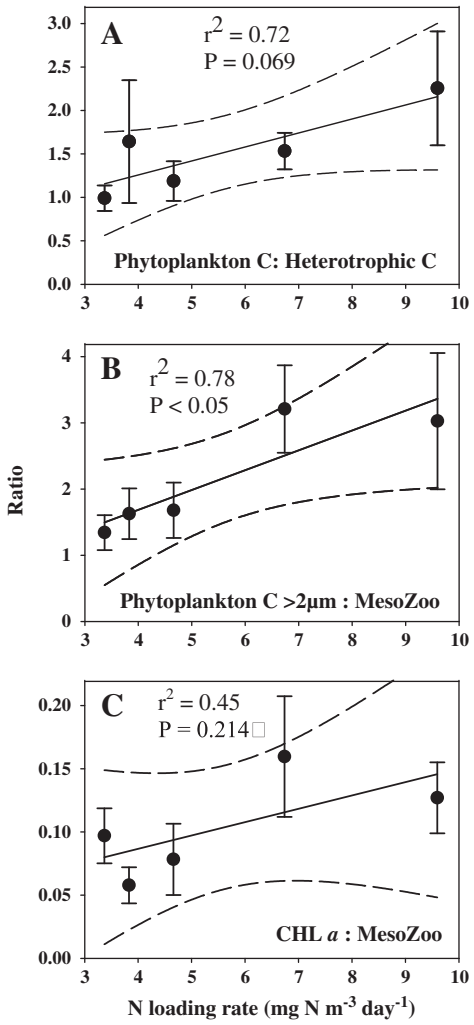

Fig. 6. Different mean seasonal ratios of autotrophic to heterotrophic biomass (n = 16–18,) as functions of the total loading rate of N to euphotic waters (0–10 m) in lagoon Hopavaagen through 1996 to 2000. A: autotrophic carbon: heterotrophic carbon (Phytoplankton C: Heterotrophic C), B: eukaryotic autotrophic carbon: mesozooplankton carbon (Phytoplankton C > 2 µm: MesoZoo C) and C: Chlorophyll *a*: mesozooplankton carbon (CHL *a*: MesoZoo C). Bars express 1SE and the dotted lines the 95% CL of regression curves.

Table 1). The responses of PON and POP in the open lagoon and the stagnant mesocosm systems were much the same, meaning that the water exchange rate did not affect the concentrations of PON and POP so much following the sustained nutrient perturbation. This pattern is in agreement with a rapid uptake of DIN and DIP just after fertilisation and a relatively long lifetime of PON and POP thereafter. Our data demonstrated this very clearly (Fig. 5A, B) by the fact that the slopes of the response curves for PON and POP in the mesocosms and for the lagoon were not statistically different (P > 0.05, Table 4). This means that PON and POP are not very dependent on hydrodynamics.

The responses of CHL *a* and Phytoplankton C, both expressing phytoplankton biomass, were lower in the lagoon than in the stagnant mesocosms (Fig. 5C and D). This can be expected if the growth response has a similar reaction time as the water dilution. A fraction of the phytoplankton cells will not respond in terms of growth before they are transported out of the lagoon, the growth response is too slow (Fig. 7). The pattern of water exchange of the lagoon cannot exactly be deduced, but the inflow of a volume corresponding to the volume of the mixed layer of water in the lagoon will take 5.2 ± 1.9 days (Table 1). If mixing of the inflowing water with euphotic waters of the lagoon is continuous and complete, 37% of the phytoplankton cells that were perturbed 5.2 days earlier will still remain in the lagoon, while 63% will be transported out. This corresponds well with the differences in the slopes of the responses found for phytoplankton biomass proxies in mesocosms and lagoon. The slope of the response curves in the lagoon was 42% and 27% of the corresponding slopes found in mesocosms for CHL *a* and Phytoplankton C, respectively.

Without considering the effects of grazing, we conclude that the differences in response curves of the phytoplankton biomass proxies in lagoon *versus* mesocosms can be understood based on our conceptual understanding of the process of enrichment of the food web components (Fig. 7). The long response time of the meso-zooplankton masked the response of this group almost completely in the experiment by the fact that most of their response must be expected to take place outside, downstream of the lagoon.

4.4. Indicators of ecosystem state for the summer period

The above discussion concludes that PON and POP appear to be more credible indicators of nutrient loadings and the chemical state of the pelagic coastal ecosystem than DIN and DIP during the summer period. Measurements of inorganic nutrients may provide important information in hyper-eutrophic situations and in winter situations (e.g., Cloern, 2001; Ferreira et al., 2011), but not so much in more pristine or slightly eutrophic locations where inorganic nutrients normally are taken up immediately by the phytoplankton (Pitta et al., 2009). Moreover, DIN:DIP and other aggregated indicators of inorganic nutrients included may be useful to verify whether N or P is the primary limiting nutrient (Ptacnik et al., 2010).

4.4.1. Background level of nutrients

Knowledge on the natural background level of nutrient indicators is important for assessing the chemical state of coastal waters (Topcu et al., 2011). Beside the response pattern, a stable background level of an indicator across different unaffected coastal regions will, in our view, increase its credibility. Four sampling stations in a neighbour coastal region (Nord-Møre, Table 5) showed mean concentrations of DIN, DIP, POP and CHL *a* that were all significantly different (P < 0.001) for the two regions, whereas the PON concentrations and its range of variability were equal (P > 0.05, SE/Mean value < 0.06). This supports our hypothesis that PON can be an indicator for pelagic coastal ecosystems in NE Atlantic coastal water. The concentration of POP found in the Nord-Møre stations was higher than the highest value found in the lagoon (Fig. 3B). We will, however, still include POP as a candidate indicator, because we suggest that the primary factor which determines mean seasonal PON and POP concentrations (and total nutrients) in pristine coastal waters during the summer season is the nutrient inflow from deep-water. Both DIN and DIP concentration and the N:P ratio remains fairly constant in NE Atlantic coastal water (Falkowski, 2000), which suggests that we may expect a constant PON background if N is the primary limiting factor and a constant POP background if P is the primary limiting factor, like in more freshwater affected locations.

Re-suspension may affect the coefficients of the response functions of both PON and POP (Table 4) in shallow waters which is mixed to the bottom. This means that the supply rate to mixed upper water masses becomes increased compared to that in deeper water. A consequence is a reduced capacity of assimilating anthropogenic supply, because the maximum capacity remains constant, but PON and POP are likely still reliable indicators.

4.4.2. Total N and P

The concentrations of Total N (TN) and Total P (TP) also have the potential of being credible indicators of chemical state, and these indicators are already widely used (e.g., Caruso et al., 2010; Carstensen et al.,

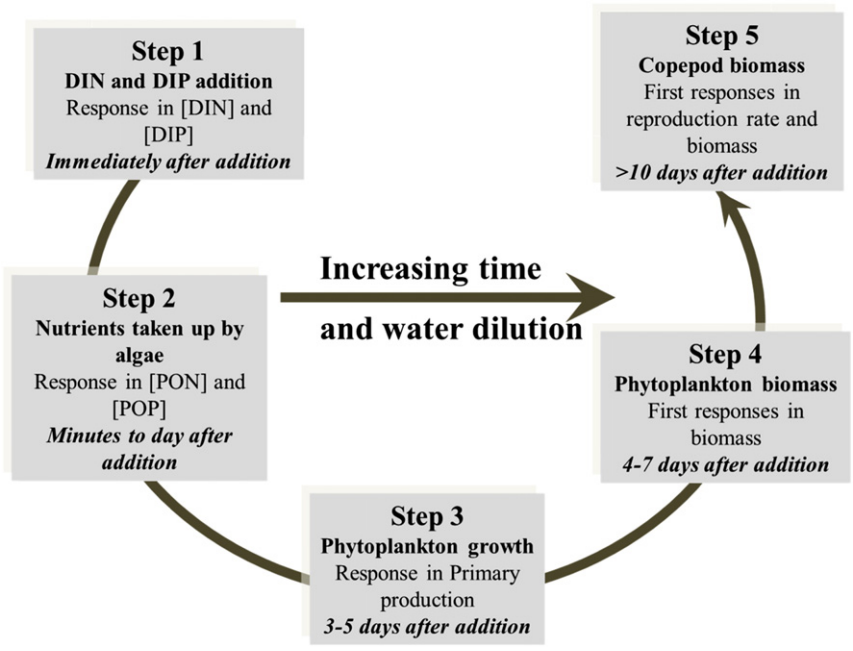

Fig. 7. Steps of the assimilation process of nutrients in functional key components of the planktonic food web following a perturbation of inorganic nutrients, with evaluated response times given for the different steps.

2011; Ferreira et al., 2011; Topcu et al., 2011). However, our experimental data did not show clear relationships with nutrient loading rate for these variables (Table 4). Dissolved organic N and P (DON and DOP, respectively) are normally main components of TN and TP, respectively, and are found in high and variable concentrations across contrasting European coastal seas (Ferreira et al., 2011). The concentration of DON in the lagoon was around 130 mg m^{-3} and therefore >4 times higher than PON, whereas the concentration of DOP was around 6 mg m^{-3} and of the same magnitude as POP. DON did not respond significantly ($P > 0.05$) to nutrient enrichment whereas DOP responded slightly but significantly in the mesocosms ($P < 0.05$, Olsen et al., 2011). The mean concentration of DON (0-15 m) in the Gulf of Finland during a four months summer period was 220 mg N m^{-3}, whereas the mean DOP concentration was 18 mg P m^{-3} (Hoikkala et al., 2012). For the Mediterranean Sea, DON and DOP were found in concentrations of 35–97 mg N m^{-3} and 1-2 mg P m^{-3}, respectively, in surface waters of the Southern Adriatic Sea (September, 0–200 m), and these values were found to be representative for the Mediterranean Sea (Santinelli et al., 2012). The contrasting values demonstrate the major differences in DON and DOP concentrations found in the main types of European coastal water (Ferreira et al., 2011), a difference that appears even more extreme for DOC (Olsen et al., 2006). The main weaknesses of TN and TP as indicators are the relatively high analytical uncertainty of DON and DOP measurements combined with the facts that they are little affected by nutrient input and that they are a main nutrient component of all types of water. TP may regrettably be relevant for the Mediterranean Sea.

It is a challenge to review PON and POP data in the literature to explore the appropriateness of PON and POP as indicators across coastal regions. Both variables are frequently measured in studies of the oceans, but according to our knowledge, neither PON nor POP has been included in many scientific studies related to coastal eutrophication (Pitta et al., 2009; Caruso et al., 2010; Ptacnik et al., 2010) or in monitoring programs.

Table 5
Comparison of global mean background values of key variables measured in lagoon Hopavaagen (see Table 4) and in 4 sampling stations in the Nord-Møre region, which is a neighbouring coastal region south of the lagoon (stations: Raudeggflua (63,1037, 7,5271), Skjerjebaan, (63,4158, 7,7009), Bremsnesfjorden (63,0111, 7,7430) and Reiraklakken (63,4622, 8,1824). The variability of mean values is also shown among years and stations (SE and range of SE). Equality of the mean global values for 5 years in lagoon Hopavaagen and 4 stations in the Nord-Møre region were compared using a Mann-Whitney Rank Sum Test.

Location		1- Lagoon Hopavaagen	2- Nord-Møre region	
		Inflowing water 5 yr time series, June-September (n = 16-18)	Integrated sample 0 - 10 m depth, 4 stations (n = 59 – 60)	1#2 P
DIN	Global mean ± SE	13.9 ± 1.5	4.9 ± 0.71	<0.001
	SE for station/yr (range)	5.4 (6.6 – 35.3)	1.2 (2.0 – 8.2)	
DIP	Global mean ± SE	4.7 ± 0.25	0.67 ± 0.13	<0.001
	SE for station/yr (range)	0.23 (4.2 – 5.4)	0.20 (0.2 – 1.2)	
PON	Global mean ± SE	45.8 ± 1.7	48.5 ± 2.1	0.343
	SE for station/yr (range)	2.6 (41.3 – 55.5)	2.0 (45.1 – 55.0)	
POP	Global mean ± SE	6.4 ± 0.36	10.2 ± 0.5	<0.001
	SE for station/yr (range)	0.61 (4.58 – 7.79)	0.34 (9.3 – 11.1)	
CHL *a*	Global mean ± SE	2.17 ± 0.11	0.86 ± 0.09	<0.001
	SE for station/yr (range)	0.13 (1.74 – 2.54)	0.07 (0.67 – 1.03)	

4.4.3. Biological indicators

Chlorophyll *a*, the main indicator to express the biological state of open waters (Andersen et al., 2011; Carstensen et al., 2011; Ferreira et al., 2011), showed low although steadily different concentrations ($P < 0.001$) between the two regions (Table 5) over the summer season. The response was similar to that of Phytoplankton C, supporting its credibility as a proxy for phytoplankton biomass. Although there is some background variability, Chlorophyll *a* is always an important indicator for evaluating the biological state of coastal planktonic ecosystems.

There is ample evidence that ratios between autotrophic to heterotrophic biomass can be more relevant as biological indicators than the biomass of the respective functional components. This is because phytoplankton biomass tends to respond more strongly to enhanced nutrient addition than heterotrophic biomass, shown in the lagoon and in mesocosms (Olsen et al., 2006, 2007). Zooplankton biomass is difficult and time consuming to measure, but it is important to explore additional biological indicators beside chlorophyll *a*, primary production, water transparency, oxygen concentration and species composition (Ferreira et al., 2011). The ratio of phytoplankton to zooplankton biomass should be among the candidates.

PON and POP are proposed as chemical indicators, but because N is limiting the phytoplankton, PON will also reflect the biological state of the plankton (< 200 μm) by the fact that it expresses the concentration of N in all small organisms and dead organic matter between 0.2 and 200 μm, and therefore the biomass potential. We therefore suggest that PON, representing a link between abiotic and biotic factors, can be a credible indicator also for ecosystem functioning when N is the primary limiting nutrient. POP can play the same role if P is primarily limiting. No negative influence on the functioning of the pelagic ecosystem was found for PON < 88 mg N m^{-3} in the lagoon experiment and for PON < 110 mg N m^{-3} in the mesocosm experiment (Olsen et al., 2006). The planktonic food web retained its high efficiency, the responses in biological components with increasing nutrient input were linear, and there was no increase in sedimentation rates. We like to emphasize that the threshold values for PON must be further challenged and evaluated.

4.5. Extension of results to the coastal ecosystems

It is never trivial to extend experimental results to the natural situation. If we imagine a similar fertilisation experiment undertaken in a whole water region that forms the ecosystem unit for coastal management, why should an extended scale of the experiment not produce the same results? Physical forcing and water movement in the lagoon were fairly strong and primarily driven by tides. Wind and sea currents may be more important in an extended case and there may be stronger gradients because mixing will likely not be that efficient and take more time. If mixing of the receiving water masses (ecosystem unit) takes place within a week, or within the times needed for the phytoplankton to respond, and the dilution rate to outside water masses from the ecosystem unit is higher than for the lagoon (>0.2 day^{-1}), we suggest that response functions of the lagoon will represent a worst case scenario for the extended coastal system. If the dilution rate of the ecosystem unit is lower and mixing is slower, the extended coastal system may exhibit a stronger response in phytoplankton biomass than the lagoon. Further studies of water residence times and mixing efficiencies in water regions may contribute to answer these questions.

4.6. Principles for management of surface waters

We have suggested that PON and POP, dependent on which nutrient is the primary limiting factor, can serve as summer indicators for both chemical and biological states of the pelagic ecosystem in contrasting coastal waters. Below we focus on PON as N was found to be the primary limiting nutrient in 4 out of 5 years of our study. PON can be expressed as a function of the N loading rate (L_N, mg N m^{-3} day^{-1}, natural plus anthropogenic) of euphotic (0–10 m) NE Atlantic coastal waters as follows (Fig. 2B and Table 4):

$$PON = (5.6 \pm 0.7) \times L_N + (32.2 \pm 3.9) \quad (1)$$

(Table 4, combined data). It then follows that the loading rate of N to euphotic waters in any geographical point can be estimated as:

$$L_N = (0.18 \pm 0.02) \times PON – (5.7 \pm 1.0) \quad (2)$$

This suggests that the N loading rate of coastal waters can be calculated in any geographical position based on measurements of PON, and we hypothesise that this relation can be valid for open coastal systems under the restrictions given. This approach is conceptually not that different from the concept derived and used for freshwater by Vollenweider (1976), which allows estimation of P loading rate in a closed water mass like a lake, as entities of a waterway, based on measurements of TP and water retention times under the assumption that the lakes are completely mixed.

Taking a conservative approach, we have concluded that the functioning of the planktonic ecosystem of euphotic coastal NE Atlantic waters was not negatively affected as long as the mean PON for the summer season was < 88 mg N m^{-3} which corresponds to a nitrogen loading rate of 10 mg N m^{-3} day^{-1}. Of this nutrient supply, the natural background supply was 4.0 mg N m^{-3} day^{-1}, meaning that the external supply was 6.0 mg N m^{-3} day^{-1} (Fig. 1).

There is ample evidence that safe limits of the PON concentration can be as high as 110 mg N m^{-3}, corresponding to a loading rate of 14 mg N m^{-3} day^{-1} to euphotic marine waters. Most functional responses of mesocosm communities remained unaffected up to this point (unpublished data and Olsen et al., 2006, 2007, 2011).

Both our background value of PON, the estimated natural N supply rate from natural sources and the suggested threshold value for what is considered to be safe limits for ecosystem functioning must be further evaluated for other regions, and values will likely be different for contrasting water types. There is accordingly an obvious need to clarify system dependency of our estimated backgrounds, response functions and critical thresholds, but we advocate that the general concept provided will be adequate. It is also important to emphasise the sampling frequencies and the temporal and spatial scales of the measurements made. Management of dynamic coastal systems cannot be based on a single or a few days sampling, and sampling must be representative for the euphotic water masses. The pronounced variability of nutrients and phytoplankton biomass require a comprehensive sampling programme in time and space, from which mean values are used as a basis for state assessments. The global mean and variability of PON (Table 4) suggest that 12 samplings of euphotic waters were needed for each sampling station over the summer season to ensure that the standard error of the mean (SM) remains <10% of the mean value. We suggest that a more standardised sampling matrix will contribute to reduce variability in measured variables.

4.6.1. Concluding remarks

Our approach will contribute to reduce the variability in biological responses following the enhanced input of inorganic nutrients by three main means. First, we suggest using data series for the mixed surface layer with 12 or more samplings per station in the summer/autumn period taken at representative stations and depths. This will reduce the scatter introduced by natural variability of phytoplankton species, phytoplankton biomass, fraction of phytoplankton of total plankton biomass (<200 μm) and the nutritional state (i.e., N:C and P:C ratios) of the phytoplankton. The average value for all sampling stations in a region will give a representative value for the water region or ecosystem

unit, and it may give information of state within the region. Second, we suggest using PON and POP as main response variable together with other well established indicators. This suggestion is for assessment of coastal Atlantic waters that are stratified during the summer period (end of spring bloom to autumn mixing), but we hypothesise that it may be valid for other water types as well.

Conflicts of interests

There are no obvious conflicts of intersect, but we suggest to use non-Norwegian reviewers.

Acknowledgements

The experimental work was undertaken by Norwegian partners in COMWEB, a project of the EU-MAST programme, and a member of the ELOISE and the MARICULT umbrella programmes, which was funded by the EC MAST Programme (MAST3-CT96-0052) and Norsk Hydro ASA, and later by the Research Council of Norway allowing a second year of fertilisation. The field work is a result of 5 years of hard work of all of the enthusiastic hard working project participants at Trondhjem Biological Station and other colleagues who were involved in the experiments carried out in Hopavaagen, Central Norway (63°36' N, 9°33' E) through 1996-2000. The comprehensive basis of results gathered, together with results from a main mesocosm experiment have unconditionally enhanced our understanding of coastal eutrophication, even though it took time to pursue.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2013.12.028. These data include Google maps of the most important areas described in this article.

References

Andersen T, Saloranta TM, Tamminen T. A statistical procedure for unsupervised classification of nutrient limitation bioassay experiments with natural phytoplankton communities. Limnol Oceanogr Methods 2007;5:111–8.
Andersen JH, Axe P, Backer H, Carstensen J, Claussen U, Fleming-Lehtinen V, et al. Getting the measure of eutrophication in the Baltic Sea: towards improved assessment principles and methods. Biogeochemistry 2011;106:137–56.
Arrigo KR. Marine microorganisms and global nutrient cycles. Nature 2005;438:122.
Booth BC. Estimating cell concentrations and biomass of autotrophic plankton using microscopy. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ, editors. Handbook of methods in aquatic microbial ecology. Boca Raton: Lewis Publishers; 1993. p. 199–205.
Børsheim KY, Bratbak G. Cell volume to cell carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Mar Ecol Prog Ser 1987;36:171–5.
Børsheim KY, Vadstein O, Myklestad S, Reinertsen H, Kirkvold S, Olsen Y. Photosynthetic algal production, accumulation and release of phytoplankton storage carbohydrates and bacterial production in a gradient in daily nutrient supply. J Plankton Res 2005;27(8):743–55.
Borum J. Shallow waters and land/sea boundaries. In: Jørgensen BB, Richardson K, editors. Eutrophication in coastal marine ecosystemsAmerican Geophysical Union; 1996. p. 179–203.
Capriulo GM, Smith G, Troy R, Wikfors GH, Pellet J, Yarish C. The planktonic food web structure of a temperate zone estuary; and its alteration due to eutrophication. Hydrobiologia 2002;475/476:263–333.
Carstensen J, Sanchez-Camacho M, Duarte CM, Krause-Jensen D, Marb N. Connecting the Dots: Responses of Coastal Ecosystems to Changing Nutrient Concentrations. Environ Sci Technol 2011;45:9122–32.
Caruso G, Leonardi M, Monticelli LS, Decembrini F, Azzaro F, Crisafi E, et al. Assessment of the ecological status of transitional waters in Sicily (Italy): First characterisation and classification according to a multiparametric approach. Mar Pollut Bull 2010;60:1682–90.
Cloern JE. Our evolving conceptual model of the coastal eutrophication problem. Mar Ecol Prog Ser 2001;210:223–53.
Colijn F, Hesse KJ, Ladwig N, Tillmann U. Effects of the large-scale uncontrolled fertilization process along the continental coastal North Sea. Hydrobiologia 2002;484:133–48.
Devlin M, Bricker S, Painting S. Comparison of five methods for assessing impacts of nutrient enrichment using estuarine case studies. Biogeochemistry 2011;106:177–205.
Droop MR. 25 years of algal growth-kinetics - A personal view. Bot Mar 1983;26:99–112.
Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole SW, Hillebrand H, et al. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 2007;10:1135–42.
Engqvist A. Long-term Nutrient Balances in the Eutrophication of the Himmerfjärden Estuary. Estuar Coast Shelf Sci 1996;42:483–507.
Falkowski P. Rationalising elemental ratios in unicellular algae. J Phycol 2000;36:3–6.
Ferreira JG, Vale C, Soares CV, Salas F, Stacey PE, Bricker SB, et al. Monitoring of coastal and transitional waters under the E.U. Water Framework Directive. Environ Monit Assess 2007;135:195–216.
Ferreira JG, Andersen JH, Borja A, Bricker SB, Camp J, Cardoso da Silva M, et al. Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. Estuar Coast Shelf Sci 2011;93:117–31.
Geider R, La Roche J. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 2002;37:1–17.
Gismervik I, Olsen Y, Vadstein O. Micro- and mesozooplankton response to enhanced nutrient input – A mesocosm study. Hydrobiologia 2002;484:75–87.
Grasshoff K, Ehrhardt M, Kremling K. Methods of Seawater Analysis. Basel: Verlag Chemie; 1983.
Grizzetti B, Bouraoui F, Aloe A. Changes of nitrogen and phosphorus loads to European seas. Glob Change Biol 2012;18:769–82.
HELCOM. Eutrophication in the Baltic Sea – An integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region. Balt Sea Environ Proc No. 115B; 2009. [http://www.helcom.fi/].
Hoikkala L, Lahtinen T, Perttil M, Lignell R. Seasonal dynamics of dissolved organic matter on a coastal salinity gradient in the northern Baltic Sea. Continental Shelf Research 2012;45:1–14.
Howarth RW, Marino R. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnol Oceanogr 2006;51(1, part 2):364–76.
Kitsiou D, Karydis M. Coastal marine eutrophication assessment: A review on data analysis. Environ Int 2011;37:778–801.
Klausmeier CA, Litchman E, Daufresne T, Levin SA. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 2004;429:171–4.
McCarthy JJ, Yilmaz A, Coban-Yildiz Y, Nevins JL. Nitrogen cycling in the offshore waters of the Black Sea. Estuar Coast Shelf Sci 2007;74:493–514.
Nixon SW, Buckley BA. "A Strikingly Rich Zone" - Nutrient enrichment and secondary production in coastal marine ecosystems. Estuaries 2002;25:782–96.
Nixon SW, Pilson MEQ. Nitrogen in estuarine and coastal marine ecosystems. In: Carpenter EJ, Capone DG, editors. Nitrogen in the marine environment. Academic; 1983. p. 565–648.
Nutman SR. A fish cultivation experiment in an arm of a sea-loch. II. Observations on some hydrographic factors in Kyle Scotnish. Proc R Soc Edinb B 1950;64:5–35.
Olsen Y. Evaluation of competitive ability of *Staurastrum luetkemuellerii* (Chlorophyceae) and *Microcystis aeruginosa* (Cyanophyceae) under P limitation. J Phycol 1989;25:486–99.
Olsen Y, Agusti S, Andersen T, Duarte CM, Gasol P, Gismervik I. A comparative study of responses in planktonic food web structure and function in contrasting European coastal waters exposed to experimental nutrient addition. Limnol Oceanogr 2006;51:488–503.
Olsen Y, Andersen T, Gismervik I, Vadstein O. Protozoan and metazoan zooplankton-mediated carbon flows in nutrient-enriched coastal planktonic communities. Mar Ecol Prog Ser 2007;331:67–83.
Olsen Y, Andersen T, Gismervik I, Vadstein O. Marine heterotrophic bacteria, protozoan and metazoan zooplankton may experience protein N or mineral P limitation in coastal waters. Mar Ecol Prog Ser 2011;436:81–100.
Orr AR. An experiment in marine fish cultivation: II. Some physical and chemical conditions in a fertilised sea-loch (Loch Craiglin, Argyll). Proc R Soc Edinb B 1947;63:3–20.
OSPAR Commission. Ecological quality objectives for the Greater North Sea with regard to nutrients and eutrophication effects. OSPAR Background Document on Eutrophication 1-904426-71-9; 200533 [Publication Number: 2005/229].
Pitta P, Tsapakis M, Apostolaki1 ET, Tsagaraki T, Holmer M, Karakassis I. Ghost nutrients' from fish farms are transferred up the food web by phytoplankton grazers. Mar Ecol Prog Ser 2009;374:1–6.
Ptacnik R, Andersen T, Tamminen T. Performance of the Redfield ratio and a family of nutrient limitation indicators as thresholds for phytoplankton N vs. P limitation. Ecosystems 2010;13:1201–14.
Putt M, Stoecker DK. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 1989;34:1097–103.
Raymont JEG. A fish cultivation experiment in an arm of a sea-loch. W. The bottom fauna of Kyle Scomish. Proc R Soc Edinb B 1950;64:65–108.
Santinelli C, Ibello V, Lavezza R, Civitarese G, Seritti A. New insights into C, N and P stoichiometry in the Mediterranean Sea: The Adriatic Sea case. Continental Shelf Research 2012;44:83–93.
Schiewer U. 30 years' eutrophication in shallow brackish waters - lessons to be learned. Hydrobiologia 1998;363:73–9.
Sommer U, Stibor H. Copepoda-Cladocera-Tunicata: The role of three major mesozooplankton groups in pelagic food webs. Ecol Res 2002;17:161–74.
Sommer U, Hansen T, Stibor H, Vadstein O. The persistence of phytoplankton responses to different Si:N ratios under mesozooplankton grazing: a mesocosm study with Northeast Atlantic plankton. Mar Ecol Prog Ser 2004;278:67–75.
Sommer U, Hansen T, Blum O, Holzner N, Vadstein O, Stibor H. Copepod and microzooplankton grazing n mesocosms fertilised with different Si:N ratios: no overlap between food spectra and Si:N-influence on zooplankton trophic level. Oceologia 2005;142:274–83.
Stibor H, Vadstein O, Lippert B, Roederer W, Olsen Y. Calanoid copepods and nutrient enrichment determine population dynamics of the appendicularian *Oikopleura dioica*: a mesocosm experiment. Mar Ecol Prog Ser 2004a;270:209–15.

Stibor H, Vadstein O, Diehl S, Gelzleichter A, Hansen T, Hantzsche F, et al. Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. Ecol Lett 2004b;7:321–8.
Stibor H, Gelzleichter A, Hantzsche F, Sommer U, Striebel M, Vadstein O, et al. Combining dialysis and dilution techniques to estimate gross growth rate of phytoplankton and grazing by micro- and mesozooplankton in sit. Arch Hydrobiol 2006;167(1–4):403–19.
Strathman RR. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 1967;12:411–8.
Strickland JDH, Parsons TR. A Practical Handbook of Seawater Analysis. 2nd ed. Ottawa: Fisheries Research Board of Canada; 1972310.
Sundt-Hansen LE, Olsen Y, Stibor H, Heldal M, Vadstein O. Trophic cascades mediated by copepods, not nutrient supply rate, determined the development of picocyanobacteria. Aquat Microb Ecol 2006;45:207–18.
Tett P. Fish farm wastes in the ecosystem. In: Holmer M, Black K, Duarte CM, Marbà N, Karakassis I, editors. Aquaculture in the ecosystem. Dordrecht: Springer; 2008.
Thingstad TF, Krom MD, Mantoura RFC, Flaten GAF, Groom S, Herut B, et al. Nature of phosphorus limitation in the Ultraoligotrophic Eastern Mediterranean. Science 2005;309:1068–71.
Topcu D, Behrendt H, Brockmann U, Claussen U. Natural background concentrations of nutrients in the German Bight area (North Sea). Environ Monit Assess 2011;174:361–88.
Turner RE. Element Ratios and Aquatic Food Webs. Estuaries 2002;25:694–703.
Vadstein O, Olsen Y. Chemical composition and PO4 uptake of limnetic bacterial communities cultured in chemostat under P limitation. Limnol Oceanogr 1989;34:939–46.
Vadstein O, Stibor H, Lippert B, Løseth K, Roederer W, Sundt-Hansen L, et al. Moderate increase in the biomass of omnivorous copepods may ease grazing control of planktonic algae. Mar Ecol Prog Ser 2004;270:199–207.
Vadstein O, Andersen T, Reinertsen H, Olsen Y. Carbon, nitrogen and phosphorus resource supply and utilisation for coastal planktonic heterotrophic bacteria in a gradient of nutrient loading. Mar Ecol Prog Ser 2012;447:55–75.
Verity PG, Sieracki ME. Use of colour image analysis and epifluorescence microscopy to measure plankton biomass. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ, editors. Handbook of methods in aquatic microbial ecology. Boca Raton, FL: Lewis Publishers; 1993. p. 327–37.
Vollenweider RA. Advances in defining critical load levels for phosphorus in lake eutrophication. Mem Ist Ital Idrobiol 1976;33:53–83.
Wassmann P. Relationship between primary and export production in the boreal coastal zone of the North-Atlantic. Limnol Oceanogr 1990;35:464–71.