Pergamon

Marine Pollution Bulletin Vol. 39, Nos. 1–12, pp. 212–223, 1999

Printed in Great Britain
0025-326X/99 $ - see front matter

PII: S0025-326X(99)00025-9

Bioassessment Techniques for Monitoring of Eutrophication and Nutrient Limitation in Coastal Ecosystems

JENS ERIK LYNGBY*, SVERRE MORTENSEN and NICK AHRENSBERG
VKI Institute for the Water Environment, 11 Agern Alle, DK-2970 Hørsholm, Denmark Agern Alle 11, DK-2970 Hørsholm, Denmark

Bioassesment by the use of the macroalga, *Ulva lactuca* L., was carried out in the Limfjord, Denmark, to assess the significance of nitrogen and phosphorus as limiting factors for primary production during 1985, 1993, 1994 and 1995 and for the detection of changes in eutrophication levels.

Minimum and critical tissue concentrations for nitrogen and phosphorus in macroalgae were identified. The concentrations of nitrogen were generally below the critical concentration level in June–October in 1985, 1993, and 1995 but in 1994 nitrogen was only limiting for primary production in short periods. Only in early spring in 1985 and 1993 were the tissue concentrations of phosphorus below the critical concentration level, whereas in 1994 up to 3–4 months showed phosphorus limited growth, indicating that significant changes in limitation patterns can occur between different years.

It was concluded that the use of biomonitoring techniques is well suited as a bioassessment method for direct detection and for providing a time-integrated measure of nutrient availability in coastal waters, and thus for assessing ecosystem health with regard to eutrophication. It is recommended that biomonitors and the concept of critical tissue concentrations should be used in environmental management and incorporated in future monitoring programmes. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: nutrient limitation; eutrophication; ulva lactuca; phytoplankton; nitrogen; phosphorus.

Introduction

The eutrophication of coastal waters caused by nitrogen and phosphorus has made it relevant to develop more sensitive techniques than the presently indirect methods for assessing changes in the eutrophication levels. Experience has shown that the significance of nutrients as limiting factors for production cannot be assessed from water chemistry data and traditional chlorophyll and productivity measurements (Lyngby and Mortensen, 1994). Such information can therefore not be incorporated into management decisions for which reduction measures need to be implemented. Another aspect is that after implementation it is not possible to assess the actual effect on the coastal ecosystems. From 1981 onwards different biomonitoring techniques have been used for assessing eutrophication levels and changes in different coastal zones in Denmark. The techniques are based on a study of natural phytoplankton assemblages, selected macroalgae species (*Ulva lactuca*, *Ceramium rubrum*) and the use of established critical tissue concentrations. The use of these techniques will be demonstrated in this paper.

Low levels of dissolved nitrogen and phosphorus can be found in coastal waters during the growth season. Little information is, however, available concerning their relative and quantitative importance in limiting growth and productivity in such areas (Nyholm and Lyngby, 1988). Based on water chemistry data and traditional chlorophyll and productivity measurements, it cannot be concluded which nutrient(s) is(are) kinetically limiting (Nyholm and Lyngby, 1988). In reality, only the following negative conclusion can be drawn, "that if a nutrient is found present in a biologically available form in a significant concentration, this nutrient is not kinetically limiting".

Gerloff and Krombholz (1966) used information concerning critical levels of nitrogen and phosphorus in aquatic plants to evaluate the significance of nitrogen and phosphorus as limiting factors for macrophyte growth in lakes. Several studies have been carried out in the laboratory to establish critical and minimum nutrient concentrations for different species of algae (e.g., Chapman *et al.*, 1978; Hanisak, 1979; Gordon *et al.*, 1981; Schramm *et al.*, 1988). The data show in

*Corresponding author.

general a very low variation in the levels recorded between the different species.

Only a few attempts have been made to apply critical tissue concentrations in order to evaluate *in situ* the significance of nitrogen and phosphorus as limiting factors in coastal waters (Chapman and Craigie, 1977; Birch *et al.*, 1981; Lapointe, 1987; Schramm *et al.*, 1988; Ho, 1998; Lyngby, 1990; Lyngby and Mortensen, 1994; Lyngby and Mortensen, 1995).

The aim of the present study was to investigate the relative importance of nitrogen and phosphorus as limiting factors for primary producers during the production season by using the macroalga *Ulva lactuca* as a biomonitor. Furthermore, the study should elucidate site dependent differences in nutrient availability in the area and changes from year to year.

Materials and Methods

Study area

The investigation was made in the Limfjord, a rather shallow and enclosed water area located in the northern part of Jutland, Denmark. The Limfjord is highly eutrophic and is influenced by sewage outlets and agricultural run-off. One of the major eutrophication problems are the phytoplankton blooms causing oxygen deficiency and related detrimental effects on benthic flora and fauna, as well as aesthetic problems and unpleasant odours (e.g. Jørgensen, 1980). Three locations were selected for the study. Nissum Broad is located in the western part of the Limfjord and least affected by eutrophication. The Logstor Broad is affected to a certain extent but is located in one of the more open parts of the inner fjord. Skive Fjord is located in the innermost parts and is affected by a number of sources.

Sampling and analytical procedures

At three locations, Nissum Broad, Logstor Broad and Skive Fjord, buoys were installed (Fig. 1). At each location, ten discs (5.7 cm^2) of *Ulva lactuca* were transplanted to perforated plexiglas cages (diameter: 5.2 cm, volume: 530 cm^3) hanging from the buoy. The cages were incubated at 1 m below the surface at all stations and additionally at 3.5 m depth at one station.

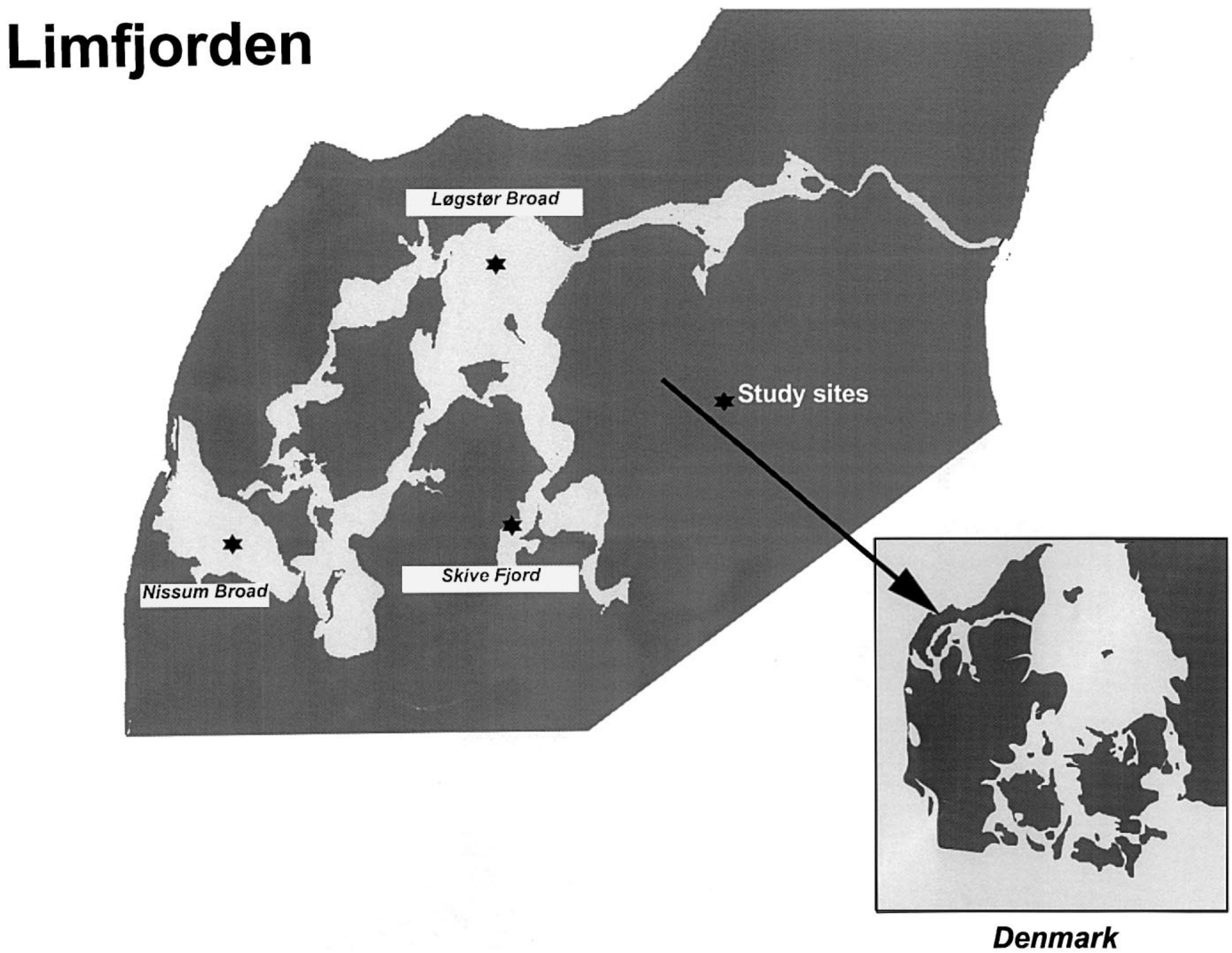

Fig. 1 Map of the Limfjord, Denmark, showing the sampling locations.

In the period May–October 1993–1995 sampling was carried out at fortnightly intervals. At each site, all discs were collected and replaced with new discs for the following period. The samples were put in plastic bags and kept cold and dark during transportation to the laboratory and then frozen. After thawing, the number and diameter of the discs were determined. The samples were dried to constant weight at 60°C and the biomass was determined. Then the samples were ground in a porcelain mortar. The homogenized samples were acid-digested and analyzed for total-nitrogen and total-phosphorus following the procedure given by Nordforsk (1975). Nitrogen was measured titrimetically and phosphorus colometrically (Pye Unicam SP8-500 UV/VIS) by the ascorbic acid method.

Statistical analyses

Statistical computations (analysis of variance and Duncan multiple range test) were performed on the log-transformed data.

The tissue concentrations are evaluated in relation to the critical tissue concentrations (CTC) and minimum tissue concentrations (MTC) of nitrogen and phosphorus, where the critical tissue concentration (CTC) of a nutrient is defined as the minimum internal concentration which is necessary for maximum growth (Gerloff and Krombholz, 1966). Furthermore, the minimum tissue concentration (MTC) is defined as the minimum concentration at which no growth occurs and all nutrients are bound in structural components. The information on the critical and minimum tissue concentrations used are collected from several studies conducted on different species (e.g., Gerloff and Krombholz, 1966; Chapman *et al.*, 1978; Hanisak, 1979; Gordon *et al.*, 1981; Schramm *et al.*, 1988; Lyngby, 1990; Lyngby and Mortensen, 1994). The results, however, obtained for the different species show little difference.

Results

Dissolved nutrients

The seasonal variation in the concentrations of dissolved nitrogen and phosphorus in 1994 at the three locations is shown in Figs. 2 and 3. The concentrations of dissolved nitrogen varied between 1440 μg N l^{-1} in April to 30 μg N l^{-1} in July. At all locations a significant drop in concentrations took place from April to middle of June.

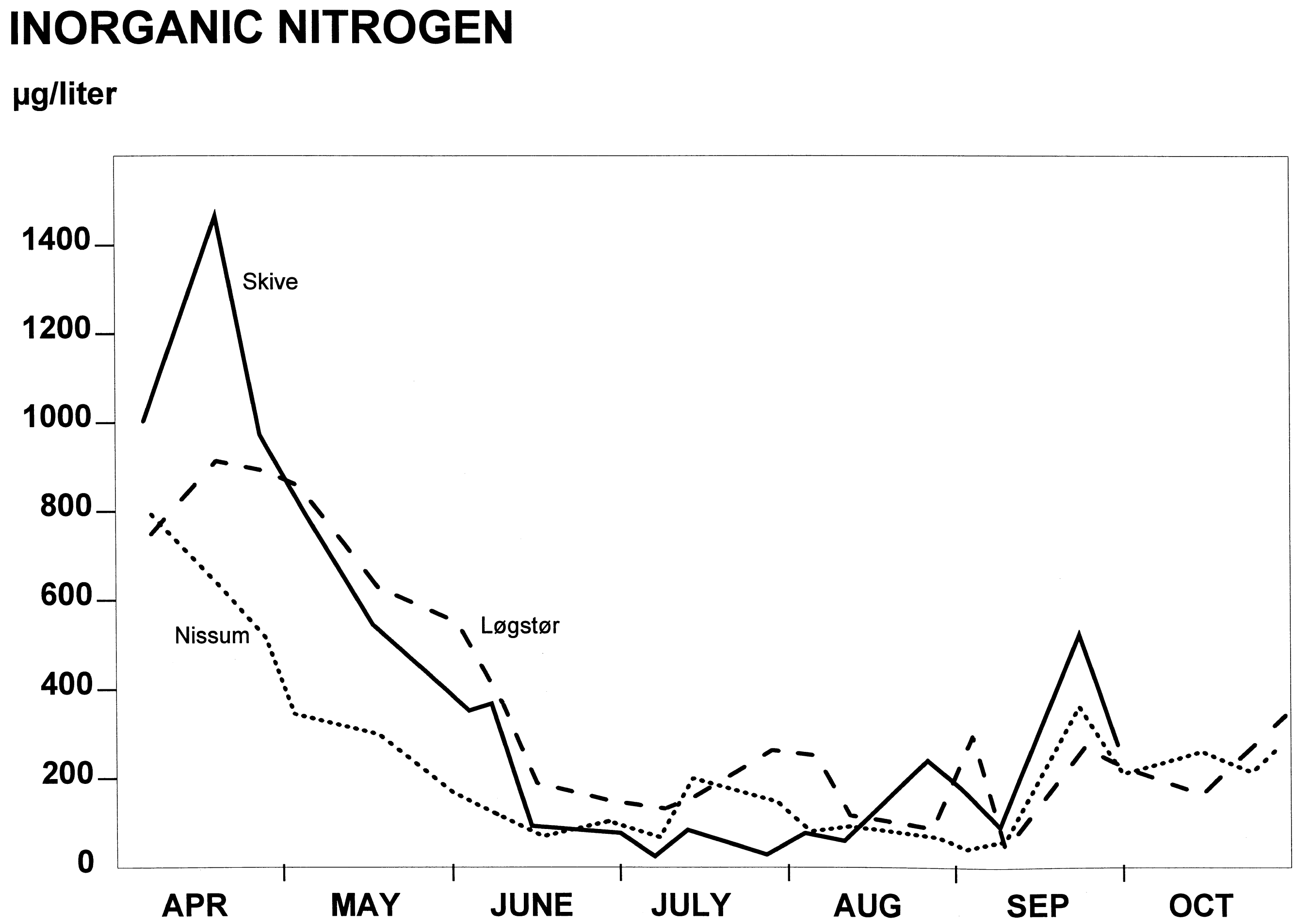

Fig. 2 Concentrations (μg l^{-1}) of dissolved nitrogen in the surface water at the three locations.

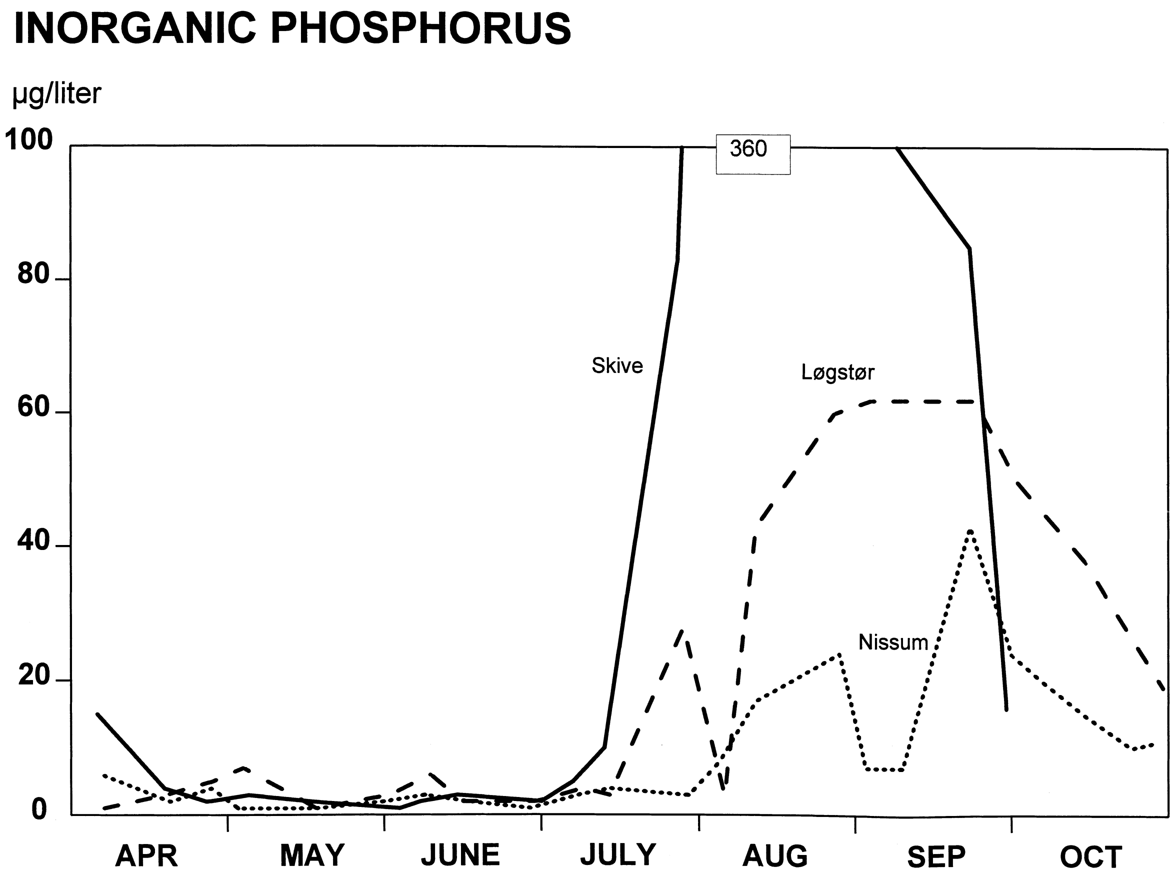

Fig. 3 Concentrations (µg l^{-1}) of dissolved phosphorus in the surface water at the three locations.

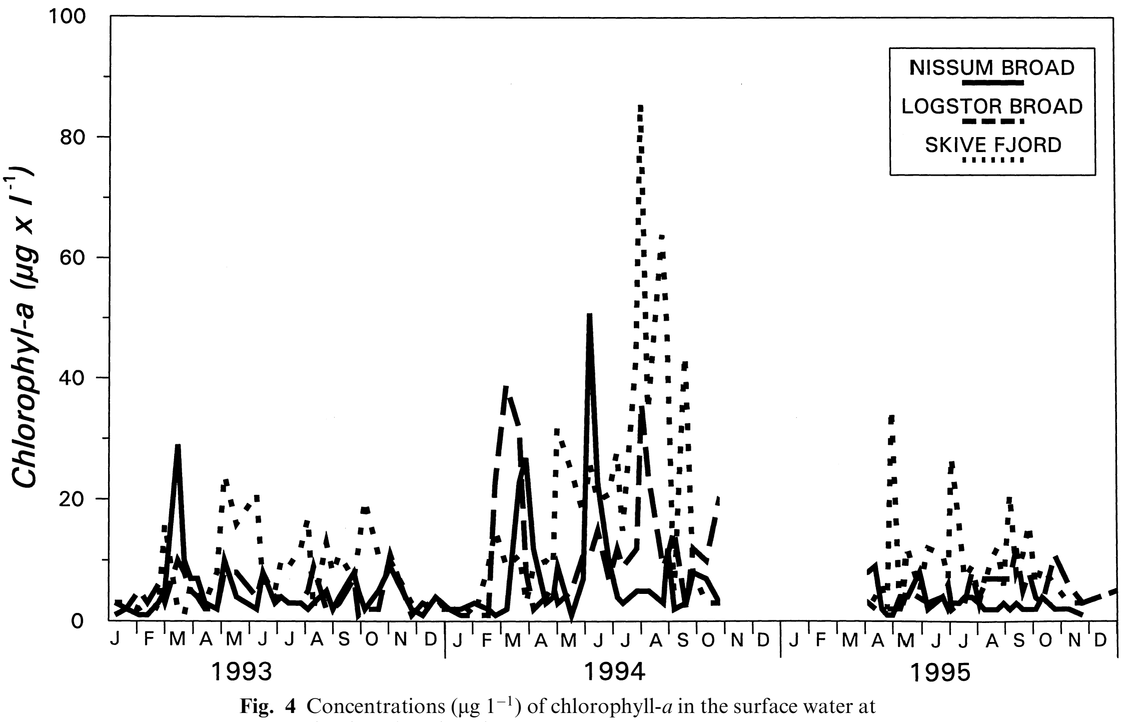

Fig. 4 Concentrations (µg l^{-1}) of chlorophyll-*a* in the surface water at the three locations in 1993–1995.

The variation in dissolved phosphorus concentrations was pronounced, varying from 2 μg P l^{-1} in May–June to 360 μg P l^{-1} in August. Significant differences can be seen in the concentration levels between the three locations in July–August. These high concentrations of inorganic phosphorus in 1994 were attributed to the severe oxygen deficiency seen in large areas of the Limfjord.

Chlorophyll-a

In Fig. 4 the chlorophyll-*a* concentrations recorded in 1993–95 at the three locations are shown. The highest chlorophyll-*a* concentrations at all locations were found in 1994. In Skive Fjord the average chlorophyll-*a* level from June–September was 35 μg l^{-1} compared to 11 μg l^{-1} in 1993. On single occasions values of almost 90 μg l^{-1} were recorded.

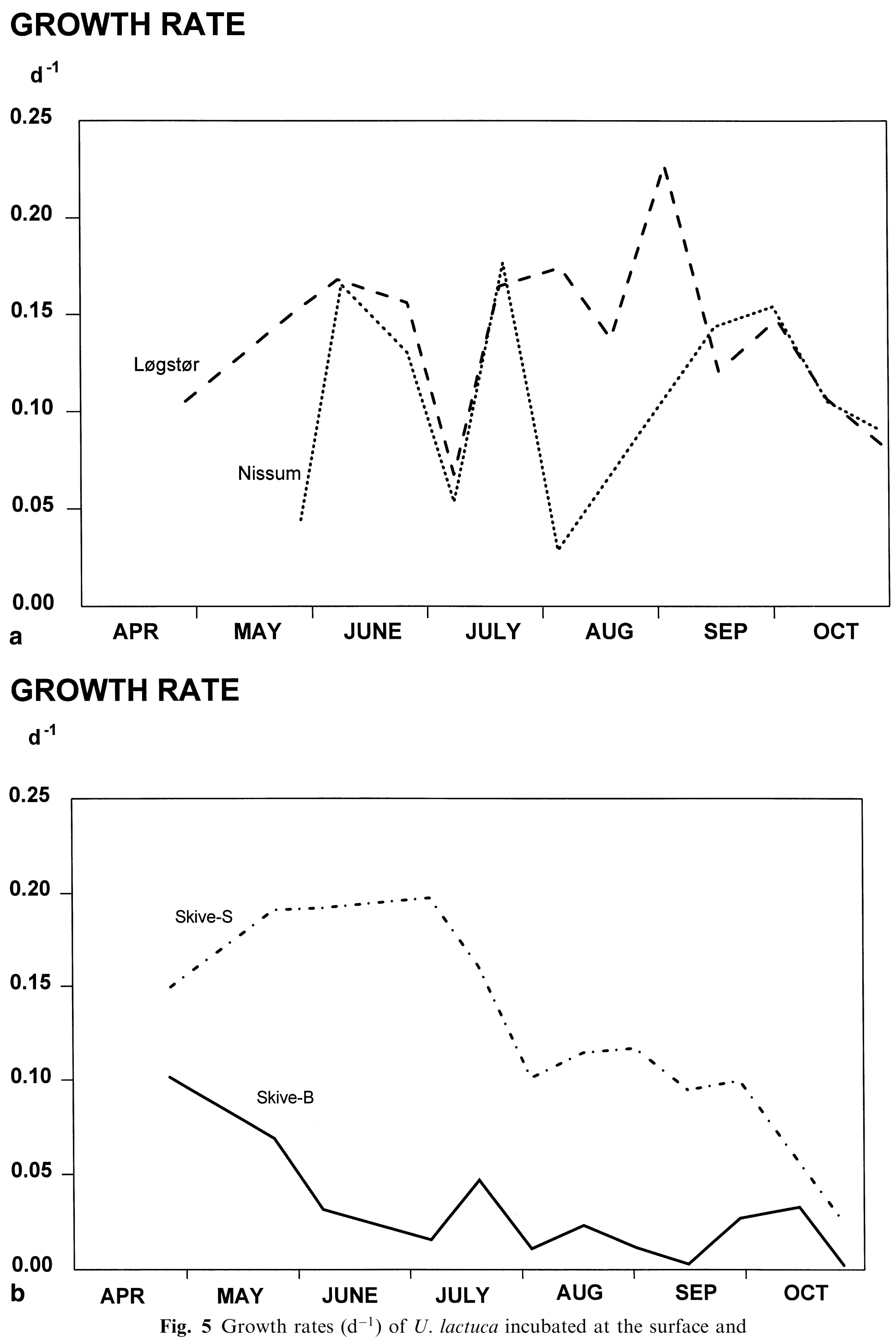

Fig. 5 Growth rates (d^{-1}) of *U. lactuca* incubated at the surface and bottom at the three locations in 1994.

Growth of U. lactuca

In Fig. 5 A and B the growth rates of *U. lactuca* incubated in 1994 at the surface and on the bottom at the three locations during the different periods are shown. The growth rates of the discs incubated at the surface were significantly ($p < 0.01$) greater than on the bottom. During the period May to middle July the growth rates were significantly greater in Skive Fjord compared to the two other locations, whereas in the remaining part of the growth period it was lower than in Logstor Broad and partly Nissum Broad. The great difference between growth rates in the surface and bottom in Skive Fjord was due to the limited light available caused by the great biomass of phytoplankton.

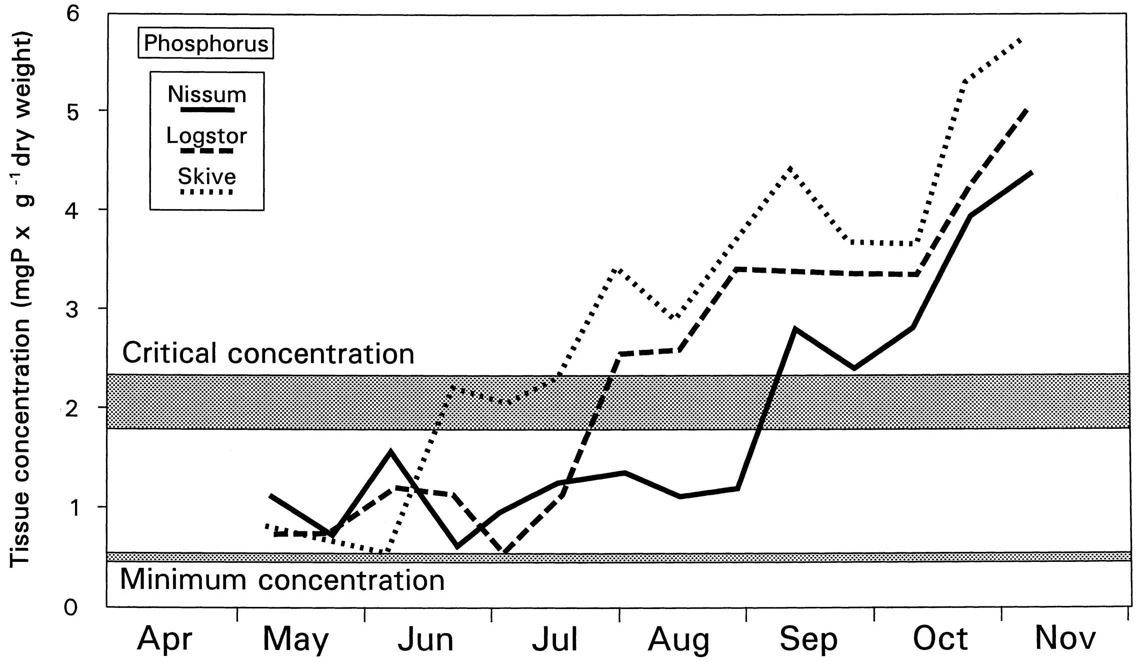

Fig. 6 Seasonal variation in the tissue concentrations (mg g^{-1} dry weight) of phosphorus in *U. lactuca* incubated at the three locations in 1995. Shaded area: critical tissue concentration (CTC), solid line: minimum tissue concentration (MTC).

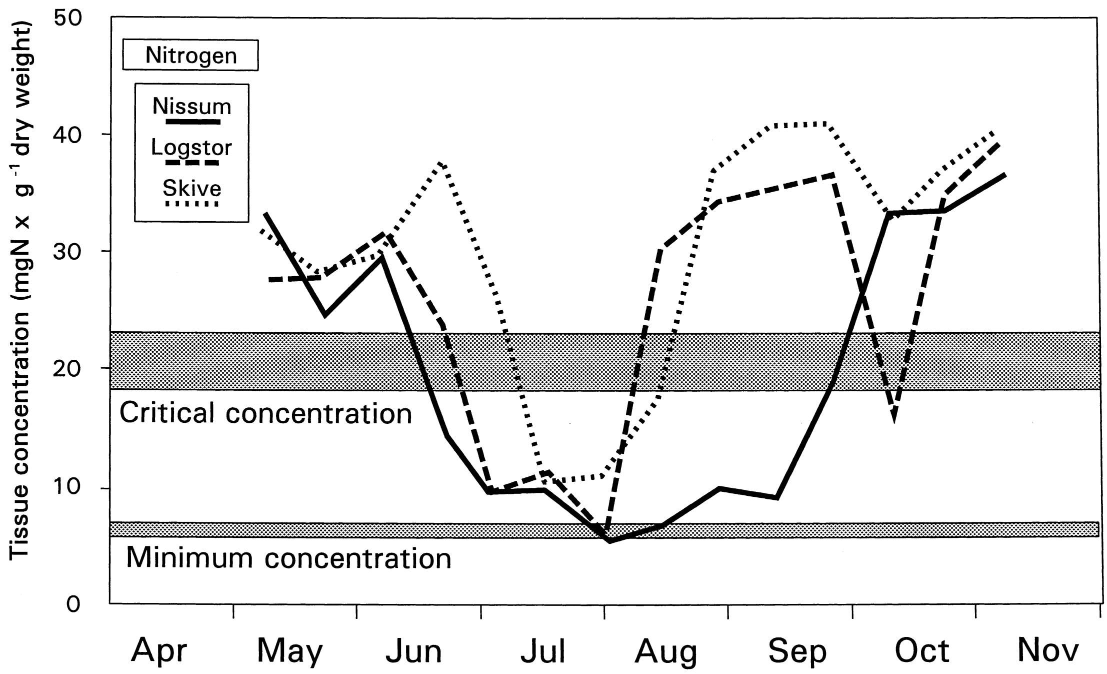

Fig. 7 Seasonal variation in the tissue concentrations (mg g^{-1} dry weight) of nitrogen in *U. lactuca* incubated at the three sites in 1995. Shaded areas: Critical tissue concentration (CTC), solid line: minimum tissue concentration (MTC).

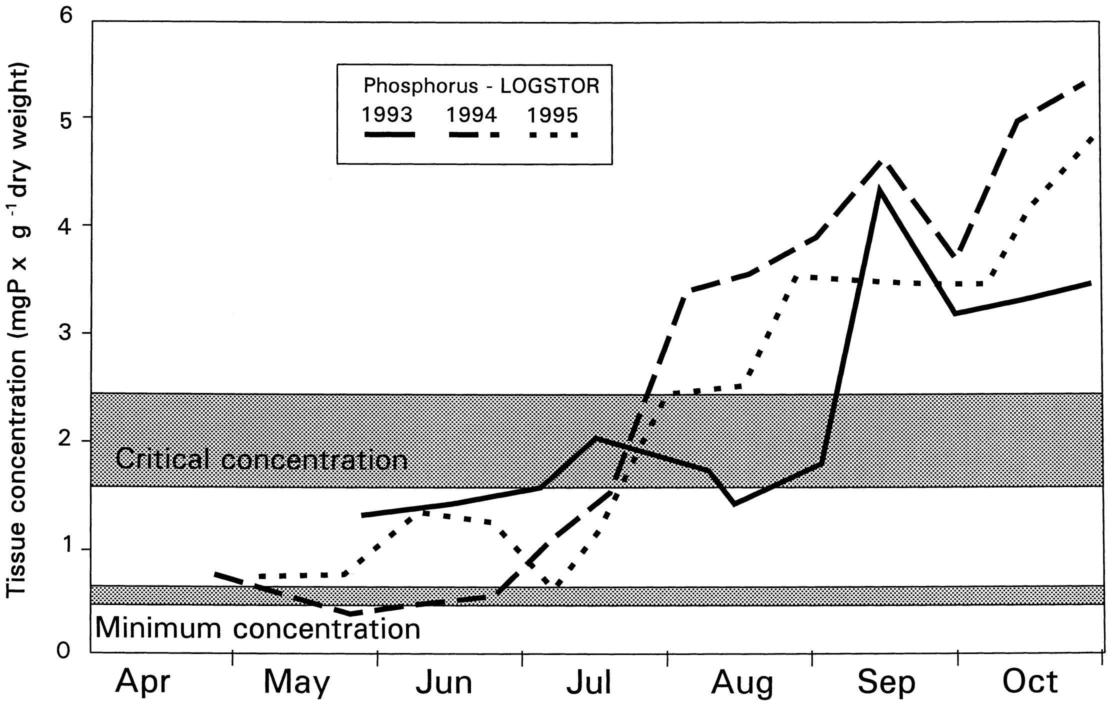

Fig. 8 Seasonal variation in the tissue concentrations (mg g^{-1} dry weight) of phosphorus in *U. lactuca* incubated at Logstor Broad in 1993–1995. Shaded area: critical tissue concentration (CTC), solid line: minimum tissue concentration (MTC).

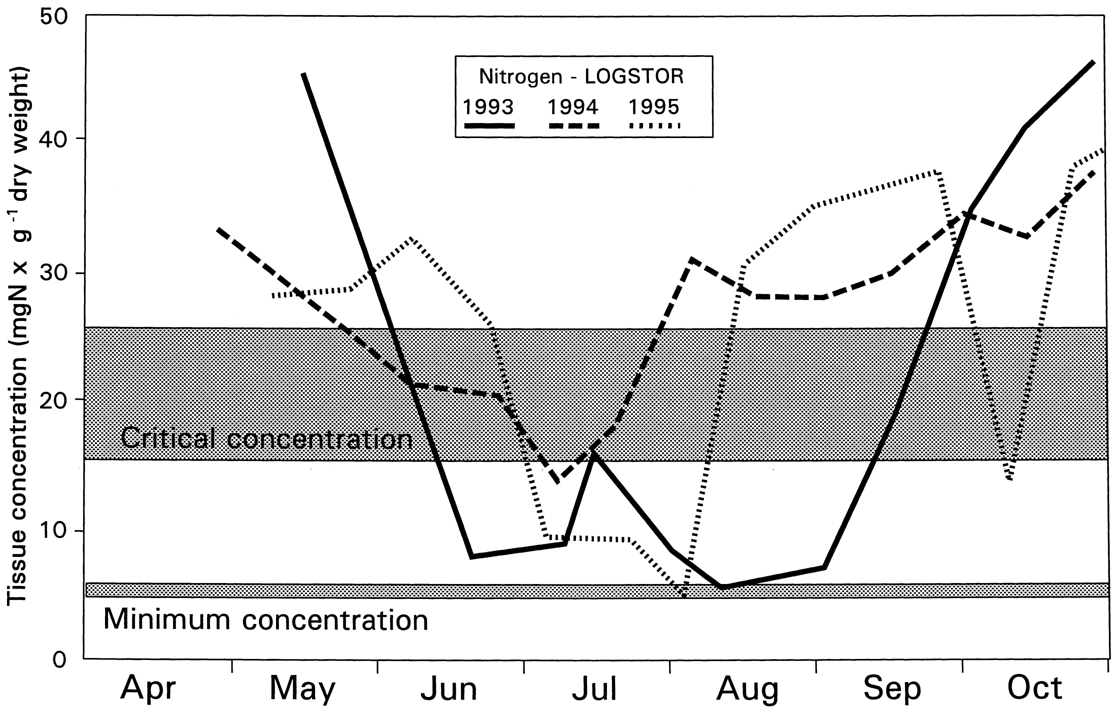

Fig. 9 Seasonal variation in the tissue concentrations (mg g^{-1} dry weight) of nitrogen in *U. lactuca* incubated at Logstor Broad in 1993–1995. Shaded areas: Critical tissue concentration (CTC), solid line: minimum tissue concentration (MTC).

The greatest growth rates were recorded from June to September. The observed growth rates which correspond to a doubling time of *U. lactuca* was 3–5 days showing the great growth potential of the macroalgae.

Tissue concentrations and nutrient limitation

In Fig. 6 the variation in internal concentrations of phosphorus in *Ulva* in 1995 from the three locations is shown. The critical (CTC) and minimum tissue concentrations (MTC) of phosphorus are indicated on the figure. In Nissum Broad the tissue concentrations are below the CTC from May until the end of August, whereas in Skive Fjord the tissue concentrations were only lower than the CTC until early June. The location in Logstor Broad show an intermediary situation. The concentration levels at Skive Fjord were significantly higher than Logstor Broad and then followed by

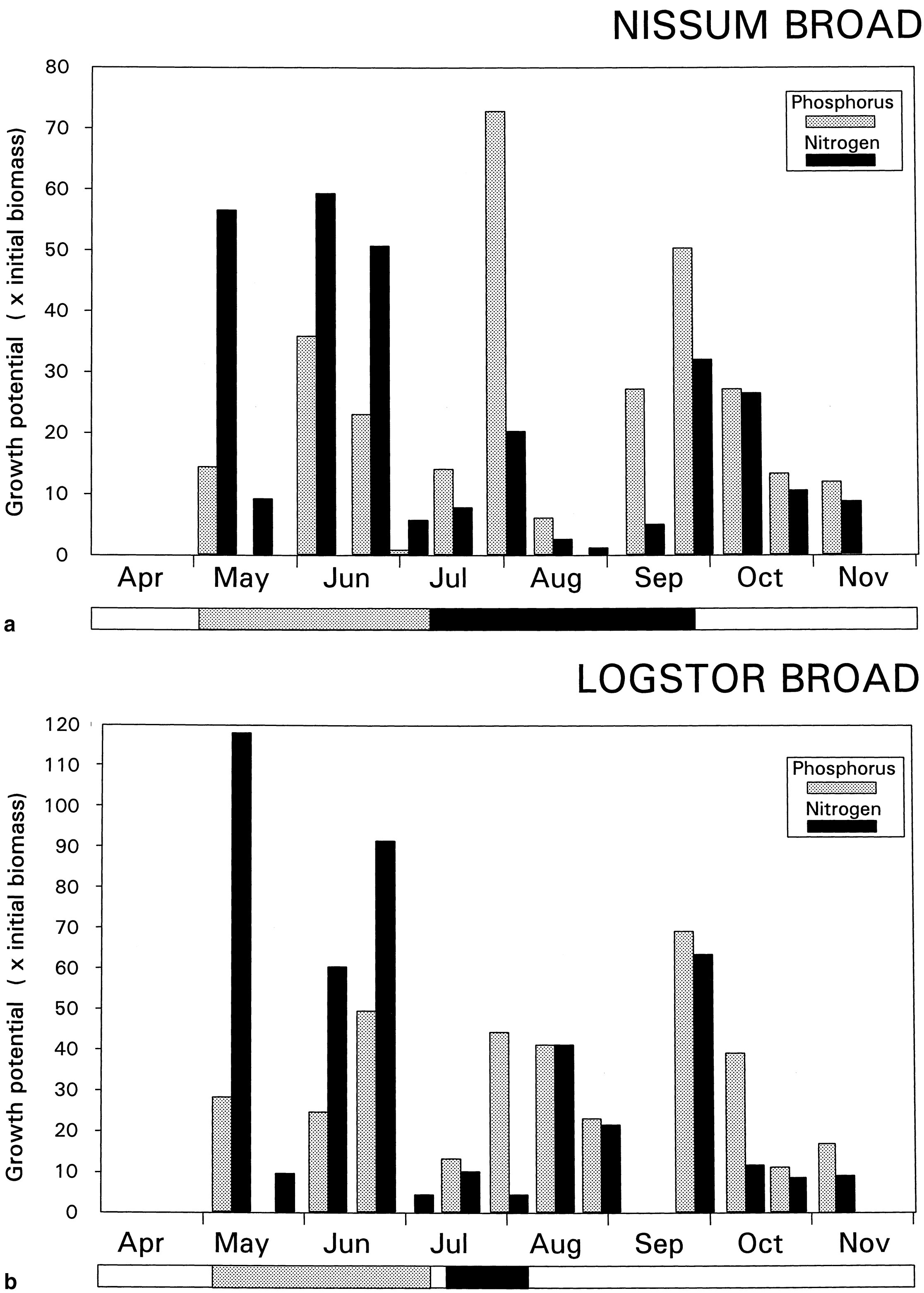

Fig. 10 (a) (b) *Caption overleaf.*

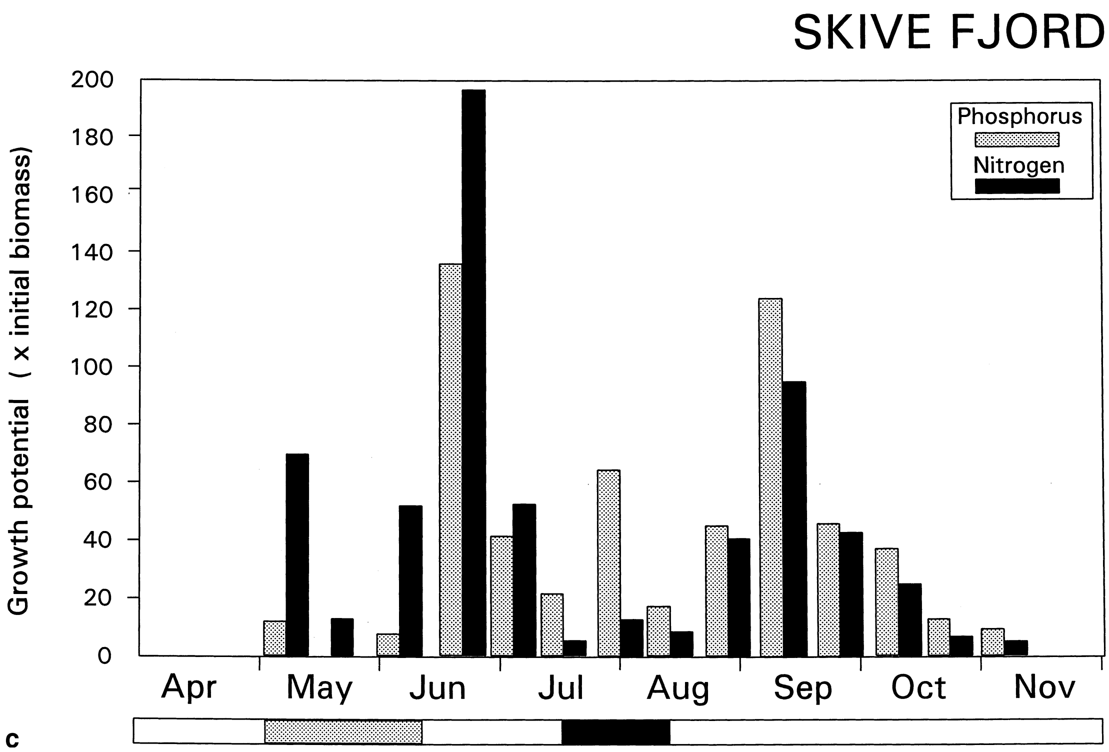

Fig. 10 Growth potential of *U. lactuca* incubated at the three locations. The figure shows how many times *Ulva* could increase the initial biomass based on the amount of nitrogen and phosphorus taken up.

Nissum Broad. The concentrations of phosphorus in *Ulva* show that this element was of significance as a limiting factor for algal production at the three locations, mainly in spring and early summer of 1995.

The variation in internal tissue concentrations of nitrogen in 1995 from the three locations is shown in Fig. 7. The variation in nitrogen concentrations through the study period showed significant differences ($p < 0.01$) between the locations. At Nissum Broad, the concentrations of tissue nitrogen were lower than the CTC from the middle of June to end of September, whereas the levels in Logstor Broad and Skive Fjord only were lower than the CTC for 1–1.5 months in July–August. However, the levels were lower than the CTC, showing that nitrogen was a potential limiting factor at all locations. In Nissum Broad, the concentrations of nitrogen were located around the MTC for approx 2.5 months

To illustrate the difference in nutrient availability and limitation between different years the results from 1993–1995 for tissue phosphorus concentrations in Logstor Broad are shown in Fig. 8. In 1993 the tissue concentrations are located at or above the CTC showing that phosphorus was not a significant limiting factor for production. In 1994–95 the tissue concentrations were lower than the CTC for the spring and early summer, indicating that phosphorus was a significant limiting factor. However, in all years the tissue concentrations of phosphorus from July to October showed that phosphorus was available in a quantity to sustain maximum growth rate of *Ulva*.

The tissue concentrations of nitrogen in 1993 at Logstor Broad was lower than the CTC from middle of June to the middle of September and in 1995 from July to early August (Fig. 9). In 1994, however, the tissue nitrogen concentration at no occasion was lower than the CTC, showing that nitrogen did not limit production during that year.

Flux of nutrients and growth potential

In Fig. 10, the growth potential of *Ulva* at the three locations during the different incubation periods is shown. The growth potential indicates how many times the macroalga can produce its initial biomass based on the amount of nitrogen or phosphorus taken up. An evaluation of this information in combination with the internal tissue concentrations shows which nutrient is the actual limiting factor during the incubation period. In Nissum Broad the greatest growth potential of nitrogen was recorded in May–June, where *Ulva* could increase its initial biomass up to 60 times based on the amount of nitrogen taken up. Considering the tissue concentrations, it could be seen that phosphorus was the actual limiting factor during this period in Nissum Broad. From July to end of September nitrogen was the limiting factor. The calculated growth potential during this period show a very limited availability of nitrogen.

In Logstor Broad *Ulva* could take up nitrogen so it could increase the initial biomass 120 times. The data showed that phosphorus was the actual limiting factor from May to early July. Phosphorus was the actual limiting factor in May – early June in Skive Fjord. Nitrogen was only limiting growth for one month in July–August.

Comparison of nutrient limitation between different years
In Fig. 11, the duration of nitrogen and phosphorus limitation in the years 1985, 1993–1995 in the three areas is shown. These data clearly show the differences between the areas and also the different limitation patterns between the different years. The results from 1995 are based on a bioassay technique using natural phytoplankton. The two methods give comparable results (Lyngby and Mortensen, 1995). The results show that in 1985 and 1993 nitrogen was the main limiting factor for primary production, whereas in 1994 phosphorus was the limiting factor.

Discussion

Significant differences in the internal concentrations of nitrogen and phosphorus in *Ulva* were recorded between the different locations. The results demonstrated the ability of *Ulva* to reflect the availability of nutrients during the growth season. Compared with the great variation in nutrient concentrations in coastal waters, the internal concentrations in macroalgae are more suitable than water quality data for monitoring, and provide a time-integrated measure for nutrient availability in coastal areas. Similar findings have been reported for *Cladophora* (Birch *et al.*, 1981) and *Ceramium rubrum* (Lyngby, 1990).

In Nova Scotia, Canada, studies with *Laminaria longicruris*, indicated that nitrogen was the main growth

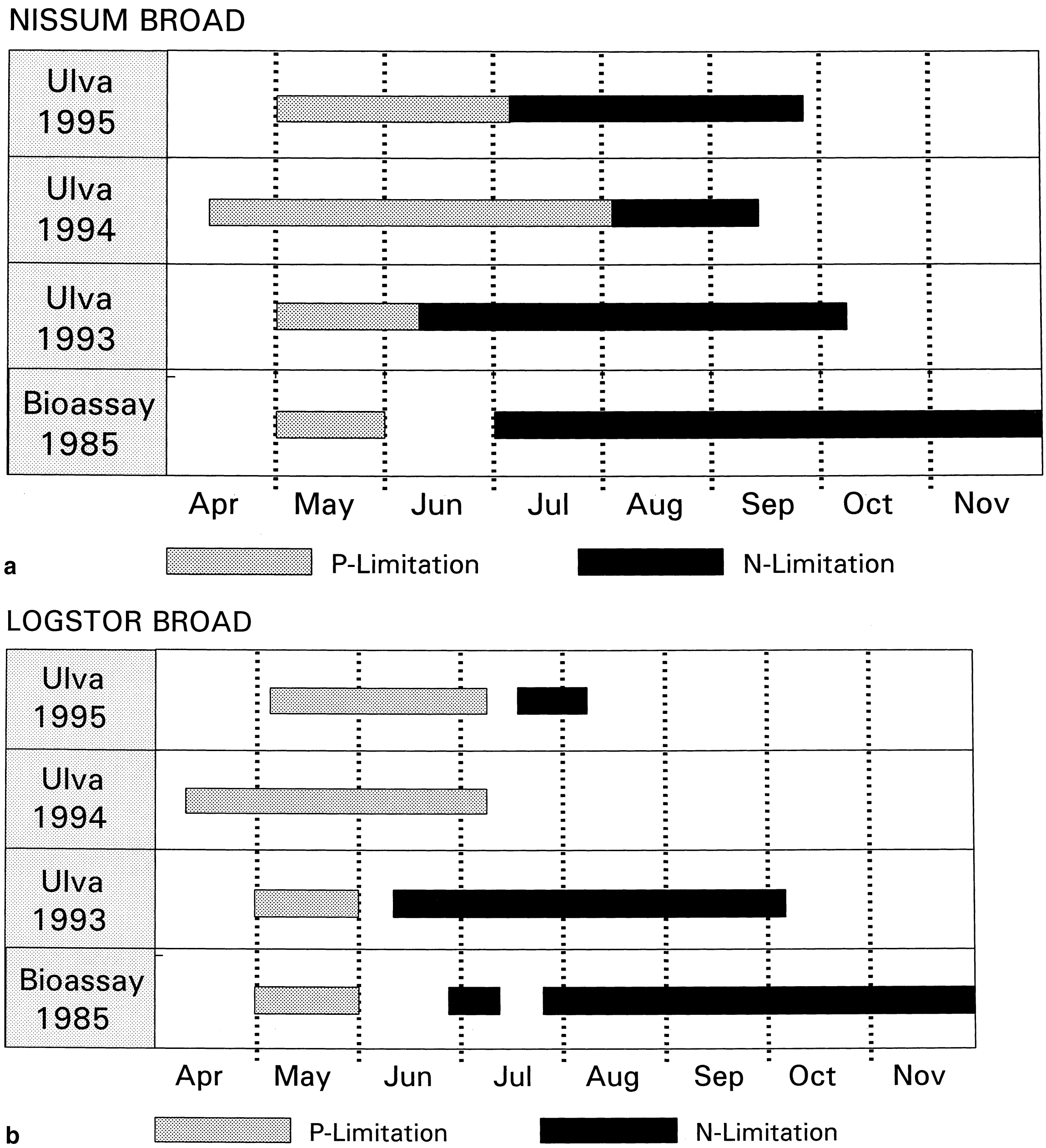

Fig. 11 (a) (b) *Caption overleaf.*

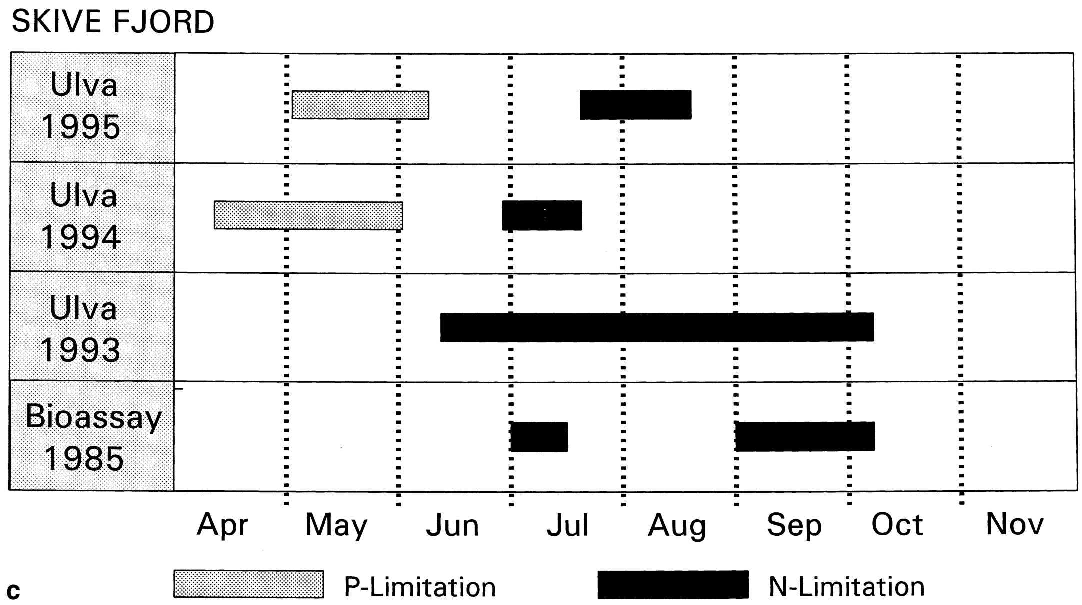

Fig. 11 Nitrogen and phosphorus limitation at the three locations in 1985 and 1993–1995.

controlling factor during the summer growth (Chapman and Craigie, 1977). Studies in the Kiel Bight, Germany, with *Fucus vesiculosus* and *Phycodrys rubens* indicated nitrogen limitation during summer (Schramm *et al.*, 1988). *Cladophora* has been shown to be phosphorus limited in an Australian estuary (Birch *et al.*, 1981). Lapointe (1987) showed that *Gracilaria tikvahiae* was phosphorus limited during summer in the Florida keys, and during winter, both nitrogen and phosphorus limited the growth. In a previous study (Lyngby, 1990) in the Limfjord, Denmark, it was found that phosphorus limited the growth of *Ceramium rubrum* in May, and that nitrogen was the main limiting factor during summer. Similar results using *Ulva* have been seen in other parts of Denmark (Lyngby and Mortensen, 1994, 1995). These data together with the present study indicate that site-specific data is necessary to identify the main limiting nutrient for a specific coastal area.

During the growth season, the majority of nutrients taken up are used to sustain a maximum growth rate, and therefore the tissue concentrations will only increase for nutrients available in sufficient quantities. The macroalgae have a great storage capacity of both nitrogen and phosphorus. In *Ulva*, nitrogen concentrations of approximately 55 mg g^{-1} dry weight have been reported (Ho, 1988; Lyngby, unpublished data) which is nine times higher than the MTC and three times higher than the concentration which is necessary to sustain a maximum growth rate. Similarly, tissue phosphorus concentrations of 3.5 mg g^{-1} dry weight were reported, being approximately seven times higher than the MTC. In the present study tissue phosphorus concentration of approx. 6 mg g^{-1} was recorded in Skive Fjord, being 12 times higher than the MTC.

The results obtained showed a pronounced nitrogen limitation in the study period in 1985 and 1993, whereas phosphorus showed limitation in 1994. The data also clearly show an increased eutrophication from the open western part of the Limfjord to the inner parts.

In the period from 1988 to 1991 the waste water treatment plants were extended to include full nutrient removal thus reducing the annual load of nitrogen and phosphorus approx. 10% and 65% compared to the load in 1985. The major load of nitrogen now comes from diffuse sources and is mainly due to the intensive agriculture around the fjord. The reduction of phosphorus has resulted in the period in spring with phosphorus limitation being increased compared to the situation in 1985, but it also seems to have resulted in nitrogen that could have been used for production in spring now being available for production during summer. The water quality data show high and even increasing concentrations of phosphorus during summer due to the release of phosphorus from the sediment. It will not, therefore, be possible to make phosphorus limiting for primary production during this period.

The biomass of phytoplankton in the summer was up 2–10 times higher in 1994 with phosphorus limitation compared to 1993 with nitrogen limitation. The nitrogen load was 16500 tons in 1993 and 22 500 tons in 1994. The results of the present study clearly show that for managing and improving the water quality in this area it is necessary to achieve a significant reduction in the nitrogen load. Otherwise, the water quality in the future will only be controlled by the annual run-off of nitrogen.

In conclusion, the data of the present investigation show that the use of critical tissue nutrient concentrations in macroalgae, offers a valuable tool for determining nutrient limitation and eutrophication levels in shallow coastal areas. The present study showed that both nitrogen and phosphorus were limiting factors for primary production during the growth season. To achieve a reasonable water quality, it will be necessary to reduce the nitrogen load. The results also clearly illustrate that this kind of information will be essential for effective water quality management and decisions regarding load reduction of nutrients.

The authors thank Limfjordsovervågningen for financing the study and providing the data on water chemistry and chlorophyll-*a*.

Birch, P. B., Gordon, D. M. and McComb, A. J. (1981) Nitrogen and phosphorus nutrition of *Cladophora* in the Peel-Harvey estuarine system. *Western Australia. Bot. Mar.* **24**, 381–387.

Chapman, A. R. O. and Craigie, J. S. (1977) Seasonal growth in *Laminaria longocruris*: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* **40**, 197–205.

Chapman, A. R. O., Markman, J. W. and Luning, K. (1978) Effects of nitrate on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *Mar. Biol.* **14**, 195–198.

Gerlof, G. C. and Krombolz, P. H. (1966) Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnol. and Oceanogr.* **11**, 529–537.

Gordon, D. M., Birch, P. B. and McComb, A. J. (1981) Effects of inorganic phosphorus and nitrogen on the growth of the estuarine Cladophora in culture. *Bot. Mar.* **24**, 93–106.

Hanisak, M. D. (1979) Growth patterns of *Codium fragile* spp. *tomentosoides* in response to temperature, irradiance, salinity and nitrogen source. *Mar. Biol.* **50**, 319–322.

Ho, Y. B. (1988) *Ulva lactuca* (Chlorophyta, Ulvales) in Hong Kong intertidal waters – its nitrogen and phosphorus contents and its use as a bioindicator of eutrophication. *Asian Mar. Biol.* **4**, 97–102.

Jørgensen, B. B., Seasonal oxygen depletion in the waters of a Danish Fjord and its effect on the benthic community. *Oikos* **34**, 68–76.

Lapointe, B. E. (1987) Phosphorus and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida keys : an experimental field study. *Mar. Biol.* **93**, 561–568.

Lyngby, J. E. (1990) Monitoring of nutrient availability and limitation using the marine macroalga *Ceramium rubrum* (Huds.) *G. Ag. Aquat. Bot.* **38**, 153–161.

Lyngby, J. E. and Mortensen, S. M. (1994) Assessment of nutrient availability and limitation using macroalgae. *J. Aquat. Ecosys. Health* **3**, 27–34.

Lyngby, J. E. and Mortensen, S. M. (1995) Biomonitoring of eutrophication levels in shallow coastal ecosystems. In: Eleftheriou, A., *et al.*, (Ed.), *Biology and Ecology of Shallow Coastal Waters*, Olsen & Olsen, Fredensborg, Denmark, pp. 39–44.

Nordforsk (1975) *Intercalibration of Sediment Chemical Analytical Procedures*, Nordforsk Publ., p. 6,.

Nyholm, N. and Lyngby, J. E. (1988) Algal bioassays in eutrophication research. A discussion in the framework of a mathematical analysis. *Water Res.* **22**, 1293–1300.

Schramm, W. D., Abele and Brewar, G. (1988) Nitrogen and phosphorus nutrition and productivity of two community forming seaweeds (*Fucus vesiculosus, Phycodrys rubens*) from the western Baltic (Kiel Bight) in the light of eutrophication processes. *Kieler Meeres Forsch. Sonderh. G* 221–240.