

Copyright ©

Es gilt deutsches Urheberrecht.

Die Schrift darf zum eigenen Gebrauch kostenfrei heruntergeladen, konsumiert, gespeichert oder ausgedruckt, aber nicht im Internet bereitgestellt oder an Außenstehende weitergegeben werden ohne die schriftliche Einwilligung des Urheberrechtinhabers. Es ist nicht gestattet, Kopien oder gedruckte Fassungen der freien Onlineversion zu veräußern.

German copyright law applies.

The work or content may be downloaded, consumed, stored or printed for your own use but it may not be distributed via the internet or passed on to external parties without the formal permission of the copyright holders. It is prohibited to take money for copies or printed versions of the free online version.

Kieler Meeresforschungen	Sonderheft 4	Kiel 1978
--------------------------	--------------	-----------

Nitrogen fixation by blue-green algae in the Baltic Sea

I. Rinne¹, T. Melvasalo², Å. Niemi³ and L. Niemistö³

¹ Water Conservation Laboratory, City of Helsinki Building Office

² National Board of Waters, Water Research Office

³ Institute of Marine Research,
Helsinki, Finland

Abstract

Nitrogen fixation measurements carried out in 1974 and 1975 in the Baltic Sea showed that one heterocyst fixed nitrogen c. 3.5 pg (2h)⁻¹. According to our preliminary calculations the amount of nitrogen fixed in 1974 in the northern and central Baltic proper was c. 100000 tons.

Zusammenfassung

Stickstoff-Fixierung durch Blaualgen in der Ostsee

Messungen der Stickstoff-Fixierung in den Jahren 1974 und 1975 ergaben, daß eine Heterocyste etwa 3,5 pg (2h)⁻¹ Stickstoff bindet. Nach unseren vorläufigen Berechnungen betrug die 1974 in der nördlichen und zentralen Ostsee gebundene Stickstoff-Menge etwa 100000 Tonnen.

Introduction

The blooms of nitrogen-fixing blue-green algae evidently play an important role in the nitrogen budget of the Baltic Sea. The dominant nitrogen-fixing blue-green algae seem to be favoured by upwelling of Baltic deep water rich in phosphorus and by the phosphorus load discharged from the coast, which contribute to lower the N: P ratio (cf. TARKIAINEN et al., 1975). The aim of the present study was to obtain information about the blooms of blue-green algae, the intensity of nitrogen fixation and the influence of environmental conditions.

Material and methods

The investigations of this project, "MERININNI", were performed onboard R/V Aranda. Preliminary measurements were carried out in August 1974 in the Baltic proper. In 1975 several areas, including a transect extending from the east of the Gulf of Finland to the Gotland deep, were studied in early August (Fig. 1). During this cruise the weather was sunny, calm and exceptionally warm (surface water > 19 °C). Blue-green algal blooms of varying intensity were observed throughout the study area. The sampling was performed during the light part of the day (4.00 a. m.–4.30 p. m.).

The chemical analyses were made on board by the chemical staff of the Institute of Marine Research (KOROLEFF, 1976). Primary production ability was measured *in vitro* (LASSIG and NIEMI, 1972). Nitrogen fixation was measured by the acetylene reduction method, according to BURRIS (1972) and VUORIO (1977). The samples were concentrated to about 100/1 with a 25 μm plankton net. Subsamples of 5 ml were injected into serum bottles of 12 ml. Three parallel subsamples were prepared.

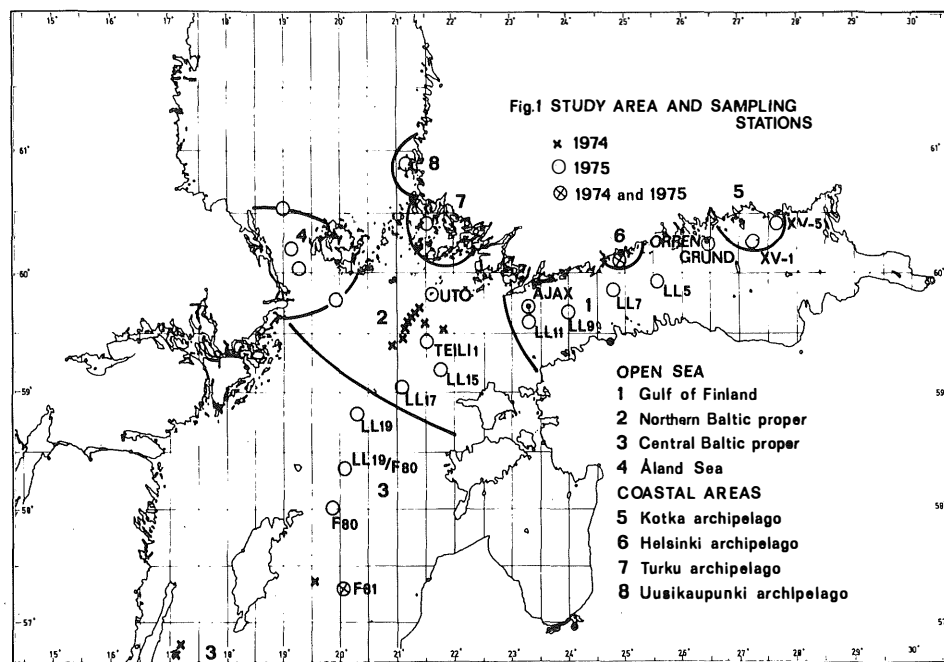


Figure 1
Areas studied and sampling stations

The acetylene concentration was 20% and the incubation time 2 h (18°C, 5000 lx). After that the reaction was stopped by adding Lugol's solution. The ethylene concentration was measured using a Carlo Erba Fractovap gas chromatograph. One mole of ethylene produced corresponds to 2/3 mole of NH_3 produced (KLUCAS, 1969). The phytoplankton was analysed on both water samples and net samples, which were preserved with Keefe's solution and counted by Utermöhl's technique (for details, see MELVASALO et al., 1973). From the net samples taken for the acetylene reduction measurements only the heterocystic blue-greens and the heterocysts were counted.

Environmental conditions

From late June 1975 the surface temperature of the northern Baltic Sea (Utö) gradually increased, reaching a maximum (23°C) in early August (Fig. 2). By then stable thermal stratification had developed throughout the areas studied (Fig. 3). The 15°C isotherm was found at c. 15 m in the Gulf of Finland and somewhat deeper in the northern Baltic proper. Cold winter water occurred between c. 40 and 60 m.

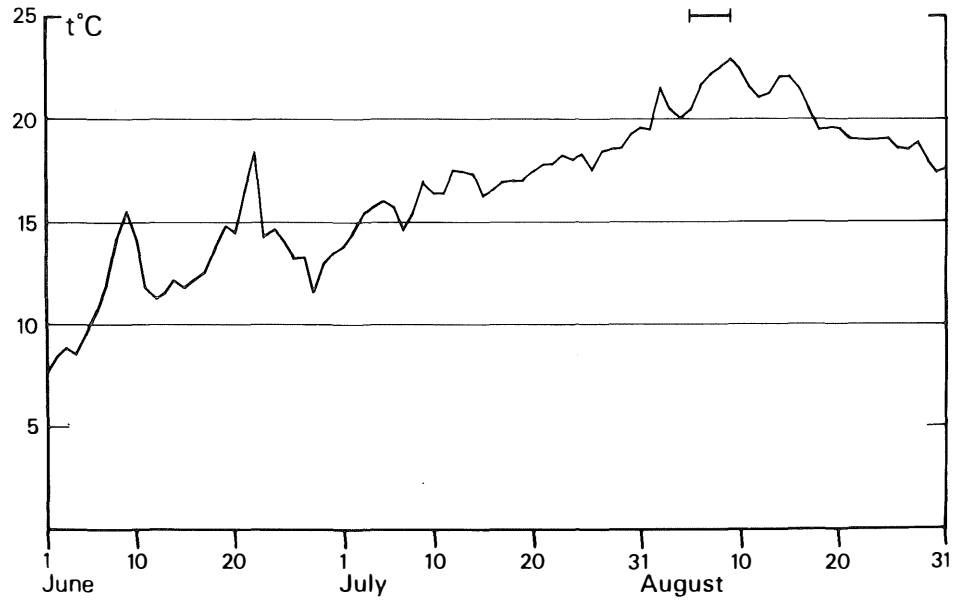


Figure 2
Surface water temperature at 2.00 p.m. in June–August 1975 at the Utö station, area 2 (Bar = cruise 1975)

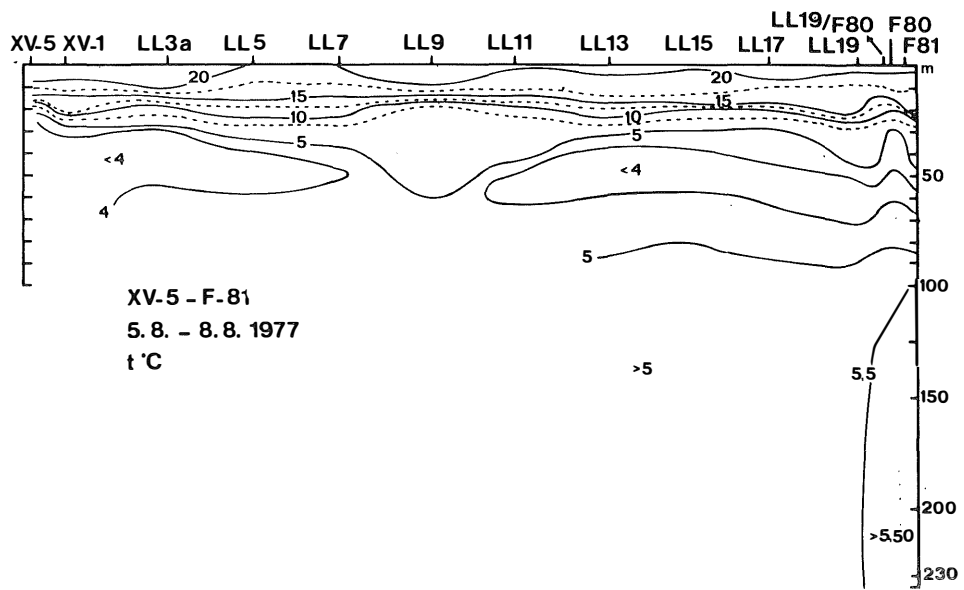


Figure 3
Temperature on transect XV-5 – F 81 in 1975

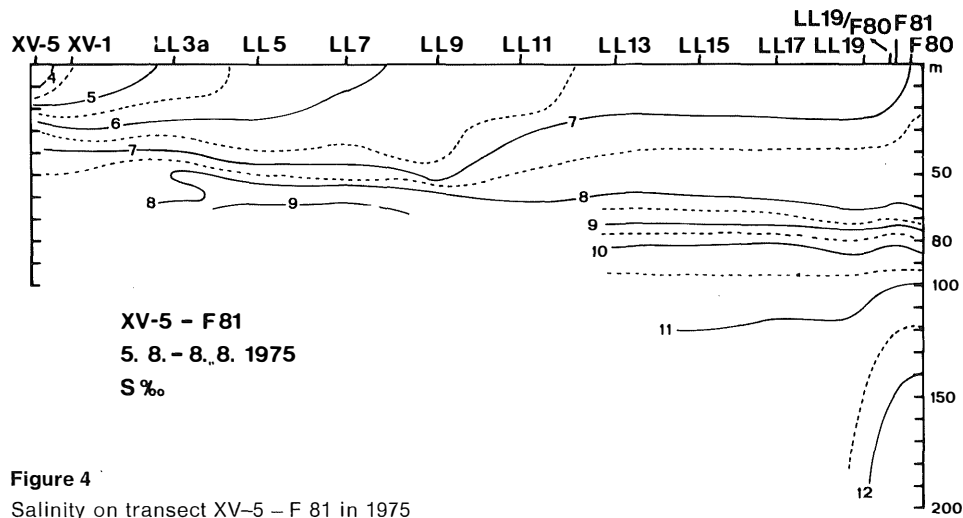


Figure 4
Salinity on transect XV-5 - F 81 in 1975

The surface salinity (Fig. 4) showed a gradual increase from the Gulf of Finland ($< 4\text{‰}$ station XV-5) to the central Baltic proper ($> 7\text{‰}$ F 81). The permanent halocline occurred at a depth of c. 60–70 m. At the entrance of the Gulf of Finland the isohalines were depressed downwards (stat. LL 9). A tendency to upwelling (F 80) was also revealed by the isotherms and isohalines. On the transect across the eastern Gotland Basin (stat. IV b 1 – IV b 7) the stratification was uniform. The levels of total phosphorus ($< 10 \mu\text{g l}^{-1}$) and total nitrogen (c. 300–400 $\mu\text{g l}^{-1}$) and the low concentrations of inorganic phosphorus and nitrogen ($< 6 \mu\text{g l}^{-1}$ $\text{PO}_4\text{-P}$, 3–1 $\mu\text{g l}^{-1}$ $\text{NO}_3\text{-N}$, 10 $\mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$) in the surface water can be considered typical and normal for this stage of production in late summer in the northern Baltic Sea (Figs. 5 and 6).

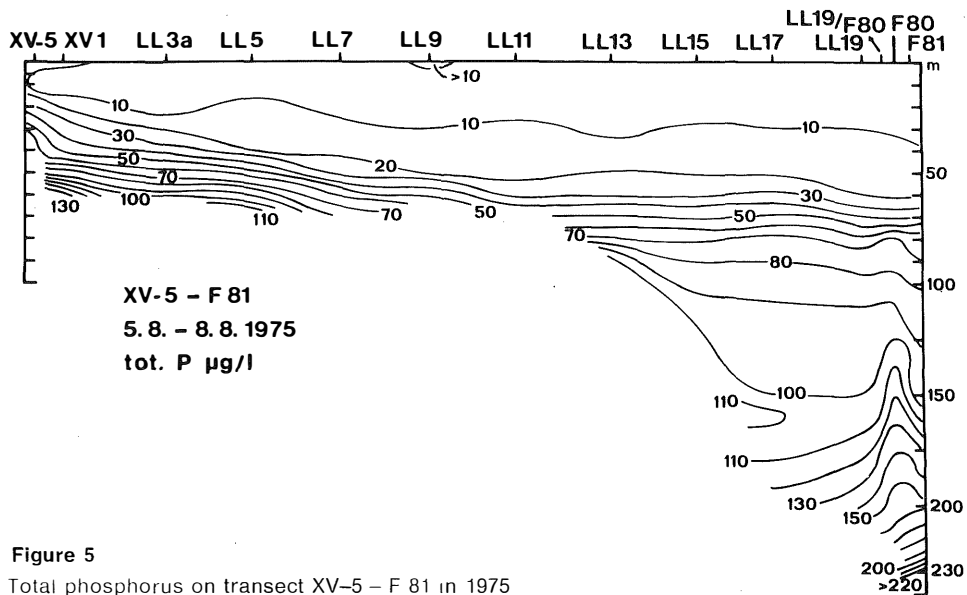


Figure 5
Total phosphorus on transect XV-5 - F 81 in 1975

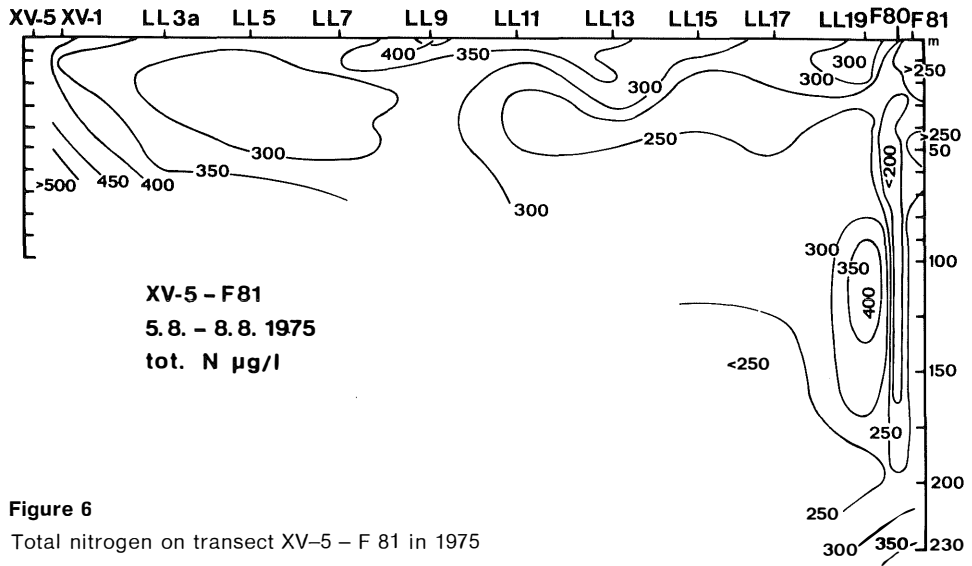


Figure 6
 Total nitrogen on transect XV-5 - F 81 in 1975

Results

In the northern Baltic proper and the Gulf of Finland, the dominant blue-green algae were the nitrogen-fixing species *Aphanizomenon flos-aquae* (L.) RALFS, *Nodularia spumigena* MERT. and *Anabaena lemmermannii* P. RICHT, the last-named being abundant only in the Gulf of Finland (Fig. 7).

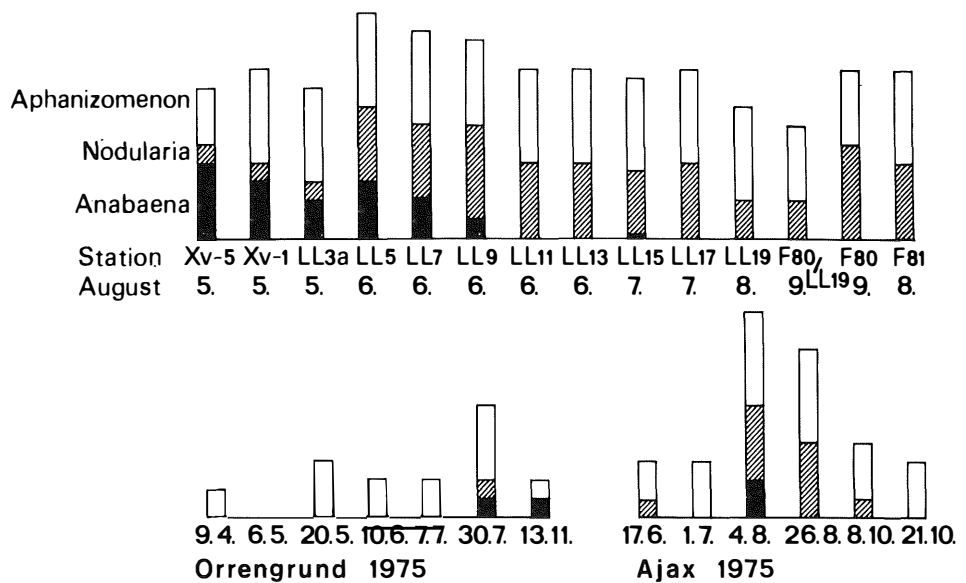


Figure 7
 The abundance of *Aphanizomenon flos-aquae*, *Nodularia spumigena* and *Anabaena lemmermannii* in net samples from 15-0 m (25 µm) on transect XV-5 - F 81 and at Orrergrund and Ajax in 1975

The results of the nitrogen fixation studies of the surface water at stat. XV 5–F 81 are given in Fig. 8. The ethylene production rate was highest at stat. LL 5–LL 17 ($6.0\text{--}23.5 \mu\text{M C}_2\text{H}_4 (2 \text{ h})^{-1} \text{ m}^{-3}$), where the biomass of heterocystic blue-greens and the number of heterocysts were also highest ($191\text{--}387 \text{ mg m}^{-3}$ and $15.8\text{--}65.3 \times 10^6$ heterocysts m^{-3} , respectively). A dense *Nodularia* bloom was visually observed near LL 9. The ethylene production rate, the biomass of heterocystic blue-greens and the number of heterocysts in this bloom were about ten times as high as in the other areas studied. However, the heterocystic activity (acetylene reduction/heterocyst) and primary production ability were of the same order of magnitude as at the other stations.

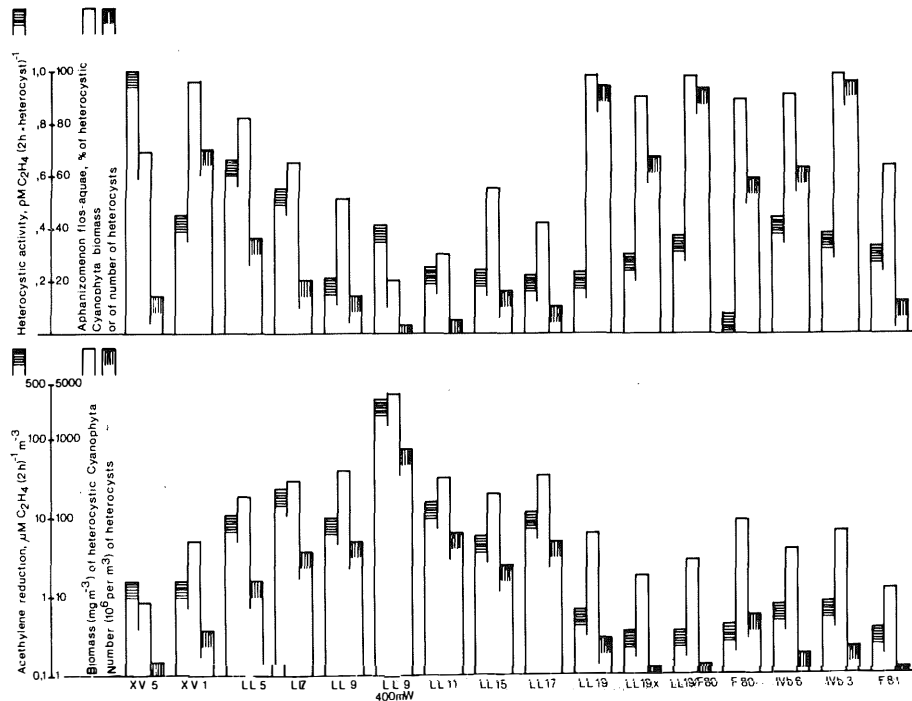


Figure 8

Ethylene production and related data of surface water on transect XV-5 – F 81 in 1975

At LL 19–F 81 the ethylene production rate ($0.4\text{--}0.9 \mu\text{M C}_2\text{H}_4 (2 \text{ h})^{-1} \text{ m}^{-3}$) as well as the biomass of heterocystic blue-greens and the number of heterocysts ($19\text{--}93 \text{ mg m}^{-3}$ and $1.2\text{--}5.8 \times 10^6$ heterocysts m^{-3} , respectively) were low. The highest heterocystic activity was found in the eastern part of the Gulf of Finland, the lowest values in the area LL 9–F 80.

At stat. LL 11 the measurements were carried out twice, on 6.8. and 14.8. (Table 1). On August 14 the *Nodularia* bloom had disappeared and the ethylene production rate was only 14% of that of August 6. The biomass of heterocystic blue-greens on August 14 was about 60%, but the number of heterocysts only 11% of the value found on August 6. However, the primary production ability ($6.8: 14.9 \text{ mg C } (4 \text{ h})^{-1} \text{ m}^{-3}$, $14.8: 40.1 \text{ mg C } (4 \text{ h})^{-1} \text{ m}^{-3}$) had increased about 2.5 times during these 6 days.

Table 1

Ethylene production, primary production ability, biomass of blue-greens, number of heterocysts and heterocystic activity at stat. LL 11 and Teili 1 in 1975.

Station	Unit	LL 11	LL 11	Teili 1
Date		6. 8.	14. 8.	14. 8.
Ethylene prod.	$\mu\text{M C}_2\text{H}_4 (2\text{h})^{-1} \text{m}^{-3}$	16.4	2.3	1.8
Primary production ability	$\text{mgC ass.} (4\text{h})^{-1} \text{m}^{-3}$	14.9	40.1	25.5
Biomass				
<i>Aphanizomenon</i>	mg m^{-3}	92	183	171
<i>Nodularia</i>	mg m^{-3}	218	3.9	14.2
Total	mg m^{-3}	310	187	185
No. of heterocysts				
<i>Aphanizomenon</i>	10^6m^{-3}	3.5	6.3	5.1
<i>Nodularia</i>	10^6m^{-3}	61.8	0.8	2.9
Total	10^6m^{-3}	65.3	7.1	6.0
Heterocystic activity	$\text{pM C}_2\text{H}_4 (2 \text{ h het.})^{-1}$	0.251	0.331	0.225

Table 2

Vertical distribution of ethylene production and heterocystic activity at Sts. LL 17, F 81, Teili 1 and LL 11 in 1975.

Depth m	Ethylene production $\mu\text{M C}_2\text{H}_4 (2\text{h})^{-1} \text{m}^{-3}$				Heterocystic activity $\text{pM C}_2\text{H}_4 (2\text{h heterocysts})^{-1}$			
	LL 17	F 81	Teili 1	LL 11	LL 17	F 81	Teili 1	LL 11
0	11.4	0.39	1.78	2.33	0.22	0.33	0.23	0.33
10	6.0	3.4	1.29	2.33	0.27	0.23	0.22	0.39
20	0.66	0.47	0.64	0.74	0.12	0.30	0.14	0.23

The vertical distribution of ethylene production was studied at stations LL 17, F 81, Teili 1 and LL 11 (Table 2). The ethylene production was obviously less intense at a depth of 20 m than in the euphotic layer. At 0 and 10 m the heterocystic activity was almost the same, but at 20 m the activity was c. 70% (on average) of that in the euphotic layer. Although the ethylene production rate followed the abundance of blue-green algae, the relative decrease in ethylene production from 0 to 20 m was greater than that in the amount of blue-green algae.

Measurements of ethylene production made in the study areas in 1974 and 1975 are given in Table 3. The means of the areas varied between 0.6 and 28 $\mu\text{M C}_2\text{H}_4 (2\text{h})^{-1} \text{m}^{-3}$. The highest mean was found in the central Baltic proper in 1974, the lowest mean in the same area in 1975. In the northern Baltic proper the mean of the ethylene production was also much higher in 1974 than 1975. In both these areas the means of the N : P ratio were higher in 1975 than in 1974.

Table 3
Nitrogen fixation and related data of surface water in different areas in 1974 and 1975 (mean values and standard deviations).

Parameter	Areas (Fig. 1)								
	1 1975	2 1974	2 1975	3 1974	3 1975	4 1975	5 1975	7 1975	8 1975
Date	6., 14.8	2.8	7., 14.8	4., 9.8	7.-9.8	13.8	5.8	12.8	12.8
No. of observations	5	9	3	6	7	4	2	1	7
Ethylene production $\mu\text{M C}_2\text{H}_4$ (2h) ⁻¹ m ⁻³	12.5	13.9	6.4	28.1	0.56	10.3	1.6	1.9	5.6
s	7.9	5.5	4.8	16.4	0.22	4.2	0.0		2.8
Nitrogen fixation $\mu\text{g N (24h)}^{-1}$ m ⁻³	117	130	60	262	5	96	15	18	52
s	74	51	45	153	2	39	0		26
Primary production ability mg C (2h) ⁻¹ m ⁻³	12.5	7.7	10.5	10.6	7.7	12.5	9.8	17.8	21.6
s	4.7	2.0	2.3	3.1	3.4	2.0	0.4		15.3
Total nitrogen, mg N m ⁻³	355	241	303	237	307	328	392		319
s	41	20	37	19	43	58	45		48
Total phosphorus, mg P m ⁻³	7	18	10	11	9	7	12		27
s	2	1	1	4	1	2	1		23
N/P (w/w)	53.9	13.6	32.1	25.2	35.5	54.3	34.3		25.2
s	9.1	1.7	6.4	9.3	4.3	17.7	6.0		23.6
Total biomass of heterocystic blue-green algae, mg m ⁻³	275	232	241	285	46	264	30	21	73
s	86	27	83	41	29	54	30		49
Total number of heterocysts 10 ⁶ m ⁻³	35.0	42.5	28.3	43.8	2.39	33.8	2.54	4.85	12.6
s	23.9	9.3	22.0	11.5	1.66	4.2	1.44		9.2
Heterocystic activity pM C ₂ H ₄ (2 h het.) ⁻¹	0.399	0.328	0.227	0.619	0.289	0.301	0.741	0.396	0.502
s	0.197	0.137	0.007	0.290	0.119	0.097	0.414		0.105
<i>Aphanizomenon flos-aquae</i> : % of total biomass	65.0	65.8	63.0	69.5	89.8	63.7	82.1	55.4	71.9
s	26.6	5.4	26.2	17.3	12.4	6.8	19.0		9.3
% of total no. of heterocysts	32.9	40.3	30.4	44.4	69.1	25.2	42.1	32.8	49.1
s	33.4	9.7	44.6	20.8	29.9	6.0	39.3		6.6
<i>Nodularia spumigena</i> : % of total biomass	34.9	33.7	37.0	29.6	10.1	36.0	6.5	33.8	20.3
s	26.5	5.5	26.2	17.6	12.3	7.3	5.6		13.0
% of total no. of heterocysts	66.7	57.2	61.5	55.3	30.4	73.8	11.1	37.7	29.4
s	33.2	9.6	44.0	22.2	28.7	7.9	0.5		20.5

The means of the heterocystic activity varied between 0.23 and 0.74 $\mu\text{M C}_2\text{H}_4$ (heterocyst) $^{-1}$ in two hours, the highest means occurring in the eastern Gulf of Finland and in the central Baltic proper (1974). A high mean value was also found in the Uusikaupunki archipelago (area 8, Fig. 1). In the surface water samples, the correlation between ethylene production and the number of heterocysts was very significant (correlation coefficient of logarithms = 0.938, $n = 45$), and, if ethylene production is y ($\mu\text{M C}_2\text{H}_4$ (2h) $^{-1}$ m $^{-3}$) and the number of heterocysts is x (10^6 per m 3), the equation is:

$$y = 0.38 x^{0.99}$$

So one heterocyst produced about 0.38 $\mu\text{M C}_2\text{H}_4$ (2h) $^{-1}$, which corresponds to nitrogen fixation of 3.5 $\mu\text{g N}$ (2h) $^{-1}$. This is somewhat smaller than values obtained in the Helsinki (VUORIO, 1977) and Stockholm archipelagoes (BRATTBERG, 1975).

Observations made by Ilkka RINNE and Hanna VUORIO (unpublished) have shown that measurements of nitrogen fixation in standard conditions are fairly comparable to measurements carried out using incubation bottles in situ.

In our calculations we have assumed that

- the seasonal succession of nitrogen-fixing blue-green algae and the diurnal and vertical variation in nitrogen-fixing activity in our study areas are similar to those found in the sea area outside Helsinki (VUORIO, 1977)
- c. 2% of the nitrogen fixed during June–August is fixed during one day in early August

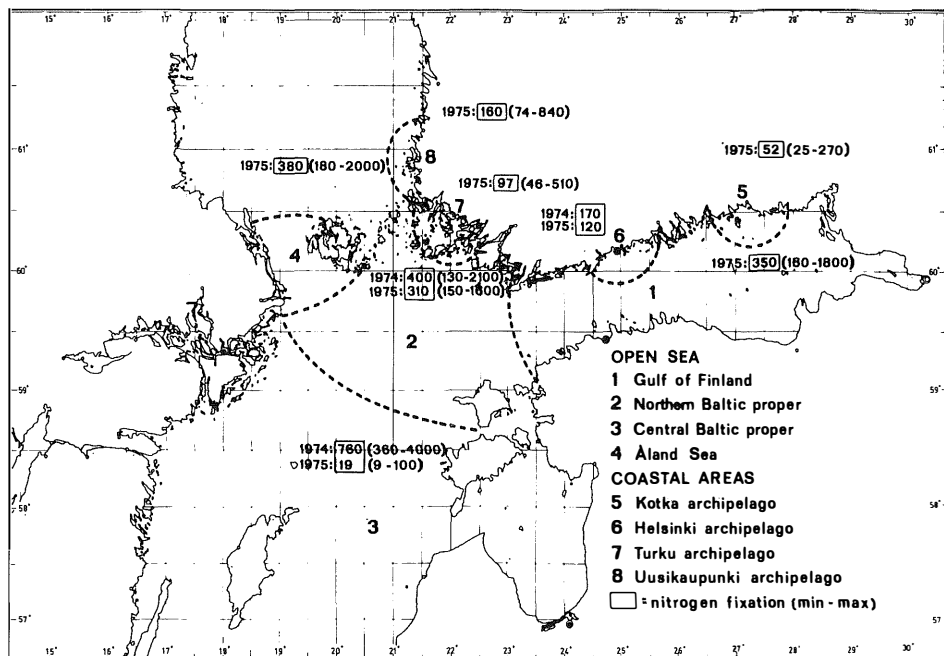


Figure 9

Calculated nitrogen fixation (kg km^{-2} (3 months) $^{-1}$) by planktonic algae in the areas studied in 1974 and 1975. (The values from the Helsinki area according to Rinne, 1976 and Vuorio, 1977.)

- the surface fixation is c. 12% of that in the whole water column
- the fixation in one hour in early August corresponds to 2–7% of the daily fixation (the rate depends on which time of the day the measurements are made)

The estimates are presented in Fig. 9. According to our preliminary calculations the amount of nitrogen fixed in 1974 in the northern and central Baltic proper was c. 100000 tons.

References

- BRATTBERG, G.: Nitrogen fixation in a polluted brackish water archipelago. – 3rd Soviet-Swedish Symposium on the Baltic Sea Pollution, Stockholm, September 1975, 12 pp (1975)
- BURRIS, R. M.: Measurements of biological N_2 fixation with $^{15}N_2$ and acetylene. In: Techniques for the Assessment of Microbial Production and Decomposition in Fresh Waters. Ed. by Y. I. SOROKIN and H. KADOTA. IBM Handbook No. **23**, 3–14 (1972)
- KLUCAS, R. V.: Nitrogen fixation assessment by acetylene reduction. – In: Proc. Eutrophication Biostimulation Assessment Workshop, pp. 109–116. Berkeley, Calif. (1969)
- KOROLEFF, F.: Determination of nutrients. – In: Methods of sea water analyses, 317 pp. Ed. by K. GRASSHOFF. Weinheim, New York (1976)
- LASSIG, J. and Å. NIEMI: Standardization of techniques for measuring phytoplankton primary production by the ^{14}C method, recommendations for Finnish scientists working on the Baltic. – Merentutkimuslait. Julk./Havsforskningsinst. Skr. **237**, 27–30 (1972)
- MELVASALO, T., H. VILJAMAA and M. HUTTUNEN: Plankton methods in the Water Conservation Laboratory in 1966–1972. – Vesiensuojelulaboratorion tiedonantoja (Rep. Wat. Conserv. Lab.) **5** (2), 1–21 (1973)
- RINNE, I.: Plankisten sinilevien typensidonta (Nitrogen fixation by planktonic blue-green algae). In: Investigation of Helsinki and Espoo Sea Areas in 1975 (English summary). Ed. by E. TARKIAINEN. – Vesiensuojelulaboratorion tiedonantoja (Rep. Wat. Conserv. Lab.) **8** (1) 42–44, 145 (1976)
- TARKIAINEN, E., I. RINNE and L. NIEMISTÖ: On the chemical factors regulating the primary production of phytoplankton in the Baltic proper. – Merentutkimuslait. Julk./Havsforskningsinst. Skr. **238**, 39–52 (1974)
- VUORIO, H.: Plankisten syanobakteerien ilmakehän typensidonta Helsingin merialueilla 1974. (Summary: Molecular nitrogen fixation by planktonic cyanobacteria in Helsinki Sea Area in 1974). – Vesiensuojelulaboratorion tiedonantoja (Rep. Wat. Conserv. Lab.) **9** (1), 1–65 (1977)