# Phytoplankton monitoring: the effect of sampling methods used during different stratification and bloom conditions in the Baltic Sea

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Investigation of the phytoplankton community is an important part of the overall water quality monitoring. Different countries and research institutes have different methods and traditions in sampling methods. In this study, the applicability of five different types of samplers — a hose, rosette, small and large bottle sampler and ship-of-opportunity-like pump sampling — were investigated in phytoplankton monitoring. Also, the effect of arithmetic integration of samples was studied. Sampling took place at three stations at the entrance to the Gulf of Finland and in the northern Baltic Proper during weak stratification and phytoplankton minimum, and strong stratification and a cyanobacterial bloom in the summer of 2005. Chlorophyll *a* and the number of selected species were analysed. There were some significant differences between the applied samplers but they were not consistent and — although there was a weak indication of the largest bottle sampler being the most reliable — the differences between the samplers were not large if they were operated correctly. However, ship-of-opportunity samples may underestimate the biomass of phytoplankton during cyanobacterial blooms. Chlorophyll *a* concentration estimated from arithmetically-integrated samples did not differ from that estimated from pooled samples.

## Introduction

Analysis of the phytoplankton community structure is an important part of the overall water quality monitoring. The Baltic Marine Environment Protection Commission (Helsinki Commission, HELCOM) has coordinated a joint monitoring programme — called COMBINE — of the Baltic Sea since 1979. HELCOM publishes monitoring guidelines (HELCOM 2008) prepared by the member country experts. In these guidelines, phytoplankton monitoring is divided into three components: phytoplankton primary production, chlorophyll (chl) *a*, and phytoplankton composition, abundance and biomass. In this study, we concentrated on the chl *a* component and gave two examples of the abundance and biomass components (small flagellated algae and

*Nodularia spumigena*).

Several methods have been used in phytoplankton sampling. Venrick (1978), Beers (1978) and Tangen (1978) discussed advantages and disadvantages of water bottles, pump systems and nets, respectively. Samplers and sampling methods may vary in neighbouring countries because of the traditions in different research institutes. This is also evident in the HELCOM guidelines (HELCOM 2008: Annex C-6). The guidelines recommend usage of an integrating hose-sampler (Lindahl 1986) for phytoplankton composition sampling and for taking samples from the surface to the 10-m depth. The Swedish monitoring is performed using this method (e.g. Rolff *et al.* 2007). Alternatively, pooling of discrete samples taken with a bottle sampler from 1, 2.5, 5, 7.5 and 10-m depths is recommended. This method is used in the other countries around the Baltic Sea (Wasmund and Uhlig 2003, Suikkanen *et al.* 2007, M. Huttunen, FIMR, pers. comm.). For the ship-of-opportunity automated high frequency sampling (Rantajärvi and Flinkman 2003) and for helicopter sampling a single sample from the mixed surface layer is adequate (HELCOM 2008).

Chl *a* is a widely used proxy for algal biomass. According to HELCOM (2008: Annex C-4) guidance, chl *a* should be measured from the integrated or pooled samples collected for the phytoplankton composition analyses. However, discrete samples for chl *a* determination are also acceptable, and often these discrete samples are used; in analyses individual measurements are averaged to represent the uppermost 10-m layer (henceforth arithmetically-integrated samples) (e.g. Wasmund and Uhlig 2003, Suikkanen *et al.* 2007).

Despite the multitude of methods in HELCOM (2008) guidelines and elsewhere, only few comparisons between them have been done. Youngbluth *et al.* (1983) and Zhang and Prepas (1993) did not find any difference in chl *a* concentrations between arithmetically-integrated and integrated samples. Studies also show that comparable results can be obtained with different samplers (Youngbluth *et al.* 1983, Sutherland *et al.* 1992, Zhang and Prepas 1993, Kononen *et al.* 1999 and Gollasch *et al.* 2003). However, larger deviations in chl *a* concentrations may occur

depending on the sampler used and integration techniques when water mass is strongly stratified and when vertically migrating flagellated algae or surface accumulations of cyanobacteria occur (Zohary and Ashton 1985, Ahn *et al.* 2008). In the Baltic Sea, these two phytoplankton groups constitute a substantial part of the plankton communities of the Gulf of Finland and the northern Baltic Proper during summers (Gasiūnaitė *et al.* 2005, Suikkanen *et al.* 2007). They form distinctive population maxima at different depths (Lindholm 1992 and references therein, Olli and Seppälä 2001, Hajdu *et al.* 2007) and can, therefore, cause problems in getting representative and comparable results when different samplers are used.

In this study, the applicability of five types of samplers — a hose, rosette, small and large bottle sampler and ship-of-opportunity-like pump sampling — for phytoplankton monitoring was investigated. Also, the effect of pooling and arithmetic integration of water samples was studied. As getting representative samples of bloom-forming cyanobacteria in summer have been demonstrated (Zohary and Ashton 1985) to be the most problematic, as well as season being important to the phytoplankton community composition and structure, the study was conducted during two separate periods: the first sampling was carried out during weak stratification and the phytoplankton summer minimum in mid-June 2005, while the two later samplings were performed in mid-July 2005 when stratification was strong and surface accumulations of filamentous cyanobacteria were abundant. The hypotheses were that differences in the sampler structures and the integrative or pooling nature of the samplers do not have an effect on the results, and that results obtained by pooling and arithmetic integration are similar.

# Material and methods

## Study area

The Baltic Sea is a brackish waterbody and the salinity at the studied sites varies between 5‰ and 7‰. The general water circulation in the area is cyclonic: more saline water of the north-
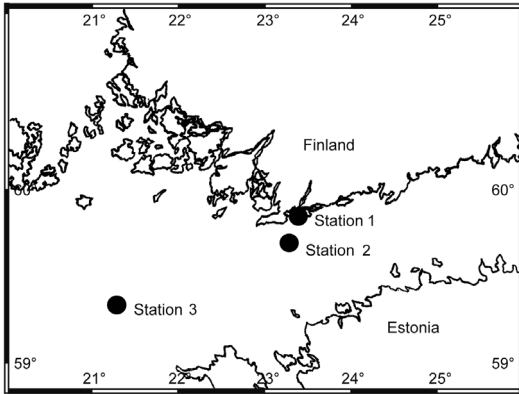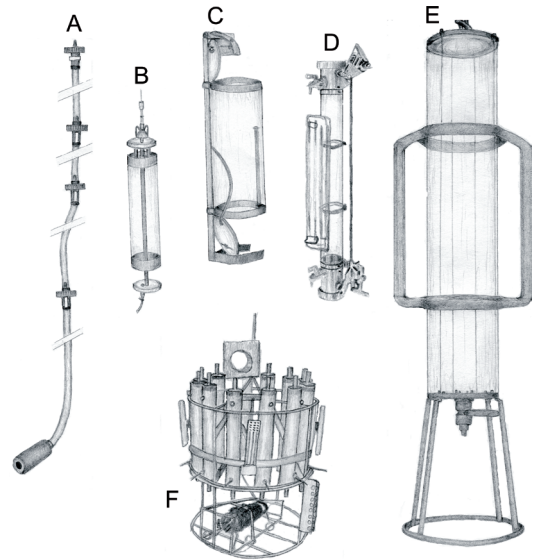
Fig. 1. Location of the study sites.



Fig. 2. (A) Hose, (B) Ruttner, (C) Limnos, (D) serial and (E) 30-l Jussi samplers are in about the same scale. Rosette (F) used at stations 2 and 3 is in a smaller scale than the other samplers. There are no pictures of the rosette used at station 1 and the flow-through device used at stations 2 and 3.

ern Baltic Proper flows into the Gulf of Finland along the Estonian coast and less saline water from the Gulf of Finland flows out of the Gulf along the Finnish coast. These upper-layer flows create a quasi-permanent front at the entrance to the Gulf of Finland. The predominant wind field regulates the location of the front (Pavelson *et al*. 1997). Sampling stations 1 (59°51.34´N, 23°15.73´E) and 2 (59°26.52´N, 23°7.98´E) were located near the Hanko peninsula at the entrance to the Gulf of Finland and station 3 (59°11.52´N, 21°12.12´E) was located further offshore in the northern Baltic Proper (Fig. 1). Samples were taken at station 1 on 16 June 2005, and at stations 2 and 3 on 12 and 14 July 2005, respectively.

## Compared samplers

A hose sampler (Fig. 2A) was the only sampler that was used at all three stations (Table 1). It was built for this study from a PVC hose and ball valves by adapting Lindahl's (1986) and Sutherland *et al*.'s (1992) designs. The sampler consists of four five-meter long segments that are interconnected with ball valves. The inner diameter of the hose is 25 mm and that of the ball valves 18 mm. Operation of the sampler is easy: when it is at the desired depth, the uppermost ball valve is closed and the sampler is raised; subsequently emerging ball valves are closed as well. If samples are taken from the uppermost 10 m the sample volume is 4.9 l.

The 2.5 l Ruttner sampler (Fig. 2B) — a small bottle sampler — was used at station 1. Water flows through top- and bottom-circular end openings of 73 mm in diameter, each divided into two halves by a 13-mm-wide bar. A large bottle sampler was the Limnos sampler (Limnos Ltd., Turku, Finland) (Fig. 2C) with the inner diameter of 127 mm and the volume of 5.5 l. The Hydro-Bios Kiel rosette with six bottles (not shown), each with the inner diameter of 75 mm and the volume of about 4 l was used at station 1 (Table 1).

At stations 2 and 3 the Hydro-Bios Kiel manufactured Nansen-typed serial sampler (Fig. 2D) was used as a small bottle sampler. In this study, four samplers of this kind were placed in a row on the same wire. Each bottle has the inner diameter of 39 mm and the volume of 1.7 l. A large bottle sampler at stations 2 and 3 was the 30-l Jussi sampler (FIMR, Helsinki, Finland) (Fig. 2E). Water flows through top- and bottom-circular end openings of 140 mm in diameter, each divided into two halves by a 30-mm-wide bar. The rosette used at the two latter stations was a General Oceanics Inc. (Miami, FL, USA) rosette

with 12 GoFlo bottles (Fig. 2F), which have inner diameters of 85 mm and volumes of 5 l. The ship-of-opportunity type of pump sampling was performed only at stations 2 and 3 with a flow through device (Fluorometer Turner 10 AU, Turner Designs, Inc., Sunnyvale, CA, USA; and an automatic sequence sampler Isco 3700 R, Teledyne Isco, Inc., Lincoln, NE, USA) connected to a centrifugation pump (Grundfos CRN2-30, Grundfos Pumput Oy Ab, Vantaa, Finland). The pump lifts water from below the bottom of the ship, i.e. from the depth of 4 m (Table 1).

## Sampling

The hose was the only truly integrative sampler in the study. The other compared samplers sampled discrete depths. These discrete depth samples were pooled in a bucket to represent the same layer that hose samples were taken from. Two kinds of samples were taken: (1) samples that were integrative (hose) or pooled (the other samplers); these samples are referred later in the text as pooled samples (or integrated samples if referring to hose samples only); and (2) discrete depth samples, which were arithmetically integrated after the chl *a* and cell abundance analyses (later in the text referred as arithmetically-integrated samples). The flow-through device took samples from only one depth, but these samples were treated as pooled in the study. Three parallel samples were taken at every station with each sampler. With the small and large bottle samplers water was taken separately for both arithmetically-integrated and pooled samples. With the rosettes and the hose samplers only pooled samples were taken. The sampling depths were 1, 2.5, 5 and 10 m both for the arithmetically-integrated and pooled samples and 1–10 m for the hose samples (Table 1). In order to reduce the laborious phytoplankton analyses, 7.5 m depth [included in the HELCOM (2008) guidelines] was left out from the sampling, which led to the under-representation of the deep layer in pooled samples. At stations 2 and 3, the rosette samples were taken in reverse order, which is the established practice onboard r/v *Aranda* (discussed later). The flow through device took samples from the depth of 4 m. Water from the samplers was emptied into a bucket and mixed thoroughly before subsampling for both chl *a* and phytoplankton analyses. The flow through device samples were taken directly from the pump hose. The samples were taken with one sampler at a time because multiple operations were impossible. Both chl *a* and cell abundances were analysed from all the samples.

**Table 1**. Used samplers and chl *a* medians. The inner diameter stands for the dimension through which water flows into a sampler. Note that the compared samplers were different at station 1 and at stations 2 and 3.

| Sampler | Sampler details | | | | Chl *a* median (mg m⁻³) | | |
|---|---|---|---|---|---|---|---|
| | Inner diameter (mm) | Height (cm) | Volume (litres) | Sampling depth (m) | Station 1 | Station 2 | Station 3 |
| Hose | 25 | 1000 | 4.9 | 0–10 | 2.56 | 8.67 | 4.35 |
| **Small samplers** | | | | | | | |
| Ruttner | 73[a] | 35 | 2.5 | 1, 2.5, 5,10 | 2.21 | – | – |
| Hydro-Bios Kiel | 39 | 72 | 1.7 | 1, 2.5, 5,10 | – | 8.14 | 4.68 |
| **Large samplers** | | | | | | | |
| Limnos | 127 | 42 | 5.5 | 1, 2.5, 5,10 | 2.26 | – | – |
| 30-l Jussi | 140[a] | 102 | 30 | 1, 2.5, 5,10 | – | 9.68 | 5.15 |
| **Rosette samplers** | | | | | | | |
| Hydro-Bios Kiel | 75 | 45 | 4 | 1, 2.5, 5,10 | 2.43 | – | – |
| General Oceanics | 85 | 71 | 5 | 1, 2.5, 5,10 | – | 7.87 | 4.55 |
| **Ship-of-opportunity** | | | | | | | |
| Flow-through device | 18 | – | – | 4.0 | – | 9.19 | 3.49 |

[a] The largest dimension through which water flows in the sampler.

## Chl *a* measurements, cell counts and biovolumes

A duplicate sample of 100 ml at station 1 and 50 ml at stations 2 and 3 were filtered through the 25 mm Whatman GF/F filters. The filters were extracted in 10 ml of 96% ethanol overnight and during the next day chl *a* concentration was measured from re-filtered samples against reference pure chl *a* (Sigma, USA) using either Shimatzu spectrofluorometer or Jasco-750 fluorometer.

Samples for the determination and enumeration of phytoplankton taxa were taken from the bucket into 200–250 ml glass bottles and fixed with 0.5–1 ml acidic Lugol's solution (Willén 1962). The samples were stored in the dark at +4 °C until 50 ml subsamples were settled in 50 ml chambers for circa 24 h and examined using an inverted microscope (Leica Diavert equipped with 12.5× oculars and 10×, 25× and 40× objectives). The larger taxa were counted from maximum of 60 fields or until 100 counting units were reached using the 10× objective. The small flagellated algae were counted from 20 fields using the 40× objective. From each sample about fifteen most abundant phytoplankton taxa were counted and their biovolumes were estimated according to Olenina *et al.* (2006).

## Statistical analyses

Due to non-normality of the data set, the nonparametric Kruskall-Wallis test of the SPSS 13 software was first applied in order to find out if the chl *a* concentrations and the cell abundances of the studied taxa differed among samplers or between pooled and arithmetically-integrated samples. The nonparametric analogues of Tukey's tests were used in post hoc analyses in order to determine between which of the samplers or samples differences occurred (Zar 1999, and references therein). Pair-wise comparisons in the sampler comparisons were performed with eq. 11.22 of Zar (1999: p. 223) with significance level 0.05. In the comparisons between the pooled and the arithmetically-integrated samples the pair-wise comparisons were performed with eq. 11.26 of Zar (1999: p. 224) with significance

level 0.05. Median values were used in the figures since the tests were nonparametric, and because Ahn *et al.* (2008) recommend using median values when presenting phytoplankton bloom results. The median is not as sensitive as the arithmetic mean to few high outlying values, which are typical to observations during cyanobacterial blooms, and hence the median reduces possible large differences in the sampling methods.

# Results

## Hydrography and phytoplankton communities

At station 1, the water column was only slightly stratified with a weak thermocline at the 5-m depth. The cyanobacteria *Aphanizomenon* sp. and *Anabaena* spp. were present in low numbers. *Pseudanabaena* spp. (Cyanophyceae), *Glenodinium* spp. (Dinophyceae), *Skeletonema costatum* (Diatomophyceae) and *Monoraphidium contortum* (Chlorophyceae) were found in pooled samples in notable numbers: on average about 115 000, 10 000, 200 000 and 230 000 counting units l$^{-1}$, respectively. The small flagellated algae [e.g. *Chrysochromulina* spp. (Prymnesiophyceae), *Pseudopedinella* spp. (Chrysophyceae) and *Pyramimonas* spp. (Prasinophyceae)] dominated distinctly in the phytoplankton community both in cell numbers (96%) and in biovolume (60%).

In July 2005, temperature stratification had already developed and the upper warm layer extended at station 2 to the depth of 5 m. The phytoplankton community consisted of three abundant groups. *Aphanizomenon* sp. was the dominant member of the filamentous cyanobacteria with 23% of the total phytoplankton biovolume. *Anabaena* spp. and *Nodularia spumigena* were present with lower biovolumes, 7% and 4%, respectively. Small aggregates of filamentous cyanobacteria floated around the site but there was no clear surface accumulation. Also dinoflagellates were abundant, especially *Heterocapsa triquetra* and some 15–35-$\mu$m-sized unidentified dinoflagellate species (together 22% of the total biovolume). The third important group was the small flagellated algae [*Chryso-*
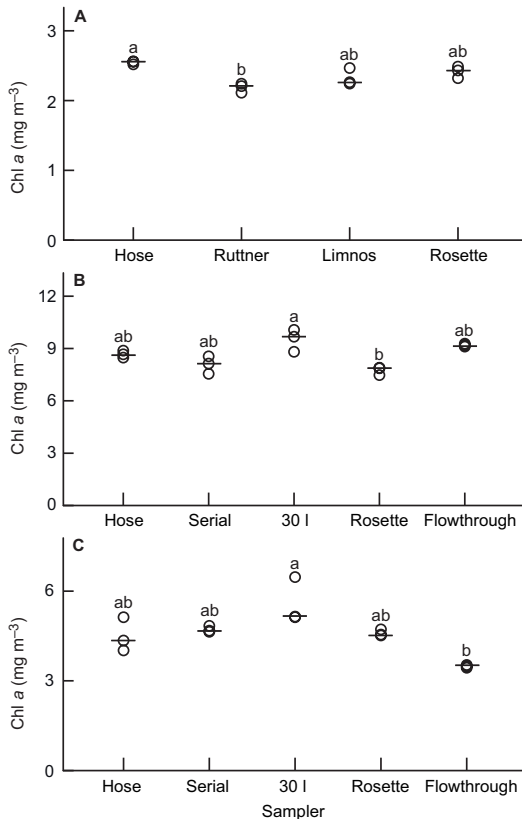
**Fig. 3**. Chl *a* concentrations in the samples taken with the different samplers at (**A**) station 1, (**B**) station 2, and (**C**) station 3. Three samples were collected with each sampler. The average chl *a* measurements are marked with circles. Black horizontal lines mark the median chl *a* values. Samplers marked with the same letter do not differ significantly from each other at $p < 0.05$.

*chromulina* spp., *Uroglena* spp. (Chrysophyceae), *Pseudopedinella* spp., *Pyramimonas* spp. and *Chlamydomonas* spp. (Chlorophyceae)], which constituted 27% of the total phytoplankton biovolume.

At station 3, the primary thermocline was at the 16-m depth and a steep secondary thermocline was at 7 m. Chl *a* fluorescence had a distinctive peak at 9 m. Unidentified dinoflagellate species (15–20 $\mu$m in size) were the only abundant dinoflagellates at this site constituting 9% of the total biovolume. Filamentous cyanobacteria dominated, and in the upper 2.5 m the biovolume of *Nodularia spumigena* was especially high (72% of the total phytoplankton biovolume at 1 m, and 47% at 2.5 m) and surface accumulation

stripes extended to the horizon. *Aphanizomenon* sp. and small flagellated algae were the most abundant groups at the depth of 10 m: 29% and 37% of the total biovolume, respectively. The latter group was also abundant at 5 m: 57% of the total biovolume.

## Sampler comparisons

The nonparametric Kruskall-Wallis test showed that there were statistically significant ($p < 0.05$, $H = 9.67$, 11.60 and 10.91 at stations 1, 2 and 3, respectively) differences between the samplers at every station. The chl *a* median concentration in the samples taken with the different samplers varied between 2.21 mg m$^{-3}$ (Ruttner sampler) and 2.56 mg m$^{-3}$ (hose sampler) at station 1 (Table 1 and Fig. 3A). The pair-wise comparison showed that the Ruttner sampler differed significantly ($p < 0.05$) from the hose sampler. The median cell numbers of the dominant small flagellated algae were $19 \times 10^6$ cells l$^{-1}$ in the Ruttner, $20 \times 10^6$ cells l$^{-1}$ in the Limnos and rosette, and $23 \times 10^6$ cells l$^{-1}$ in the hose samples. At station 2, the chl *a* median varied between 7.87 mg m$^{-3}$ (rosette sampler) and 9.68 mg m$^{-3}$ (30-l Jussi sampler) (Table 1 and Fig. 3B). The chl *a* median concentrations in the samples taken with the rosette and Jussi samplers differed significantly ($p < 0.05$) from each other. At station 3, the chl *a* median concentrations varied between 3.49 mg m$^{-3}$ (flow-through device) and 5.15 mg m$^{-3}$ (30-l Jussi sampler) (Table 1 and Fig. 3C). The 30-l Jussi sampler differed significantly ($p < 0.05$) from the flow-through device. The counting unit numbers of *Nodularia spumigena* were significantly higher [Kruskall-Wallis: $H = 10.03$, $p < 0.05$, pair-wise comparison according to eq. 11.22 of Zar (1999: p. 223)] in samples taken with the 30-l Jussi sampler than in samples taken with the rosette sampler or flow-through device at station 3 (Fig. 4).

## Comparison between chl *a* in pooled and arithmetically-integrated samples

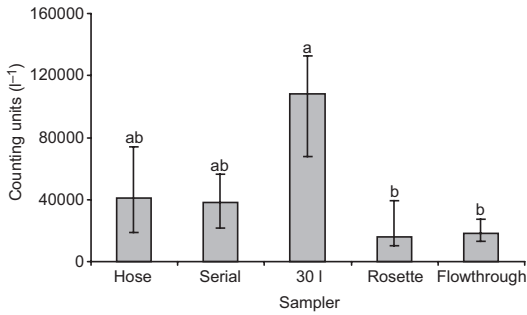The nonparametric Kruskall-Wallis test showed that there were statistically significant ($p < 0.05$,

**Fig. 4**. Medians of *Nodularia spumigena* counting units l$^{-1}$ (a counting unit was 100 µm of filament) at station 3 in samples taken with different samplers ($n = 3$). Error bars represent 95% confidence intervals. Samplers marked with the same letter do not differ significantly from each other at $p < 0.05$.

$H = 33.50$, 19.77 and 23.37 at stations 1, 2 and 3, respectively) differences between the chl *a* samples taken from different depths at every station. At station 1, the chl *a* median concentrations were 2.38 mg m$^{-3}$ and 2.20 mg m$^{-3}$ in the pooled and arithmetically-integrated samples, respectively (Fig. 5A). At station 2, the concentrations were 8.67 and 8.51 mg m$^{-3}$, respectively; and at station 3, 4.64 and 6.16 mg m$^{-3}$, respectively (Fig. 5B and C). There were no significant ($p > 0.05$) differences between the pooled samples and the arithmetically-integrated samples or the pooled samples and the samples from 5 and 10 m depths at any station [pair-wise comparison using eq. 11.26 of Zar (1999: p. 224)].

# Discussion

## Sampler comparisons

The aim in phytoplankton monitoring is to determine the extent and the effects of anthropogenic inputs on phytoplankton and resulting eutrophication. Therefore, the most extensive sampling effort is carried out during periods when chl *a* values are high. In this light, the statistically significant difference in chl *a* concentrations between the Ruttner and the hose taken samples at station 1 (Fig. 3A) may seem negligible, as the difference is only 0.36 mg m$^{-3}$ and the total chl *a* concentration is low. On the other hand, the chl *a* content in the hose samples is 15.8% higher than
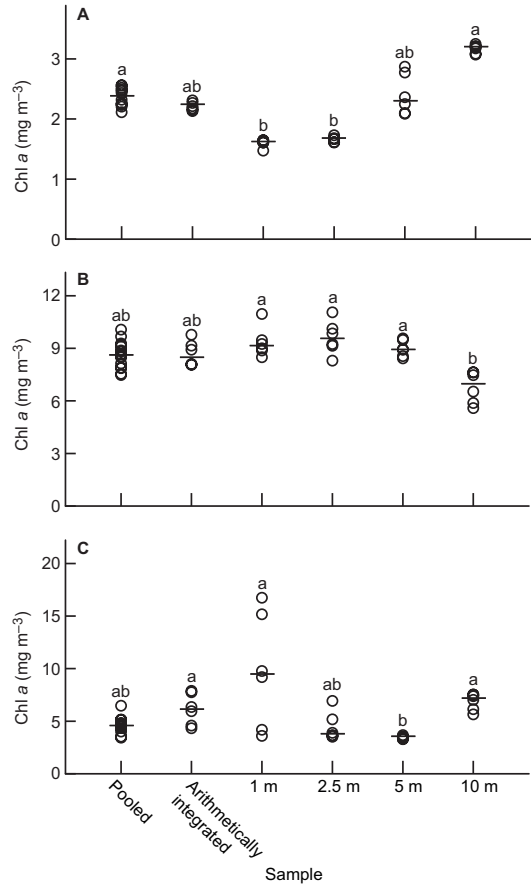


**Fig. 5**. Chl *a* concentrations in the pooled and arithmetically-integrated samples, and discrete depth samples at (**A**) station 1, (**B**) station 2, and (**C**) station 3. Average chl *a* measurements are marked with circles, and black horizontal lines mark chl *a* medians. Sample types marked with the same letter do not differ significantly from each other at $p < 0.05$.

in the samples taken with the Ruttner. Moreover, during the sampling in June, the small flagellated algae were by far the most abundant group at station 1 and their number was 21% higher in the samples taken with the hose than with the Ruttner. The hose sampler takes samples from the entire 10-m layer unlike the discrete depth bottle samplers. Due to the omission of the HELCOM (2008) recommended 7.5-m depth in the sampling, the deeper layer was under-represented in the pooled samples. Therefore, possible abundant layers of the small flagellated algae between 5 and 10 m were certainly sampled with the hose but not with the other samplers. During stratifica-

tion, these abundant and usually thin layers of algae are more pronounced (Lindholm 1992 and references therein), but the samples taken with the hose were identical to the samples taken with the other samplers at the two other study sites (Fig. 3B and C) where stratification was more obvious than at station 1. This is in accordance with the results of Youngbluth *et al.* (1983) that the chl *a* concentration is similar in tube and bottle samples even when the water mass is stratified. However, when Zohary and Ashton (1985) compared integrated samples taken with hoses with different diameters and basal weights against the pooled samples taken with a bottle sampler, the difference in the chl *a* concentrations were largest when cyanobacteria were abundant. Using a hose that has a large diameter (63 mm) and a streamlined weight, the pooled samples were, however, similar (Zohary and Ashton 1985). We found some differences between the hose and the other samplers in cyanobacterial numbers but they were not consistent. Even more interesting, there was no difference between the hose and the other samplers in *Nodularia spumigena* numbers at station 3 where this species was abundant enough for analysis (Fig. 4). This is in accordance with Rolff *et al.* (2007, citing unpublished data of Almesjö and Rolff) who did not find any difference in numbers of *Nodularia* and *Aphanizomenon* sp. sampled with hoses of variable diameters.

The difference between the rosette and 30-l Jussi samplers at station 2 (Fig. 3B) and the fact that the chl *a* median concentration was highest in the pooled samples taken with the 30-l Jussi sampler at stations 2 and 3 (Fig. 3B and C) might be explained by the bow-wave of the samplers as in different basal weights of hoses in Zohary and Ashton (1985). The 30-l Jussi sampler is a large bottle sampler that has a very small bow-wave (J. Rapo, FIMR, pers. comm.) as water can flow freely through the large diameter of the sampler in descent. The rosette, on the other hand, is a large construction with bottles and CTD equipment under the bottles and has distinctively larger bow-wave — although taking samples with the rosette when it is slowly lifted upwards minimizes the effect of the bow-wave. The smaller bow-wave of the 30-l Jussi sampler might also be the cause for the higher *Nodularia*

*spumigena* numbers in the samples taken with this sampler than with the other samplers at station 3 (Fig. 4). On the other hand, the 30-l Jussi sampler is higher than the other samplers (Table 1). The *Nodularia spumigena* surface accumulation at station 3 was concentrated in the uppermost meter or two of the water column and, unlike the other samplers, the 30-l Jussi sampler may have reached accumulation with in case of 1 and 2.5 m samples.

The significant difference between the 30-l Jussi sampler and the flow-through device at station 3 (Fig. 3C) is clearly an effect of vertical distribution of filamentous cyanobacteria that dominated at this station. *Nodularia spumigena* concentrated near the surface and *Aphanizomenon* sp. around the depth of 10 m. The flow-through device takes samples from the 4-m depth where these algae were least abundant. Several studies (Niemistö *et al.* 1989, Kononen *et al.* 1998 and Hajdu *et al.* 2007) showed the vertical separation of *Nodularia spumigena* and *Aphanizomenon* sp. during summer, which may be explained by different ecological preferences of the species: *Nodularia spumigena* benefits from a shallow mixed layer with high temperature and irradiance (Kononen *et al.* 1996, Wasmund 1997) and *Aphanizomenon* sp. from phosphorus pulses from deeper water layers (Kononen *et al.* 1996).

Overall variances of the pooled samples were clearly higher at stations 2 and 3 than at station 1, indicating larger variability between the different sampling methods during cyanobacterial blooms which was also found by Zohary and Ashton (1985), and which should be taken into account during blooms.

## Comparison between chl *a* content in pooled and arithmetically-integrated samples

The results from comparison of pooling and arithmetic integration (Fig. 5) justify use of arithmetic integration as a valid method. Youngbluth *et al.* (1983) and Zhang and Prepas (1993) obtained similar results when comparing the chl *a* concentrations in arithmetically-integrated water-bottle samples and integrated tube-samples. However, Youngbluth *et al.* (1983) recom-

mend using discrete depth samplers in stratified conditions if nutrient samples are to be taken.

According to Rantajärvi *et al.* (1998), a ship-of-opportunity sample from a single depth can represent the productive layer in the Baltic Sea, as the layer is only 10–20 meters thick and the moving ship mixes the layer. They argue that the depth of 5 m is the most suitable sampling depth and this study gives support to that: there is no statistical difference between the pooled samples and the samples from 5 m at any station (Fig. 5), despite the fact that the sampling was performed from non-moving vessels. Hence, the high frequency sampling with flow-through devices onboard merchant ships gives valuable chl *a* data for monitoring. During cyanobacterial mass occurrences 5-m samples may, however, underestimate the biomass of cyanobacteria that concentrate near the surface and/or around the depth of 10 m (Figs. 3C and 5C) (Ahn *et al.* 2008).

## Conclusions

This study shows that, despite the differences, the results are remarkably similar for all the applied sampling devices. Two out of three statistically significant differences between the samplers are such that they can be avoided with proper sampling: at station 1 the under-representation of the deeper layer in the pooled samples may account for the difference between the integrative hose and the pooling Ruttner sampler, and at station 3 the difference between the Jussi sampler and the flow-through device is easily recognisable — the biomass was concentrated near the surface and around the depth of 10 m. Furthermore, it is possible to analyse discrete depth samples independently for more detailed information, and afterwards make arithmetically-integrated samples from the results. However, during the dominance of cyanobacteria, chl *a* results are more sensitive to the use of proper sampler. A large sampler, like the 30-l Jussi sampler, should be considered to ensure reliable sampling, and results from a ship-of-opportunity-like pump sampling with a flow-through device should be treated with caution during cyanobacterial blooms. As a conclusion it can be said that the applied methods are all useful for monitoring purposes and the rec-ommendations in the HELCOM (2008) do not need adjustment.

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