Benthic diatoms in the Gulf of Bothnia

Community analysis and diversity

BY

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ABSTRACT


Benthic diatoms are valuable tools for biological monitoring and paleo-ecological reconstruction of past environmental conditions. This thesis aims at describing size-related properties of benthic diatoms and suggests that data assessment for community analysis can be improved by considering the importance of scale. It investigates which environmental factors structure epilithic diatom communities on the coast of the Gulf of Bothnia and identifies environmental factors correlated with phytobenthic biomass. It also contributes to the floristic knowledge of the Baltic Sea.

Diatom species show large variation in size. The responses of large species (≥1000 µm³) in diatom communities to environmental factors are underestimated if solely measured as relative abundance, as is the common practice. However, relative abundance gives the best gradient resolution, as compared to surface area and biovolume, if species are counted separately in two biovolume classes. Small and large species in the same community may respond differently to the same environmental factors.

To assess the principal environmental factors structuring diatom communities in the Gulf of Bothnia, 270 quantitative samples were collected from submerged stones. Sampling was carried out in spring in four areas of the Bothnian Bay, characterized by a stable north-south salinity gradient (0.4-3.3 psu), and in three areas of the Bothnian Sea which has a rather uniform salinity of ca. 5 psu. Canonical correspondence analysis (CCA) identified salinity and exposure to wave action as the principal factors structuring the diatom communities of the Bothnian Bay, whereas exposure to wave action was the principal factor in the Bothnian Sea. Measurements of relative ignition loss suggested that the cover of macroalgae, and thereby the higher abundance of epiphytic diatoms in the epilithic samples, was positively correlated with salinity in the Bothnian Bay and with water movement in the Bothnian Sea.

Two new brackish water species are described, *Navicula sjoersii* S. Busse & Snoeij and *N. bossvikensis* S. Busse & Snoeijs. The new species are compared with *N. perminuta* Grunow, a common brackish-water species.

Key words:
Baltic Sea, Biodiversity, Biomass, Community ecology, Diatoms, Multivariate analysis, *Navicula*, Phytobenthos, Salinity gradient, Scale, Size, Water movement

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To my parents

Derrière les ennuis et les vastes chagrins
Qui chargent de leur poids l'existence brumeuse
Heureux celui qui peut d'une aile vigoureuse
S'élancer vers les champs lumineux et sereins

(Charles Baudelaire, *Elévation*)
This thesis is based on the following four papers, referred to by their Roman numerals:


III Busse, S. & Snoeijjs, P. (Submitted manuscript): Gradient responses of diatom communities in the Bothnian Sea (northern Baltic Sea), with emphasis on responses to water movement.


In paper **I**, I carried out parts of the laboratory work, analyzed the species composition of the epilithic diatoms and took part in compiling the manuscript.

In paper **II**, I participated in planning the analysis, carried out all the laboratory work, analyzed the diatoms for species composition, analyzed the data and compiled the manuscript.

In paper **III**, I analyzed the species composition, analyzed the data and compiled the manuscript.

In paper **IV**, I carried out the measurements, participated in the photographic documentation and compiled the manuscript.
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INTRODUCTION

Benthic diatoms as monitoring tools

Benthic diatoms are widely used as indicators in biological monitoring and paleo-ecological reconstruction of environmental conditions. Their indicator value is based on the high diatom species richness in almost all aquatic habitats and on their specific gradient responses. Different diatom species have different ecological requirements and they all respond sensitively to various environmental factors. Each species’ response to a given environmental factor, recorded as the species’ relative abundance in a diatom community along the factor’s gradient, is expected to have a unimodal distribution curve with a defined tolerance and optimum (KOLBE 1927, TER BRAAK 1987). In habitats with strong variation in salinity, this factor is expected to have an overriding importance in structuring diatom communities; other factors such as water movement predominate when salinity varies only marginally (SNOEIJIS 1999a). Papers II and III describe typical gradient responses in the Gulf of Bothnia in the presence (II) and absence (III) of a salinity gradient.

Community analyses are based on the results of cell counts. A defined number of specimens is identified so as to assess how strongly every species is represented within the diatom community. Conventional counting methods use abundance scores that ignore differences in size. However, diatom valves have a size range from several µm to several mm. It can therefore be assumed that size is important when species of so different size classes co-exist, and this probably affects various ecological responses at the opposite ends of the scale. While the length of the apical axis only provides a very rough measure of size, surface area or biovolume are probably more relevant measures for the majority of ecological questions related to bioindication. Since large species are usually rare in diatoms communities, ecologists face the problem that these species are underrepresented in data collection by means of counts (SIMOLA 1990). Paper I provides the methodological basis for Papers II and III. It addresses the importance of size in community analysis, considering diatoms from the entire Baltic Sea.

Community analysis can only be as good as the floristic knowledge it is based on. The diatom flora of the Baltic Sea has been the subject of many floristic works (e.g. CLEVE-EULER 1951-1955, PANKOW 1990, WITKOWSKI et al. 2000). Nevertheless, some species have only recently been described and many more are suspected to be yet undescribed. Paper IV makes a contribution to the knowledge of the Baltic Sea diatom flora by describing two new species.

The Baltic Sea: a model system for salinity gradient studies

The Baltic Sea (Fig. 1) has a year-round stable salinity gradient of more than 1500 km length, ranging from 0.4 psu (practical salinity units = gram salt per kg water = °/oo) in the northern Gulf of Bothnia and the eastern Gulf of Finland to 15-25 psu in the Kattegat and continuing up to 25-34 psu in the Skagerrak (WITTING 1912, EHLIN 1981). This gradient provides a unique possibility to study the optima and ranges of diatom
species in relation to salinity. In comparison, salinity gradients in estuaries of tidal coasts are shorter in length and subject to diurnal, seasonal and irregular salinity fluctuations. In estuaries, contrary to the Baltic Sea with its stable salinity gradient, euryhalinity of organisms (the ability to cope with salinity fluctuations) is more important than salinity optima for the distributions of species (SNOEJIS 1999a).
At the North Sea’s entrance to the Baltic Sea proper (south of the Kattegat), three narrow and shallow straits (the Large Belt, the Small Belt and the Öresund), the two larger ones with underwater thresholds (the Darß Sill, max. sill depth 18 m, and the Drodgen Sill, max. sill depth 7 m), represent bottle-necks for inflowing salty deepwater (KÖSTER & LEMKE 1996). Large salt water intrusions are rare events, depending on certain weather conditions. They have no immediate effect on surface salinity because of complex density stratification by salinity as well as by temperature (MATTHÄUS 1995, FENNEL 1996), but maintain the salinity gradient over a longer time period.

The Gulf of Bothnia, particularly studied in this thesis, has no lunar tides, but it does have meteorologically induced average water level fluctuations of 1.2-1.6 m, max. 3 m in the north (LISITZIN 1957, JERLING 1999). It is characterized by a large seasonal variation and severe climatic conditions. The Gulf of Bothnia consists of two sub-basins of different sizes. The Bothnian Bay in the north has a fairly stable north-south surface salinity gradient (0.4-3.3 psu) and represents the lower end of the Baltic Sea’s salinity-gradient. It has a volume of 1500 km³ and a residence time of 4 years. The Bothnian Sea in the south has a rather constant surface salinity of ca. 5 psu along the Swedish coastline. It has a volume of 4700 km³ and a water residence time of 3 years (FONSELIUS 1986). Both sub-basins receive freshwater from Swedish and Finnish rivers with a nival (snow-dependent) water regime, carrying high loads of silica and humic substances (VOIPIO 1961). While the Bothnian Bay freezes every winter, only the coasts of the Bothnian Sea regularly become ice-covered (though in some years the whole Bothnian Sea may freeze).

On a smaller scale, the Gulf of Bothnia’s salinity gradient results from a hydrographic situation similar to that of the whole Baltic Sea: a net outflow of fresher surface water as a result of higher river-runoff and precipitation than evaporation, combined with a restricted inflow of deepwater of higher salinity due to the basin entrance’s bottom topography (HÅKANSSON et al. 1996). An underwater ridge, the Southern Quark, restricts denser deepwater from the Baltic Sea proper to enter the Bothnian Sea. The Bothnian Sea’s deep water below the halocline is therefore only slowly renewed, mainly by denser surface water from the Baltic Sea proper (WULFF et al. 1994). Most inflowing brackish water enters through the Åland Sea (Area 6 in Fig. 1), which is deeper and more open than the shallow Archipelago Sea (KULLENBERG 1983). Further north, the intrusion of deepwater from the Bothnian Sea into the Bothnian Bay is also restricted by a sill, the Northern Quark. Again, it is denser surface water that renews the Bothnian Bay’s deepwater, maintaining the salinity gradient.

Besides salinity, the Gulf of Bothnia’s archipelago coast also provides ideal conditions for studying gradients of water movement. Islands in the outer archipelago are strongly exposed to wind and wave action, while those in the middle and inner archipelago are usually more sheltered. However, even on a single island, one shore can be strongly exposed and the other only weakly. The archipelago coast (LAMPE 1996) is a result of the ongoing land uplift. During the last ice age, the vast ice masses compressed the Fennoscandian Shield which, up to this day, is subject to isostatic rebound (KÖSTER 1979).
Though the Gulf of Bothnia is still an oligotrophic water body (Jonsson et al. 1996), eutrophication problems are reported from other parts of the Baltic Sea (Elmgren 1989, Jansson & Dahlberg 1999). The eutrophication status is reflected by measures of biological productivity. These measures are often related to nutrients in the water, absolute nutrient concentrations as well as their ratios in comparison to the Redfield Ratio (Redfield 1958). The latter is the ratio in which algae generally need certain nutrients (N, P, C, Si) for optimal growth. The Bothnian Bay has an N:P ratio above 16, indicating P-limitation (Kangas et al. 1993), the Bothnian Sea’s is near or equal to the Redfield Ratio (Fonselius 1986), whereas the Baltic Sea proper has an N:P ratio below 16 on molar basis, indicating N-limitation (Snoeijis 1999b). Since most of the P-discharge into the Gulf of Bothnia is contributed by rivers and coastal (municipal and industrial) wastewaters (Ahl 1977), and thus by point-sources, the nutrient situation at the coast can locally differ from that in the off-shore surface water.

Eutrophication promotes sedimentation of organic material. The resulting soft bottom coverage is a significant constraint for the macroalgal vegetation in the Bothnian Sea (Eriksson 2002) because macroalgae need bare substratum to settle on. Soft bottom coverage is expected to indirectly affect the diatom community because macroalgae serve as hosts to many epiphytic species. It may, however, also have a direct effect on the relative abundance of those diatom species common on soft bottom.

Background of this thesis

The gradient analysis studies of Gulf of Bothnia presented in this thesis were designed as part of a larger interdisciplinary project (“DIACAL”) with the goal of building a model for reconstruction of brackish water paleo-environments (Andrén et al. 2000). The goals of this ongoing diatom calibration project are to gather ecological information on living diatoms affected by different environmental constraints, notably salinity, eutrophication and temperature, to calibrate the different diatom species to the measured environmental factors measured and to build a model (on the basis of living diatoms) for inferring environment from diatoms for use in bioindication of current (ecological monitoring) as well as past environments (geological surveys). Collaboration between biologists and geologists from Uppsala and Stockholm University will make it possible to base the model on a large number of recent diatom samples, both geological (from surface sediment) and biological (from stone surfaces as well as plankton) from the entire Baltic Sea area.

The model can be applied to infer paleoecological conditions from Baltic sediment cores. Similar attempts for reconstructions have recently been made (Smol & Cumming 2000, Battarbee et al. 2001), but none of these is based on a stable salinity gradient and on a similarly large data set. Since the last Weichselian deglaciation, the Baltic Sea has undergone several stages, both lacustrine and marine. So far, no exact quantification of these changes in salinity has been achieved through lack of data, intercalibration and appropriate multivariate statistics. Especially the quantification of paleo-salinity will be essential for understanding the Baltic Sea’s post-glacial development. Moreover, inferences of temperature and nutrient concentrations
can provide valuable reference data to frame present-day climatic change and eutrophication, respectively, by human impact in the wider context of natural fluctuations.

OBJECTIVES AND HYPOTHESES

The aims of this thesis were (1) to describe size-related properties of diatoms, (2) to make practical suggestions for community analysis considering the importance of scale, (3) to investigate which environmental factors structure diatom communities in the presence and absence of a surface salinity gradient, (4) to identify which environmental factors best explain variation in dry weight and relative ignition loss, different measures of phytobenthos biomass, and (5) to enhance the floristic knowledge of the Baltic Sea.

The specific hypotheses to be examined were: (1) Diatom species with a small biovolume show different responses to environmental factors from those with a large biovolume when co-occurring in the same diatom community. (2) Salinity and water movement are significant environmental factors structuring benthic diatom communities in the Gulf of Bothnia.

MATERIAL AND METHODS

Sampling procedure

Paper I includes epilithic, epiphytic and pelagic diatom data from the entire Baltic Sea and Papers II-IV are exclusively based on epilithic samples from the Gulf of Bothnia (Fig. 1). Four sampling areas were chosen in the Bothnian Bay (10-13; Holmön, Skellefetåhamn, Bergöfjärden and Rånefjärden; Paper II), three in the Bothnian Sea (6-8; Gräsö, Gävlebuken and Hornslandet; Paper III) and one in the transition area near the Northern Quark (9, Omnefjärden, Paper IV).

Within each area (Papers II+III), ca. 10 sites were selected so as to represent a maximum variation in exposure to wave-action, degree of soft bottom coverage and macroalgal cover. At each site, normally 4 replicate samples were collected. Sampling dates were: 13-14 May 1990 (Area 6), 24 April to 16 May 1991 (Areas 7-9) and 27 May to 1 June 1991 (Areas 10-13). Along with the biological sampling, salinity and temperature were recorded in all sites and NO2-N, NO3-N, PO4-P and SiO2-Si concentrations of sites were determined later in the laboratory. Exposure to wave action (Exposure) and Soft bottom coverage (Soft) were estimated using ordinal scales (1-5 and 1-6, respectively) and the beach type (Beach) was classified (scale: 1-6).

For the first time, the quantitative sampling method developed by Snoeij & Snoeij (1993, Fig. 2) has been applied successfully on a large scale. The method allows the removal of all benthic microalgae, small macroalgae etc. on a defined (55.4 cm²) circular surface area of a flat stone. The sampled stones were taken from the upper hydrolittoral zone (0.2-0.7 m of depth) and had a diameter of ca. 25-30 cm.
They were carefully lifted out of the water and carried to the beach. A plexiglass tube was pressed against the stone surface by means of two strong straps attached to a bottom plate. A flexible ring on the bottom of the cylinder completely sealed the enclosed area. A brush was then inserted and whirled around. Repeated rinsing with distilled water removed remaining biomass within the cylinder. The samples were fixed with formalin in the field.

Fig. 2. Quantitative sampling method (SNOEJS & SNOEJS 1993): (a) sampling device, consisting of a rigid plexi cylinder with a flexible rubber ring (left) and a hand brush attached to a led (right); (b) brushing off a defined surface area of a stone from the hydrolittoral (distilled water added); (c) quantitative sample; (d) stone after sampling. © Schweizerbart’sche Verlagsbuchhandlung, reproduction with the publisher’s courtesy.
Fig. 3. Quantitative subsampling method (BUSSE & SNOEJS 2002): (a) sedimentation; (b) supernatant drained through holes in the outer and inner cylinders; (c) biomass transferred to trays, using a plastic Pasteur pipette with a wide opening. © UNESCO, reproduction with the editor’s courtesy.
Subsampling procedure and biomass measurement

The epilithic samples were treated in the laboratory in 1998/99. After sedimentation in the original sampling containers, most of the supernatant was removed. Varying amounts of macroalgae, microalgae, zoobenthos, silt and sand occurred in the samples from stone surfaces and had created the micro-environment for the diatom communities. First, zoobenthos organisms larger than 1 mm were sorted out. Macroalgae and filamentous cyanobacteria were finely cut. After vigorous shaking, sand grains were separated from the suspended fraction by repeated manual rotation and 5 seconds sedimentation. The sand was dried, burned and weighed separately to give a measure of substrate availability for epipsammic diatoms (growing on or between sand grains). It can be assumed that the biomass was well separated from the sand in the samples because the weight of sand after drying and the weight after incineration differed minimally.

To divide the remaining (biomass) fraction of the sample into subsamples a quantitative subsampling device (Fig. 3) was developed. It consisted of a cylindric polystyrene container with a water-tight screw lid. Enclosed in the large container of 9.9 cm diameter were five small cylinders of 2.6 cm diameter each, open on the top and attached to the bottom with epoxy glue. Biomass sedimenting inside one of the small open containers gives a subsample. Each of the five subsamples represents 6.9% of the original sample and thus refers to a brushed-off stone surface of 3.82 cm². A subsample’s biomass (g), multiplied by the factor 0.262, gives a standardized mass-per-area measure (g cm⁻²) that allows comparisons with the literature.

The samples from which zoobenthos and sand grains had already been removed were transferred to the subsampling container, shaken and then kept still for 36 h to sediment evenly. After sedimentation, most of the water column was removed from the subsampler without resuspension. The water was drained through holes at 2 cm height from the bottom of the large container as well as each of the inner containers, thus emptying the small inner cylinders simultaneously with the big enclosing cylinder. The algal biomass, together with a little water residue, was taken out of the small cylinders with a plastic Pasteur pipette. Of the five subsamples obtained, one was dried on a filter and preserved as herbarium material. Another was used for diatom species identification. Three subsamples were used for analysis of dry weight (DW) and ash weight (AW), yielding ash-free dry weight (ADW = DW – AW) and relative ash-free dry weight (= relative ignition loss, ADW% = ADW* 100 / DW).

Species identification and community analysis

Subsamples used for diatom species identification were dried, oxidized with H₂O₂ and K₂Cr₂O₇ to remove all organic components and washed several times (24h sedimentation, removal of the supernatant, resuspension of the pellet in distilled water). The diatom valve suspension was dried on cover glasses and mounted in highly refractive medium (Naphrax™). The relative abundance of diatom species was analyzed at 1000x magnification, using mainly SNOEIJJS et al. (1993-1998) and KRAMMER & LANGE-BERTALOT (1986-1991) as identification literature.
Biovolume calculations were carried out using rectangularity, the area of a valve expressed as a proportion of the area of its enclosing rectangle (DROOP 1994), measured on electron and light micrographs. For diatoms with an uneven pervalvar axis (PA), a mean value of the PA was used in combination with rectangularity. As small and large diatoms show different responses to the same environmental constraints (Paper I), diatoms were counted separately in two size (biovolume) categories: small (<1000 µm³) and large species (≥1000 µm³). Thus, different species data sets were obtained that were combined with the environmental data set. Multivariate gradient analysis techniques were applied using the computer program CANOCO (TER BRAAK & ŠMILAUER 1998).

RESULTS AND DISCUSSION

The importance of cell size in diatom community analysis (Paper I)

In Paper I, morphometric measurements of 515 epiphytic, epilithic and planktonic species from the Baltic Sea were summarized and their size- and shape-related properties were discussed. The study showed that conventional community analysis, ignoring differences in cell size, underestimates the responses of large species to environmental factors in two ways: (1) the weight of a large species with 10,000 times higher biomass than a small species has higher importance in the community and (2) large species usually have low abundance, and their occurrence in diatom counts is therefore stochastic and appears as not ecologically relevant in the analysis results. Moreover, responses of small and large species were compared using different measures of their “weight” in the diatom community. Based on these responses, suggestions for an improved counting method were made.

The largest mean cell dimension (mostly the apical axis) varied between 4.2 and 653 µm, cell surface area between 46 and 63,000 µm², and cell volume between 22 and 735,000 µm³. Shape (length/width) varied by a factor of 15 and biovolume by a factor of 3600 in >95% of the species. Differences in size are thus enormous, and so species’ relative importance in the community analysis differs enormously, depending on how its occurrence in relation to other diatom species is recorded: by relative abundance, log transformed abundance, or relative abundance corrected by length of the apical axis, shape, surface area, surface to volume ratio or volume. All shapes are represented in almost every size group, but shape was weakly correlated with size, i.e. large diatom species have a slight tendency to be longer and thinner species than small ones.

By dividing up the species pool into two biovolume classes and placing the threshold level at 1000 µm³, close to the median, two almost equally species-rich data sets were obtained. However, since no shape groups were restricted to any given size, a division of the species pool at just that level is artificial. Subsequently, the weight of large species in the community was compared according to the measure used. The large species are weakly (10%) represented if counted by abundance, more strongly (ca 40%) if counted by surface area and very strongly (60%) on the basis of biovolume (Fig. 4). This suggests the question which measure (or size correction factor) gives
large species the ecologically right “weight” in the community, reflecting its biological activity and interaction with the environment. Relative abundance does not consider any difference in size. In log transformed abundance counts, all rare species are upgraded, even the small ones with just stochastic occurrence. It therefore seems reasonable that some measure related to surface area or volume best reflects a diatom cell’s ecological and physiological constraints.

Fig. 4. Proportions of large diatom species (>1000 µm³) in epiphyton communities from Area 6 in the Bothnian Sea, expressed as (a) relative abundance, (b) surface area and (c) cell volume. CERA, *Ceramium gobii*; CLAD, *Cladophora glomerata*; PILA, *Pilayella littoralis*. The numbers 63, 64, 68 and 69 represent the four sampling sites. Error bars show the SD of n = 4 samples.

Different dimensions probably express different functional aspects, so that the question of “correct weight” of large species in the community cannot be definitely answered. Surface area is probably relevant for photosynthetic light harvest and nutrient uptake, whereas the volume of protoplast and vacuole has an impact on the metabolic rate and storage capacity. Biovolume might, however, be easier to handle in community analysis since it is not affected by colony formation as surface area is.

One may further ask why there is such a diversity of shape in all size classes. This problem cannot fully be assessed by statistical means and was therefore left as a side issue of this statistical survey. Shape is a very complex phenomenon. It reflects life history strategies and specific habitat adaptations. For instance, shape affects
sinking velocity by generating rotation movements of the cell in the water column (e.g. *Asterionella* and *Campylodiscus* spp). Grazing resistance, though volume-related, might also have a shape impact, as spiny cells or colonies are probably more difficult to engulf than spheric cells. For solitary, motile epipelic diatoms such as *Navicula rhynchotella* Lange-Bertalot, a spindle shape is probably advantageous.

When the species pool was divided into two biovolume classes which were separately analyzed, relative abundance scores yielded the best resolutions by some strongly varying test factors in a Correspondence Analysis CA survey comparing abundances, surface area and volume. Abundance is also simpler to score than for example biovolume. For the latter variable, measurements on every sample would become necessary. To assign species to one of the two groups, “Small” or “Large”, data based on means of measurements from several populations are sufficient.

**Fig. 5.** Ordination plots for two correspondence analyses (CA) of epiphyton data from Area 6 in the Bothnian Sea, using relative abundance scores, showing centroids of host/site combinations (multiple regression: $P<0.05$). (a) Small species, (b) large species. The eigenvalues of the axes are given in parentheses.

Counting the large species separately yields reproducible and not just random stochastic results without the need to count thousands of specimens per sample. This approach reduces the size differences within each data set and thus allows comparisons between species within an ecologically reasonable size range. It also yields additional information on community responses, since small and large diatom species responded differently to the same environmental constraints when co-occurring in the same diatom community. Large epiphytic diatoms (Fig. 5) responded most strongly to macro-
algal host species, whereas small epiphytic diatoms responded most strongly to the environmental conditions at the sampling site. Large epilithic diatoms responded strongly to salinity while small epilithic diatoms did so less clearly. The results from the test data show that important ecological information from diatom data can be missed if the large species are neglected or underestimated.

Large species have a slower reproductive rate (RAVEN & GEIDLER 1988, MIZUNO 1991) and are probably $K$-strategists. Their surface-volume ratio is more unfavorable for nutrient uptake. They also tend to be more stenoikoous and thus give the sharp gradient responses that are most predicative in the context of bioindication and paleo-ecological reconstruction.

Compared to biovolume calculation approaches of other authors, the approach used in this study is rather precise on a species level. Despite considerable variation of shape within the genera, HILLEBRAND et al. (1999) base their biovolume calculations on standardized geometric shapes, which represent compromises at the genus level. In the study made by BRZEZINSKI (1985), biovolume and surface were estimated based on measurements of one cell dimension only. CASTENHOLZ (1960) presented biovolume factors obtained by a method similar to rectangularity (DROOP 1994), but only for 53 freshwater species including few that are found in the Baltic Sea. These factors relate to the smallest species in his list which is *Achnanthes minutissima* Kützing [syn. *Achnanthidium minutissimum* (Kützing) Czarnecki], receiving factor 1. For some species, the results of CASTENHOLZ’s calculations match very well with those of Paper I (compiled in a biovolume list in the Appendix), but for others, especially *Navicula* spp., they differ strongly.

The biovolume calculations of this study are based on averages of size measurements from several populations. Size and shape can, however, change within a species life cycle (POTAPOVA & SNOEIJS 1997), depending on the seasonal variability of the nutrient supply and the timing of the reproductive phase. They may also vary between populations as a result of modification determined by environmental factors or genetic differentiation.

Any higher number of biovolume classes would make counting routines unnecessarily difficult in relation to the additional information obtained. One could, however, ask whether it would be more economic to only analyze the large species and neglect the small ones (or vice versa). Large species are generally easier to identify and usually included in the standard Floras. They are hard to overlook, and their valves are not so easily covered by other valves. However, this tempting simplification cannot be recommended, at least not without previous testing on a smaller data set, because in certain habitats richness of large species could, for instance, be far lower than in the Baltic Sea. Moreover, as the results of Papers II and III showed, small and large species do not respond in the same predictable way to variation in environmental factors in all habitats.

**Community responses to salinity in the Bothnian Bay (Paper II)**

Paper II applies the method proposed in Paper I, to analyze separately diatoms species in two different biovolume classes, to a large data set of epilithic diatom communities
Table 1. Area means of environmental and community variables of the Bothnian Sea and Bothnian Bay and their coefficients of variation (CV). Means are based on measurements per site or site means of 2-4 samples. Temp = water temperature; Salin = salinity; NO$_3$-N = nitrate nitrogen concentration; NO$_2$-N = nitrite nitrogen concentration; Ntot = sum of NO$_3$-N and NO$_2$-N; PO$_4$-P = phosphate phosphorus concentration; SiO$_2$-Si = silicate silicon concentration; Si:P = ratio of silicon to (phosphate) phosphorus; N:P = ratio of (nitrate + nitrite) nitrogen to (phosphate) phosphorus; RichSL = richness of species <1000 μm$^2$ and ≥1000 μm$^2$ together for 250 small + x large valves (see text); RichL = richness of species ≥1000 μm$^2$ for 125 counted valves; RichSL = ratio of number of specimens <1000 μm$^2$ to specimens ≥1000 μm$^2$. Note that counting methods were slightly different between Areas 6, 7, 8 and Areas 10, 11, 12, 13, which may marginally affect species richness and the ratio of small to large specimens.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Area 7 (n = 10)</th>
<th>Area 8 (n = 10)</th>
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<td>82</td>
<td>0.14</td>
<td>24</td>
<td>0.26</td>
</tr>
<tr>
<td>NO$_2$-N</td>
<td>μmol l$^{-1}$</td>
<td>0.64</td>
<td>31</td>
<td>0.49</td>
<td>92</td>
<td>0.26</td>
<td>54</td>
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<tr>
<td>Ntot</td>
<td>μmol l$^{-1}$</td>
<td>0.86</td>
<td>31</td>
<td>0.65</td>
<td>85</td>
<td>0.40</td>
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<tr>
<td>PO$_4$-P</td>
<td>μmol l$^{-1}$</td>
<td>0.04</td>
<td>73</td>
<td>0.17</td>
<td>98</td>
<td>0.15</td>
<td>79</td>
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<tr>
<td>SiO$_2$-Si</td>
<td>μmol l$^{-1}$</td>
<td>6.54</td>
<td>22</td>
<td>11.18</td>
<td>46</td>
<td>15.32</td>
<td>15</td>
<td>19.23</td>
</tr>
<tr>
<td>Si:P</td>
<td>(by moles)</td>
<td>188</td>
<td>35</td>
<td>209</td>
<td>112</td>
<td>179</td>
<td>81</td>
<td>252</td>
</tr>
<tr>
<td>N:P</td>
<td>(by moles)</td>
<td>25</td>
<td>42</td>
<td>8</td>
<td>97</td>
<td>5</td>
<td>87</td>
<td>36</td>
</tr>
<tr>
<td>Si:N</td>
<td>(by moles)</td>
<td>8</td>
<td>33</td>
<td>25</td>
<td>56</td>
<td>43</td>
<td>38</td>
<td>10</td>
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<tr>
<td>Sand</td>
<td>mg cm$^{-2}$</td>
<td>399.7</td>
<td>208</td>
<td>95.8</td>
<td>115</td>
<td>1087.8</td>
<td>208</td>
<td>193.6</td>
</tr>
<tr>
<td>DW</td>
<td>mg cm$^{-2}$</td>
<td>15.3</td>
<td>99</td>
<td>7.9</td>
<td>69</td>
<td>19.2</td>
<td>90</td>
<td>9.2</td>
</tr>
<tr>
<td>ADW</td>
<td>mg cm$^{-2}$</td>
<td>3.0</td>
<td>40</td>
<td>2.4</td>
<td>60</td>
<td>4.0</td>
<td>48</td>
<td>2.2</td>
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<tr>
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<td></td>
<td>26.4</td>
<td>35</td>
<td>34.5</td>
<td>31</td>
<td>32.5</td>
<td>45</td>
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<tr>
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<td>31</td>
<td>22.2</td>
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<td>24.3</td>
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<tr>
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<tr>
<td>RichL</td>
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<td>24</td>
<td>20.6</td>
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<td>28.5</td>
<td>22</td>
<td>18.4</td>
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<tr>
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<td>16.0</td>
<td>21</td>
<td>12.6</td>
<td>18</td>
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from the Bothnian Bay. Species richness was high; 290 diatom taxa were recorded in the 151 samples. Diatom species richness (small and large together) differed significantly between areas (ANOVA: p<0.001) and had a minimum in Area 11 (at ca. 2.4 psu).

Table 1 summarizes area means of environmental factors and community variables from the two subsequent gradient studies (Papers II and III). It shows that phytobiomass dry weight (DW) in the Bothnian Bay varied between areas, but also strongly within areas, so that differences between areas were not significant (ANOVA: p>0.05). However, ADW% in the Bothnian Bay varied between areas (ANOVA: p<0.001) and increased from north to south, indicating a relative increase of macroalgal biomass in the samples from north to south. Relative ignition loss is usually high when macroalgae are abundant and low when diatoms dominate the algal communities.

Phytobiomass dry weight and the weight of sand grains on the sampled stones were positively correlated (Fig. 6a). Relative ignition loss (ADW%) was positively
Correlated with salinity and negatively with dry weight (DW). For epiphytic diatom communities from the Baltic Sea, Snoeijis (1994) regarded ADW% values of ca. 40% as typical, as compared to ca. 80% for macroalgae. In comparison, the ADW% values for epilithon from the Bothnian Bay were extremely low, especially in the northernmost area with only 10.8 (Table 1).

The correlation of ADW% with salinity probably does not reflect a causal relationship; instead ADW% is probably affected by factors co-varying with salinity. The low macroalgal abundance in the northernmost study area is probably due to harsh climatic conditions (ice scour) and water turbidity. Diatom dominance, especially in the north, may also be related to a high absolute silica concentration in the water. Mean ratios of Si:N (above 7) and Si:P (above 69) in the Bothnian Bay were always higher than the Redfield Ratios (Si:N:P = 48:16:1 on molar basis, Redfield 1958).

Silica, an essential component of the diatom frustule, was not limiting in the Bothnian Bay, providing a competitive advantage for diatoms in the benthic communities. Most other organisms in these communities have higher energy costs for
building up their walls from cellulose or murein since these substances, unlike silica for diatoms, first need to be synthesized. For diatoms, the possession of a silicified cell wall saves a substantial amount of energy (MARTIN-JEZEQUEL et al. 2000). Unusually thick silicification of diatom frustules may also factor into the low ADW% values measured in the Bothnian Bay.

Canonical correspondence analysis (CCA) revealed that small diatoms responded to the same principal environmental factors as large ones. However, the two most important factors had a reversed order of importance for the two biovolume classes (Fig. 7). Small diatoms were most strongly affected by exposure to wave action, whereas large species responded most strongly to salinity which co-varied with sampling area. The factor sampling area probably also includes biological factors such as grazing, competitive exclusion and the presence or absence of host species for epiphytes, apart from the physical and chemical constraints affecting community composition. Both size classes also showed strong responses to the Si:N ratio.

Community responses to water movement in the Bothnian Sea (Paper III)

For the Gulf of Bothnia, SNOEIJS (1990, 1994, 1995) showed that exposure to wave action has an effect on epilithic and epiphytic diatom species composition, biomass and species richness. However, in these studies Exposure was not the major factor varying among sampling sites. Paper III focussed on this factor’s impact on diatom communities of the Bothnian Sea where salinity, usually having an overriding effect over other factors (cf. Paper II), did not vary much.

Altogether, 218 diatom taxa were recorded in the 120 samples of the Bothnian Sea. Species richness was negatively correlated with exposure to wave action. Table 1 shows that area means of all three biomass measures (DW, ADW and ADW%) in the Bothnian Sea varied strongly within areas. None of these measures differed significantly between areas (ANOVA: p>0.05). For DW, variation within the areas was particularly high. Dry weight (Fig. 6b), as well as ADW, were most strongly (positively) correlated with Sand, ADW% was most strongly correlated with exposure to wave action (and the related factors beach type and soft bottom coverage). A correlation between DW and Sand does not necessarily mean that sand grains occurring on the sampled stones provided a suitable habitat for benthic algal communities. An abundant vegetation of filamentous macroalgae may simply trap sand grains that water movement would otherwise wash away, or these factors may only co-vary, having no causal relationship.

Canonical correspondence analysis (CCA) revealed that exposure to wave action was the main factor structuring the composition of diatom communities. All nutrient measures were significant in the ordination. Small and large species not only responded to almost the same set of environmental factors, but Exposure also had the strongest impact on both biovolume classes.

One difference between responses of the two biovolume classes, however, was that small species showed a “threshold” response, marking a clear difference between Exposure level 1 (stagnant water) and all the higher levels, whereas the large species’ response to Exposure was continuous, separating in CCA three groups of Exposure
levels (1, 2+3 and 4+5). Relative abundance scores of species at the different Exposure levels revealed that a “threshold” response was only found in species with a typically epipsammic or epipelagic life form.

Some evidence of a “threshold response” phenomenon was found in the literature: Wind velocities >4 m s\(^{-1}\) caused significant resuspension of particulate organic carbon (POC) into the water column, while winds >6 m s\(^{-1}\) resulted in no further POC increase (DEMERS & THERRIAULT 1987). Measurements of high water column primary productivity at wave-exposed sites (SHAFFER & SULLIVAN 1988) suggest that resuspension creates a relationship between microphytobenthos in the sediments and the water column. A high fraction (73-76%, BAILLIE & WELSH 1980; 90%, SHAFFER & SULLIVAN 1988) of the diatoms resuspended into the water column are pennates and thus not genuinely planktonic.

Sampling in Area 6 was carried out in a warmer year, plus two weeks later in the season than in Area 7, not compensating for any latitudinal difference. However, no large successional shifts in diatom species composition take place at this time of the year (SNOEIJS 1989, 1994). This suggests that even in the present study any possible differences in stage of succession between areas only had a minor effect on the community composition. Indeed, sampling years yielded no significant regression coefficients in CCA.

Two new *Navicula* species from brackish water (Paper IV)

Paper IV is a floristic contribution on two small *Navicula* species that were repeatedly encountered in the course of the gradient studies presented in this thesis. Previously, these species had been assigned working names (*N. spec “rond”* and *N. spec “loaf”*, see Appendix of Paper I) and are now described as new species, *N. sjoersii* S. Busse & Snoeijs and *N. bossvikensis* S. Busse & Snoeijs. They are compared to a closely related species from the same habitat, *N. perminuta* Grunow, a well-known species occurring in high relative abundances in several of the areas studied here. Morphological details, illustrated in light microscopy (LM, Fig. 8) and scanning electron microscopy (SEM) are shown in Fig. 8.

![Fig. 8. Light (LM) micrographs of *Navicula* spp.: (a) *N. sjoersii* S. Busse et Snoeijs; (b) *N. bossvikensis* S. Busse et Snoeijs; (c) *N. perminuta* Grunow. Scale bar = 10 µm.](image-url)
Fig. 9. Scanning electron (SEM) micrographs of *Navicula* spp.: (a) *N. sjoersii* S. Busse et Snoeij; (b) *N. bossvikensis* S. Busse et Snoeij; (c) *N. perminuta* Grunow. Scale bars = 2 µm.
microscopy (SEM, Fig. 9), and measurements for all three species are presented.

Small specimens of these two species can easily be confused with one another. A clear distinctive feature is, however, that the lineolae in *N. sjoersii* (ca. 40 in 10 µm) usually render a punctate appearance in LM, while in *N. bossvikensis* (ca. 50 lineolae in 10 µm) they never appear punctate. Voigt discordances were not observed in *N. sjoersii*, but were often present in *N. bossvikensis*.

The two new species clearly fit into the genus concept of *Navicula* Bory, as revised by COX (1999), even though their linear-elliptic shape with broadly rounded apices is rather uncommon in the genus, as opposed to a linear-lanceolate shape with obtusely rounded apices in *N. perminuta*. Length of the apical axis is 6.8-(10.0)-12.6 µm (n = 66) in *N. sjoersii*, 8.7-(12.8)-16.7 µm (n = 18) in *N. bossvikensis* and 9.7-(12.4)-15.7 µm (n = 73) in *N. perminuta*. The new species’ distinction from *N. perminuta* becomes even more clear in scan electron microscopy, e.g. the external polar raphe fissure describes a gently curved hook in *N. sjoersii*, a large square hook in *N. bossvikensis* and a triangular to gently curved hook in *N. perminuta*.

Both *N. sjoersii* and *N. bossvikensis* are typical brackish-water species, thriving at a salinity around 5 psu. It was suspected that the two new species might have a tube-dwelling life form like the related *N. ramosissima* (COX 1981, BASSON & SIMS 1998) and *N. perminuta* (KUYLENSTIERNA 1993), but this could not be confirmed by LM observations of formalin fixed samples.

Paper IV clarifies the taxonomic identity of *Navicula perminuta*, based on comparisons of Bothnian Sea material with published light micrographs of the type material. *N. perminuta* and the two new ones, respectively, show some likelihood or have been confused with at least the following species: *N. hansenii* Møller, *N. tropicoidea* Witkowski, Metzeltin & Lange-Bertalot in Metzeltin & Witkowski 1996, *N. celinei* Witkowski, Metzeltin & Lange-Bertalot in Metzeltin & Witkowski 1996, *N. diserta* Hustedt and *N. pseudoincertata* Giffen.

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REFERENCES


CLEVE-EULER, A. (1951-1955): Die Diatomeen von Schweden und Finnland. Teil I-V. – Kungliga Svenska Vetenskapsakademiens Handlingar, Ser. 4, 2(1) 1-163, Fig. 8-294, Taf. I-VI (I: 1951), Ser. 4, 4(1): 1-158, Fig. 292-483 (II: 1953a), Ser. 4, 4(5): 1-255, Fig. 484-970 (III: 1953b), Ser. 4, 5(4): 1-231, Fig. 971-1306 (IV: 1955), Ser. 4, 3(3): 1-153, Fig. 1318-1582 (V: 1952).


LISITZIN, E. (1957): The annual variation of the slope of the water surface in the Gulf of Bothnia. – Societas Scientiarum Fennica, Commentationes Physico-Mathematicae 20.6: 2-20.


REDFIELD, A. C. (1958): The biological control of the chemical factors in the environment. – American Scientist 46: 205-221.


