



A novel approach for estimating phytoplankton biodiversity

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Maintaining biodiversity is one of the main priorities in environmental protection. The biodiversity of phytoplankton, the key primary producers in the marine ecosystem, is, however, often very difficult to estimate, since the phytoplankton assemblage includes a vast number of taxa, many of which occur in such small quantities that they may not be recorded in routine sampling. Moreover, many taxa cannot be identified to species level by routine methods such as light microscopy of preserved samples, even by a skilled taxonomist. This means that, in general, we cannot assume to have a complete list of species in the ecosystem at any given point in time. In this paper, we present an approach for evaluating phytoplankton biodiversity in spite of this challenge. Since eutrophication, which increases phytoplankton biomass, has been identified as the most important factor causing degradation of the Baltic Sea ecosystem, the proposed approach was evaluated against total phytoplankton biomass. When phytoplankton biomass was low, both low and high biodiversity values were observed, and, as the phytoplankton biomass increased, the high biodiversity values disappeared. These results were consistent both using data based on individual samples and using yearly sampling station averages.

Keywords: Baltic Sea, biodiversity, eutrophication, phytoplankton, Shannon's index.

Introduction

Maintaining biodiversity has been recognized as one of the main priorities in environmental protection (European Union, 1992; United Nations, 1992; HELCOM, 2007). The Water Framework Directive (European Union, 2000) and the Marine Strategy Framework Directive (European Union, 2008) of the European Union, as well as the HELCOM Baltic Sea Action Plan (HELCOM, 2007) specifically mention phytoplankton as an ecological component to be addressed in the assessment of the ecological status of the sea.

Protecting biodiversity requires the ability to quantify it and track changes in it. Phytoplankton biodiversity is, however, notoriously difficult to estimate (Carstensen *et al.*, 2005). The plankton community comprises a large number of species, many present only in very low abundance, and many such that their identification requires specialized methods (such as electron microscopy) and a high level of taxonomical expertise. Moreover, the turnover rate of phytoplankton is fast, and the assemblage quickly reacts to

changes in the environment, making the community structure spatially and temporally variable (Dybern and Hansen, 1989); a large number of samples is required to overcome this high natural variability. Phytoplankton assemblage structure and biodiversity indices have been used as part of several indicators related to ecosystem health and ecological status, especially in relation to eutrophication (Arhonditsis *et al.*, 2003; Lacouture *et al.*, 2006; Sagert *et al.*, 2008; Tett *et al.*, 2008; Devlin *et al.*, 2009; Kane *et al.*, 2009; Revilla *et al.*, 2009), but the focus has largely remained on assessing water quality and eutrophic status rather than the biodiversity itself.

The Baltic Sea is a sea area with many unique features: it is a geologically young semi-enclosed brackish-water basin with strong temperature and salinity gradients, and a biota of both freshwater and marine origin (Leppäranta and Myrberg, 2009). Despite the long history of marine research in the Baltic Sea, many facets of its biodiversity remain unknown (Ojaveer *et al.*, 2010). Hällfors (2004) lists a total of 1700 phytoplankton species

reported from the Baltic Sea, with the highest species diversity (1565 species) in the Gulf of Finland (cf. Ojaveer *et al.*, 2010). These figures must be considered indicative however; the number of species identified within a sea area depends not only on the actual number of species present, but also on the skill, dedication, and focus of the analysts, as well as on the efforts allocated to investigate the particular area (Carstensen *et al.*, 2005). This is also true for individual samples, and further complicates the quantitative assessment of phytoplankton biodiversity.

The Baltic Sea phytoplankton assemblage has a strongly seasonal character (Hällfors *et al.*, 1981; Jaanus, 2011). In the central Gulf of Finland, the annual succession includes a spring bloom period, a summer minimum, a late summer bloom period, and sometimes also a modest autumn bloom. The predominant summertime taxa (in terms of biomass) in the central Gulf of Finland are typically filamentous cyanobacteria (*Aphanizomenon flos-aquae*, *Dolichospermum lemmermannii*, and *Nodularia spumigena*), dinoflagellates (*Heterocapsa triquetra* and *Dinophysis acuminata*), the autotrophic ciliate *Mesodinium rubrum*, and nanoflagellates of different taxonomical affiliations (Jaanus, 2011). The summertime autotrophic biomass usually peaks in July when cyanobacteria comprise >50% of total biomass; heavy cyanobacterial blooms are an almost annually recurrent phenomenon in the area (Jaanus, 2011).

Eutrophication, i.e. an increase of carbon especially in the form of primary producers, caused by anthropogenic nutrient loading (Nixon, 1995), has been identified as the most important factor causing degradation of the Baltic Sea ecosystem (HELCOM, 2009). In the pelagic habitat of the Baltic Sea, eutrophication has resulted in increases in summer phytoplankton abundance and biomass (Carstensen and Heiskanen, 2007; Fleming-Lehtinen *et al.*, 2008; Jaanus *et al.*, 2011) and more intense and frequent blooms (Finni *et al.*, 2001; Carstensen *et al.*, 2007). Also the phytoplankton species composition has been observed to change with different nutrient levels and ratios (Gasiūnaitė *et al.*, 2005; Carstensen and Heiskanen, 2007; Suikkanen *et al.*, 2007; Jurgensone *et al.*, 2011). It is expected that the diversity of the Baltic phytoplankton assemblage is sensitive to eutrophication, as has been shown at other locations (Gilmartin and Revelante, 1980; Moncheva *et al.*, 2002; Chalar, 2009).

In previous studies, phytoplankton biodiversity has been related to community function, primary production and community structure, eutrophication, and physical conditions (Vadrucci *et al.*, 2003; Duarte *et al.*, 2006; Spatharis *et al.*, 2007, 2008). Moreover, the applicability of phytoplankton biodiversity as a measure for eutrophication has also been assessed (Karydis and Tsirtsis, 1996; Tsirtsis and Karydis, 1998; Spatharis and Tsirtsis, 2010; Spatharis *et al.*, 2011). However, these studies have not addressed the problems concerning phytoplankton sampling and identification that lead to uncertainties as regards the actual species count in the ecosystem.

In this paper we present a novel, robust approach for detecting changes in the alpha diversity of phytoplankton. The development of the approach was made possible by access to data sufficiently extensive to assess changes of biodiversity in the phytoplankton assemblage. The aim of this work is to demonstrate and assess the behaviour of the approach using an example case from the northern Baltic Sea. The results are evaluated especially against total phytoplankton biomass, since eutrophication is the dominant pressure in the study area.

Material and methods

Study area

The Gulf of Finland is an elongated estuary (length ~ 400 km, width 48–135 km) in the north-eastern Baltic Sea. To the west, the gulf is a direct continuation of the Baltic Proper, whereas the eastern end receives the largest single freshwater inflow to the Baltic Sea from the River Neva (Soomere *et al.*, 2008). The study area is located in the central Gulf of Finland, on the transect between Tallinn, Estonia and Helsinki, Finland (24°39′–24°54′ E; Fig. 1). Water depth at the sampling stations varies from 25–30 m near the coasts to 85 m in the open part of the gulf. Surface salinity varies between 4.5 and 6.5 PSU. The Gulf of Finland and especially its entrance area is known as an important upwelling area (Leppäranta and Myrberg, 2009). Upwelling brings to surface colder, more nutrient-rich, and more saline water from the deeper layers, hence affecting the phytoplankton assemblage structure (Lips and Lips, 2010; Wasmund *et al.*, 2012).

Sampling and analyses

The data comprised a total of 547 samples from seven stations collected in June–September during 6 years (1997–2002). The number of samples per year varied between 82 and 101. All measurements and sampling were conducted on board the passenger ferries Wasa Queen and Finnjet in 1997–2002. Water was pumped through an inlet from a depth of ~ 5 m on board the moving ship, but the samples represent the entire productive layer well (Rantajärvi *et al.*, 1998). Temperature and salinity were recorded quasi-continuously with a spatial resolution of ~ 150–200 m using an Aanderaa thermosalinograph. Water samples, collected automatically from the fixed sampling stations (Figure 1), were kept refrigerated (4°C; 2–12 h depending on the schedule) in the dark before fixation with acid Lugol's solution (0.5–1 ml per 200 ml sample) in preparation for phytoplankton analysis.

Phytoplankton species composition was analysed using the inverted microscope technique (Utermöhl, 1958) within 2 weeks of sampling. The sedimented volume was mainly 25 ml. Wet weight biomass of phytoplankton was based on cell geometry and derived from publications (HELCOM, 1988; Hillebrand *et al.*, 1999) or, for those species not in these lists, calculated from cell geometry according to cell size measurements. All taxa were identified to the level achievable in fixed material using light microscopes (Olympus IM, Olympus IMT-2, and Leitz Fluvert FU). Most taxa were identified and counted using × 200 magnification, except for small flagellates (<20 µm) and small centric diatoms, for which × 400 magnification was used. Autotrophic picoplankton was not included in the analysis, nor was their biomass included in the estimate of total biomass.

The taxonomic unit used in the analysis was species where possible; however, all of the specimens cannot be determined to species or even genus level (Ojaveer *et al.*, 2010). The greatest possible accuracy was used, meaning that the analysis included different taxonomic units: of 140 taxa, 86 were determined to species level, 45 to genus level, and 9 to higher than genus level. This approach was also adopted by Vadrucci *et al.* (2003). When deemed relevant, we distinguished between autotrophic and heterotrophic individuals in genus or higher level taxa. The taxonomical nomenclature was updated (mainly according to Hällfors, 2004).

To obtain biomass results which were as accurate as possible, several of the phytoplankton taxa were recorded in two or more

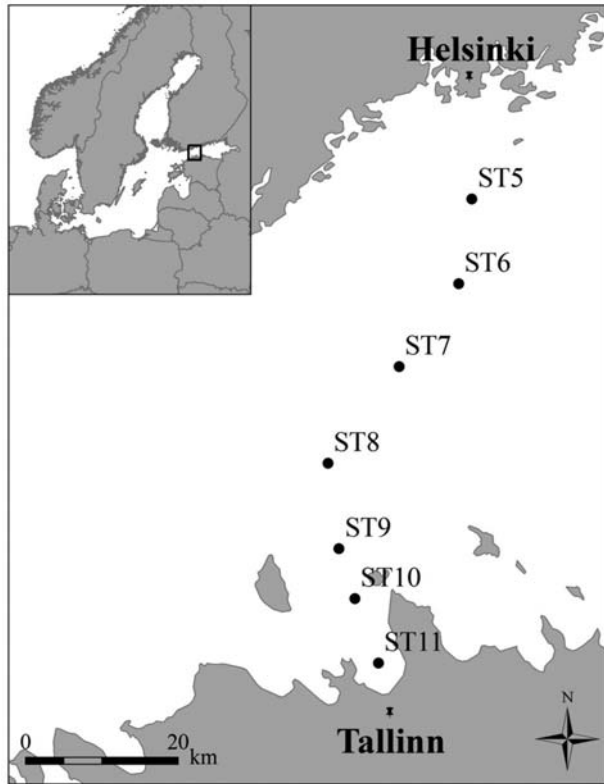


Figure 1. The sampling stations in the Gulf of Finland, along the ship route from Helsinki to Tallinn. Coastline data from the Digital Chart of the World.

size classes when the samples were analysed. The biomass of each taxon was calculated based on these records. Due to a counting software-related technicality, taxa with a very low biomass, recorded as 0, were recalculated as $0.1 \mu\text{g l}^{-1}$ to facilitate their inclusion in the analysis.

Biodiversity estimation

Metrics were developed to describe the diversity of the main part of the phytoplankton assemblages, emphasizing the evenness of dominating taxa, i.e. those present in the highest biomasses. Measurements of biomass, rather than abundance, were used since they can be readily translated into understanding of biogeochemical cycles, they link to eutrophication, and are considered to give a more accurate picture of the phytoplankton community (Paasche, 1960; Olenina *et al.*, 2006). The aim was to find a metric that would robustly capture the difference between communities dominated by one or few taxa (i.e. assemblages with a low biodiversity), and those with more even species distributions (i.e. assemblages with a higher biodiversity). R software (R Development Core Team, 2011) and its ‘plyr’ package (Wickham, 2011) were used for calculations.

In order to examine the extent to which the total biomass constitutes just one or few taxa, the taxa were ordered in a descending order of biomass, and their proportion of the total biomass was computed (Figure 2). The proportion of the most dominant taxon in a single sample varied from $> 80\%$ to $< 20\%$, while the proportion of the second most dominant taxon was usually between 5% and 30% of the total biomass. To describe the

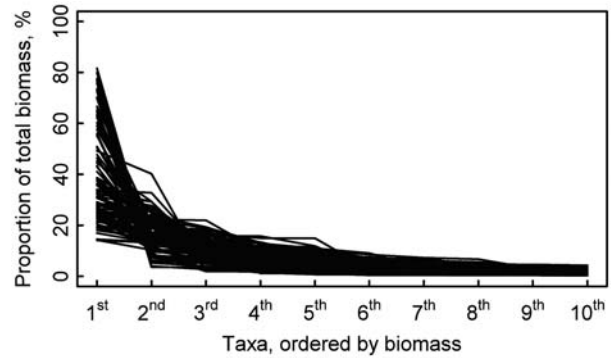


Figure 2. The relative biomasses of the ten most dominant taxa in the samples taken in 2002. Each line in the graph describes one sample. The taxon with the highest biomass is on the left, the one with the second highest biomass is the second from left, etc. The y-axis represents the proportion of the total biomass that each taxon constitutes.

evenness of the species distribution, we examined the following biodiversity measures as indicator candidates: (i) the cumulative proportion of biomass of the three, five, or ten most dominant taxa; (ii) the number of taxa that together account for 75, 90, 95, or 99% of the total biomass; and (iii) a derivative of Shannon’s index henceforth referred to as Shannon95.

The Shannon95 index is a derivative of the classical Shannon’s diversity index (Shannon, 1948). Originally, the Shannon index was developed to be used with abundance data as input, and under the assumption that the abundance of all species is known. The current version, Shannon95, was computed using the Shannon index equation, but using the biomass data of the taxa that together constitute 95% of the total recorded biomass (Figure 3). In other words, we include taxa in descending order of biomass until the cumulative biomass reaches 95%, and compute the Shannon index using the biomasses of these taxa. Biomass of phytoplankton has been used as the basis of Shannon’s index calculations previously by Spatharis *et al.* (2011). Natural logarithm was used when computing Shannon’s index.

The biodiversity measures were computed for each sample separately, and year- and station-wise averages were computed from these values weighing each sample equally.

Upwelling index

In the Gulf of Finland, upwelling occurs when alongshore winds are blowing: northeastern and eastern winds cause upwellings near the Estonian coast, and western and southwestern winds near the Finnish coast. The intensity of the upwelling was estimated for June–September 1999–2002, using an upwelling index (ΔT_{si}) described by Lips and Lips (2008). Years 1997–1998 were excluded due to insufficient data. Temperature observations from the transects, divided into 40 equal sections, were used in order to identify upwelling events. The near-coast water temperature (sections 1–10 and 31–40) was compared with the mean water temperature in the open part of the gulf (sections 11–30), and the temperatures measured in each section during successive transects were analysed to describe initiation and development of upwelling events. The intensity of upwelling events was calculated for every crossing.

Results

The eight examined biodiversity measures correlated well with each other (Table 1). The proportion of biomass of the top three, five, and ten taxa had negative correlation coefficients with the Shannon95 index and the number of taxa in 75, 90, 95, and 99% of total biomass, which was expected: the more the community is dominated by one or few taxa, the higher the biomass of the top three, five, and ten taxa and the lower the Shannon95

index and the number of taxa found in 75, 90, 95, and 99% of biomass.

The summer averages of the biodiversity measures, as well as total biomass, followed the same year-to-year pattern in all stations (Figure 4, station numbers in Figure 1). There was higher variance, as well as a larger difference between the largest and smallest observed value, between different years in one station than between stations during any one year. This

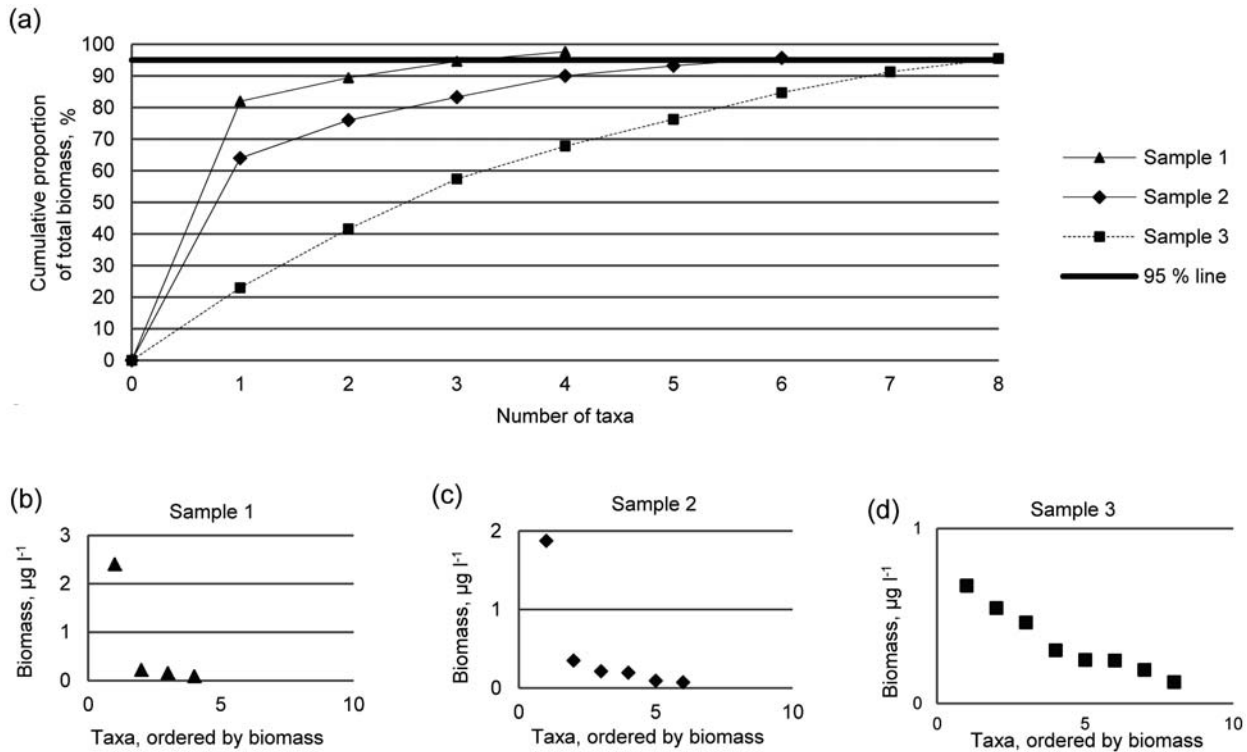


Figure 3. Computation of the Shannon95 metric. (a) The taxa to be taken into the calculations are defined by arranging the taxa in the order of decreasing biomass, and starting from the most dominant taxa, including it and the following taxa until their cumulative biomass reaches 95% of the total biomass. (b–d) The Shannon index was computed using the biomasses of these taxa; the resulting figure is the Shannon95 metric.

Table 1. Correlation coefficients between the biodiversity measures computed from each sample separately ($n = 547$).

	No. of taxa in 75%	No. of taxa in 90%	No. of taxa in 95%	No. of taxa in 99%	Biomass of top 3 taxa	Biomass of top 5 taxa	Biomass of top 10 taxa	Shannon95	Total biomass
No. of taxa in 75%	1.00	0.90	0.83	0.65	-0.94	-0.96	-0.90	0.94	-0.49
No. of taxa in 90%	0.90	1.00	0.97	0.82	-0.86	-0.92	-0.96	0.89	-0.49
No. of taxa in 95%	0.83	0.97	1.00	0.90	-0.79	-0.86	-0.94	0.83	-0.47
No. of taxa in 99%	0.65	0.82	0.90	1.00	-0.60	-0.69	-0.82	0.65	-0.28
Biomass of top 3 taxa	-0.94	-0.86	-0.79	-0.60	1.00	0.96	0.81	-0.96	0.54
Biomass of top 5 taxa	-0.96	-0.92	-0.86	-0.69	0.96	1.00	0.91	-0.93	0.50
Biomass of top 10 taxa	-0.90	-0.96	-0.94	-0.82	0.81	0.91	1.00	-0.82	0.40
Shannon95	0.94	0.89	0.83	0.65	-0.96	-0.93	-0.82	1.00	-0.58
Total biomass	-0.49	-0.49	-0.47	-0.28	0.54	0.50	0.40	-0.58	1.00

The correlation coefficients with absolute values ≥ 0.8 are in bold.

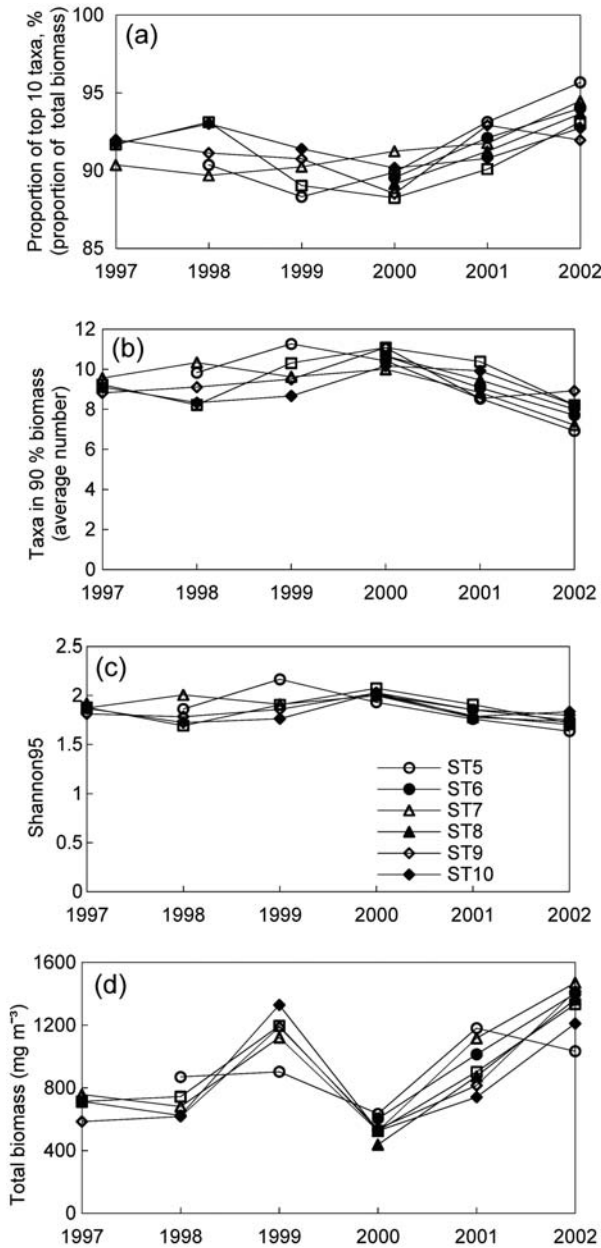


Figure 4. The yearly summer season averages for each station for three biodiversity measures and total biomass. (a) The proportion of total biomass that the ten taxa with the highest biomasses constitute; (b) the number of taxa that together constitute >90% of the total biomass; (c) Shannon95 index; (d) total biomass. These biodiversity measures (a–c) are shown as examples; all examined measures displayed the same pattern.

implies that there are no major differences between the stations, and they can all be considered to represent the same pelagic ecosystem in the central Gulf of Finland. The pattern displayed by the yearly station averages reflects the same behaviour as the sample-wise correlations (Table 1): as the proportion of the top ten taxa went up, the Shannon95 index and the number of taxa that constitute 90% of the biomass went down, correlating negatively with it.

The biodiversity measures had a clear relationship with total phytoplankton biomass: the highest observed biodiversity values

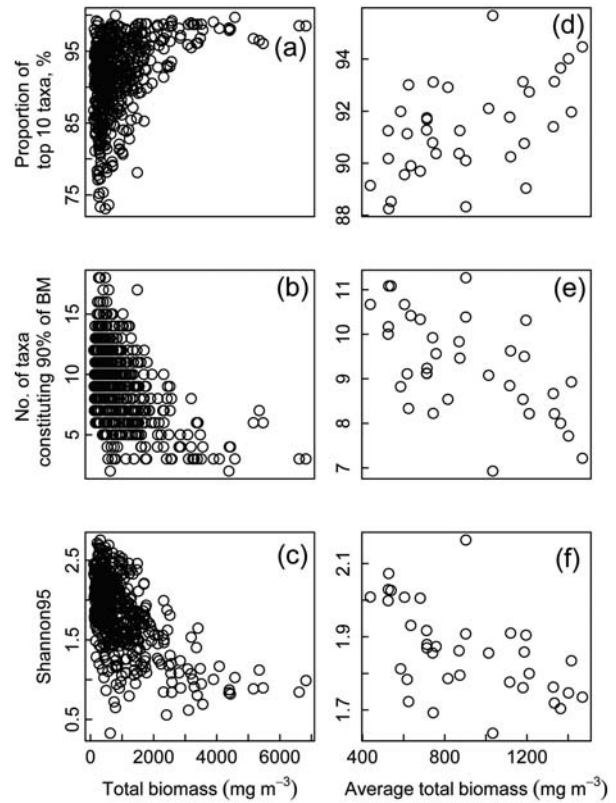


Figure 5. The biodiversity measures and total phytoplankton biomass. (a) Proportion of the top ten taxa for all observations separately. (b) Number of taxa constituting 90% of the biomass for each observation separately. (c) Shannon95 index for each observation separately. (d) Proportion of top ten taxa, yearly averages for each station. (e) Number of taxa constituting 90% of the biomass yearly averages for each station. (f) Shannon95 index yearly averages for each station.

decreased steadily as total biomass increased (Figures 4 and 5). Such a clear division line could not be found in the yearly station averages (Figure 4d–f), but some correlation between biomass and biodiversity measures remained; the correlation coefficient was 0.52 for total biomass and the proportion of the top ten taxa, -0.59 for total biomass and the number of taxa in 90% of the total biomass, and -0.55 for total biomass and Shannon95 index, when only those year and station combinations with > 9 observations were considered. The correlation coefficients increased significantly if stations with fewer observations were also included, but we considered this misleading: the averages calculated with only a small number of observations can be more extreme due to the bigger role of random variation (the Law of Large Numbers). In the case of these data, these values were more extreme in the direction that would strengthen the argument that biodiversity decreases as biomass increases; however, the argument was considered to be more reliable when the averages computed from only a small number of values were excluded.

Upwelling, indicated by negative upwelling index values, did not have a clear and consistent connection with the Shannon95 index on either coast (Figure 6). The correlation coefficients between upwelling (on the same day and as the average of the preceding 3, 5, or 10 days) and Shannon95 index and total biomass had absolute values between 0.25 and 0.29 (Table 2).

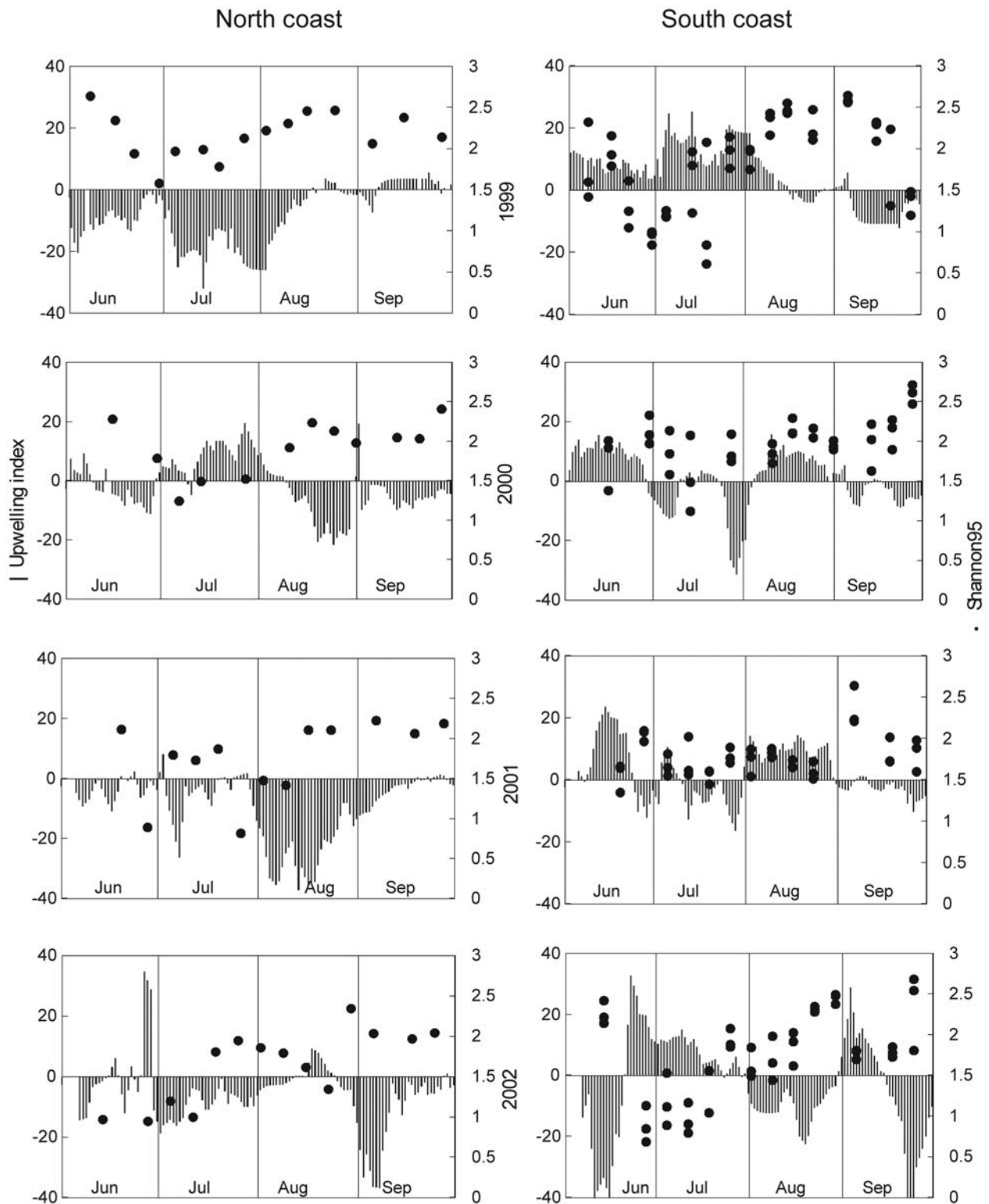


Figure 6. Upwelling index (bars) and Shannon95 (dots) on the northern (left) and southern (right) coast. Negative values indicate upwelling. On the northern coast, there was just one offshore station (station 5) in the upwelling zone; on the southern coast, there were three (stations 9–11). Stations 6–8 were not considered here due to their position in the open sea.

Discussion

We present a robust approach for assessing the level of and tracking the changes in the alpha biodiversity of the phytoplankton

assemblage. This approach focuses on the taxonomic diversity of the main body of the phytoplankton biomass, i.e. the taxa that together comprise 95% of the phytoplankton biomass at any given

Table 2. The correlation coefficients between upwelling, Shannon95 index, and total phytoplankton biomass, in all samples separately ($n = 278$).

	Shannon95 index	Total observed biomass
Upwelling index, same day	-0.26	0.29
Upwelling index, last 3 days' average	-0.26	0.28
Upwelling index, last 5 days' average	-0.27	0.28
Upwelling index, last 10 days' average	-0.25	0.28

time. In other words, this approach gives an indication of the extent to which the phytoplankton community is dominated by one or few taxa.

Another component of biodiversity, the number of different species present in the ecosystem, is not covered by this approach. The phytoplankton assemblage consists of a very large number of species, and the number of species (or taxa) recorded in a sample is dependent not only on what is present in the sea or the skill, dedication, and focus of the analysts, or on the efforts allocated to investigate the particular area (Carstensen *et al.*, 2005), but also on the restrictions of the routine analysis methodology; many taxa simply cannot be identified to species level by light microscopy of preserved samples (Ojaveer *et al.*, 2010). Therefore, it is considered that this aspect of biodiversity cannot be reliably measured.

There are three ways in which to approach the problem of having to work with data with variable taxonomic detail: to exclude all except species-level observations; to summarize all data into genus- (or higher) level information; or to use the data as they are and accept the fact that the taxonomic units vary. All of these options have their downsides: losing data in the first option, and losing accuracy of data in the second, and having to deal with data where all entries are not entirely comparable in the third. We consider the third approach the best of the available options, since discarding parts of the data or aggregating them means losing information related to biodiversity, precisely the property we aim to estimate. Using all of the available data, with some taxa identified to higher taxonomic level than species, means that we may underestimate the biodiversity in cases where the genus or higher unit actually includes several species. As the taxa were identified to as good a level as possible, the analysis result will, however, be the best available estimate of taxonomic diversity.

We examined three categories of biodiversity measures: those focusing on the number of taxa constituting the majority (75, 90, 95, or 99%) of the biomass; the proportion of biomass consisting of a small, selected number (three, five, or ten) of dominating taxa; and the Shannon95 index. These approaches provide slightly different slants to the same question: to what extent is the community dominated by just one or a small number of taxa? The aim of comparing these approaches was to examine their behaviour and see if any of these metrics differ from the others, or display particularly robust behaviour. The number of taxa constituting the majority of the biomass is indicative of the extent to which the community is dominated by the top taxa, but the examined proportion of the total biomass needs to be selected with care. If the chosen percentage is too small, the results are likely to be constricted between one and three taxa, and the results may not be very informative (cf. Figure 2). On the other hand, if the chosen

percentage is too large, the proportions of the taxa that are present in small biomasses might be given too much weight in the result: whether we get 8 or 11 taxa in the top 99% may in the end be due more to chance than to the real situation in the sea. The proportion of biomass consisting of a small, selected number of dominating taxa avoids these problems, but does not consider the relative biomasses of these taxa. The Shannon95 index combines these aspects since it considers both the number of taxa in 95% of the biomass and the evenness of these taxa. Therefore, the Shannon95 index is somewhat less prone to random variation than either of the simpler approaches.

Spatharis *et al.* (2011) examined the behaviour of the Shannon's index on real and simulated phytoplankton data. They pointed out that there was much unexplained variance in the index value in real-world data, suggesting that the index is sensitive to stochastic processes. The Shannon95 index suggested here may reduce the effect of this stochasticity, as it decreases the effect that rare species may have on the index value, as they may or may not appear in the samples even though they are present in the assemblage.

The strength of correlations between the different measures and biomass across the whole data are in the neighborhood of 0.5 (positive for the relative biomass of the top three, five, and ten taxa, and negative for the other measures); the number of taxa in 99% of the total biomass had a lower correlation of -0.28 with the total biomass, while the Shannon95 index had a slightly higher correlation of -0.58 (Table 1). The examined biodiversity measures, including the Shannon95 index, indicate low biodiversity in populations of high total biomass (Figures 4 and 5), while the biodiversity had a wide range of values when biomasses were lower. No clear threshold could, however, be observed where the biodiversity values would change greatly; rather, the disappearance of high biodiversity values, as the total biomass increases, appears to be gradual (Figure 5).

Theory suggests that biodiversity should have a unimodal, dome-shaped, relationship with productivity, i.e. biodiversity should peak at intermediate levels of productivity (Grime, 1973; Irigoien *et al.*, 2004). The current results can be assumed to include only the right-hand side of this shape however: eutrophication has been identified as a problem in the Baltic Sea since the 1980s (e.g. Larsson *et al.*, 1985; Elmgren, 1989), and hence the current data do not cover non-eutrophied, low-productivity conditions. Spatharis *et al.* (2011) pointed out that while this theory of a unimodal relationship is strong, the area below the unimodal curve is often filled with data points. The phytoplankton assemblage is highly receptive to alterations in temperature, salinity, the relative abundance of different nutrients, and predation, among others (e.g. Gasiūnaitė *et al.*, 2005; Muylaert *et al.*, 2010), which are also reflected in the biodiversity assessment (Spatharis *et al.*, 2011).

Upwelling is known to affect the phytoplankton assemblage by bringing water that is colder, more saline, and rich in dissolved nutrients to the surface (Blasco *et al.*, 1981; Lips and Lips, 2010), and smaller sized phytoplankton have shown the ability to respond faster to upwelled nutrients compared with the larger sized fraction (Kovaldina *et al.*, 2010). The dominance of small-sized taxa usually means that the proportion that the dominant taxa constitute of the total biomass is relatively low, meaning that the biodiversity values are higher than when large-sized taxa dominate. This suggests that biodiversity values might be higher after an upwelling event. In the current results, however, no consistent effect of upwelling events on the biodiversity estimates could be observed (Figure 6).

The indices presented here are currently restricted to describing alpha diversity, i.e. biodiversity at one place and time. For example, when assessing the year- and station-wise averages, we computed the diversity measure for each sample, and then used these values to compute the averages. This means that the value of the biodiversity measures would be the same whether we had the same taxa in all of the samples during the summer, or dominant taxa changing from sample to sample, as long as the diversity values of the individual samples were the same.

Due to the potentially very quick changes that can occur in the phytoplankton communities as a response to changes in their environment, even this robust approach for estimating the phytoplankton biodiversity requires rather intensive sampling. The current work is based on samples taken, generally, every 2 weeks. Our results indicate that in an open sea area such as the Gulf of Finland, this sampling frequency is sufficient to give results that are reliable on a yearly average basis: the yearly averages computed for each station (Figure 4), covering the open water area of the transect across the gulf (Figure 1), were very similar for all stations each year. The time series of 6 years is relatively short, and no trends in the biodiversity can be reliably discerned, although year-to-year variation can be detected (Figure 4).

Our results suggest that a robust assessment of phytoplankton biodiversity is possible, provided that it is accepted that the biodiversity measure does not include the whole phytoplankton assemblage, but is based on the taxa that dominate the assemblage, forming a large majority, e.g. 95%, of the total biomass. The exact percentage of biomass to be included in the metric can be defined according to the area, season, etc.; based on current experience, something lower than 99% would be advisable, but this proposition needs to be further tested on data from other areas. While keeping in mind its limitations, this approach can be refined to function as a biodiversity indicator to aid marine protection, including the Marine Strategy Framework Directive of the European Union (European Union, 2008). To act as an indicator, the measure needs to be complemented with a target or a reference value, defining the range of accepted values, and the range where restoration is needed. In optimal circumstances, reference values are reconstructed using data from a time or site unaffected by human-induced pressures; in the absence of such data, another approach or method would be needed. In addition, an examination of the required sampling frequency and spatial resolution is needed to define the required monitoring programme.

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