Phytoplankton Species Richness along Coastal and Estuarine Salinity Continua

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Abstract: The high number of freshwater species at low salinity and the correspondingly high number of marine species at high salinity enveloping a conspicuous richness minimum at intermediate salinities has shaped our basic understanding of biodiversity along a coastal salinity gradient for almost 80 years. Visualized as the Remane curve, this iconic concept was originally based on sedentary macroinvertebrates in the Baltic Sea. To what extent the concept can be generalized, particularly to free-drifting organisms, is currently debated. Here we use approximately 16,000 phytoplankton samples from two large coastal ecosystems—the Baltic Sea and Chesapeake Bay—to analyze the relationship between salinity and phytoplankton species richness. Alpha diversity showed a consistent variation along the salinity gradient, with a minimum at mesohaline salinities of around 7–9. Rarefied species pools at narrow salinity intervals also showed reduced diversity at intermediate salinities, surrounded by high richness toward both ends of the gradient. The cumulative likelihood of species presence validated the minimum at intermediate salinities. Community composition changed abruptly at the α diversity minimum in the Baltic Sea, while it changed gradually along the salinity gradient in Chesapeake Bay. We conclude that the Remane concept is in every respect valid for phytoplankton.

Keywords: biodiversity, biogeography, phytoplankton, Remane curve, salinity gradient.

Introduction

It has been more than 80 years since Adolf Remane (1934) published his seminal Artéménimum concept describing minimum species diversity at intermediate salinities along a continuum from full oceanic salinity to fresh water. The concept was visually coined as the Remane curve, a graphical generalization of species richness distribution along the salinity gradient. The curve disclosed a high number of freshwater species at low salinities, a concomitant equally high number of marine species at high salinity, and a conspicuous richness minimum at intermediate salinities of about 6 (Remane 1934). In essence, the Remane diagram visualizes the inability of many freshwater and marine species to tolerate low salinities, while the limited number of true brackish species cannot compensate for the absence of freshwater and marine taxa (Logares et al. 2009; Whitfield and Elliott 2011).

Two mutually nonexclusive perspectives may explain the artéménimum. First, as brackish habitats necessarily border marine and freshwater habitats, they are isolated from each other on a global scale. The low diversity at intermediate salinity thus may emerge from limited connectivity between these dispersed habitats. Second, we may postulate a universally reduced biodiversity (limited species pool size) due to the limited number and overall size of habitats and the ephemeral nature of oligohaline and mesohaline conditions that does not promote speciation on a large scale (Whitfield 1994).

Besides these general patterns that have been described for macroscopic taxa, complexity is added by the vast differences in dispersal rates, evolutionary speciation timescales, and global distribution models when it comes to comparing microbial and macrobial organisms (Martiny et al. 2006). It has been postulated that microbial diversity follows fundamentally different rules (Finlay 2002; Fenchel and Finlay 2004), while others found indication of global scaling rules for macrobial and microbial diversity (Telford 2006; Vervier et al. 2007). The applicability of ecological theory regarding metacommunity dynamics and emergence of regional species pools is debated for microbial organisms. Fi-
nally, diversity of planktonic taxa may be affected by unfavorable salinity per se, while sessile benthic organisms could be more constrained by the range of fluctuation in salinity (Attrill 2002).

Originally described for macrozoobenthos in the Baltic Sea, the Remane diagram legend does not refer specifically to invertebrate diversity, suggesting that the diagram was intended for general rather than specific use—hence the ease with which it has been used as a paradigm to cover the biota in all estuaries (Elliott and Whitfield 2011; Whitfield et al. 2012). While the pattern is widely quoted as accounting for this species minimum, there is an obvious need for evidence that it can be applied to other organisms and not just the sedentary invertebrate taxa used by Remane (Snoeijs-Leijonmalm 2017).

Although it is accepted that communities in transitional waters are characterized by a low taxonomic $\alpha$ diversity, there is a high diversity of other aspects, particularly physiological diversity, where euryoecious species can tolerate highly variable conditions (Elliott and Whitfield 2011; Basset et al. 2013). Low taxonomic $\alpha$ diversity in combination with high physiological diversity is termed the “estuarine diversity paradox” (Basset et al. 2013). The low but significant number of brackish species is a reflection of the stress subsidy—they thrive, often forming high biomass and productivity, partly due to low interspecific competition (Elliott and Quintino 2007).

The species richness–salinity relationship can be expressed by two fundamentally different approaches. First, $\alpha$ diversity or the sample species richness can be considered. Second, we can think of the species pool within a salinity range. The latter also accounts for the heterogeneity and size of the habitat. Species pool tends to increase as habitat size increases, as predicted by the species-area relationship—one of the most universal and fundamental laws in ecology (Lomolino 2000; Azovsky 2011; Šťirling et al. 2011). For instance, the Baltic Sea has the largest area around salinity 5–7 (the central Baltic Sea), suggesting that the species pool could peak near this range.

The consequences of the salinity-diversity functional relationship become increasingly significant in the era of climate change and sea-level rise, as estuaries and other transitional waters may undergo major changes. Recent studies from Chesapeake Bay revealed that variable climatic conditions—for example, alternating dry and wet years—dominate the phytoplankton dynamics and taxonomic composition in a predictable way (Harding et al. 2015, 2016). Climate change models predict a long-term increase in precipitation in northern Europe (BACC II Author Team 2015), which affects surface salinity in the Baltic Sea—one of the largest brackish ecosystems. This, in turn, may impact species diversity and ecosystem productivity and functioning (Ptacnik et al. 2008; Olli et al. 2014). Therefore, understanding the vulnerability of freshwater and marine taxa in transitional waters and the role and colonization potential of brackish species has never been more important.

The aim of this study is to use empirical data from long-term environmental monitoring programs to analyze the relationship between phytoplankton species richness and salinity in two large coastal ecosystems (fig. 1). Our purpose here is also to investigate the validity of the Remane concept for the dynamic phytoplankton communities. We hypothesize that species richness of phytoplankton is not uniformly distributed along the salinity gradient but conceptually follows the pattern of the Remane curve. We explicitly address $\alpha$ diversity in our study as estimated from

![Figure 1: Spatial distribution of phytoplankton sampling locations in the regional data sets. A, Baltic Sea; B, Chesapeake Bay. Maps were created in R with the rworldmap (ver. 1.3-6) and rworldextra (ver. 1.01) libraries.](image)
single phytoplankton samples, which is most directly related to ecosystem processes carried out by phytoplankton.

Methods

Study Areas and Data Sets

We used light microscopy–derived species-specific quantitative phytoplankton data from two coastal ecosystems of the world ocean (Olli et al. 2015; fig. 1). Both ecosystems are influenced by river inflows, with time-varying mixtures of seawater and fresh water, pronounced seasonality, and salinity gradients ranging from fresh water to near oceanic salinity of 32. Chesapeake Bay is the largest estuary in the United States (∼11,000 km²), with nearly 16 million people inhabiting the 165,000-km² watershed. The Baltic Sea is a semi-enclosed brackish water body (377,000 km²), with a drainage area that is more than four times larger (∼2 million km²) and highly populated (∼80 million people). Both have suffered from nutrient overenrichment since World War II, which has led to deterioration of the ecosystems caused by increased phytoplankton biomass and spreading of anoxic bottom waters (Elmgren 2001; Harding et al. 2016).

Detailed data compilation and harmonization methods are presented elsewhere (Olli et al. 2013, 2015). Sampling and sample processing details are given in respective program manuals (Michaels and Kahrr 2010; HELCOM 2014). Shortly, aliquots of surface layer samples were preserved with acid Lugol’s solution or formalin, and cells were enumerated from aliquots of 10–50 mL using inverted microscopes. Our study involves 7,896 samples from Chesapeake Bay, taken from fixed statutory monitoring stations between 1984 and 2006. From the Baltic Sea, 7,744 samples represented a combination of fixed and spatially scattered locations and were collected between 1984 and 2008. The counting effort varied but in general was better than 100 cells of the most numerous taxon. We used only phytoplankton samples that were accompanied by salinity measurements. The nomenclature was harmonized according to the World Register of Marine Species (Costello et al. 2013) by using Web Services through the R interface (R library taxizesoap). Data tables needed to reproduce the results in this study are deposited in the Dryad Digital Repository (https://dx.doi.org/10.5061/dryad.q6m40r0; Olli et al. 2019).

α Diversity along the Salinity Gradient

The association between α diversity, measured as sample species richness, versus salinity was nonlinear. The relationship was visualized and examined with generalized additive model smoothers (GAM; mgcv library in R). The analysis is based on a penalized regression-spline approach with automatic smoothness selection. Degrees of smoothing were selected by the package to minimize the generalized cross-validation score (Wood 2006). The explained deviance of the GAM indicates the strength of the effect of salinity gradient on species richness (model I; table 1).

In addition, we analyzed the effect of potential confounding factors, such as seasonal and decadal scale trends, and temperature on α diversity. First, we added the confounding factors into the GAM model (model II) and compared directly their effects (F values). Second, we fitted a GAM model with α diversity as a dependent variable and only the confounding factors as independent variables. The residuals of this GAM model—that is, the detrended α diversity values—were then analyzed against salinity (model III).

Species Pool along the Salinity Gradient

To compare the species pools along a salinity gradient, we used an arbitrary two-unit salinity window and moved it in 0.5-unit increments over the salinity continuum. The

Table 1: Generalized additive model outputs of the regionally explicit data sets

|                      | Chesapeake Bay | Baltic Sea      |
|----------------------|----------------|-----------------|
|                      | edf            | F               | edf            | F               |
| Model I              | n = 7,896      | 32% deviance explained | n = 7,744      | 19% deviance explained |
| Salinity             | 7.6            | 440.0           | 8.8            | 204.1           |
| Model II             | n = 7,895      | 38% deviance explained | n = 7,719      | 30% deviance explained |
| Salinity             | 7.5            | 415.2           | 8.8            | 218.8           |
| Day of year          | 8.6            | 43.3            | 8.0            | 22.5            |
| Temperature          | 7.0            | 30.3            | 5.0            | 9.5             |
| Year                 | 8.6            | 27.7            | 8.8            | 69.6            |
| Model III            | n = 7,895      | 29% deviance explained | n = 7,719      | 19% deviance explained |
| Salinity             | 7.5            | 375.8           | 8.8            | 203.0           |

Note: Model I = species richness as a function of salinity; model II = species richness as a function of salinity, seasonal trend (day of year), temperature, and long-term trend (year); model III = species richness detrended with respect to temperature, day of year, and year as a function of salinity. Due to the high sample size (n), all effects are highly significant (P < .001). The explanatory power is shown by the deviance explained by each model. edf = estimated degrees of freedom for the model terms.
overlapping but discrete groups of phytoplankton samples within the increments were subjected to rarefaction. The number of samples per salinity interval ranged from 108 to 3,941 in the Baltic Sea and from 138 to 730 in Chesapeake Bay. The highest number of samples in the Baltic Sea corresponded to a salinity interval of 4–6, reflecting the large surface area within this salinity range. The lowest number of samples, 108 at a Baltic Sea salinity interval of 11–13, set the limit for sample-based rarefaction to approximate the species pool in each specific two-unit salinity window without extrapolation.

Further, we used coverage-based rarefaction, which standardizes samples based on equal completeness of assemblages (Chao and Jost 2012). Completeness in our context is the proportion of the true total species richness (including undetected species) represented by the pooled species richness detected by the sample. The true total species richness remains unknown, but completeness of the sample can be judged by looking at the final slope of the rarefaction curve calculated from the species frequency data. First, we calculated the final slopes of sample-based rarefaction curves of 108 random samples over all salinity windows. The final slopes varied from 0.4 to 1.0, depending on the salinity window, with a mean of 0.6 added taxa per added phytoplankton sample. Second, we calculated coverage-based rarefaction curves (iNEXT function of the iNEXT library in R, with incidence_raw data type and Hill number 0; Hsieh et al. 2016). Finally, the species richness of the coverage-based rarefaction curves, closest to the curve slope of 0.6, was used as a coverage-based standardized pooled diversity estimate.

Community Composition along the Salinity Gradient

We used nonmetric multidimensional scaling (NMDS) to visualize the variation of phytoplankton community composition along the salinity continuum. First, we constructed community matrices from quantitative biovolume values (species in columns, samples in rows). The community matrices were square root transformed to stabilize variability, followed by Wisconsin double standardization (species biovolumes were first divided by their maxima and thereafter by sample total biovolumes). From the community matrix, a Bray-Curtis dissimilarity matrix was calculated and subjected to NMDS (metaMDS with two axes; vegan library in R). Finally, the sample scores of the first ordination axis, which captures most of the variability in the community composition, were plotted against salinity and superimposed by a fitted GAM smoother to reveal the shape of the relationship.

Due to seasonality in phytoplankton community composition, NMDS was restricted to the summer season (May–September). Further, the spatial distribution of salinity can be highly uneven in coastal ecosystems. For example, in the central Baltic Sea, large areas have a surface salinity between 5 and 7, while other salinities have a lower areal coverage. This unevenness is also reflected in sampling frequency and may obscure NMDS analysis. To alleviate the unevenness and allow fair representation of different salinity regions, we down-sampled areas with high sampling frequency proportionally to the inverse of the salinity frequency distribution.

Species-Specific Cumulative Likelihood of Presence along the Salinity Gradient

We predicted the likelihood of presence for the observed taxa along the salinity gradient (GAM on presence-absence data; binomial error distribution with logit link). Rare species, which were present in less than three samples, were excluded. The taxa were ordered according to their salinity optima, that is, the salinity where the likelihood of presence was highest. Thereafter, the ordered likelihoods were plotted cumulatively and color coded according to the salinity optima.

Habitat Specialists and Generalists

We divided the salinity gradient into two to represent two habitats—one in fresh water and the other on the marine side. Next, we used a multinomial model (clamtest in the vegan library of R, using the default two-thirds supermajority rule for classification threshold) to categorize the phytoplankton species into habitat specialists, generalists, and species too rare to be classified (Chazdon et al. 2011). The model is insensitive to unequal sampling intensities and within-habitat heterogeneity. However, clamtest uses count data to judge species abundances, which is not how phytoplankton data are archived. We thus retreated to binary presence-absence data, which is a conservative approach ignoring the uneven species abundances and, as a consequence, increases the proportion of rare species at the expense of other categories.

Results

The combined phytoplankton data set from both regions contained 1,366 taxa—800 in Chesapeake Bay and 963 in the Baltic Sea, with 397 taxa shared by both regions. Alpha diversity, measured as the sample species richness, showed a predictable and systematic variation along the salinity gradient, with a minimum at intermediate salinities of around 7–9 (fig. 2). In both ecosystems, a diversity was higher at the marine end of the salinity gradient. In Chesapeake Bay, salinity explained more variation in species richness (32%) than in the Baltic Sea (19%; model I in table 1). Adding temperature, seasonal, and long-term trends into the GAM model increased the explanatory power of the models, but salinity
remained the most influential predictor of $\alpha$ diversity (compared as $F$ values; model II in table 1). Long-term trend was an important predictor of $\alpha$ diversity in the Baltic Sea, which we have shown previously (Olli et al. 2014), but not in Chesapeake Bay. Finally, the detrended $\alpha$ diversity (the effect of confounding variables removed; model III in table 1) revealed a similar relationship with salinity as the raw data (the shape of the relationship), and the explained deviance dropped only marginally. We therefore conclude that the salinity effect on $\alpha$ diversity is genuine and not due to known confounding variables.

Rarefied species pools showed reduced diversity at mesohaline salinities, enveloped with high species richness at the saline and fresh limbs (fig. 3). Both ecosystems—the Baltic Sea and Chesapeake Bay—showed partial match in the peaks of the species pools and had uneven surface areas along the salinity gradient. In the Baltic Sea, the rarefied species pool was at a minimum at a salinity of around 9, which is close to the $\alpha$ diversity minimum. A similar pattern was evident in Chesapeake Bay (fig. 3). A notable feature was the high rarefied species pool at the low-salinity branch of the Baltic Sea, with no corresponding match in Chesapeake Bay. In

Figure 2: Regional scatterplots of species richness versus salinity. Symbols are plotted with transparency to visualize overlapping data points as darker, data-rich areas. The smooth line represents the generalized additive model prediction with standard errors (dashed lines). The vertical dashed point to the salinity of the minimum $\alpha$ diversity. A, Baltic Sea ($n = 7,744$); B, Chesapeake Bay ($n = 7,896$).

Figure 3: Approximation of species pools within two-unit salinity intervals, moved in 0.5-unit increments along the estuarine salinity gradient. Filled and open symbols represent sample-based and coverage-based rarefaction; error bars show bootstrap standard errors. The vertical dashed lines show the salinity of the minimum $\alpha$ diversity, as in figure 2. Shaded areas show the relative spatial coverage of surface salinity. Note the different scales along the Y-axes. A, Baltic Sea; B, Chesapeake Bay.
Chesapeake Bay, the rarefied species pool at the freshwater end matched the marine branch, which contrasted with the discrepancy in α diversity (fig. 2). The sample- and coverage-based standardizations of species pools agreed in the general shape of the relationship. The discrepancy in the Baltic Sea was due to systematically higher completeness of the samples from the marine branch, possibly due to the narrow straits and very restricted area at this end, resulting in a relatively higher species pool on the freshwater side when standardized to fixed coverage (fig. 3).

The Baltic Sea phytoplankton community structure changed abruptly at a salinity matching the α diversity minimum (fig. 4). The conspicuous threshold-type pattern in community structure at salinity 7–9 was in contrast to the gradual change above and below. Notably, a correspondingly abrupt change in community structure was not shown in Chesapeake Bay (fig. 4), where phytoplankton community changed gradually along the entire salinity continuum.

The cumulative likelihood of presence also revealed a minimum at mesohaline salinities, very close to the α diversity minimum (fig. 5). In the Baltic Sea, the minimum was narrow and distinct, while in Chesapeake Bay, the cumulative likelihood minimum was in a wider range. The Baltic Sea showed a characteristic local maximum at a salinity of around 5, which corresponded to the salinity of the largest habitat size and, correspondingly, the highest sampling frequency.

By using the salinity at the minimum α diversity (fig. 2), we confined 1,671 and 6,073 Baltic Sea samples to habitats from the marine and freshwater sides, respectively. The corresponding sample distribution was more even in Chesapeake Bay, with 4,601 belonging to marine-side habitats and 3,295 to freshwater-side habitats. Of the 963 taxa considered in the Baltic Sea, 565 were found exclusively on the freshwater side and 111 taxa exclusively on the marine side, including species too rare to be classified as marine or freshwater specialists. Of the 800 Chesapeake Bay taxa, only 141 were observed exclusively on the freshwater side and 205 on the marine side.

Categorizing the observed taxa into habitat specialists and generalists reflected the broad evolutionary origin of major phytoplankton lineages—green algae and cyanobacteria were more species rich on the freshwater side and dinoflagellates on the marine side, while diatoms were relatively evenly split between the two habitat types (table 2). The most frequently observed taxa in each category are listed in table A1 (available online).

Discussion

Our cross-ecosystem comparison supports the Remane concept of the salinity-species diversity relationship and broadens it to the dynamic realm of coastal phytoplankton. The low phytoplankton α diversity at intermediate salinities seems

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**Figure 4:** Change of the phytoplankton community composition (Y-axis) along the estuarine salinity gradient (X-axis). The community composition is represented by scores of the first ordination axis (nonmetric multidimensional scaling [NMDS]). The sample sizes (n) are reduced to include only summer samples (May–September) and to provide fair representation of different salinity regions. The symbol colors represent species richness and are plotted in increasing order. The vertical dashed lines show the salinity of the minimum α diversity, as in figure 2. The superimposed generalized additive model smoother (red line) illustrates the threshold-type change in community composition in the Baltic Sea (A), in contrast with the smooth transition in Chesapeake Bay (B). The rapid change in community composition in the Baltic Sea coincides with the salinity of the lowest species richness.
to be a strong pattern, supported by rich data sets collected from two of the world’s largest transitional water bodies with a full salinity range. Despite some difference in the salinity range at the species-richness minima of phytoplankton and benthic invertebrates, the overall shape of the relationship appears to be a robust feature.

There were important dissimilarities in the salinity-diversity relationship between the two coastal ecosystems. The abrupt change in community structure at the salinity corresponding to the diversity minimum in the Baltic Sea had no equivalent in Chesapeake Bay (fig. 4). As a long-term average, the habitat with salinity of 7–9 in the Baltic Sea forms

![Graph A and B](#)

**Figure 5:** Cumulative likelihood of species presence along the salinity gradient in the Baltic Sea (A) and Chesapeake Bay (B). The cumulative likelihood of each species is plotted in the order of their respective salinity optima, shown with the color code. The vertical dashed lines show the salinity of the minimum $\alpha$ diversity, as in figure 2. Black (A) and red (B) smooth lines denote taxa with a salinity optima closest to the salinity of the minimum $\alpha$ diversity.

### Table 2: Classification of the observed taxa according to habitat preference

|                   | Diatoms | Chlorophytes | Cyanobacteria | Dinoflagellae | Others | Total |
|-------------------|---------|--------------|---------------|--------------|--------|-------|
| **Chesapeake Bay:** |         |              |               |              |        |       |
| Fresh exclusive   | 3       | 9            | 1             | 0            | 1      | 14    |
| Fresh             | 40      | 80           | 28            | 1            | 8      | 157   |
| Generalists       | 48      | 9            | 6             | 11           | 16     | 90    |
| Marine            | 52      | 1            | 0             | 49           | 5      | 107   |
| Marine exclusive  | 9       | 0            | 0             | 8            | 0      | 17    |
| Rare              | 156     | 81           | 28            | 104          | 46     | 415   |
| Total             | 308     | 180          | 63            | 173          | 76     | 800   |
| **Baltic Sea:**   |         |              |               |              |        |       |
| Fresh exclusive   | 39      | 63           | 33            | 6            | 25     | 168   |
| Fresh             | 21      | 13           | 24            | 7            | 15     | 80    |
| Generalists       | 21      | 8            | 8             | 22           | 25     | 84    |
| Marine            | 40      | 1            | 1             | 35           | 13     | 90    |
| Marine exclusive  | 24      | 0            | 0             | 17           | 4      | 45    |
| Rare              | 135     | 151          | 59            | 58           | 93     | 496   |
| Total             | 280     | 238          | 125           | 145          | 175    | 963   |

Note: Rows designate generalists, habitat specialists of either the freshwater or marine side, and rare taxa. Habitat specialists observed exclusively on the marine or freshwater side are shown separately ("fresh exclusive," "marine exclusive"). Columns distinguish major algal lineages—diatoms, chlorophytes (pooling classes form the Streptophyta and Chlorophyta clades), cyanobacteria, dinoflagellae, and other taxa.
an ecological transition in the southwestern part of the central Baltic Sea, apparently separating two distinct phytoplankton communities. Recent studies have shown that this salinity interval also forms a boundary for intraspecific genetic diversity, separating two genetically differentiated populations of *Skeletonema marinoi*—an ubiquitous bloom-forming planktonic diatom species (Godhe et al. 2016).

The regional dissimilarity between these two water bodies could be related to their very different hydrologies and topographies. The topography of Chesapeake Bay is more open, also at the diversity minimum, and the salinity distribution along the estuarine gradient is more gradual (Urquhart et al. 2012). Chesapeake Bay, with its short water residence time of about 0.5 years (Du and Shen 2016), acts more like a one-way flushing channel compared to the more stable (residence time ~25 years), spatially dominant low-salinity regime of the Baltic main basin. Accordingly, Chesapeake Bay exhibits a continuous phytoplankton community change at the diversity minimum and the Baltic Sea a threshold-type change (fig. 4). Also, the low number of taxa observed exclusively on the marine or freshwater side (table 2) reflects the dynamic nature of Chesapeake Bay hydrology, compared to the stable salinity gradient and long retention times in the Baltic Sea.

With its large volume and long retention time, the Baltic Sea is not a typical estuary. However, for studying the effects of salinity gradients on planktonic organisms, the rather littoral nature of the Baltic Sea provides ideal conditions, as the phytoplankton communities are close to a steady state with local salinity conditions. The community pattern observed in the Baltic Sea indicates a salinity band at 7–9, where the species richness minimum coincides with a distinct transition zone between two relatively homogeneous assemblages, suggesting a repelling edge effect, where both of the two adjacent communities find stressful conditions. Such a threshold is in contrast with the smooth pattern seen in Chesapeake Bay as well as in other, more dynamic estuaries, such as for benthic macroinvertebrates in the Thames River estuary and for phytoplankton in the Scheldt estuary in the North Sea (Attrill and Rundle 2002; Muylaert et al. 2009). The comparison of our very exhaustive data sets from two ecosystems with contrasting hydrology gives support to the idea that smooth transitions observed in other estuaries may be misleading in interpreting how salinity affects community composition. Rather, such smooth patterns as seen in Chesapeake Bay and elsewhere seem to reflect averaging effects caused by dynamic hydrology, coupled with limited system size per salinity interval.

It has been suggested that on an evolutionary timescale, the marine-freshwater boundary has been one of the most difficult barriers to cross for both macro- and microorganisms (Logares et al. 2009). For protists, the marine-freshwater cross-colonization is physiologically hampered by the energetic costs and metabolic pathways related to osmoregulation and ionic concentration (Hoef-Emden 2014; Suescún-Bolívar and Thomé 2015). A rigid cell wall, in many cases, resists cell expansion caused by water influx from a hypertonic environment. But this does not work for flagellated cells, as even with a strong cell wall, the flagella membrane remains an unprotected area. Flagellates rely on contractile vacuoles that pump out excess water and intracellular accumulation of biologically compatible osmolytes, like floridoside, to counter the high osmotic pressure of the environment (Welsh 2000). Lineages confined to fresh water may lack the pathways to produce intracellular osmolytes, whereas marine lineages may not be able to downregulate their osmolyte production; euryhaline species, meanwhile, use both mechanisms (Hoef-Emden 2014).

Our results imply that the negative edge effects in intermediate salinity zones are a strong and robust feature in phytoplankton communities. High species invasion rate (Olenina et al. 2010) indicates that the geologically young Baltic Sea is not saturated with species. Further, the recent decadal-scale increase in phytoplankton species richness suggests connectivity enhancement through anthropogenic activities (Olli et al. 2014). A corresponding pattern has not been observed in other well-studied coastal waters (e.g., Chesapeake Bay), and the low species richness in intermediate salinity may thus reflect lack of colonizers due to limited diversity in the global brackish water species pool.

In spite of the differences regarding smooth versus abrupt transition, overall the pattern we show here for phytoplankton matches surprisingly well those reported earlier for higher organisms. Marine and freshwater species pools contribute overproportionally to populating the transitional zone with species but do not compensate for the lack of better-adopted brackish taxa—eventually resulting in a diversity minimum. A high degree of differentiation at the subspecies level seems to be characteristic for transitional waters both in macroscopic and microscopic organisms (Cognetti and Maltagliati 2000). Moreover, for the Baltic Sea, where ongoing invasion of species is known as a general phenomenon for macroscopic taxa (e.g., Olenina et al. 2010), the same applies to phytoplankton (Olli et al. 2014). This adds further support to the existence of general scaling rules for both macrobial and microbial diversity (Martiny et al. 2006).

Recent studies have used genetic markers and metabarcoding to reveal considerable variability in prokaryotic and protist diversity along the Baltic Sea salinity gradient (Herlemann et al. 2011; Hu et al. 2016). These works have identified specific brackish water assemblages but have not verified either elevated or reduced diversity at intermediate salinities. Yet Herlemann et al. (2011) show that salinity band 8–10 represents a major transition for most phyla they studied (cluster analysis; fig. 2). In the near future, genetic studies will likely become mainstream in environmental monitoring, with a strong potential to provide better reso-
olution to resolve recurrent patterns in the highly variable diversity data.

There are many ways to express species richness in transitional waters, including α, β, and γ diversity and species pool over homogeneous salinity areas. The intrinsic spatial salinity gradient is likely to support high taxonomic turnover (β diversity) and, subsequently, high regional diversity (γ) compared to the adjacent marine and freshwater habitats (Basset et al. 2013). In a recent cross-ecosystem comparison, we estimated phytoplankton α and γ diversity for a range of large coastal ecosystems with long salinity gradients (Olli et al. 2015). Although α diversity was comparable between regions (see also fig. 2), γ diversity scaled with the ecosystem size, confirming that the ubiquitous species-area relationship (Azovsky 2011; Sízlíng et al. 2011) is valid also at the spatial scale of large marine ecosystems.

We expected that within an ecosystem, the species pool size in homogeneous salinity intervals is a combination of two processes: the environmental filtering in line with the Remane concept and the spatial scale of the salinity interval in line with the species-area relationship. Due to the latter, it is reasonable to assume an elevated species pool at salinity intervals with the highest surface area (Telesh et al. 2015). Here we show that the large area of the central Baltic Sea does not overcompensate for low diversity at intermediate salinities (even though the rarefied species pool is higher near the minimum α diversity threshold than toward higher salinities; fig. 3). We thereby conclude that the Remane concept is in every respect valid for phytoplankton.

The Remane model suggests equal species richness of the marine and freshwater components. Later inventories of the Baltic Sea macrozoobenthos suggest a higher diversity at the saline side (Zettler et al. 2014). Our data also show a higher α diversity of the marine phytoplankton component, which seems to be prevalent in many organism groups like fish, zooplankton, and macrophytes but contrasts with lineages of predominantly freshwater origin, such as aquatic angiosperms and rotifers (reviewed in Whitfield et al. 2012). This does not imply low diversity in fresh water per se. Inland water bodies are collectively more heterogeneous than oceans. The low α diversity reflects the limited subset of freshwater sources and is compensated (Chesapeake Bay) or overcompensated (Baltic Sea) for when using the rarefied species richness. In particular, the conspicuous large species pool at the freshwater side of the Baltic Sea is partly due to two disjunct low-salinity limbs—the eastern Gulf of Finland and the northern Bothnian Bay. While comparable in salinity, other gradients such as temperature and eutrophication introduce large heterogeneity between these two habitat types, which is reflected in elevated rarefied species richness in the low-salinity segment.

Species richness is an important property, which determines the conservational value of the ecosystems as well as many essential ecosystem functions (Snøeijis-Leijonmalm 2017). Our results point to predictable trajectories of how shifts in salinity—for example, driven by climate change (BACC II Author Team 2015; Harding et al. 2015, 2016)—will impact coastal biodiversity with important consequences on ecosystem functioning (Ptacnik et al. 2008; Olli et al. 2014).

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Monoraphidium, a planktonic green algal genus of freshwater origin, which thrives also in the brackish waters of the Baltic Sea. Scale bar = 50 µm. Photo credit: Kalle Olli.