Diversity and distribution across a large environmental and spatial gradient: evaluating the taxonomic and functional turnover, transitions and environmental drivers of benthic diatom communities

Leena Virta[1,2*], Janne Soininen[1] & Alf Norkko[2,3]

[1] Department of Geosciences and Geography, PO Box 64, FIN-00014 University of Helsinki, Finland

[2] Tvärminne Zoological Station, University of Helsinki, J.A. Palméns väg 260, FI-10900 Hangö, Finland

[3] Baltic Sea Centre, Stockholm University, Stockholm, Sweden

[*] leena.virta@helsinki.fi

Acknowledgements

We are thankful for Mervi Sjöblom and Jaana Koistinen at Tvärminne Zoological Station for the nutrient analyses, and for Kaarina Weckström at the University of Helsinki for providing species identification literature. L.V. acknowledges Walter and Andrée de Nottbeck Foundation, Societas pro Fauna et Flora Fennica, and the Finnish Society of Sciences and Letters, and A.N. the Academy of Finland (project ID 294853) for financial support.

Biosketch

Leena Virta is a doctoral student studying the functioning of marine ecosystems. Particular interests include diversity and distribution of microphytobenthic organisms in the Baltic Sea ecosystem.

Janne Soininen is a professor of spatial environmental research and widely interested in causes and consequences of aquatic biodiversity.

Alf Norkko is interested in the biodiversity of sea floor habitats and their importance for ecosystem functioning across environmental gradients. He is particularly interested in how disturbance may influence relationships between biodiversity and ecosystem functioning.

Diversity and distribution across a large environmental and spatial gradient: evaluating the taxonomic and functional turnover, transitions and environmental drivers of benthic diatom communities

Running title: Diversity across a large gradient

Abstract

Aim: Global biodiversity loss has raised interest in understanding variation in diversity at different scales. Especially studies conducted across large spatial gradients are crucial, because they can increase perspectives on how ecological patterns change relative to environmental factors, and facilitate predictions of possible responses to environmental change. We explored the full extent of a brackish sea to test the hypotheses that (i) benthic communities are defined by species' limited ranges, controlled by varying drivers along a large environmental gradient, (ii) the responses of taxonomic and functional community composition and turnover to the environmental gradient are different, thus highlighting the need to include both measures in ecological studies, and (iii) diversity reaches the minimum at intermediate salinities (Remane curve) due to the low adaptation of freshwater and marine species.

Location: A large environmental and spatial gradient spanning the entire Swedish coastline (ca. 2300 km; salinity 1.2-27.6), the Baltic Sea

Time period: August 2018

Major taxa studied: Benthic diatoms

Methods: We assessed environmental drivers for the communities and calculated the taxonomic and functional alpha and beta diversity along the gradient. We also compared the taxonomic and functional composition and diversity of communities between areas with different salinity.

Results: We found support for the hypothesis of limited species ranges, as taxonomic beta diversity, mainly induced by changes in salinity and climate, was high, whereas functional beta diversity remained considerably lower, and the composition and diversity of communities, as well as environmental drivers controlling the communities, differed between regions with different salinity. The lowest taxonomic diversity was found at intermediate salinities of 5-6.

Main conclusions: These findings advance understanding of large-scale patterns of benthic diversity, emphasize the importance of large gradient studies for a better understanding of general ecological patterns, and highlight the vulnerability of brackish water ecosystems as ecologically important tipping point realms.

Keywords

beta diversity, brackish, diatoms, diversity, functional, gradient, limited ranges, Remane curve, salinity, tipping points

Introduction

The ongoing biodiversity crisis has increased the need for understanding the variation of diversity. This, in turn, has raised interest and triggered substantial efforts for studying the different kinds of gradients that often are associated with changing diversity patterns (Mittelbach et al., 2007). However, our understanding of gradients underlying diversity patterns is hampered by different obstacles: i) natural gradients are almost always complex, with several physical, chemical and biological factors interacting and correlating with each other (Huston, 1994; Willig, Kaufman, & Stevens, 2003), ii) diversity varies constantly along environmental, spatial and temporal gradients, although the rate of this variation depends on the type of organisms (Willig et al., 2003), iii) physical and chemical gradients are also constantly changing, and the ongoing climate change accelerates this change (The BACC II Author Team, 2015).

Despite the challenges listed above, studies conducted along gradients facilitate a better understanding on how ecological patterns, such as variation in biodiversity, change relative to the environment (Thrush et al., 2006). Spatial gradients, including gradients in latitude, have received considerable attention as drivers of diversity (Willig et al., 2003). However, many environmental variables vary in concert with spatial gradients and, hence, spatial gradients can act as surrogates for primary environmental gradients, such as the ones of climate or productivity (Fuhrman et al., 2008). Here we focus on the responses of benthic diatom communities to environmental factors along a large-scale gradient of salinity, climate and space. For aquatic organisms, salinity is often a major environmental driver that can vary along spatial gradients (Villnäs, & Norkko, 2011; Ji et al., 2019). This applies for both freshwater and marine realms but is particularly pronounced in ecosystems with strong salinity gradients, such as estuaries or semi-enclosed seas (Remane, 1934; Olli, Ptacnik, Klais, & Tamminen, 2019).

As a study area, we used the Baltic Sea, which is a good example of a semi-enclosed, high-latitude sea, where salinity forms a strong gradient but interacts and correlates with other environmental factors, such as climatic and latitudinal forces. Due to several reasons, the Baltic Sea provides an excellent platform for studying the responses of biotic communities to environmental factors across gradients. First, the environmental gradient of the Baltic Sea is large, spanning from freshwater to brackish and finally marine conditions. Studies conducted across such large gradients can be particularly useful for increasing understanding and building predictions of possible community responses to environmental change (Thrush et al., 2006). However, most of the studies in the Baltic Sea, with consistently collected data have focused on regional or local areas, whereas only few have utilized the whole salinity gradient (see Ulanova, Busse, & Snoeijs, 2009; Villnäs & Norkko, 2011; Olli et al., 2019 for large gradient studies). Furthermore, sampling of large gradients is notoriously time-consuming and arduous and, thus, large datasets have usually been collected in several parts (but see Herlemann et al., 2011 for a large bacterial investigation). This may not cause a problem while studying large organisms in stable climatic conditions, but may compromise the results of studies conducted on quickly evolving communities, such as microphytobenthic communities, at high latitudes, where strong seasonality leads to a seasonal succession of changing species composition and diversity among short-lived organisms (Huston, 1994).

Secondly, across the Baltic Sea gradient, species typically have limited ranges that follow the pattern suggested by the central-marginal hypothesis, i.e., lower and more variable abundances towards the margins of their distribution dictated by environment, typically salinity (Gaston, 2009; Westerbom, Mustonen, Jaatinen, Kilpi, & Norkko, 2019). Because the effects of environmental change on biotic communities and their distributions can often first be detected at the edges of distribution areas, studying gradients that include such marginal areas may further facilitate understanding population responses to future environmental change (Sorte et al., 2017). In the Baltic Sea, as well as other basins with strong salinity gradients, the limited ranges of species often result in species richness minimum at intermediate salinities. This pattern is called the Remane curve (Remane, 1934), and it is due to the inability of freshwater or marine species to tolerate such salinities. Thirdly, the Baltic Sea has been predicted to respond strongly to climate change due to increasing temperatures and river flow, thus lowering salinity and changing the salinity gradient (The BACC II Author Team, 2015). And fourthly, the Baltic Sea has a long history of eutrophication, and species are strongly affected by anthropogenic drivers (Gustafsson et al., 2012; Andersen et al., 2017).

Diversity and distribution of species along gradients have most commonly been described using taxonomic metrics, e.g., species richness and community composition. However, including the functional characteristics, i.e. traits, of communities can provide a more effective way to describe diversity and facilitate establishing links between environment and the community (Hooper et al., 2005). Functional traits describe differences in resource acquisition, environmental preferences, and tolerance to stressors and consumers (Passy, 2007), thus linking communities effectively to small- and large-scale environmental gradients.

Here, while keeping in mind the theories of limited ranges of species, central-marginal hypotheses and Remane curve (Remane, 1934), we sought to find new insights into the effects of environment on communities and, thus, provide ideas of possible community responses to environmental change. We did this by means of assessing the taxonomic and functional composition and diversity of benthic diatoms in relation to environmental drivers along a large salinity, climatic and spatial gradient in the Baltic Sea. Diatoms are eukaryotic microorganisms that have been estimated to produce ~20% of oxygen in the atmosphere (Nelson, Treguer, Brzezinki, Leynaert, & Queguiner, 1995; Field, Behrenfeld, Randerson, & Falkowski, 1998). They are an integral part of the microphytobenthos in all aquatic systems, and in regions with strong environmental gradients, diatoms exhibit large variation in community composition and diversity (Passy, Pan, & Lowe, 1999) and, hence, provide an excellent model group for large gradient studies. We performed the sampling during a short time period using consistent methods, and verified the results with re-sampling of certain sites to control for temporal variance. We predicted that (i) despite continuous water flow between sites and consequent free dispersal of organisms, differences in the environment and consequent species' limited ranges would result in significant spatial variation in the composition and diversity of communities (Busse & Snoeijs, 2003), thus identifying potential tipping points that may indicate mechanisms and drivers of community change in response to environmental change (Westerbom et al., 2019). We also hypothesized that different environmental factors would structure the communities in the different parts of the gradient (Ulanova et al., 2009), showing that studies conducted at large gradients are necessary for the understanding of general ecosystem processes, and also possibly suggesting that climate change induced changes in physical gradients may change environmental drivers and ultimately the communities in ways that we cannot yet predict; (ii) the taxonomic and functional community composition would respond differently to the environment (Virta et al., 2019), which would highlight the advantages of including both community measures in ecological analyses; and (iii) lowest taxonomic diversity of communities would be found at brackish water salinities of ca. 6 (Remane, 1934), thus emphasizing the uniqueness and vulnerability of brackish water ecosystems.

Materials and methods

Study area and sampling

Our study area spanned the entire coastline of Sweden, in northern Europe. It extended from the northern to southern Baltic Sea and finally to the transition zone from the Baltic Sea to the North Sea (Fig. 1). The sampling gradient covered ca. 2300 km of coastline between latitudes 55.73°N and 65.79°N and longitudes 11.17°E and 23.90°E. Environmental conditions in this area are highly variable. Climate-related factors, such as air and water temperature, precipitation and the duration of ice cover, follow a north-south gradient. Due to the restricted water exchange between the Baltic Sea and the North Sea, and the abundance of rivers discharging into the Baltic Sea, salinity follows a north-south and east-west gradient and increases from ~1 in the northeastern part of the sampling gradient to ~28 in the western part of the gradient. Thus, biotic communities represent a transition from freshwater to brackish and finally to marine species. Nutrient concentrations are also highly variable but site-dependent without a clear gradient.

We conducted our sampling over a short time period in late summer, 9 - 24 August 2018, to minimize the potential for temporal variability. We had a total of 46 study sites, focusing on areas where the change in environment, such as salinity, is known to be most pronounced, and then re-sampled the first six sites to explore the potential bias due to temporal variation. To minimize the effect of terrestrial factors, such as the effect of land use, we chose sites at far ends of peninsulas or islands, and avoided close proximities of river mouths. We conducted the sampling following the modified recommendations by Kelly et al. (1998). At each site, we randomly selected twenty cobble-sized stones along the shoreline from depths of 20-50 cm. We collected the biofilm by scraping the surfaces of stones with a sponge (25 cm^2 per stone) and pooled the accumulated suspension into a composite sample, which was then stored in cold (+4 °C) and dark conditions until further processing. To account for the stability of the substrate, which may affect the growing conditions of microphytobenthic organisms, we measured the volume (length × width × height) of each sampling stone. We also measured salinity, pH and water temperature *in-situ*, and collected water samples from each site. We froze the water samples immediately after sampling and later analyzed them for nutrients, namely $NO_2^- + NO_3^-$, NH_4^+, PO_4^{3-}, and Si. To evaluate habitat characteristics, we classified the bottom type according to the amount of sediment (classes 1 to 3; class 1 denotes a rocky bottom, class 2 a sedimentary or sandy bottom covered with stones, and class 3 a sedimentary or sandy bottom with only a few stones) and the amount of macroalgae or vegetation on stones (classes 1 to 3; class 1 denotes no macroalgae/vegetation, and class 3 stones totally covered by macroalgae/vegetation) at each site.

Diatom and laboratory analyses, trait characteristics, wind exposure, and climatic variables

Diatom samples were boiled with hydrogen peroxide (30% H_2O_2) to remove organic material, and the cleaned diatoms were mounted on slides using Naphrax (Brunel Microscopes Ltd, United Kingdom). We used a phase contrast light microscope with a 1000× magnification to identify 500 valves per sample to the lowest possible taxonomic level (typically species level) following Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Snoeijs (1993), Snoeijs and Vilbaste (1994), Snoeijs and Potapova (1995), Snoeijs and Kasperovicienè (1996), and Witkowski (2000). After the identification of diatoms, we transformed species counts into relative abundances and verified taxonomic names according to AlgaeBase (Guiry, 2020).

To account for the functional composition of communities, we used the abundances of traits that are robust indicators of ecological behavior (Westoby, Falster, Moles, Vesk, & Wright, 2002). We classified diatom species according to their size (biovolume classes: large > 1000µm³ / small < 1000µm³), mobility (mobile / non-mobile), type of attachment (adnate / pedunculate [which was further divided to pad-attached / stalk-attached] / non-attached), colonization (colonial / non-colonial), guild (low-profile / high-profile / motile / planktonic) (Rimet & Bouchez, 2012), nitrogen-fixing abilities (nitrogen-fixer / non-nitrogen-fixer) (Passy, 2007), and salinity preference (freshwater / brackish / marine; each species may belong to 1-3 salinity categories depending on the breadth of distribution). Each species was classified according to all seven classifications, which resulted in numerous possible combinations for classifying a certain species. As the measure of trait composition, we used the combination of traits of all the species present in the community. To identify traits for each diatom species, we used, in addition to above mentioned species and trait literature, Snoeijs, Busse and Potapova (2002) and Diatoms of North America (2019). Traits used here indicate morphological characteristics of species and are related to ecosystem functioning. For example, grazers are dependent on high-profile diatom species, whereas low-profile species persist at low nutrient levels, where high-profile and motile species cannot survive (Passy, 2007).

Nutrient analyses of the water were conducted with an automated photometric analyzer (Thermo Scientific Aquakem 250 [Thermo Fisher Scientific Oy, Vantaa, Finland]) ($NO_2^- + NO_3^-$, PO_4^{3-}, and Si), except for NH_4^+, which was analyzed manually. To account for the wind exposure of our sampling sites, we calculated fetch, i.e., the distance over which wind can travel across open water. We did this using the ruler tool in Google Earth (Google). We placed the beginning of the ruler at the exact study site and measured the distance to next shore, island or islet along 36 lines, which were 10° apart from each other. Thus, we obtained 36 values for each site, and used the sum of these values as the measure of the fetch of the sampling site (Mason, Riseng, Layman, & Jensen, 2018). To account for climate, we extracted the average annual temperature and average annual precipitation (representative of 1950-2000, spatial resolution ~ 1km²) from WorldClim database

(Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and calculated climatic values for sampling sites with the ArcGIS 10.2.1, Arcmap-application.

Statistical analyses

Prior to statistical analyses, we log10-transformed environmental variables $NO_2^- + NO_3^-$, NH_4^+, PO_4^3, Si, salinity, stone volume and fetch to reduce their skewed distributions. We assessed statistical dependence between explanatory variables using Spearman's rank correlation coefficients. Si, air temperature, precipitation, latitude and longitude were highly correlated (> 0.7) with salinity. Thus, these variables were excluded from analyses, and salinity was used as a proxy for climatic and spatial variables, reflecting the joint effect of these factors for biotic communities.

To validate the results of the spatial sampling, we calculated the degree of temporal beta diversity at the six sites that had been visited twice. Our aim was to see whether the communities changed significantly during our sampling project. This was done using temporal beta diversity indices (TBI) (Winegardner, Legendre, Beisner, & Gregory-Eaves, 2017). TBI computes the total temporal beta diversity and its components (species gain or species loss) at each sampling site. We calculated the TBI for taxonomic composition using binary data and Sørensen dissimilarities, and computed significances with a parametric paired t-test with 9999 permutations. TBI was done with the R package adespatial (Dray et al., 2019).

To study the spatial beta diversity of communities, we calculated all pairwise dissimilarities in taxonomic and functional community composition using abundance data and the Bray-Curtis dissimilarity index (Bray, & Curtis, 1957). Then, we examined the relationship between dissimilarities and the salinity gradient, which was identified by generalized dissimilarity models (GDM) as the only environmental variable to significantly affect the communities (see below), using linear models. The significance of the relationship was determined using Mantel tests with Pearson's correlation and 999 permutations.

We studied the effects of individual environmental variables on taxonomic and functional beta diversity with generalized dissimilarity modelling (GDM) (Ferrier, Manion, Elith, & Richardson, 2007). GDM is a technique for studying spatial variation in biodiversity between pairs of geographical locations, and for defining the importance of predictor variables for beta diversity. We performed GDMs with taxonomic and functional abundance data and Bray-Curtis distance, and used function *gdm.varImp* with 50 permutations to estimate p-values for the whole model and each of the predictor variables. We constructed GDMs using R package gdm (Manion et al., 2018).

To study the variation in communities along the environmental gradient, we classified our sampling sites according to salinity, which was indicated by GDM as the only environmental variable to significantly explain taxonomic and functional beta diversity. The classification of samples was conducted, because it enabled us to visualize the taxonomic and functional community composition at different salinities, and also enabled us to indicate, which environmental variables control the communities at areas with different salinities. This is important for, e.g., conservation perspectives, because if different factors affect the communities at different salinities, conservation measures, such as nutrient reductions, need to be modified accordingly. No consensus of common salinity categories has been reached in literature (Snoeijs, & Weckström, 2019), but we used salinity categories of > 20 to indicate brackish to marine water with high salinity (11 samples), 10-20 to indicate brackish water with higher salinity (6 samples), 4-10 to indicate brackish water with lower salinity (21 samples), and 0-4 to indicate fresh to brackish water with low salinity (14 samples) (modified from Snoeijs, 1993 and Snoeijs, & Weckström, 2010). We then used pie charts to illustrate diatom species assigned to major taxonomic groups, i.e., genus, and functional traits in different salinity categories. For the pie charts, we used the R package ggplot2 (Wickham, 2019). We also used distance-based redundancy analyses (dbRDA) with Hellinger-transformed data to examine the influence of environmental variables on the taxonomic and functional community composition within different salinity groups (Legendre, & Gallagher, 2001). We used Bray-Curtis distance for species composition and Gower's distance for trait composition (Legendre, & Anderson, 1999). To ensure an adequate number of samples in each salinity group, salinities 10-20 and > 20 were combined in dbRDA analyses. We used the R package vegan for the dbRDA analyses (Oksanen, 2019).

We studied the relationship between taxonomic and functional community composition and species richness and salinity using regression models. Taxonomic and functional community composition was described as multidimensional scaling (nMDS) 1 scores, and the nMDS were calculated with Bray-Curtis similarity index and two dimensions. To visualize the relationships, we used the R package ggplot2 (Wickham, 2019).

All statistical analyses were calculated using R version 3.6.1 (R Development Core Team, 2019).

All data used in the analyses are available through the Dryad Repository (https://doi.org/10.5061/dryad.bvq83bk6p).

Results

Community composition did not change significantly at any of the twice-visited six sites between the first sampling at the beginning of August and the second sampling at the end of August (Appendix, table 1).

Across the broad-scale spatial gradients, communities were taxonomically highly different at different parts of the sampling gradient (Fig. 2). Pairwise mean taxonomic dissimilarity was 0.732 (max 0.990, min 0.206). Functional dissimilarities were considerably lower, as pairwise mean functional dissimilarity was 0.230 (max 0.577, min 0.032). Taxonomic and functional dissimilarities changed significantly (p: 0.001) along the salinity gradient, and the relationships between community similarity and the salinity gradient were statistically significant (taxonomic dissimilarity: R: 0.549, p: 0.001; functional dissimilarity: R: 0.403, p: 0.001) (Fig. 2).

Environment explained taxonomic and functional spatial beta diversity significantly ($p < 0.001$), although the deviance of taxonomic beta diversity was explained more efficiently than the deviance of functional beta diversity (Table 1). Salinity was the only significant variable for both taxonomic and functional beta diversity. However, this may indicate the combined influence of salinity, climate and spatial factors, because climatic and spatial variables were excluded from analyses due to high correlation with salinity.

The genus *Navicula* and *Nitzschia* were the dominant taxonomic groups along the environmental gradient (Appendix, fig. 1) and formed 16.9% and 28.5% of the communities, respectively, when all salinity categories were considered. The contribution of different taxonomic groups varied between salinity categories, with salinity category < 4 featuring the most even distribution of groups with no clear dominance. Many functional traits showed a gradient from high to low salinity (Fig. 3). The relative proportion of large versus small diatoms increased towards low salinity, as well as the relative proportion of pedunculate versus mobile diatoms, high-profile versus motile diatoms, and colonial versus non-colonial diatoms. The relative proportions of diatoms with preference for freshwater, brackish or marine environment did not show a clear pattern.

Taxonomic and functional community composition was significantly explained by environmental variables in all salinity groups (Table 2). Nutrients were among the significant environmental variables in all salinity groups for taxonomic composition and in two of three salinity groups for functional composition. Wind exposure, i.e. fetch, and stone volume were significant in almost all the groups, and water temperature, pH, salinity, vegetation amount, and sediment amount were significant in at least one of the salinity groups for either taxonomic or functional community composition.

The relationships between salinity and taxonomic/functional community composition or species richness were non-linear (Fig. 4, Fig. 5). Lowest species richness (26, 28 and 29 species per sample) was found in brackish water with intermediate salinity (5.4, 5.2 and 5.3, respectively), whereas highest species richness (109, 98 and 83 species per sample) was found in marine water with high salinity (26.0, 26.6 and 26.7, respectively).

Discussion

We investigated the general hypotheses that benthic communities along a large environmental gradient are defined by species' limited ranges (Gaston, 2009), controlled by varying drivers along a large environmental gradient, and that diversity reaches the minimum at intermediate salinities due to the low adaptation of freshwater and marine species (Remane, 1934). To our best knowledge, this is one of the first studies to investigate aquatic microorganismal communities on an environmentally and spatially large gradient (across ca. 2300 km) with a coherent sampling project that minimized the influence of temporal variability on the communities.

As suggested by the theories of limited species ranges and the central-marginal hypothesis (Gaston, 2009), we found an almost complete spatial taxonomic beta diversity, i.e. turnover of communities from one end of the gradient to the other end, in other words, only a few same species were found at both ends. Due to the strong gradient in environment, especially salinity, this was expected. However, functional beta diversity remained low, i.e., species were replaced by taxonomically new but functionally similar species along the gradient. Similar results of low functional despite high taxonomic beta diversity have been found before across short spatial gradients in estuaries (Villéger, Miranda, Hernandez, & Mouillot, 2012 in a tropical estuary; authors' unpublished data in a Baltic Sea estuary), but our large spatial gradient makes the result interesting. It seems that the functional characteristics of microphytobenthos needed for efficient benthic ecosystem functioning are very similar in very different environments. In our study, the number of species per site (54 on average) was considerably higher than the number of traits (7 per site), and this confounding effect may affect the notable difference between taxonomic and functional beta diversity. However, due to our method of categorizing each species according to seven different classifications, the number of possible functional combinations per site was high and closely comparable to the species number.

Salinity was the only environmental variable that significantly explained taxonomic and functional beta diversity of the communities. Due to the large range of salinity (1.2-27.6) in our sampling area, the effect of salinity was to be expected, as salinity has regularly been found as a major driver for biotic communities in aquatic systems (Cognetti, & Maltagliati, 2000; Villnäs, & Norkko, 2011), even in studies with considerably smaller range (Svensson, Norberg, & Snoeijs, 2014 on a salinity gradient of 0.5-7.8). However, salinity was also used as a proxy for highly correlated climatic and spatial variables, and hence, climate and spatial distance probably also contributed to beta diversity. There are a number of climatic variables that can affect aquatic communities at high latitudes, such as air temperature (Chu, Jones, Mandrak, Piggott, & Minns, 2008), precipitation (Primo, Azeiteiro, Marques, Martinho, & Pardal, 2009), day-time length, and ice cover duration (Virta, Soininen, & Norkko, 2020). These variables are usually highly inter-correlated, which supports the use of only one or two of them to represent climate, which we did. Our finding of climate as an important factor for determining species distribution is supported by several previous studies at the regional scale (Pearson, Dawson, & Liu, 2004; Pajunen, Luoto, & Soininen, 2016). Although spatial variables (latitude and longitude) were also correlated with salinity and climate, we think that they are not a major driver for the communities in this study, because there is a continuous water flow and, thus, free dispersal of species between sites.

Our classification of sites according to salinity divided the sampling area to four different areas: 1. the western area of Kattegat and the North Sea, with salinity of > 20; 2. the southwestern area of the Belt Sea, with salinity of 10-20; 3. the eastern area of the Bornholm Basin, the Western Gotland Basin, the Åland Sea and the Bothnian Sea, with salinity of 4-10; and 4. the northernmost area of the Bothnian Bay, with salinity of < 4. As was hypothesized following the ideas of limited species ranges and the central-marginal hypothesis (Gaston, 2009), the taxonomic composition of communities differed between these areas. All the main taxonomic groups were present in all salinity areas, but their relative proportions varied across areas, and the area with the lowest salinity differed from the other areas by having the most even distribution of taxonomic groups. Thus, it seems that marine and brackish waters share characteristics of taxonomic groups, whereas there is a clear distinction and a threshold between brackish and fresh waters. This may indicate potential changes in the benthic communities at the transition zone between brackish and freshwater conditions, if future climate change decreases salinity in the Baltic Sea as has been predicted.

Many functional characteristics also showed a gradient along salinity areas. The general trend was the increase of large and high-growing species or colonies (traits large, pedunculate, high-profile and colonial) towards low salinities. This was surprising, because other organisms, such as benthic macrofauna, grow in size from the low salinities of the Baltic Sea to the higher salinities of the North Sea (Furman, Pihlajamäki, Välipakka, & Myrberg, 2014). The different behavior of diatoms in relation to other organisms may be due to several abiotic factors, such as different responses to salinity and other environmental factors by small and large diatom species (Snoeijs et al., 2002), varying nutrient conditions along the gradient, or increasing disturbance by wind exposure towards marine conditions (Passy, 2007). We also speculate that, due to the increase of the species richness and abundance of benthic fauna towards marine conditions (Norkko et al., 2015), biotic disturbances, i.e., grazers, may have played a key role in shaping the functional characteristics of diatom communities along the salinity gradient and in favoring smaller species with better resistance to grazers at high salinities. The distribution of species with preference to fresh, brackish or marine water was surprisingly constant across salinity areas. This may be due to a high number of species that were included in two or three preference groups because of their large geographical distribution areas.

Our hypothesis of different environmental drivers for community composition at different salinity areas was partly confirmed, because many of the variables appeared as significant at only one or two salinity areas. This emphasizes the importance of large gradient studies for the understanding of ecosystem processes. However, certain environmental drivers seemed to affect communities everywhere. Wind exposure and stone volume, both describing physical disturbance, were important in almost all areas. Physical disturbance has been shown to affect all kinds of benthic communities, such as diatoms (Passy, 2007), macroalgae (Underwood, 1998), and macrofauna (Cai, Gong, & Qin, 2012), and this effect may increase in the future with intensifying winds and more severe storms (The BACC II Author Team, 2015). Nutrients were also important for the communities in all salinity groups, but with varying contributions. The areas with lowest (< 4) and highest (> 10) salinity were limited by both nitrogen and phosphorus, and the brackish waters (salinity 4-10) by phosphorus. These results disagree with several other studies that have shown phosphorus limitation in freshwater realms and in the northern parts of the Baltic Sea and nitrogen limitation in brackish and marine waters (Tamminen, & Andersen, 2007). This disagreement may possibly be due to the seasonally varying nutrient compositions in the Baltic Sea, or the effect of eutrophication, as suggested by Tamminen and Andersen (2007).

All the relationships between community metrics (taxonomic and functional community composition, and species richness) and salinity were non-linear but considerably different from each other. Change in the taxonomic and functional community composition remained quite even through salinities 0-ca. 20 before levelling off at salinities >20. This seems to indicate that even small changes in salinity have strong effects on communities in fresh and brackish waters, which may lead to considerable changes in communities if climate change alters the salinity, as has been expected. The relationship between species richness and salinity indicated high diversity in samples with low and high salinity, whereas diversity in the brackish water samples was lower. This agrees with the ideas of Remane (1934) and many other studies after him that have described the lowest diversity of organisms at intermediate salinities of ca. 5-8 (e.g. Olli et al., 2019). The species richness is at its lowest at these salinities probably because estuaries and brackish water seas are universally small, ephemeral and isolated from each other, which has prevented the development and speciation of actual brackish-water species (Olli et al., 2019).

To conclude, resolving patterns in biotic communities and their drivers along large environmental gradients is important for understanding variation in biodiversity, especially given concerns over environmental change and consequent biodiversity loss. Large gradients can reveal patterns that remain hidden in smaller areas. We showed that, across a large environmental and spatial gradient, benthic diatom communities can exhibit a total taxonomic turnover and be controlled by different environmental factors in different parts of the gradient, thus supporting the hypotheses of species' limited ranges and central-marginal theory. Functional turnover remained considerably lower, suggesting that functional characteristics needed for stable ecosystem functioning are fairly similar in very different environments. The minimum diversity occurred at intermediate salinities, which confirmed the validity of the Remane curve and suggested that areas with intermediate salinities are a threshold and a tipping point for biotic communities. This emphasizes the vulnerability and uniqueness of brackish water areas and the need to apply measures to preserve biodiversity in these ecosystems in the course of human-induced climate change.

References

Andersen, J.H., Carstensen, J., Conley, D.J., Dromph, K., Fleming-Lehtinen, V., Gustafsson, B.G., … Murray, C. (2017). Long-term temporal and spatial trends in eutrophication status of the Baltic Sea. *Biological Reviews* 92, 135-149. doi: 10.1111/brv.12221

Bray, J.R. & Curtis, J.T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs* 27, 326-349.

Busse, S. & Snoeijs, P. (2003). Gradient responses of diatom communities in the Bothnian Sea (northern Baltic Sea), with emphasis on responses to water movement. *Phycologia* 42, 451-464. doi: 10.2216/i0031-8884-42-5-451.1

Cai, Y.J., Gong, Z.J. & Qin, B.G. (2012). Benthic macroinvertebrate community structure in Lake Taihu, China: Effect of trophic status, wind-induced disturbance and habitat complexity. *Journal of Great Lakes Research* 38, 39-48. doi: 10.1016/j.jglr.2011.12.009

Chu, C., Jones, N.E., Mandrak, N.E., Piggott, A.R. & Minns, C.K. (2008). The influence of air temperature, groundwater discharge, and climate change on the thermal diversity of stream fishes in southern Ontario watersheds. *Canadian Journal of Fisheries and Aquatic Sciences* 65, 297-308. doi: 10.1139/F08-007

Cognetti, G. & Maltagliati, F. (2000). Biodiversity and adaptive mechanisms in brackish water fauna. *Marine Pollution Bulletin* 40, 7-14. doi: 10.1016/S0025-326X(99)00173-3

Diatoms of North America. (2020). https://diatoms.org/. Accessed 28.1.2020.

Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., … Wagner, H.H. (2019). Package ‘adespatial’. https://cran.r-project.org/web/packages/adespatial/adespatial.pdf. Accessed 28 January 2020.

Ferrier, S., Manion, G., Elith, J. & Richardson, K. (2007). Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions* 13, 252-264.

Field, C.B., Behrenfeld, M.J., Randerson, J.T. & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281, 237-240. doi: 10.1126/science.281.5374.237

Fuhrman, J.A., Steele, J.A., Hewson, I., Schwalbach, M.S., Brown, M.V., Green, J.L. & Brown, J.H. (2008). A latitudinal diversity gradient in planktonic marine bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 105, 7774-7778. doi: 10.1073/pnas.0803070105

Furman, E., Pihlajamäki, M., Välipakka, P. & Myrberg, K. (Editors). (2014). The Baltic Sea, Environment and Ecology. www.syke.fi. Accessed 29.1.2020.

Gaston, K.J. (2009). Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B-Biological Sciences* 276, 1395-1406. doi: 10.1098/rspb.2008.1480

Google. Google Earth. Accessed 11 December 2019.

Guiry, M.D. (2020). AlgaeBase. http://algaebase.org/. Accessed 28.1.2020.

Gustafsson, B.G., Schenk, F., Blenckner, T., Eilola, K., Meier, H.E.M., Muller-Karulis, B., ... Zorita, E. (2012). Recontructing the development of Baltic Sea eutrophication 1850-2006. *Ambio* 41, 534-548. doi: 10.1007/s13280-012-0318-x

Herlemann, D.P.R., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J.J. & Andersson, A.F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal* 5, 1571-1579. doi: 10.1038/ismej.2011.41

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25, 1965-1978. doi: 10.1002/job.1276

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., … Wardle, D.A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* 75, 3-35.

Huston, M.A. (1994). *Biological Diversity*. Cambridge: Cambridge University Press.

Ji, M.K., Kong, W.D., Yue, L.Y., Wang, J.B., Deng, Y. & Zhu, L.P. (2019). Salinity reduces bacterial diversity, but increases network complexity in Tibetan Plateau lakes. *FEMS Microbiology Ecology* 95, fiz190. doi: 10.1093/femsec/fiz190

Kelly, M.G., Cazaubon, A., Coring, E., Dell’Uomo, A., Ector, L., Goldsmith, B., … Vizinet, J. (1998). Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology* 10, 215-224. doi: 10.1023/A:1008033201227

Krammer, K. & Lange-Bertalot, H. (1986). *Bacillariophyceae 1. Teil: Naviculaceae*. In H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa (p. 876). Stuttgart: Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1988). *Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae*. In H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa (p. 596). Stuttgart: Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1991a). *Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae*. In H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa (p. 576). Stuttgart: Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1991b). *Bacillariophyceae 4. Teil: Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema*. In H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer (Eds.), Süßwasserflora von Mitteleuropa (p. 437). Stuttgart: Gustav Fischer Verlag.

Legendre, P., & Anderson, M.J. (1999). Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69, 1-24. doi: 10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2

Legendre, P. & Gallagher, E.D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* **129**, 271-280. doi: 10.1007/s004420100716

Manion, G., Lisk, M., Ferrier, S., Nieto-Lugilde, D., Mokany, K. & Fitzpatrick, M.C. (2018). Package ‘gdm’. https://cran.r-project.org/web/packages/gdm/gdm.pdf. Accessed 28.1.2020.

Mason, L.A., Riseng, C.M., Layman, A.J. & Jensen, R. (2018). Effective fetch and relative exposure index maps for the Laurentian Great Lakes. *Scientific Data* 5, 180295.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., … Turelli, M. (2007). Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters* 10, 315-331. doi: 10.1111/j.1461-0248.2007.01020.x

Nelson, D.M., Treguer, P., Brzezinki, M.A., Leynaert, A. & Queguiner, B. (1995). Production and dissolution of biogenic silica in the ocean – Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biogeochemical Cycles.* 9, 359-372. doi: 10.1029/95GB01070

Norkko, J., Gammal, J., Hewitt, J.E., Josefson, A.B., Carstensen, J. & Norkko, A. (2015). Seafloor ecosystem function relationships: In situ patterns of change across gradients of increasing hypoxic stress. *Ecosystems* 18, 1424-1439. doi: 10.1007/s10021-015-9909-2

Oksanen, J. (2018). Package ‘vegan’. https://cran.r-project.org/web/packages/vegan/vegan.pdf. Accessed 28.1.2020.

Olli, K., Ptacnik, R., Klais, R. & Tamminen, T. (2019). Phytoplankton species richness along coastal and estuarine salinity continua. *American Naturalist* 194, E41-E51. doi: 10.1086/703657

Pajunen, V., Luoto, M. & Soininen, J. (2016). Climate is an important driver for stream diatom distributions. *Global Ecology and Biogeography* 25, 198-206. doi: 10.1111/geb.12399

Passy, S.I. (2007). Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany* 86, 171-178. doi: 10.1016/j.aquabot.2006.09.018

Passy, S., Pan, Y. & Lowe, R.L. (1999). Ecology of the major periphytic diatom communities from the Mesta River, Bulgaria. *International Review of Hydrobiology* 84, 129-174.

Pearson, R.G., Dawson, T.P. & Liu, C. (2004). Modelling species distributions in Britain: a hierarchical integration of climate and land-cover data. *Ecography* 27, 285-298. doi: 10.1111/j.0906-7590.2004.03740.x

Primo, A.L., Azeiteiro, U.M., Marques, S.C., Martinho, F., & Pardal, M.A. (2009). Changes in zooplankton diversity and distribution pattern under varying precipitation regimes in a southern temperate estuary. *Estuarine Coastal and Shelf Science* 82, 341-347. doi: 10.1016/j.ecss.2009.01.019

R Development Core Team. (2020). The R project for statistical computing. Retrieved from http://www.R-project.org.

Remane, A. (1934). Die Brackwasserfauna. *Verhandlunge Der Deutschen Zoologischen Gesellschaft* 36, 34-74.

Rimet, F. & Bouchez, A. (2012). Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. *Knowledge and Management of Aquatic Ecosystems* 406.

Snoeijs, P. (1993). *Intercalibration & Distribution of Diatom Species in the Baltic Sea Volume 1*. Sweden: Opulus Press.

Snoeijs, P., Busse, S. & Potapova, M. 2002. The importance of diatom cell size in community analysis. *Journal of Phycology* 38, 265-272. doi: 10.1046/j.1529-8817.2002.01105.x

Snoeijs, P. & Kasperovicienè, J. (1996). *Intercalibration & Distribution of Diatom Species in the Baltic Sea Volume 4*. Sweden: Opulus Press.

Snoeijs, P. & Potapova, M. (1995). *Intercalibration & Distribution of Diatom Species in the Baltic Sea Volume 3*. Sweden: Opulus Press.

Snoeijs, P. & Vilbaste, S. (1994). *Intercalibration & Distribution of Diatom Species in the Baltic Sea Volume 2*. Sweden: Opulus Press.

Snoeijs, P. & Weckström, K. (2010). *Diatoms and environmental change in large brackish-water ecosystems*. In J. Smol & E. Stoermer (Eds,), The Diatoms: Applications for the environmental and earth sciences (pp. 287-308). Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511763175.016

Sorte, C.J.B., Davidson, V.E., Franklin, M.C., Benes, K.M., Doellman, M.M., Etter, R.J., … Menge, B.A. (2017). Long-term declines in an intertidal foundation species parallel shifts in community composition. *Global Change Biology* 23, 341-352. doi: 10.1111/gcb.13425

Svensson, F., Norberg, J. & Snoeijs, P. (2014). Diatom cell size, coloniality and motility: Trade-offs between temperature, salinity and nutrient supply with climate change. *Plos One* 9, e109993. doi: 10.1371/journal.pone.0109993

Tamminen, T. & Andersen, T. (2007). Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication. *Marine Ecology Progress Series* 340, 121-138. doi: 10.3354/meps340121

The BACC II Author Team. (2015). *Second Assessment of Climate Change for the Baltic Sea Basin*. Springer Open Access.

Thrush, S., Dayton, P., Cattaneo-Vietti, R., Chiantore, M., Cummings, V., Andrew, N., … Schwarz, A.M. (2006). Broad-scale factors influencing the biodiversity of coastal benthic communities of the Ross Sea. *Deep-Sea Research Part II – Topical Studies in Oceanography* 53, 959-971. doi: 10.1016/j.dsr2.2006.02.006

Ulanova, A., Busse, S. & Snoeijs, P. (2009). Coastal diatom-environment relationships in the brackish Baltic Sea. *Journal of Phycology* 45, 54-68. doi: 10.1111/j.1529-8817.2008.00628.x

Underwood, A.J. (1998). Grazing and disturbance: and experimental analysis of patchiness in recovery from a severe storm by the intertidal alga *Hormosira banksii* on rocky shores in New South Wales. *Journal of Experimental Marine Biology and Ecology* 231, 291-306. doi: 10.1016/S0022-0981(98)00091-4

Villéger, S., Miranda, J.R., Hernandez, D.F. & Mouillot, D. (2012). Low functional beta-diversity despite high taxonomic beta-diversity among tropical estuarine fish communities. Plos One 7, e40679. doi: 10.1371/journal.pone.0040679

Villnäs, A. & Norkko, A. (2011). Benthic diversity gradients and shifting baselines: implication for assessing environmental status. *Ecological Applications* 21, 2172-2186. doi: 10.1890/10-1473.1

Virta, L., Gammal, J., Järnström, M., Bernard, G., Soininen, J., Norkko, J. & Norkko, A. (2019). The diversity of benthic diatoms affects ecosystem productivity in heterogeneous coastal environments. *Ecology* 100. doi: 10.1002/ecy.2765

Virta, L., Soininen, J. & Norkko, A. (2020). Stable seasonal and annual alpha diversity of benthic diatom communities despite changing community composition. *Frontiers in Marine Science* 7. doi: 10.3389/fmars.2020.00088

Westerbom, M., Mustonen, O., Jaatinen, K., Kilpi, M. & Norkko, A. (2019). Population dynamics at the range margin: Implications of climate change on sublittoral blue mussels (*Mytilus trossulus*). *Frontiers in Marine Science* 6, UNSP 292. doi: 10.3389/fmars.2019.00292

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002). Plant ecological strategies: Some leading dimensions of variation between species. *Annual Reviews of Ecology, Evolution and Systematics* 33, 125-159.

Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Kohske, T., Wilke, C., … RStudio. (2019). Package 'ggplot2'. https://cran.r-project.org/web/packages/ggplot2/ggplot2.pdf. Accessed 28.1.2020.

Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003). Latitudinal gradients of biodiversity: Patterns, process, scale, and synthesis. *Annual Review of Ecology, Evolution and Systematics* 34, 273-309. doi: 10.1146/annurev.ecolsys.34.012103.144032

Winegardner, A.K., Legendre, P., Beisner, B.E. & Gregory-Eaves, I. (2017). Diatom diversity patterns over the past c. 150 years across the conterminous United States of America: Identifying mechanisms behind beta diversity. *Global Ecology and Biogeography* 26, 1303-1315. doi: 10.1111/geb.12640

Witkowski, A. (2000). *Iconographia Diatomologica: annotated diatom micrographs / Vol. 7, Diatom flora of marine coasts I.* – Frankfurt am Main, Germany: Ruggell, A.R.G. Gantner,.

Figures

Fig. 1. Our sampling sites and the salinity along the coastline of Sweden, in the Baltic Sea. Salinity values have been obtained by interpolation from field data. Source of the European coastline shapefile: European Environmental Agency

Fig. 2. Pairwise relationships between taxonomic and functional similarities (Bray-Curtis dissimilarity index, abundance data) and the salinity gradient. The dashed line denotes linear model fitted to data.

Fig. 3. Relative proportions of functional traits in different salinity categories. Towards low salinity, there was an increase in the relative proportion of large versus small, pedunculate versus mobile, high-profile versus motile, and colonial versus non-colonial diatoms. The relative proportions in salinity preference did not show a clear pattern.

Fig. 4. The relationship between taxonomic and functional community composition (described as NMDS1 scores) and salinity.

Fig. 5. Species richness of diatom communities along the salinity gradient.

Appendix, fig. 1. Relative proportions of diatom species assigned to major taxonomic groups in different salinity categories. Groups that had less than hundred frustules in the whole data, are featured in the combined group of Other.

Data accessibility statement

The data (diatom species table, diatom trait table, environmental data, and R code) supporting the results of this article are available in Dryad Repository (https://doi.org/10.5061/dryad.bvq83bk6p).

Tables

Table 1. Results of generalized dissimilarity models (GDM) to explain taxonomic and functional spatial beta diversity. Fetch denotes wind exposure. 0.001 ***, < 0.01 **, < 0.05 *, < 0.1 .

	Diatom species			**Diatom traits**	
Deviance explained	52.50 %			28.24 %	
Model p-value	< 0.001 ***			< 0.001 ***	
	Importance	p-value		Importance	p-value
Salinity	74.31	< 0.001 ***		73.96	< 0.001 ***
PO_4^{3-}	3.12	0.08		1.10	0.40
Fetch	1.13	0.14		0.84	0.30
$NO_2^- + NO_3^-$	0.89	0.16		0.00	0.98
Water temperature	0.48	0.36		6.46	0.14
Sediment amount	0.41	0.20		2.93E-07	1.00
Vegetation amount	0.20	0.22		0.00	0.92
Stone volume	0.11	0.64		0.74	0.44
Ammonium	0.00	0.98		1.27	0.36
pH	0.00	1.00		0.00	1.00

Table 2. Results of the distance-based redundancy analyses (dbRDA) to examine the effect of environmental variables on taxonomic and functional community composition in different salinity groups. Only significant variables indicated by the dbRDA are listed. Fetch denotes wind exposure. < 0.001 ***, < 0.01 **, < 0.05 *, < 0.1 **.**

Salinity < 4

Taxonomic composition

Eigenvalues: CAP1: 10.378, CAP2: 0.344

Proportion explained: CAP1: 0.187, CAP2: 0.170

Model p: 0.001 ***

	SumOfSqs	*F*	*p-value*
PO_4^{3-}	0.284	3.191	0.001 ***
$NO_2^- + NO_3^-$	0.282	3.163	0.001 ***
Salinity	0.198	2.227	0.003 **
NH_4^+	0.162	1.813	0.029 *
Water temperature	0.161	1.805	0.029 *
pH	0.141	1.584	0.062 .
Vegetation amount	0.15	1.685	0.053 .

Functional composition

Eigenvalues: CAP1: 0.308, CAP2: 0.238

Proportion explained: CAP1: 0.359, CAP2: 0.2770

Model p: 0.011 *

	SumOfSqs	*F*	*p-value*
Fetch	0.216	7.416	0.001 ***
Stone volume	0.131	4.523	0.007 **
pH	0.125	4.3	0.009 **
$NO_2^- + NO_3^-$	0.07	2.421	0.067 .
PO_4^{3-}	0.064	2.202	0.087 .

Salinity 4-10

Taxonomic composition

Eigenvalues: CAP1: 0.533, CAP2: 0.395

Proportion explained: CAP1: 0.194, CAP2: 0.144

Model p: 0.001 ***

	SumOfSqs	*F*	*p-value*
Fetch	0.329	3.422	0.002 **
PO_4^{3-}	0.275	2.857	0.002 **
Sediment amount	0.247	2.576	0.005 **
Salinity	0.207	2.151	0.013 *
Stone volume	0.145	1.506	0.091 .

Functional composition

Eigenvalues: CAP1: 0.561, CAP2: 0.150

Proportion explained: CAP1: 0.612, CAP2: 0.163

Model p: 0.014 *

	SumOfSqs	*F*	*p-value*
PO_4^{3-}	0.321	7.311	0.002 **
Fetch	0.169	3.856	0.017 *
pH	0.099	2.259	0.083 .
Salinity	0.098	2.242	0.091 .

Salinity > 10

Taxonomic composition

Eigenvalues: CAP1: 0.769, CAP2: 0.517

Proportion explained: CAP1: 0.253, CAP2: 0.170

Model p: 0.001 ***

	SumOfSqs	*F*	*p-value*
NH_4^+	0.457	4.27	0.001 ***
Water temperature	0.37	3.458	0.001 ***
Salinity	0.358	3.352	0.001 ***
Fetch	0.239	2.24	0.017 *
Stone volume	0.226	2.116	0.020 *
$NO_2^- + NO_3^-$	0.222	2.074	0.023 *
PO_4^{3-}	0.196	1.832	0.050 *

Functional composition

Eigenvalues: CAP1: 0.760, CAP2: 0.096

Proportion explained: CAP1: 0.641, CAP2: 0.081

Model p: 0.025 *

	SumOfSqs	*F*	*p-value*
Fetch	0.478	13.483	0.001 ***
Stone volume	0.098	2.777	0.096 .

Appendices

Appendix, table. 1. Results of the temporal beta diversity indices (TBI). TBI denotes the level of change in community composition between two sampling occasions, p-value the significance of the change (values of < 0.05 are considered significant), Change species gain (+) or species loss (-) from the first sampling occasion to the second, SR1 species richness during the first sampling, and SR2 species richness during the second sampling.

	Site 1	*Site 2*	*Site 3*	*Site 4*	*Site 5*	*Site 6*
TBI	0.447	0.495	0.558	0.506	0.548	0.425
p-value	0.851	0.489	0.075	0.392	0.120	0.939
Change	+	+	+	-	-	-
SR1	29	35	26	53	49	47
SR2	40	35	28	51	56	65