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Seasonal variation in diversity of marine benthic invertebrates leads to a positive species–genetic diversity correlation (SGDC)

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ABSTRACT: Species–genetic diversity correlations (SGDCs) are useful indicators of processes that simultaneously affect diversity at multiple biological levels. We combined spatial and temporal sampling of 4 study sites in the Danish Isefjord–Roskilde Fjord Estuary at 4 time points over 1 yr to investigate the effect of seasonal variation on SGDCs. Species diversity was estimated as species richness from samples comprising 20 752 individuals representing 51 benthic invertebrate taxa. Genetic diversity was estimated for a single focal taxon, the polychaete *Pygospio elegans*, as mean allelic richness at 7 microsatellite loci. Combining all samples, a significant positive correlation between species richness and allelic richness was found. Median sediment grain size and mean temperature had significant effects on species richness, whereas only mean temperature had a significant effect on allelic richness of *P. elegans*. Our results show that both the benthic community as a whole and populations of *P. elegans* respond similarly to seasonal environmental variation at the study sites. The results suggest that seasonal timing of reproduction and dispersal in this temperate marine habitat might have a greater influence on diversity than spatially varying environmental variables and highlight the benefits of also investigating temporal SGDCs. Because of seasonal changes in diversity, it is important that samples are compared on the same time scale when investigating SGDCs.

KEY WORDS: Species–genetic diversity correlation · SGDC · *Pygospio elegans* · Estuarine · Species richness · Allelic richness · Polychaeta

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INTRODUCTION

Diversity can be measured within individuals, populations and/or communities, but relationships between diversity at these different levels are unclear. Ecological and evolutionary processes can have similar effects on species diversity within communities and on genetic diversity within species, and thus, positive correlations between the different levels of biodiversity can occur (see Vellend 2003, Vellend et al. 2014). These 'species–genetic diversity correlations' (SGDCs) can be useful indicators of the processes that simultaneously affect diversity at multiple biological levels. Moreover, identification of SGDCs is useful in an applied context if they allow the inference of one level of diversity based on that of another (Kahilainen et al. 2014). However, the sign of SGDCs can be difficult to both predict and interpret (Laroche et al. 2015). Positive relationships are expected when diversity is mediated by factors acting in the same way on individual species and on the entire community (Vellend 2003, Kahilainen et al. 2014, Lamy et al. 2017), for example via available habitat, productivity or shared dispersal routes. However, biological interactions can disrupt potential SGDCs, for exam-

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ple in cases of competition or facilitation between species, and could lead to either positive or negative SGDCs. Several reviews of empirical studies (Kahilainen et al. 2014, Vellend et al. 2014, Whitlock 2014) have now emphasized that an expectation of positive SGDCs in most cases might be premature. Nevertheless, investigation of the factors explaining positive (or negative) SGDCs is fruitful for understanding the ecology of the focal species and community in question.

Most studies on the relationships between species diversity and genetic diversity have focussed on terrestrial systems, whereas SGDCs in marine environments have received less attention (Messmer et al. 2012, Josefson & Göke 2013, Selkoe et al. 2016). SGDCs in α diversity (diversity at the local scale in a particular population or community) are expected to be more frequently positive in island-like systems due to clear limitations of area or available habitat on community and population size (Vellend et al. 2014). Consequently, positive SGDCs might be less likely in marine environments, where the limits of suitable habitat areas can be hard to define. Moreover, because oceans are environments of high connectivity, and both environmental conditions and behavioural characteristics of marine organisms can increase their dispersal capabilities (Cowen & Sponaugle 2009), high connectivity could contribute to increased diversity beyond what might be expected for a specific area given its size. However, restrictions to dispersal in the marine environment are also not always obvious, and there are many examples of species with limited actualized dispersal despite their potential for wider dispersal (Hellberg 2009, Weersing & Toonen 2009). Therefore, the diversity of marine communities might be affected more by environmental conditions than by area per se. For example, abiotic variables, such as water salinity (Bekkevold et al. 2005) or temperature (Banks et al. 2013), as well as different biotic factors (Cole 2010, de Juan & Hewitt 2011) impact marine communities, particularly benthic macrofauna. In some habitats, such as estuaries, fluctuations in abiotic conditions can be extreme, and dynamics in environmental conditions could also strongly influence diversity (Robinson et al. 2010, de Juan & Hewitt 2014).

At large spatial scales, variation in species diversity is often accompanied by turnover in species composition (Vellend 2005), which is more appropriately described as β diversity (diversity between different populations or communities). SGDCs in β diversity are less commonly explored, but, like SGDCs of α diversity, these also vary in strength and sign (Kahilainen et al. 2014). SGDCs in β diversity might be particularly useful as indicators of dispersal or barriers to recruitment that organisms might face in new habitats or for species that show isolation by distance. For example, when examining several focal species, seascape genetic studies have indicated characteristics of the community, specifically biological interactions and the role of coral cover in Hawaiian coral reefs that promote high diversity and connectivity (Selkoe et al. 2016). At smaller spatial scales, when connectivity between populations is expected to homogenize populations and communities, SGDCs in β diversity are not expected (see Kahilainen et al. 2014).

Turnover in species composition can also occur within a population or community as a result of immigration of ephemeral species and succession over time (e.g. see Bracken & Williams 2017), but temporal variation in diversity is not typically explored through SGDCs. This could be for several reasons. Firstly, if limited resources restrict the scale of the study, emphasis might be placed on spatial sampling rather than temporal sampling. Secondly, the factors expected to affect diversity and drive SGDCs might not show temporal variation. Thirdly, researchers might simply assume that diversity (either species diversity or genetic diversity, or both) is not temporally variable. Nevertheless, temporal variation in species or genetic diversity can occur, particularly in seasonally dynamic environments (e.g. Lamy et al. 2013, de Juan & Hewitt 2014, Hewitt et al. 2016). Long-term environmental fluctuations (such as El Niño events and increasing global climate change) also create temporal variation in species diversity (Cleary et al. 2006, Pauls et al. 2013). Therefore, studying temporal SGDCs might reveal concordant or conflicting responses to environmental variation in the focal communities. When SGDCs among temporal samples are analyzed, the same methods used for analyzing SGDCs among spatial samples typically are adopted (e.g. Cleary et al. 2006).

We expect that a combination of spatial and temporal sampling when investigating SGDCs has the potential to help clarify the most important factors affecting the diversity of communities and species living in seasonally variable environments, since the life histories and population dynamics of species living in these habitats are closely tied to seasonal variation (Kordas et al. 2011). In the present study, we examined the correlation between species diversity of benthic macrofauna at 4 sites in the Danish Isefjord–Roskilde Fjord estuary and genetic diversity of the polychaete worm *Pygospio elegans* living at

these sites with samples collected at 4 times over 1 year. *P. elegans* is common, has broad environmental tolerances (Anger 1984, Thonig et al. 2016) and shows variation in larval developmental mode, which is expected to impact its dispersal potential and population connectivity (Rasmussen 1973, Morgan et al. 1999). Our previous studies on *P. elegans* revealed seasonal population dynamics (Thonig et al. 2016) and seasonal changes in population genetic structure (Thonig et al. 2017). We hypothesized that the benthic invertebrate community might also respond to seasonally variable environmental factors and that a positive SGDC in α diversity would be found. Given the small overall spatial scale of the study area and our previous observations of chaotic genetic patchiness among *P. elegans* populations in the Isefjord–Roskilde Fjord estuary (e.g. Kesäniemi et al. 2014, Thonig et al. 2017), we did not expect to find an SGDC in β diversity among the samples.

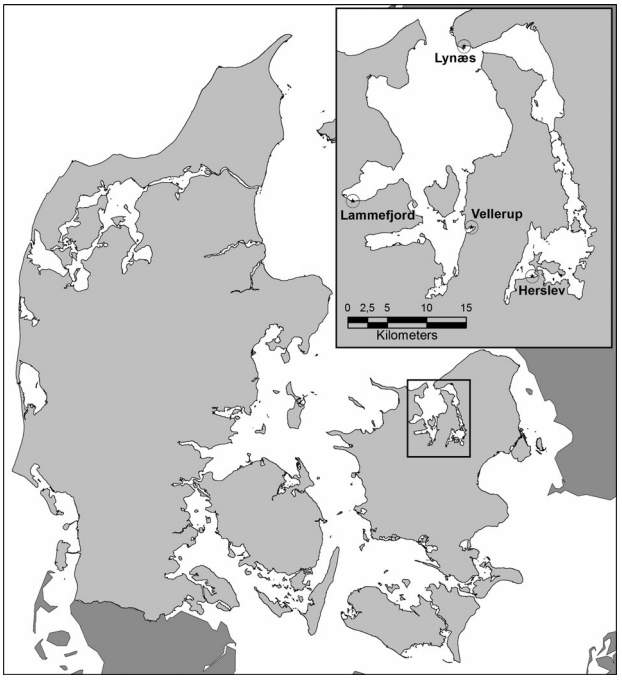

Fig. 1. The location of the Isefjord–Roskilde Fjord estuary (Denmark) is indicated by the box and is enlarged in the inset, showing the 4 sampling sites: Lynæs, Lammefjord, Vellerup and Herslev

MATERIALS AND METHODS

Data collection

We assessed seasonal variation in species diversity of benthic macrofauna at 4 time points (March, May, August and November 2014) at 4 study sites (Lynæs, Lammefjord, Vellerup and Herslev) in the Danish Isefjord–Roskilde-Fjord estuary (Fig. 1). At each sampling, 3 replicate sediment cores were collected using a hand-held corer (15 cm diameter, 30 cm length). Samples were sieved using a 1 mm mesh, and remaining material was fixed in 5% buffered formaldehyde on site. In the lab, formaldehyde was removed in several washing steps using deionized water, and the samples were stained overnight with a 2% rose bengal solution to better visualize the macrofauna. After removing the rose bengal solution, specimens were sorted and identified to the lowest reliable taxonomic level according to Barnes (1994) and Hayward & Ryland (1995), and we confirmed currently valid taxonomy using the World Register of Marine Species (WoRMS Editorial Board 2017). Sorted specimens were stored in 95% ethanol.

The samples of benthic macrofauna were collected concomitantly with a field survey performed monthly in 2014/2015, during which environmental parameters were monitored, and population dynamics of the polychaete *Pygospio elegans* were followed at the 4 sites (Thonig et al. 2016). The environmental variables measured included sediment characteristics (median grain size, sorting, porosity, water content, organic content and C:N ratio), water temperature and salinity, since these variables were expected to vary among the 4 sampled sites and, at least for temperature and salinity, were expected to show seasonal variation (Rasmussen 1973, G.T. Banta & B. Winding Hansen pers. obs.). Detailed methods can be found in Thonig et al. (2016). Briefly, sediment characteristics were determined from a mix of the top 1 cm of 3 replicate sediment cores per site and time. Median grain size (φ) and sorting (φ) were calculated as $\varphi_{50\%}$ and $(\varphi_{84\%} - \varphi_{16\%})/4 + (\varphi_{95\%} - \varphi_{5\%})/6.6$ according to Gray & Elliott (2009) using size fractions corresponding to the Wentworth scale (arithmetic phi [φ] is defined as $-\log_2$ of the size in mm). Porosity (%) and water content (%) were determined from wet weight and dry weight after 24 h at 105°C. Organic content (%) represents loss on ignition (2 h at 550°C), and carbon and nitrogen content (mol%) were obtained using an element analyser. Temperature (°C) and salinity (PSU) were logged every 10 min during the whole study period with data loggers, and the mean and standard deviation were calculated per month. During the field survey, samples of *P. elegans* were collected each month and measured, and cohorts based on size were determined (Thonig et al. 2016). Later, these worms were genotyped using 7

microsatellite loci (see detailed methods in Thonig et al. 2017, and Supplement 1 at www.int-res.com/articles/suppl/m592p129_supp1.pdf). Population genetic structure of *P. elegans* using the monthly samples is described by Thonig et al. (2017). Genetic data collected at the 4 time points chosen for surveying the benthic community (March, May, August, November) were used for assessment of genetic diversity and in analysis of SGDCs, described here.

Species diversity, genetic diversity and SGDCs

Abundance of each identified taxon was recorded in the software PRIMER-E v.6.1.16 (Clarke & Warwick 2001) for each core separately (3 replicate samples per location and sampling date). Counts were transformed using the fourth root to account for the high abundance of a single abundant taxon (i.e. *Hydrobia* spp.) and averaged over replicate sampling cores. Bray-Curtis similarity was used when constructing a resemblance matrix, and temporal and spatial differences in species abundance were visualized in a non-metric multi-dimensional scaling (NMDS) plot with the default number of restarts (1000) using PRIMER-E. Species diversity was measured as species richness: the number of species present in each core was counted and then averaged over replicate cores for each location and sampling date.

The allele frequencies of *P. elegans* at each microsatellite locus and sampling date were calculated using Fstat v. 2.9.3.2 (Goudet 1995). These were input to PRIMER-E, and a resemblance matrix was made using Euclidian distance. The spatial and temporal differences in allele frequencies were visualized in an NMDS plot constructed in PRIMER-E. Genetic diversity was represented by allelic richness, calculated for each locus based on a sample size of 26 individuals using HP-Rare v.1.1 (Kalinowski 2005) and then averaged over all loci.

A correlation between species diversity and genetic diversity (α SGDC) was calculated across all sites and time points using Spearman's rank correlation coefficient in R v. 3.4.0 (R Core Team 2017). Because the samples included in the SGDC were derived from repeated measures at 4 sites and represent a time series, there is potential for temporal autocorrelation. We investigated the temporal independence of our samples by examining a residual plot of the SGDC correlation model. In this model, we assumed that our sites are independent, but if temporal autocorrelation exists, the residuals within site should show a temporal relationship, i.e. time points close to each other should have similar residuals. We did not see such a pattern in our residual plot, however. Additionally, residuals were not more similar within site than between sites, indicating that repeated measures at the same site had no effect. Likewise, a Durbin-Watson test of ordered time points within sites did not indicate any autocorrelation (DW = 2.18, p = 0.58). Furthermore, our previous analyses indicated significant genetic variation among samples both spatially and temporally (Thonig et al. 2017), although differentiation among all samples was not always statistically significant. Therefore, we are confident that the samples are sufficiently independent to be combined in a single correlation analysis.

We calculated β SGDC by correlating the difference in species composition and allelic composition between the samples taken at all 4 sites and 4 sampling times. For species composition, this was calculated as Bray-Curtis dissimilarity, or 100 − Bray-Curtis similarity, derived from the matrix of the transformed averaged fauna counts in PRIMER-E. For allelic composition, we calculated the fixation index G'_{ST} (Hedrick 2005) using the diversity package in R (Keenan et al. 2013, R Core Team 2017). Previous studies investigating β SGDC have also used these measures (for review, see Kahilainen et al. 2014). Since the G'_{ST} values were not normally distributed, we used Spearman's rank correlation coefficient when calculating the β SGDC.

Environmental impact on diversity

Generalized linear mixed models (GLMMs) allow for the analysis of response variables that have different distributions than the normal distribution. These models can also account for dependence between samples by incorporating random effects in addition to fixed (design) effects. In this study, we used GLMM to investigate the effect of environmental parameters on both diversity measures, i.e. species diversity and genetic diversity, while accounting for repeated measures at the 4 sampling sites. Count data, such as species richness, are assumed to follow a Poisson or negative binomial distribution rather than a normal distribution. The negative binomial distribution is preferred in cases when overdispersion occurs, i.e. when the variance is larger than the mean, for example due to patchiness of species distributions, and is indicated by a small overdispersion parameter, θ. We compared a log-linear model with a Poisson error term and a log-linear model with an

error term following a negative binomial distribution for our response variable species richness. Since the latter resulted in a large estimate of θ (the overdispersion coefficient), we chose the Poisson distribution to model the error term. We checked for collinearity of our environmental variables using scatterplots and Pearson correlation coefficient, to reduce the number of explanatory variables. We detected a strong correlation between the 4 sediment characteristics median grain size, sorting, porosity and water content ($r = 0.725$, -0.818, 0.775, respectively; all $p < 0.01$), which is not surprising, given that they are by nature not independent. Since median grain size showed the strongest correlations with the other sediment variables, we kept it for the GLMM and removed porosity, sorting and water content from the explanatory data set. Additionally, the standard deviation of temperature was closely correlated with mean temperature ($r = 0.905$; $p < 0.001$). Since we did not detect extreme temperature fluctuations, we assumed that mean temperature indicating seasonality might have larger biological relevance, so we removed temperature SD from the explanatory data set. Hence, the fixed effects of our explanatory variables were median particle size, organic content, C:N ratio, mean temperature, mean salinity and standard deviation of salinity. We measured only 1 set of environmental variables per sampling; thus, the same environmental data were used for the 3 replicate measurements of species richness per sampling. As a random effect we included sample, which represents the combination of sampling time point and site, to account for the effect of season on the one hand and the repeated measures design of our study on the other hand. The GLMM was performed with glmmPQL in the R package MASS (R Core Team 2017) according to the following equation:

$$\text{Log(SpeciesRichness)} = \alpha + \beta_1 \times \text{median particle size} + \beta_2 \times \text{organic content} + \beta_3 \times \text{C:N} + \beta_4 \times \text{mean temperature} + \beta_5 \times \text{mean salinity} + \beta_6 \times \text{salinity SD} + \text{Poisson}(\lambda_{\text{Sample}}) + \text{Poisson}(\lambda_{\text{Residual}}) \quad (1)$$

Since our response variable allelic richness neither represents count data nor is normally distributed, we inspected it visually with a quantile comparison plot (qqp function in the R package 'car'), which showed that it fit best to a log-normal distribution. For that reason, we used a log-linear model with a normally distributed error term. The explanatory variables were composed of the same fixed effects as for species richness, but included only site as a random effect due to lack of replication within sample. The GLMM was performed with glmmPQL in the R package MASS (R Core Team 2017) according to the following equation:

$$\text{Log(AllelicRichness)} = \alpha + \beta_1 \times \text{median particle size} + \beta_2 \times \text{organic content} + \beta_3 \times \text{C:N} + \beta_4 \times \text{mean temperature} + \beta_5 \times \text{mean salinity} + \beta_6 \times \text{salinity SD} + \text{Normal}(0,\sigma^2_{\text{Site}}) + \text{Normal}(0,\sigma^2_{\text{Residual}}) \quad (2)$$

RESULTS

Species diversity, genetic diversity and SGDCs

In total, we collected 20 752 individuals representing 51 benthic invertebrate taxa from samples taken from 4 locations in the Danish Isefjord–Roskilde Fjord estuary at 4 times of the year (see Supplement 2 at www.int-res.com/articles/suppl/m592p129_supp2.xlsx; Fig. 2). The most abundant taxon at all sites was the gastropod *Hydrobia* spp. The focal species of our study, *Pygospio elegans,* was found at all sites, in 38 out of the 48 samples, and was the fourth most frequently found species. However, the presence of *P. elegans* was patchy, and it was not sampled in any of the replicate cores from Lynæs or Lammefjord in November, even though additional sampling at these sites in November yielded a sufficient number of *P. elegans* specimens to use in the genetic analysis. Density of *P. elegans* was highest in May and lowest in November (see Thonig et al. 2016 and Supplement 2).

We visualized the spatial and temporal variation in species abundance of the benthic macrofauna using an NMDS plot (Fig. 3A). The plot indicates good spatial differentiation (i.e. separate groupings) between all sites; Vellerup and Herslev were clearly distinct and not overlapping with other sites. Lynæs and Lammefjord were more similar to each other with some overlap, but differed from the other sites. The moderate stress value (0.15) indicates that the NMDS plot is a sufficient representation of the relations between samples based on species abundance. Polychaetes were most abundant in Vellerup, while gastropods were most abundant in Lynæs and Lammefjord (Supplement 2). Crustaceans and bivalves had relatively low abundances at all sites. No large temporal shifts in species abundance were observed, with the exception of November, which differed from the other times at all sites.

Species richness varied from a low of 5 species observed in March at Lynæs to a high of 29 species observed in August at Vellerup (Figs. 2 & 4A). Higher species richness was generally observed at Vellerup,

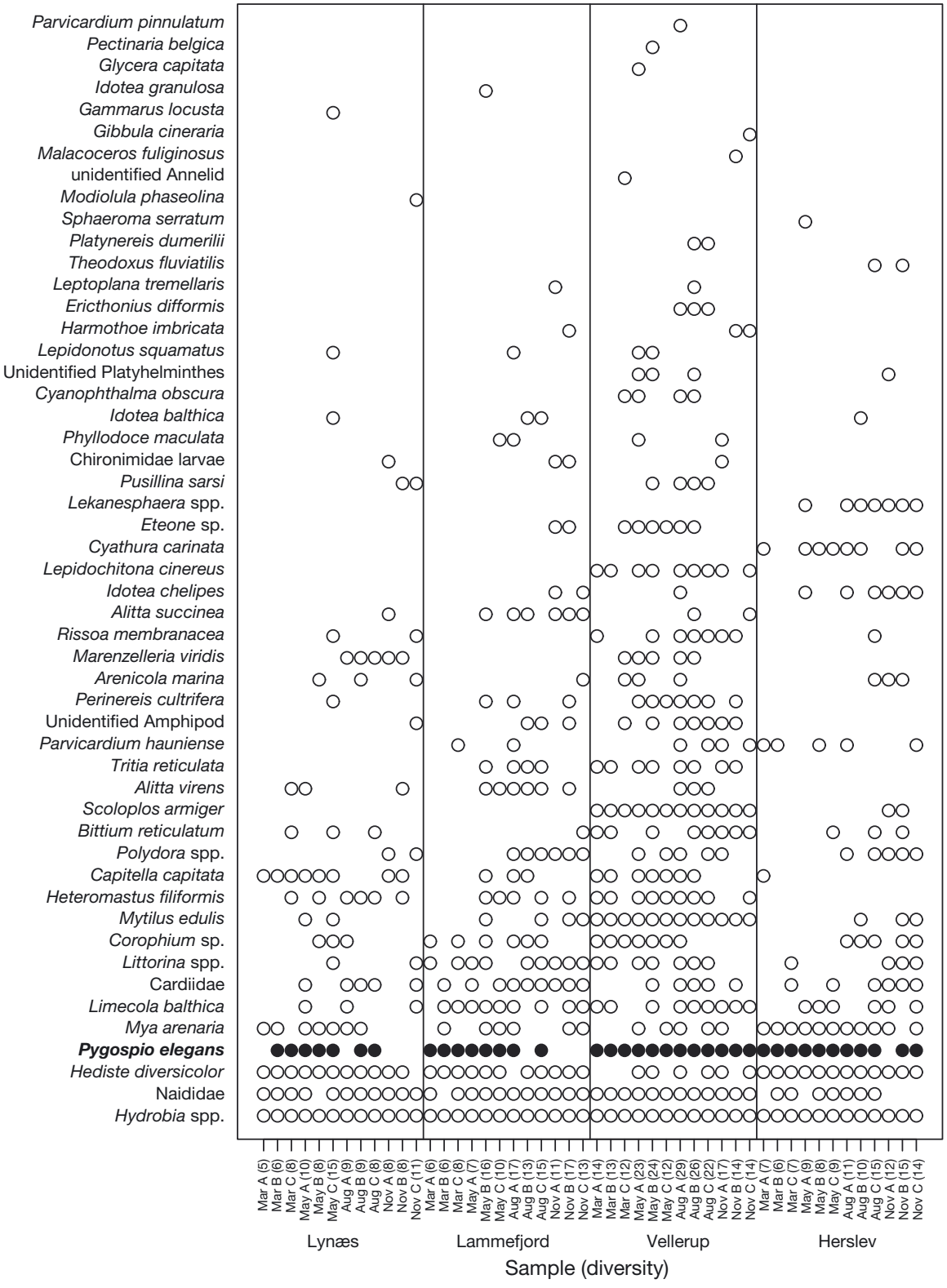

Fig. 2. Benthic macrofauna present from 4 sites in the Danish Isefjord–Roskilde Fjord estuary at 4 time points during the year (a circle indicates that a taxon was present in a particular sample). The *y*-axis lists the taxa observed ranked from least to most common (top to bottom) among the samples. The focal taxon, *Pygospio elegans*, is highlighted in **bold**. Samples are arranged on the *x*-axis according to site, time point and replicate sample. The number of taxa observed in each replicate is shown in parentheses, e.g. Lynæs Mar A (5) means replicate A collected at Lynæs in March contained 5 taxa

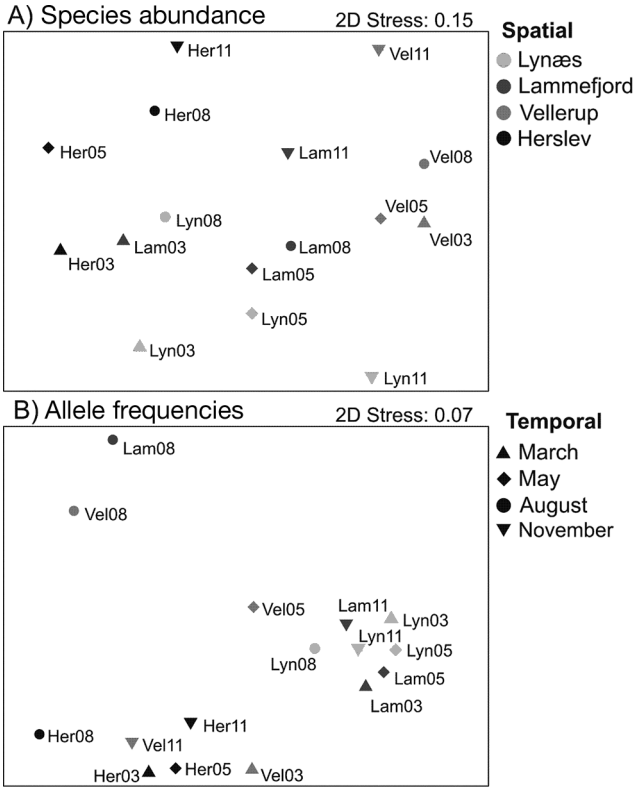

Fig. 3. Non-metric multi-dimensional scaling (NMDS) plots of (A) species abundances of benthic macrofauna and (B) allele frequencies of *Pygospio elegans* sampled at 4 sites in the Danish Isefjord–Roskilde Fjord estuary in 4 different months. Samples are coded with an abbreviated site name (Lyn: Lynæs, Lam: Lammefjord, Vel: Vellerup, Her: Herslev) and number representing sampling time (03: March, 05: May, 08: August, 11: November) and where grey shading of symbols indicates spatial sampling and different symbol shapes indicate temporal sampling

whereas the other sites had similar, lower levels of diversity. Temporal patterns at each site showed lowest richness in March, which then increased during the year. In Lammefjord and Vellerup, diversity reached a peak in August and then decreased in November. In contrast, diversity peaked in Lynæs in May and in November in Herslev.

Seasonal population genetic structure in *P. elegans* is described by Thonig et al. (2017). Allele frequencies of 7 microsatellite loci from genotyped *P. elegans* collected at the 4 study sites, and time points were visualized in an NMDS plot (Fig. 3B). Allele frequencies were similar in Lynæs and Lammefjord at all collection times excluding August at Lammefjord. Furthermore, allele frequencies in August differed markedly from those of samples taken at other times except for Lynæs. Temporal variation in allelic frequencies was greatest in Vellerup (Fig. 3B). Allelic richness averaged over all loci ranged from 2.5 in March at Vellerup to 5.7 in August at Lammefjord (Supplement 1). A seasonal pattern was observed in allelic richness, particularly for Lammefjord and Vellerup, and in general, the highest values were observed at all sites in August (Fig. 4B).

There was a significant positive correlation (α SGDC) between species richness and allelic richness (rho = 0.697, p = 0.003; Fig. 5). There was no correlation in the differences among samples in species and allelic composition (β SGDC; rho = 0.132, p = 0.152; see Supplement 2). This result is in line with the different patterns observed in the NMDS plots of species abundance and allele frequencies.

Factors explaining the pattern

Environmental variables measured for each site and sampling time are reported in detail by Thonig et al. (2016). In general, water temperature showed a similar seasonal pattern at all sites, with highest temperatures in July and lowest temperatures in February. Salinity, in contrast, differed between sites, being around 19–20 PSU at Lynæs, Lammefjord and Vellerup, and around 14 PSU at Herslev. Likewise, sediment characteristics differed between sites but did not show any consistent seasonal patterns. Sediment was fine-grained at Lynæs (mean grain size 0.18–0.25 mm) and Lammefjord (0.18–0.29 mm), medium at Herslev (0.25–0.35 mm) and coarse at Vellerup (0.44–0.62 mm). Water content and porosity were highest in fine sediment. Sediment was moderately well sorted in Lynæs, only moderately sorted in Lammefjord and Herslev, and poorly sorted at Vellerup (sorting classes derived from inclusive graphic standard deviation according to Gray & Elliott 2009). Organic content was highest at Lammefjord, followed by Lynæs, Vellerup and Herslev. At Vellerup we found the highest C:N ratio, i.e. the most refractory material, while more labile organic matter was present at Lammefjord, Herslev, and Lynæs (Thonig et al. 2016).

According to the GLMM, the variation explained by the random effects of sample and site was very low for species and allelic richness, respectively. This indicates that most of the difference between sites and times that can be predicted by the model is already captured with the fixed effects. Median sediment grain size and mean temperature had significant effects on species richness (Table 1). Considering that we used a log-linear model, an effect size of −0.5 of median grain size means that species richness decreases 0.607 (= $e^{-0.5}$) fold per unit of grain size.

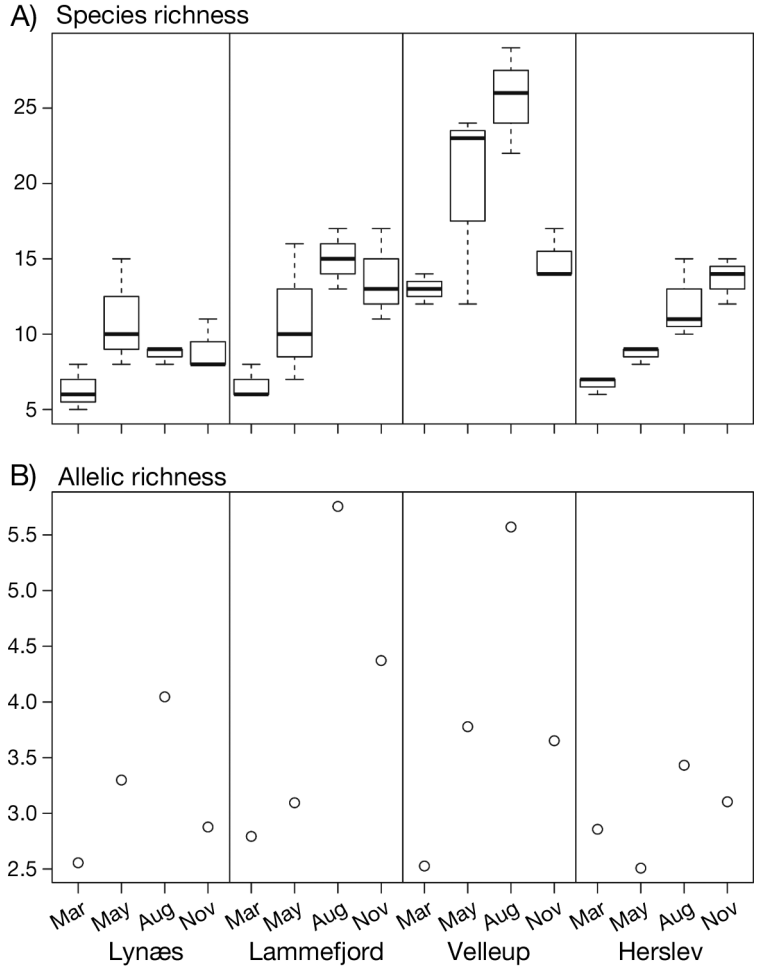

Fig. 4. (A) Species richness estimated for the benthic invertebrate communities and (B) average allelic richness of *Pygospio elegans* sampled from each sampling site and sampling time in the Isefjord–Roskilde Fjord estuary

Since median grain size is determined as φ, i.e. $-\log_2$ of grain size in mm, sediment gets finer with increasing φ. Hence, higher species richness was found in coarse and, considering the correlation with sediment sorting, poorly sorted sediments. Furthermore, species richness increases 1.034 (= $e^{0.034}$) fold per °C. Allelic richness of *P. elegans* was also affected significantly by temperature, i.e. it increased 1.044-fold per degree (Table 1). Allelic richness was not significantly related to any of the other environmental variables investigated.

DISCUSSION

We investigated SGDCs between species richness of the benthic macrofauna community in the Danish Isefjord–Roskilde Fjord estuary and allelic richness of a focal species, the polychaete *Pygospio elegans*. Our study was conducted over a small spatial scale (maximum distance between sites

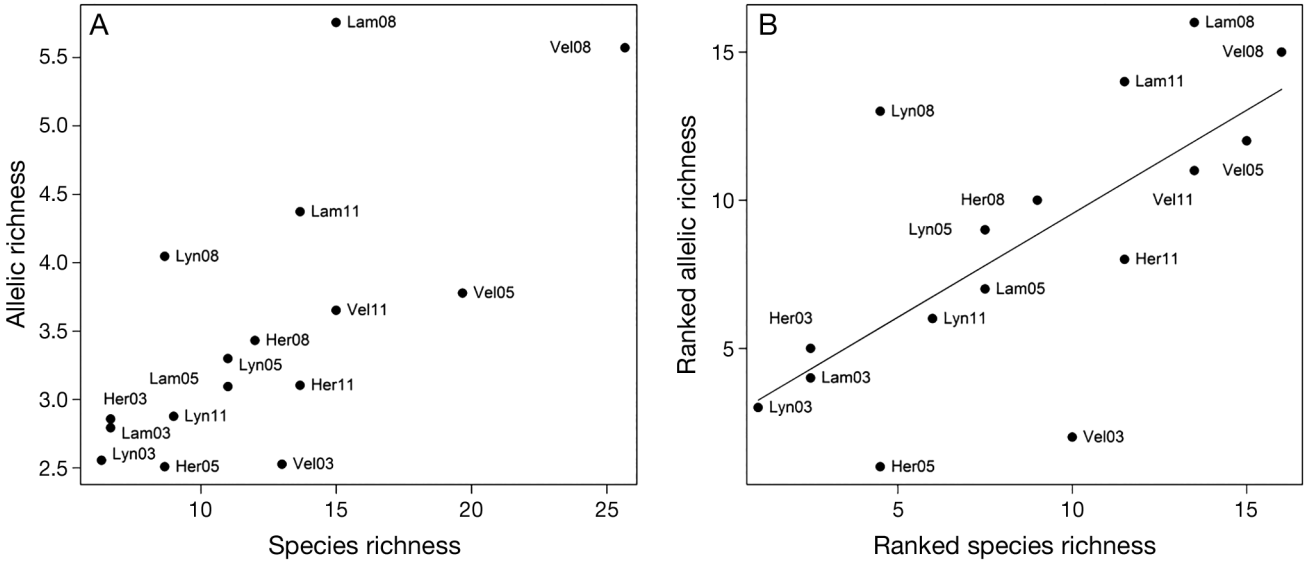

Fig. 5. (A) Average species richness at 4 sites in the Danish Isefjord–Roskilde Fjord estuary in 4 different months and average allelic richness of populations of *Pygospio elegans* from the same sites and time points. (B) Ranked average species and allelic richness and the linear regression between both variables illustrating the positive α species–genetic diversity correlation (Spearman rank: rho = 0.697, p = 0.003). Samples are denoted with abbreviated site and sampling time codes as in Fig. 3

Table 1. Environmental factors explaining species richness and allelic richness in the Isefjord–Roskilde Fjord estuary according to general linear mixed modelling (see Materials and Methods for details). Values in **bold** are significant at $p < 0.05$

Species richness						Allelic richness					
Random effects (Poisson)	SD	Variance%				**Random effects (Normal)**	SD	Variance%			
Sample	0.119	0.025				Site	1.5×10^{-6}	0.000			
Residual	0.746	0.975				Residual	0.554	1.000			
Fixed effects	Value	SE	df	*t*	p	**Fixed effects**	Value	SE	df	*t*	p
(Intercept)	2.193	0.700	32	3.134	0.004	(Intercept)	−0.475	0.842	6	−0.564	0.593
Median grain size	−0.500	0.091	9	−5.515	**0.0004**	Median grain size	−0.101	0.096	6	−1.053	0.333
Organic content	0.106	0.327	9	0.324	0.753	Organic content	0.327	0.307	6	1.066	0.327
C:N ratio	−0.011	0.055	9	−0.201	0.845	C:N ratio	0.038	0.062	6	0.612	0.563
Temperature mean	0.034	0.010	9	3.293	**0.009**	Temperature mean	0.044	0.012	6	3.607	**0.011**
Salinity mean	0.035	0.022	9	1.550	0.156	Salinity mean	0.037	0.029	6	1.252	0.257
Salinity SD	0.064	0.048	9	1.334	0.215	Salinity SD	0.023	0.055	6	0.416	0.692

~30 km) and emphasized temporal sampling in addition to spatial sampling in order to incorporate seasonal variation in population dynamics that could affect both levels of diversity. A positive correlation in α diversity was found when combining the data from all sites and collection times, suggesting that both the benthic community as a whole and populations of *P. elegans* are affected similarly by seasonal variation at the study sites. However, there was no correlation in β diversity between the studied sites and sampling times, which might indicate that the underlying metapopulation structure of the benthic community differs from that of *P. elegans* or that limitations of the sampling design precluded us from finding a correlation in β diversity.

When examining the role of abiotic environmental factors in explaining the patterns of diversity, we found that mean temperature and median sediment grain size helped explain the patterns of species richness. Species richness was higher at warmer (and more variable) temperatures and in coarser sediments (with greater porosity and water content and poorer sorting). Temperature is a good predictor of seasonal change because it is related to changes in, for example, primary productivity. Furthermore, seasonal variation in species richness has been documented for other benthic communities similar to that which we observed here, e.g. in the Baltic Sea (Blomquist & Bonsdorff 1986, Bonsdorff & Blomquist 1989) and in the North Sea (Reiss & Kröncke 2004). The association between temperature (season) and species richness likely stems from seasonal variation in food supply (e.g. vertical transport of matter originating from phytoplankton blooms, Cloern & Jassby 2010), which can support larger communities. Sediment factors, on the other hand, are not expected to vary seasonally, but represent habitat preferences of the benthic taxa that can also affect community diversity. However, an indirect relationship between sediment factors and seasonal variation might exist, for example in the biotic communities inhabiting sediments (microbial or algal population dynamics, e.g. Quero et al. 2017), that was not measured during our study. Although salinity typically has a major role in explaining patterns of species diversity in the Baltic Sea on a large spatial scale (Zettler at al. 2014), salinity mean and standard deviation did not explain patterns of species richness in the present study. This could indicate that the differences in salinity among the 4 studied sites and the sampled seasons do not fluctuate at a level that alters this estuarine community (which is made up of euryhaline species generally tolerant to salinity fluctuations). Also, there might have been insufficient power for finding an effect of salinity due to the small number of studied sites. Robinson et al. (2010) also found little support for a role of salinity in driving SGDCs in estuaries in the southeastern USA. However, when comparing regions along the North Sea–Baltic Sea transition, where salinity differences are more extreme and long-lasting, salinity significantly explained diversity patterns (Josefson & Göke 2013).

When analyzing genetic diversity of *P. elegans*, we found that, out of the environmental variables studied, only temperature (mean and its correlated standard deviation) had a significant effect explaining variation in allelic richness. Allelic richness increased in August when temperatures were warmer. Seasonal genetic variation in marine invertebrates is poorly studied, but has been observed in some line-

ages of the cryptic nematode *Pellioditis marina* as a result of (meta)population turnover (Derycke et al. 2006) and in the ascidian *Styela plicata* in North America resulting from seasonal patterns of recruitment (Pineda et al. 2016). Similarly, the variation in allelic richness of *Pygospio elegans* could also be explained by seasonal reproduction and recruitment of new, genetically differentiated cohorts that coexist with older cohorts at the sites in August (see Thonig at al. 2016, 2017). Together, these results suggest that dispersal is the driving force behind the seasonal pattern. *P. elegans* shows variation in larval developmental mode, producing planktonic, benthic and intermediate larvae that differ in their capability for dispersal (Rasmussen 1973, Morgan et al. 1999, Thonig et al. 2016). At our study sites, all types of larvae were observed, except in Herslev, where only benthic and intermediate larvae were noted (Thonig et al. 2016). Most of the taxa sampled in the benthic community also show life history strategies incorporating planktonic larvae and seasonal population dynamics, with an increased number of larvae present in summer (June, July and August) and reductions in population sizes in winter (Thorson 1946, Rasmussen 1973).

A lack of a correlation in β diversity among the sites and sampling times was not surprising, given our hypotheses based on our previous studies of *P. elegans* that showed chaotic genetic patchiness among samples and no relationship between genetic structure and geographic distance (e.g. Kesäniemi et al. 2014, Thonig et al. 2017). A broader (spatial) study might reveal such a correlation and allow for investigation of the environmental variables, both abiotic and biotic, that could affect β diversity relationships. For example, Kesäniemi et al. (2012) found isolation by distance among *P. elegans* populations greater than 100 km distant from each other, suggesting that a larger spatial scale could possibly reveal a β diversity relationship. Also, it would be interesting to know whether the lack of a SGDC in β diversity in this study is more likely due to the limited spatial scale of the study or the choice of focal taxon used for assessing genetic diversity. Other species lacking developmental mode polymorphism in the sampled study sites might be more appropriate for investigating a β SGDC.

Inter-annual temporal variation in SGDCs has been described for butterflies in rainforests and freshwater snails in a pond network (Cleary et al. 2006, Lamy et al. 2013), but seasonal variation in SGDCs has not been a focus in previous studies. Our finding of a significant SGDC with a combination of spatial and temporal sampling suggests that seasonal environmental changes and associated life histories are relevant for understanding diversity patterns in temperate marine benthic communities. Seasonal changes in diversity of marine fauna are common, particularly at latitudes where temperature and other abiotic factors vary predictably (Valiela 2015). Moreover, many marine organisms have adapted to life in seasonal environments, and are known to time their reproductive events to follow seasonal variation (Coma et al. 2000, Smart et al. 2012). Considering the small geographic distances between our study sites and the negligible differences in temperature among sites (Thonig et al. 2016), temporal sampling was needed to reveal the effects of temperature (seasonality) on species and genetic diversity. Previously, Kesäniemi et al. (2014) could not relate genetic diversity of *P. elegans* (local F_{ST}) to any environmental variables in a study in which *P. elegans* was collected from a large number of sites in the Isefjord–Roskilde Fjord estuary at a single time point (April). Due to limited resources, we could only sample 4 study sites at 4 different times, which prohibited us from investigating site-level diversity and SGDCs at each time point separately. Nevertheless, our results indeed highlight an important temporal effect and help inform a relevant sampling scale for future larger-scale studies. Namely, when investigating patterns of diversity, it is important that samples are compared on the same time scale. Timing of sampling can have a significant effect on results of SGDCs and should be clearly reported, particularly for meta-analyses and when the samples used for calculating species richness and allelic richness are not collected concomitantly. Because of seasonal changes in diversity, Reiss & Kröncke (2005) have also cautioned against comparing diversity indices of different data sets collected in different seasons. In our study, we saw clear evidence of a positive SGDC related to seasonal factors that affect diversity, most likely through seasonal reproduction and dispersal, and highlight the importance of life history strategies on broader ecological patterns that could also be relevant at other time scales.

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