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

Year: 2018

Version: Accepted version (Final draft)

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Please cite the original version:

Knott, E., Thonig, A., Heiskanen, S., Hansen, B. W., & Banta, G. T. (2018). Seasonal variation in diversity of marine benthic invertebrates leads to a positive species-genetic diversity correlation (SGDC). *Marine Ecology: Progress Series*, 592, 129-140. <https://doi.org/10.3354/meps12520>

1 Seasonal variation in diversity of marine benthic invertebrates leads to a positive species-
2 genetic diversity correlation.

3

4 Running head: Seasonal variation and SGDC

5

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14 Keywords: SGDC, *Pygospio elegans*, estuarine, species richness, allelic richness, Polychaeta

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25

26 Abstract

27

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

44

45 Introduction

46

47 Diversity can be measured within individuals, populations, and/or communities, but relationships
48 between diversity at these different levels are unclear. Ecological and evolutionary processes can
49 have similar effects on species diversity within communities and on genetic diversity within
50 species, and thus, positive correlations between the different levels of biodiversity can occur (see
51 Vellend 2003, Vellend et al. 2014). These “species-genetic diversity correlations” (SGDCs) can be
52 useful indicators of the processes that simultaneously affect diversity at multiple biological levels.
53 Moreover, identification of SGDCs is useful in an applied context if they allow the inference of one
54 level of diversity based on that of another (Kahilainen et al. 2014). However, the sign of SGDCs can
55 be difficult to both predict and interpret (Laroche et al. 2015). Positive relationships are expected
56 when diversity is mediated by factors acting in the same way on individual species and on the
57 entire community (Vellend 2003, Kahilainen et al. 2014, Lamy et al. 2016), for example via
58 available habitat, productivity or shared dispersal routes. However, biological interactions can
59 disrupt potential species-genetic diversity correlations, for example in cases of competition or
60 facilitation between species, and could lead to either positive or negative SGDCs. Several reviews
61 of empirical studies (Kahilainen et al. 2014, Vellend et al. 2014, Whitlock 2014) have now
62 emphasized that an expectation for positive SGDCs in most cases might be premature.
63 Nevertheless, investigation of the factors explaining positive (or negative) SGDCs is fruitful for
64 understanding the ecology of the focal species and community in question.

65 Most studies on the relationships between species diversity and genetic diversity have
66 focused on terrestrial systems, and SGDCs in marine environments have received less attention
67 (Messmer et al. 2012, Josefson & Göke 2013, Selkoe et al. 2016). SGDCs in α diversity (diversity at
68 the local scale in a particular population or community) are expected to be more frequently
69 positive in island-like systems due to clear limitations of area or available habitat on community

70 and population size (Vellend et al. 2014). Consequently, positive SGDCs might be less likely in
71 marine environments, where the limits of suitable habitat areas can be hard to define. Moreover,
72 because oceans are environments of high connectivity, and both environmental conditions and
73 behavioural characteristics of marine organisms can increase their dispersal capabilities (Cowen &
74 Sponaugle 2009), high connectivity could contribute to increased diversity beyond what might be
75 expected for a specific area given its size. However, restrictions to dispersal in the marine
76 environment are also not always obvious, and there are many examples of species with limited
77 actualized dispersal despite their potential for wider dispersal (Hellberg 2009, Weersing & Toonen
78 2009). Therefore, the diversity of marine communities might be affected more by environmental
79 conditions than by area *per se*. For example, abiotic variables, such as water salinity (Bekkevold et
80 al. 2005) or temperature (Banks et al. 2007), as well as different biotic factors (Cole 2010, de Juan
81 & Hewitt 2011) are known to impact marine communities, particularly benthic macrofauna. In
82 some habitats, such as estuaries, fluctuations in abiotic conditions can be extreme, and dynamics
83 in environmental conditions could also strongly influence diversity (Robinson et al. 2010, de Juan
84 & Hewitt 2014).

85 At large spatial scales, variation in species diversity is often accompanied by turnover in
86 species composition (Vellend 2005), which is more appropriately described as β diversity (diversity
87 between different populations or communities). SGDCs in β diversity are less commonly explored,
88 but, like SGDCs of α diversity, these also vary in strength and sign (Kahilainen et al. 2014). SGDCs in
89 β diversity might be particularly useful as indicators of dispersal or barriers to recruitment that
90 organisms might face in new habitats or for species that show isolation by distance. For example,
91 when examining several focal species, seascape genetic studies have indicated characteristics of
92 the community, specifically biological interactions and the role of coral cover in Hawaiian coral
93 reefs that promote high diversity and connectivity (Selkoe et al. 2016). At smaller spatial scales,

94 when connectivity between populations is expected to homogenize populations and communities,
95 SGDCs in β diversity are not expected (see Kahilainen et al. 2014).

96 Turnover in species composition can occur also within a population or community as a result
97 of immigration of ephemeral species and succession over time (e.g. see Bracken & Williams 2017),
98 but temporal variation in diversity is not typically explored through SGDCs. This could be for
99 several reasons. Firstly, if limited resources restrict the scale of the study, emphasis might be
100 placed on spatial sampling rather than temporal sampling. Secondly, the factors expected to affect
101 diversity and drive SGDCs might not show temporal variation. Thirdly, researchers might simply
102 assume that diversity (either species diversity or genetic diversity, or both) is not temporally
103 variable. Nevertheless, temporal variation in species or genetic diversity can occur, particularly in
104 seasonally dynamic environments (e.g. Lamy et al. 2013, de Juan & Hewitt 2014, Hewitt et al.
105 2016). Long-term environmental fluctuations (such as El Niño events and increasing global climate
106 change) are also known to create temporal variation in species diversity (Cleary et al. 2006, Pauls
107 et al. 2013). Therefore, studying temporal SGDCs might reveal concordant or conflicting responses
108 to environmental variation in the focal communities. When SGDCs among temporal samples are
109 analysed, the same methods used for analysing SGDCs among spatial samples typically are
110 adopted (e.g. Cleary et al. 2006).

111 We expect that a combination of spatial and temporal sampling when investigating SGDCs
112 has the potential to help clarify the most important factors affecting the diversity of communities
113 and species living in seasonally variable environments, since the life histories and population
114 dynamics of species living in these habitats are closely tied to seasonal variation (Kordas et al.
115 2011). In the present study, we examine the correlation between species diversity of benthic
116 macrofauna at four sites in the Danish Isefjord-Roskilde Fjord estuary and genetic diversity of the
117 polychaete worm *Pygospio elegans* living at these sites at four times over one year. *P. elegans* is
118 common, has broad environmental tolerances (Anger 1984, Thonig et al. 2016), and shows

119 variation in larval developmental mode, which is expected to impact its dispersal potential and
120 population connectivity (Rasmussen 1973, Morgan et al. 1999). Our previous studies on *P. elegans*
121 revealed seasonal population dynamics (Thonig et al. 2016) and seasonal changes in population
122 genetic structure (Thonig et al. 2017). We hypothesized that the benthic invertebrate community
123 might also respond to seasonally variable environmental factors and that a positive SGDC in α
124 diversity would be found. Given the small overall spatial scale of the study area and our previous
125 observations of chaotic genetic patchiness among *P. elegans* populations in the Isefjord-Roskilde
126 Fjord estuary (e.g. Kesäniemi et al. 2014, Thonig et al. 2017), we did not expect to find a SGDC in β
127 diversity among the samples.

128

129 Materials and Methods

130

131 *Data collection*

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

143 The samples of benthic macrofauna were collected concomitantly with a field survey
144 performed monthly in 2014/2015, during which environmental parameters were monitored, and
145 population dynamics of the polychaete *Pygospio elegans* were followed at the four sites (Thonig et
146 al. 2016). The environmental variables measured included sediment characteristics (median grain
147 size, sorting, porosity, water content, organic content and C/N ratio), water temperature, and
148 salinity, since these variables were expected to vary among the four sampled sites and, at least for
149 temperature and salinity, were expected to show seasonal variation (GTB, BWH *personal*
150 *observations*; Rasmussen 1973). Detailed methods can be found in Thonig et al. (2016). Briefly,
151 sediment characteristics were determined from a mix of the top one cm of three replicate
152 sediment cores per site and time. Median grain size [ϕ] and sorting [ϕ] were calculated as $\phi_{50\%}$
153 and $(\phi_{84\%}-\phi_{16\%})/4+(\phi_{95\%}-\phi_{5\%})/6.6$ according to Gray & Elliot (2009) using size fractions
154 corresponding to the Wentworth scale (arithmetic Phi (ϕ) is defined as $-\log_2$ of the size in mm).
155 Porosity [%] and water content [%] were determined from wet weight and dry weight after 24 h at
156 105 °C. Organic content [%] represents loss of ignition (2 h at 550 °C) and carbon and nitrogen
157 content [mol %] were obtained using an element analyser. Temperature [°C] and salinity [PSU]
158 were logged every ten minutes during the whole study period with data loggers and the mean and
159 standard deviation were calculated per month. During the field survey, samples of *P. elegans* were
160 collected each month, measured, and cohorts based on size were determined (Thonig et al. 2016).
161 Later, these worms were genotyped using seven microsatellite loci (See detailed methods in
162 Thonig et al. 2017, and Supplement 1). Population genetic structure of *P. elegans* using the
163 monthly samples is described in Thonig et al. (2017). Genetic data collected at the four time points
164 chosen for surveying the benthic community (March, May, August, November) were used for
165 assessment of genetic diversity and in analysis of SGDCs, described here.

166

167 *Species diversity, genetic diversity, and SGDCs*

168 Abundance of each identified taxon was recorded in the software PRIMER-E v.6.1.16 (Clarke &
169 Warwick 2001) for each core separately (3 replicate samples per location and sampling date).
170 Counts were transformed using the 4th root to account for the high abundance of a single
171 abundant taxon (i.e. *Hydrobia* spp.) and averaged over replicate sampling cores. Bray Curtis
172 similarity was used when constructing a resemblance matrix, and temporal and spatial differences
173 in species abundance were visualized in a non-metric multi-dimensional scaling (NMDS) plot with
174 the default number of restarts (1000) using PRIMER-E. Species diversity was measured as species
175 richness: the number of species present in each core was counted, and then averaged over
176 replicate cores for each location and sampling date.

177 The allele frequencies of *Pygospio elegans* at each microsatellite locus and sampling date
178 were calculated using Fstat v. 2.9.3.2 (Goudet 1995). These were input to PRIMER-E and a
179 resemblance matrix was made using Euclidian distance. The spatial and temporal differences in
180 allele frequencies were visualized in a NMDS plot constructed in PRIMER-E. Genetic diversity was
181 represented by allelic richness, calculated for each locus based on a sample size of 26 individuals
182 using HP-Rare v1.1 (Kalinowski 2005) and then averaged over all loci.

183 A correlation between species diversity and genetic diversity (α SGDC) was calculated across
184 all sites and time points using Spearman's rank correlation coefficient using R v. 3.4.0 (R Core
185 Team, 2017). Because the samples included in the SGDC were derived from repeated measures at
186 four sites and represent a time series, there is potential for temporal autocorrelation. We
187 investigated the temporal independence of our samples by examining a residual plot of the SGDC
188 correlation model. In this model, we assume our sites are independent, but if temporal
189 autocorrelation exists, the residuals within site should show a temporal relationship, i.e. time
190 points close to each other should have similar residuals. We do not see such a pattern in our
191 residual plot, however. Additionally, residuals are not more similar within site than between sites,
192 indicating that repeated measures at the same site has no effect. Likewise, a Durbin-Watson test

193 of ordered time points within sites did not indicate any autocorrelation (DW = 2.18, p-value =
194 0.58). Furthermore, our previous analyses indicated significant genetic variation among samples
195 both spatially and temporally (Thonig et al. 2017), although differentiation among all samples was
196 not always statistically significant. Therefore, we are confident that the samples are sufficiently
197 independent to be combined in a single correlation analysis.

198 We calculated θ SGDC by correlating the difference in species composition and allelic
199 composition between the samples taken at all four sites and four sampling times. For species
200 composition, this was calculated as Bray-Curtis dissimilarity, or 100 - Bray-Curtis similarity, derived
201 from the matrix of the transformed averaged fauna counts in PRIMER-E. For allelic composition we
202 calculated the fixation index G'_{ST} (Hedrick 2005) using the diversity package in R (Keenan et al.
203 2013, R Core Team, 2017). Previous studies investigating θ SGDC have also used these measures
204 (for review, see Kahilainen et al. 2014). Since the G'_{ST} values were not normally distributed, we
205 used Spearman's rank correlation coefficient when calculating the θ SGDC.

206

207 *Environmental impact on diversity*

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

218 linear model with an error term following a negative binomial distribution for our response
219 variable species richness. Since the latter resulted in a large estimate of theta (the over dispersion
220 coefficient) we chose the Poisson distribution to model the error term. We checked for collinearity
221 of our environmental variables using scatter plots and Pearson correlation coefficient, to reduce
222 the number of explanatory variables. We detected a strong correlation between the four sediment
223 characteristics median grain size, sorting, porosity and water content ($r = 0.725, -0.818, 0.775,$
224 respectively; p -values < 0.01), which is not surprising, given that they are by nature not
225 independent. Since median grain size showed the strongest correlations with the other sediment
226 variables we kept it for the GLMM and removed porosity, sorting, and water content from the
227 explanatory data set. Additionally, the standard deviation of temperature was closely correlated
228 with mean temperature ($r = 0.905$; p -value < 0.001). Since we did not detect extreme temperature
229 fluctuations, we assumed that mean temperature indicating seasonality might have larger
230 biological relevance and so, we removed temperature SD from the explanatory data set. Hence,
231 the fixed effects of our explanatory variables were median particle size, organic content, C/N,
232 mean temperature, mean salinity and standard deviation of salinity. We measured only one set of
233 environmental variables per sampling; thus, the same environmental data were used for the three
234 replicate measurements of species richness per sampling. As a random effect we included sample,
235 which represents the combination of sampling time point and site, to account for the effect of
236 season on the one hand and the repeated measures design of our study on the other hand. The
237 GLMM was performed with glmmPQL in the R package MASS (R Core Team, 2017) according to the
238 following equation:

$$\begin{aligned} 239 \quad (1) \text{Log}(\text{SpeciesRichness}) &= \alpha + \beta_1 * \text{median particle size} + \beta_2 * \text{organic content} + \beta_3 * \text{C/N} + \\ 240 \quad &\beta_4 * \text{mean temperature} + \beta_5 * \text{mean salinity} + \beta_6 * \text{salinity SD} + \text{Poisson}(\lambda_{\text{Sample}}) + \\ 241 \quad &\text{Poisson}(\lambda_{\text{Residual}}). \end{aligned}$$

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

$$\begin{aligned} 249 \quad (2) \text{Log}(\text{AllelicRichness}) = & \alpha + \beta_1 * \text{median particle size} + \beta_2 * \text{organic content} + \beta_3 * \text{C/N} + \beta_4 * \text{mean} \\ 250 \quad & \text{temperature} + \beta_5 * \text{mean salinity} + \beta_6 * \text{salinity SD} + \text{Normal}(0, \sigma^2_{\text{Site}}) + \text{Normal}(0, \sigma^2_{\text{Residual}}). \end{aligned}$$

251

252 Results

253

254 *Species diversity, genetic diversity, and SGDCs*

255 We collected in total 20,752 individuals representing 51 benthic invertebrate taxa from samples
256 taken from four locations in the Danish Isefjord-Roskilde Fjord estuary at four times of the year
257 (See Supplement 2; Figure 2). The most abundant taxon at all sites was the gastropod *Hydrobia*
258 spp. The focal species of our study, *Pygospio elegans*, was found at all sites, in 38 out of the 48
259 samples, and was the fourth most frequently found species. However, the presence of *P. elegans*
260 was patchy, and it was not sampled in any of the replicate cores from Lynæs or Lammefjord in
261 November, even though additional sampling at these sites in November yielded a sufficient
262 number of *P. elegans* specimens to use in the genetic analysis. Density of *P. elegans* was highest in
263 May and lowest in November (see Thonig et al. 2016 and Supplement 2).

264 We visualized the spatial and temporal variation in species abundance of the benthic
265 macrofauna using a NMDS plot (Figure 3A). The plot indicates good spatial differentiation (i.e.,

266 separate groupings) between all sites; Vellerup and Herslev were clearly distinct and not
267 overlapping with other sites. Lynæs and Lammefjord were more similar to each other with some
268 overlap, but differed from the other sites. The moderate stress value (0.15) indicates that the
269 NMDS plot is a sufficient representation of the relations between samples based on species
270 abundance. Polychaetes were most abundant in Vellerup, while gastropods were most abundant
271 in Lynæs and Lammefjord (Supplement 2). Crustaceans and bivalves had relatively low abundances
272 at all sites. No large temporal shifts in species abundance were observed, with the exception of
273 November, which differed from the other times at all sites.

274 Species richness varied from a low of five species observed in March at Lynæs to a high of 29
275 species observed in August at Vellerup (Figure 2, Figure 4A). Higher species richness was generally
276 observed at Vellerup, whereas the other sites had similar, lower levels of diversity. Temporal
277 patterns at each site showed lowest richness in March, which then increased during the year. In
278 Lammefjord and Vellerup, diversity reached a peak in August and then decreased in November. In
279 contrast, diversity peaked in Lynæs in May and in November in Herslev.

280 Seasonal population genetic structure in *Pygospio elegans* is described in Thonig et al.
281 (2017). Allele frequencies of seven microsatellite loci from genotyped *P. elegans* collected at the
282 four study sites and time points were visualized in a NMDS plot (Figure 3B). Allele frequencies
283 were similar in Lynæs and Lammefjord at all collection times excluding August at Lammefjord.
284 Furthermore, allele frequencies in August differed markedly from those of samples taken at other
285 times except for Lynæs. Temporal variation in allelic frequencies was greatest in Vellerup (Figure
286 3B). Allelic richness averaged over all loci ranged from 2.5 in March at Vellerup to 5.7 in August at
287 Lammefjord (Supplement 1). A seasonal pattern was observed in allelic richness, particularly for
288 Lammefjord and Vellerup, and in general, highest values were observed at all sites in August
289 (Figure 4B).

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

295

296 *Factors explaining the pattern*

297 Environmental variables measured for each site and sampling time are reported in detail in Thonig
298 et al. (2016). In general, water temperature showed a similar seasonal pattern at all sites, with
299 highest temperatures in July and lowest temperatures in February. Salinity, in contrast differed
300 between sites, being around 19-20 PSU at Lynæs, Lammefjord and Vellerup, and around 14 PSU at
301 Herslev. Likewise, sediment characteristics differed between sites but did not show any or
302 consistent seasonal patterns. Sediment was fine grained at Lynæs (mean grain size 0.18 – 0.25
303 mm) and Lammefjord (mean grain size 0.18 – 0.29 mm), medium at Herslev (mean grain size 0.25
304 – 0.35 mm) and coarse at Vellerup (mean grain size 0.44 – 0.62 mm). Water content and porosity
305 was highest in fine sediment. Sorting of sediment was moderately well in Lynæs, only moderately
306 in Lammefjord and Herslev and poorly at Vellerup (sorting classes derived from inclusive graphic
307 standard deviation according to Gray and Elliot (2009)). Organic content was highest at
308 Lammefjord, followed by Lynæs, Vellerup and Herslev. At Vellerup we found the highest C/N, i.e.
309 most refractory material, while more labile organic matter was present at Lammefjord, Herslev,
310 and most at Lynæs (Thonig et al. 2016).

311 According to the GLMM, the variation explained by the random effects sample and site, for
312 species and allelic richness, respectively, is very low. This indicates that most of the difference
313 between sites and times that can be predicted by the model is already captured with the fixed
314 effects. Median sediment grain size and mean temperature had significant effects on species

315 richness (Table 1). Considering that we used a log-linear model, an effect size of -0.5 of median
316 grain size means that species richness decreases 0.607 ($= e^{-0.5}$) fold per unit of grain size. Since
317 median grain size is determined as phi, i.e. $-\log_2$ of grain size in mm, sediment gets finer with
318 increasing phi. Hence, higher species richness was found in coarse and, considering the correlation
319 with sediment sorting, poorly sorted sediments. Furthermore, species richness increases 1.034 ($=$
320 $e^{0.034}$) fold per degree Celsius. Allelic richness of *P. elegans* was also affected significantly by
321 temperature, i.e., it increased 1.044 fold per degree (Table 1). Allelic richness was not significantly
322 related to any of the other environmental variables investigated.

323

324 Discussion

325 We investigated species-genetic diversity correlations (SGDCs) between species richness of
326 the benthic macrofauna community in the Danish Isefjord-Roskilde Fjord estuary and allelic
327 richness of a focal species, the polychaete *Pygospio elegans*. Our study was conducted over a small
328 spatial scale (maximum distance between sites ~30 km) and emphasized temporal sampling in
329 addition to spatial sampling in order to incorporate seasonal variation in population dynamics that
330 could affect both levels of diversity. A positive correlation in α diversity was found when
331 combining the data from all sites and collection times, suggesting that both the benthic
332 community as a whole and populations of *P. elegans* are affected similarly by seasonal variation at
333 the study sites. However, there was no correlation in β diversity between the studied sites and
334 sampling times, which might reflect either different underlying metapopulation structure of the
335 benthic community and *P. elegans* or limitations of the sampling design.

336 When examining the role of abiotic environmental factors in explaining the patterns of
337 diversity, we found that mean temperature and median sediment grain size helped explain the
338 patterns of species richness. Species richness was higher at warmer (and more variable)
339 temperatures and in coarser sediments (with greater porosity and water content and poorer

340 sorting). Temperature is a good predictor of seasonal change because it is related to changes in,
341 e.g. primary productivity. Furthermore, seasonal variation in species richness has been
342 documented for other benthic communities similar to what we observed here, e.g. in the Baltic
343 Sea (Blomquist & Bonsdorff 1986, Bonsdorff & Blomquist 1989) and in the North Sea (Reiss &
344 Kröncke 2004). The association between temperature (season) and species richness likely stems
345 from seasonal variation in food supply (e.g. vertical transport of matter originating from
346 phytoplankton blooms, Cloern & Jassby 2010), which can support larger communities. Sediment
347 factors, on the other hand, are not expected to vary seasonally, but represent habitat preferences
348 of the benthic taxa that can also affect community diversity. However, an indirect relationship
349 between sediment factors and seasonal variation might exist, for example in the biotic
350 communities inhabiting sediments (microbial or algal population dynamics, e.g. Quero et al. 2017)
351 that was not measured during our study. Although salinity typically has a major role in explaining
352 patterns of species diversity in the Baltic Sea on a large spatial scale (Zettler et al. 2014), salinity
353 mean and standard deviation did not explain patterns of species richness in the present study. This
354 could indicate that the differences in salinity among the four studied sites and the sampled
355 seasons are not fluctuating at a level that alters this estuarine community (which is made up of
356 euryhaline species generally tolerant to salinity fluctuations). Also, there might have been
357 insufficient power for finding an effect of salinity due to the small number of studied sites.
358 Robinson et al. (2010) also found little support for a role of salinity in driving SGDCs in estuaries in
359 the southeastern United States. But, when comparing regions along the North Sea-Baltic Sea
360 transition, where salinity differences are more extreme and long-lasting, salinity significantly
361 explained diversity patterns (Josefson & Göke 2013).

362 When analyzing genetic diversity of *P. elegans*, we found that, out of the environmental
363 variables studied, only temperature (mean and its correlated standard deviation) had a significant
364 effect explaining variation in allelic richness. Allelic richness increased in August when

365 temperatures were warmer. Seasonal genetic variation in marine invertebrates is poorly studied,
366 but has been observed in some lineages of the cryptic nematode *Pellioditis marina* as a result of
367 (meta)population turnover (Derycke et al. 2006) and in the ascidian *Styela plicata* in North
368 America resulting from seasonal patterns of recruitment (Pineda et al. 2016). Similarly, the
369 variation in allelic richness of *P. elegans* could also be explained by seasonal reproduction and
370 recruitment of new, genetically differentiated cohorts that co-exist with older cohorts at the sites
371 in August (see Thonig et al. 2016, Thonig et al. 2017). Together, these results suggest that dispersal
372 is the driving force behind the seasonal pattern. *Pygospio elegans* shows variation in larval
373 developmental mode, producing planktonic, benthic, and intermediate larvae that differ in their
374 capability for dispersal (Rasmussen 1973, Morgan et al. 1999, Thonig et al. 2016). In these study
375 sites all types of larvae have been observed, except in Herslev, where only benthic and
376 intermediate larvae were noted (Thonig et al. 2016). Most of the taxa sampled in the benthic
377 community also show life history strategies incorporating planktonic larvae and seasonal
378 population dynamics, with an increased number of larvae present in summer (June, July and
379 August) and reductions in population sizes in winter (Thorson 1946, Rasmussen 1973).

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

390 Other species lacking developmental mode polymorphism in the sampled study sites might be
391 more appropriate for investigating a β SGDC.

392 Inter-annual temporal variation in SGDCs has been described for butterflies in rainforests
393 and freshwater snails in a pond network (Cleary et al. 2006, Lamy et al. 2013), but seasonal
394 variation in SGDCs has not been a focus in previous studies. Our finding of a significant SGDC with
395 a combination of spatial and temporal sampling suggests that seasonal environmental change and
396 associated life histories are relevant for understanding diversity patterns in temperate marine
397 benthic communities. Seasonal changes in diversity of marine fauna are common, particularly at
398 latitudes where temperature and other abiotic factors vary predictably (Valiela 2015). Moreover,
399 many marine organisms have adapted to life in seasonal environments, and are known to time
400 their reproductive events to follow seasonal variation (Coma et al. 2000, Smart et al. 2012).
401 Considering the small geographic distances between our study sites and the negligible differences
402 in temperature among sites (Thonig et al. 2016), temporal sampling was needed to reveal the
403 effects of temperature (seasonality) on species and genetic diversity. Previously, Kesäniemi and
404 colleagues (2014a) could not relate genetic diversity of *P. elegans* (local F_{ST}) to any environmental
405 variables in a study in which *P. elegans* was collected from a large number of sites in the Isefjord-
406 Roskilde Fjord estuary at a single time point (April). Due to limited resources, we could only
407 sample four study sites at four different times, which prohibits us from investigating site-level
408 diversity and SGDCs at each time point separately. Nevertheless, our results indeed highlight an
409 important temporal effect and help inform a relevant sampling scale for future larger scale
410 studies. Namely, when investigating patterns of diversity, it is important that samples are
411 compared on the same time scale. Timing of sampling can have a significant effect on results of
412 SGDCs and should be clearly reported, particularly for meta-analyses and when the samples used
413 for calculating species richness and allelic richness are not collected concomitantly. Because of
414 seasonal changes in diversity, Reiss and Kröncke (2005) have also cautioned against comparing

415 diversity indices of different data sets collected in different seasons. In our study we saw clear
416 evidence of a positive SGDC related to seasonal factors that affect diversity, most likely through
417 seasonal reproduction and dispersal, and highlight the importance of life history strategies on
418 broader ecological patterns that could be relevant also at other timescales.

419

420 Acknowledgements

421 We are grateful to Rikke Guttesen and Anne Busk Faarborg who helped us collect the samples
422 needed for this study. This research was supported by the Academy of Finland (Project 258365 to
423 KEK) and The Centre for Ocean Life, which is a VKR centre of excellence supported by the Villum
424 foundation (to BWH). AT was financed by the Graduate School for Environmental Stress Studies of
425 Roskilde University. SH was financed by Kuopion Luonnon Ystäväin Yhdistys (KLYY). We declare no
426 conflict of interest.

427

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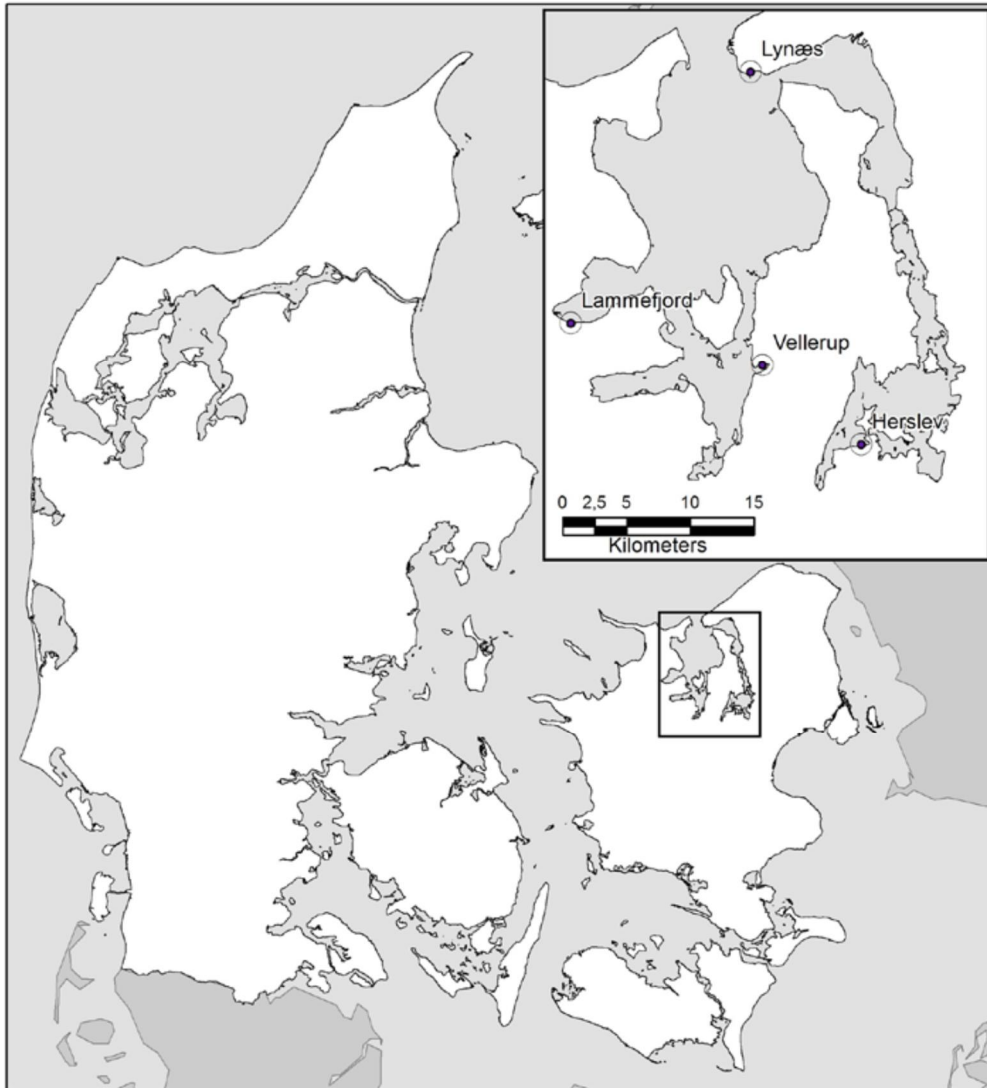
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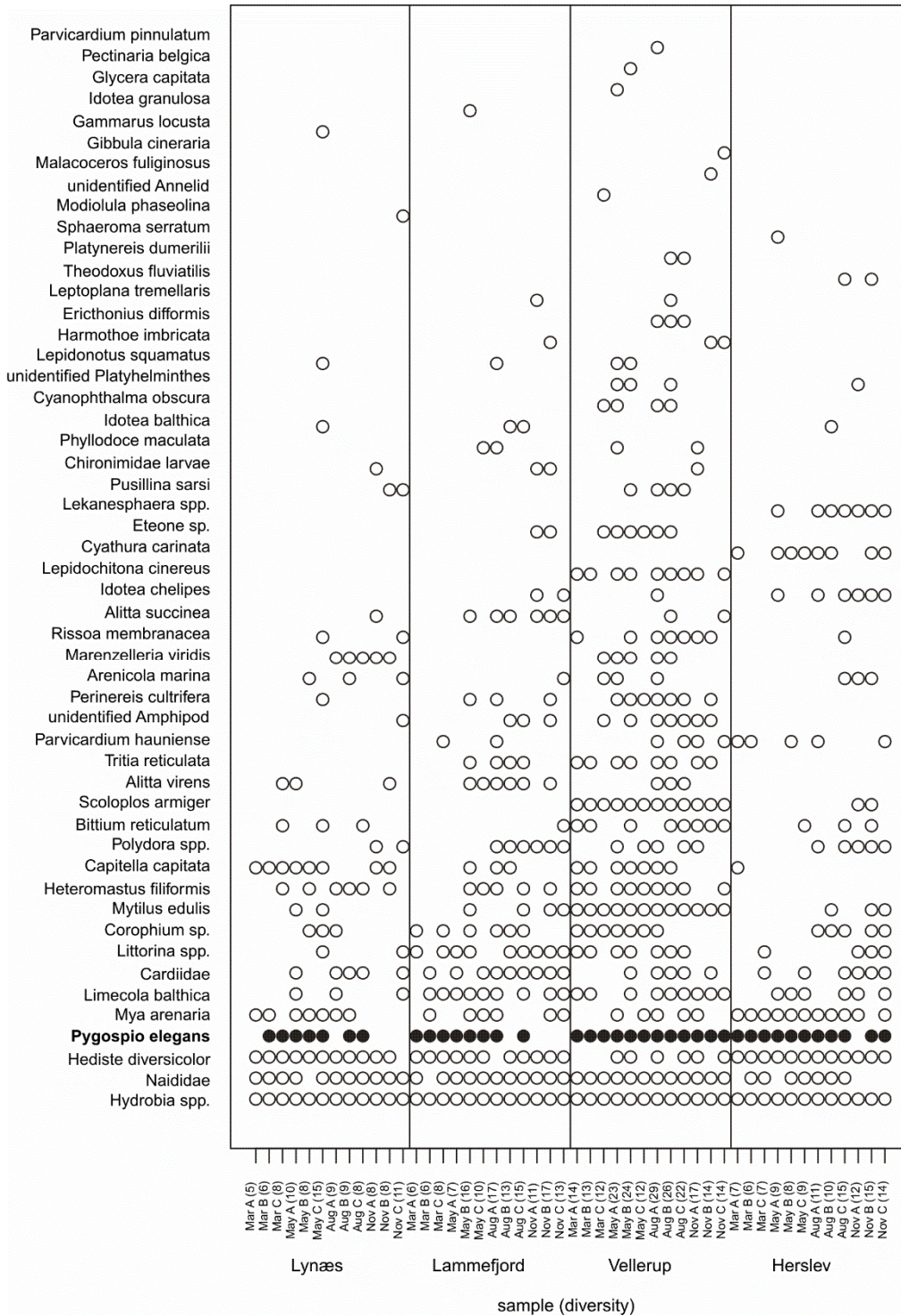
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560

561 Figure 1. Map of Denmark. The location of the Isefjord-Roskilde Fjord estuary is indicated by the
562 box and enlarged in the inset, showing the four sampling sites: Lynæs, Lammefjord, Vellerup, and
563 Herslev.



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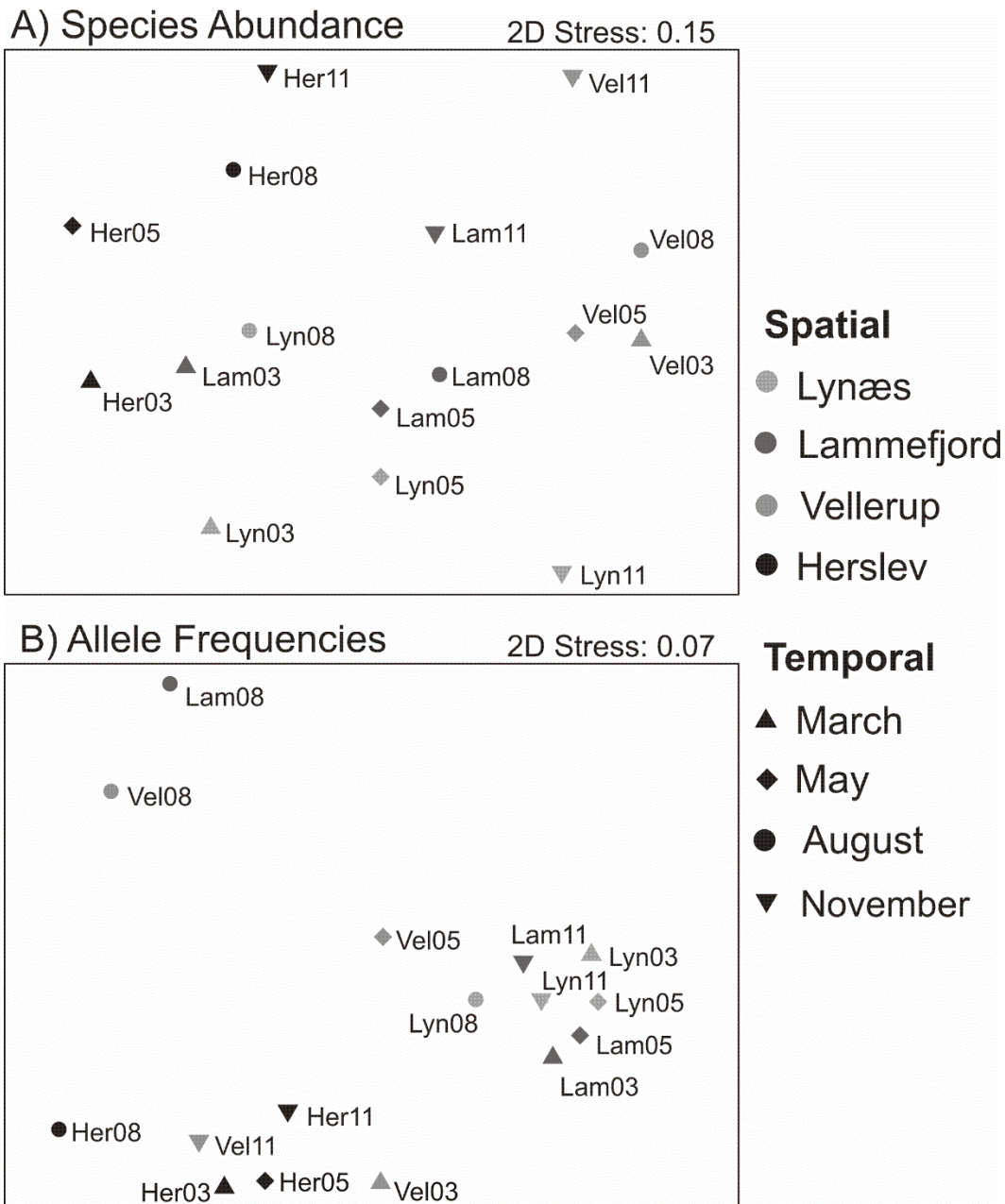
565 Figure 2. Benthic macrofauna present from four sites in the Danish Isefjord-Roskilde Fjord estuary
 566 at four time points during the year (a circle indicates that a taxon was present in a particular
 567 sample). The y axis lists the taxa observed ranked from least to most common (top to bottom)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



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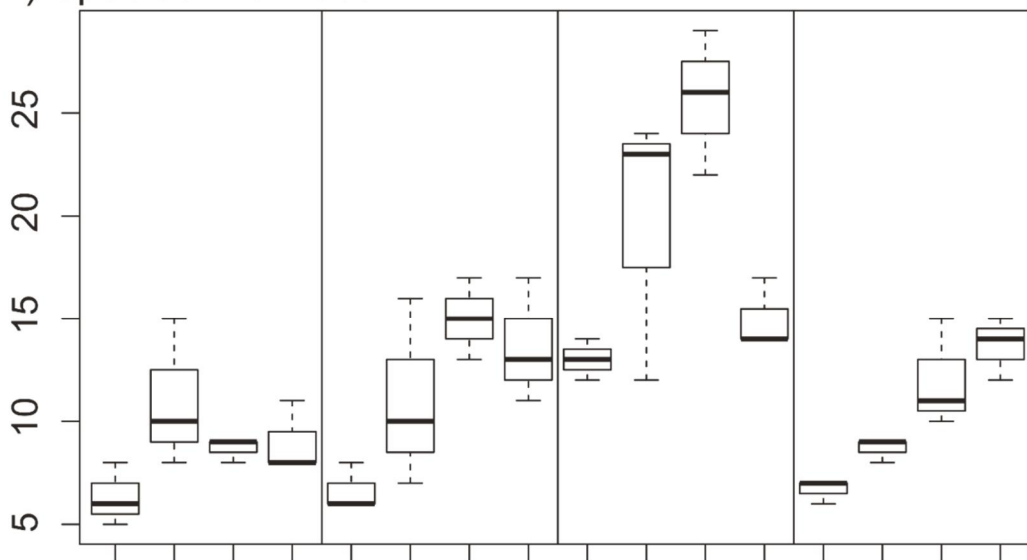
574 Figure 3. Non-metric multi-dimensional scaling (NMDS) plots of A) Species abundances of benthic
 575 macrofauna and B) Allele frequencies of *Pygospio elegans* sampled at four sites in the Danish
 576 Isefjord-Roskilde Fjord estuary at four times. Samples are coded with an abbreviated site name
 577 (Lynæs = Lyn, Lammefjord = Lam, Vellerup = Vel, and Herslev = Her) and number representing
 578 sampling time (March = 03, May = 05, August = 08, and November = 11) and with a symbol, with
 579 grey shading to indicate spatial sampling and different symbol shapes to indicate temporal
 580 sampling.



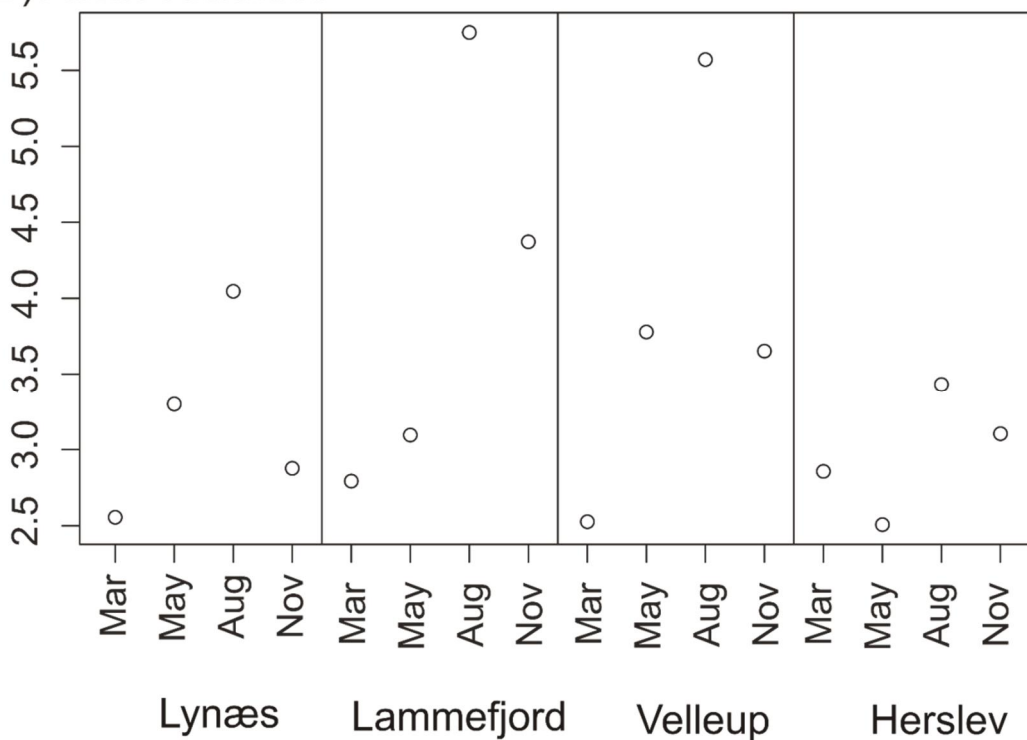
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582 Figure 4. A boxplot of species richness (A) estimated for the benthic invertebrate communities and
 583 average allelic richness (B) of *Pygospio elegans* sampled from each sampling site and sampling
 584 time in the Isefjord-Roskilde Fjord estuary.

A) Species Richness



B) Allelic Richness



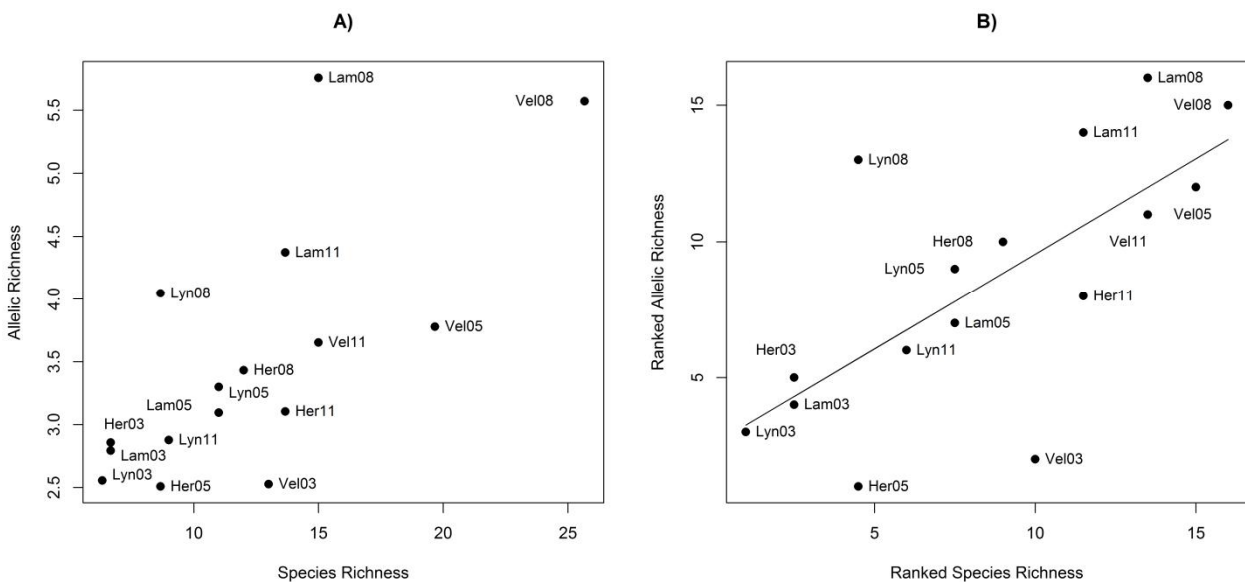
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587 Figure 5. A) Scatter plot of average species richness at four sites in the Danish Isefjord-Roskilde
 588 Fjord estuary at four time points and average allelic richness of populations of *Pygospio elegans*
 589 from the same sites and time points. B) Scatter plot of ranked average species and allelic richness
 590 and the linear regression between both variables illustrating the positive α SGDC (Spearman rank:
 591 $\rho = 0.697$, p-value = 0.003). Samples are coded with an abbreviated site name (Lynæs = Lyn,
 592 Lammefjord = Lam, Vellerup = Vel, and Herslev = Her) and number representing sampling time
 593 (March = 03, May = 05, August = 08, and November = 11).

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598 Table 1: Environmental factors explaining Species Richness and Allelic Richness in the Isefjord-Roskilde Fjord estuary according to GLMM (see
 599 Materials and Methods for details)

Species Richness						Allelic Richness					
Random effects (Poisson)						Random effects (Normal)					
	SD	Variance					SD	Variance			
		%						%			
sample	0.119	0.025				site	1.5E-06	0.000			
Residual	0.746	0.975				Residual	0.554	1.000			
Fixed effects						Fixed effects					
Groups	Value	SE	DF	t-value	p-value	Groups	Value	SE	DF	t-value	p-value
(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	-0.011	0.055	9	-0.201	0.845	C/N	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

600