

Population and reproductive dynamics of the polychaete *Pygospio elegans* in a boreal estuary complex

Thonig, Anne; Knott, K Emily; Kesäniemi, Jenni E; Winding Hansen, Benni; Banta, Gary Thomas

Published in:
Invertebrate Biology

DOI:
[10.1111/ivb.12149](https://doi.org/10.1111/ivb.12149)

Publication date:
2016

Document Version
Peer reviewed version

Citation for published version (APA):

Thonig, A., Knott, K. E., Kesäniemi, J. E., Winding Hansen, B., & Banta, G. T. (2016). Population and reproductive dynamics of the polychaete *Pygospio elegans* in a boreal estuary complex. *Invertebrate Biology*, 135(4), 370-384. <https://doi.org/10.1111/ivb.12149>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact rucforsk@kb.dk providing details, and we will remove access to the work immediately and investigate your claim.

1 Population and reproductive dynamics of the polychaete *Pygospio elegans* in a boreal
 2 estuary complex.

3 Thonig, A.^{1,2}, Knott, K. E.², Kesäniemi, J. E.^{3,2}, Winding Hansen, B.¹, Banta, G. T.¹

4 ¹ Roskilde University, Department of Science and Environment, Denmark

5 ² University of Jyväskylä, Department of Biological & Environmental Science, Finland

6 ³ University of Oulu, Department of Biology, Finland

7

8 Anne Thonig: Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box
 9 35, FI-40014 University of Jyväskylä, Finland

10 Department of Science and Environment, Roskilde University, P.O. Box 260, DK-4000
 11 Roskilde, Denmark

12 Karelyn Emily Knott: Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box
 13 35, FI-40014 University of Jyväskylä, Finland

14 Jenni Emilia Kesäniemi: Department of Ecology, University of Oulu, P.O. Box 8000, FI-90014 Oulu, Finland

15 Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box
 16 35, FI-40014 University of Jyväskylä, Finland

17 Benni Winding Hansen: Department of Science and Environment, Roskilde University, P.O. Box 260, DK-4000
 18 Roskilde, Denmark

19 Gary Thomas Banta: Department of Science and Environment, Roskilde University, P.O. Box 260, DK-4000
 20 Roskilde, Denmark

21

22

23

24 Running title: Population dynamics of *Pygospio elegans*.

25 Keywords: life history, poecilogony, development, spatio-temporal variation, environmental impact

26

27

28 ABSTRACT

29 *Pygospio elegans* is an opportunistic, wide-spread spionid polychaete that reproduces asexually via
30 fragmentation and can produce benthic and pelagic larvae, hence combining different developmental modes
31 in one species. We documented the density, size distribution and reproductive activity of *P. elegans* at four
32 sites in the Danish Isefjord Roskilde Fjord estuary complex, where all modes of reproduction were reported.
33 We compared population dynamics of this species to environmental parameters such as salinity, temperature
34 and sediment characteristics (grain size, sorting, porosity, water content, organic content, C/N). We observed
35 that new cohorts – resulting either from sexual or asexual reproduction - appeared in spring and fall and old
36 ones disappeared in late summer and winter. Sexual reproduction occurred from September until May, and
37 although their timing was variable, there were two reproductive peaks at three sites. At those sites, we also
38 observed a switch in larval developmental mode. Asexual reproduction peaked in April. While the seasonal
39 dynamics can be related to temperature to a large extent, the differences in population dynamics among sites
40 also correlated with sediment structure and salinity. Populations from sites with coarse and heterogeneous
41 sediment had high levels of sexual reproduction. At the site with lower salinity, intermediate and benthic
42 larvae were present during winter in contrast to pelagic larvae found at the other sites. However, we could
43 not identify one clear environmental factor determining the mode of development. At present it remains
44 unclear to what degree the genetic background contributes to the mode of development. Hence, whether the
45 differences in developmental mode are the result of genetically different cohorts will be further investigated.

46

47 A variety of types of larvae have evolved independently among marine taxa (Strathmann 1993). Larvae are
48 an integral part of the different life histories of invertebrates, which affect population dynamics and how
49 populations respond to environmental conditions (Marshall et al. 2012). Pelagic larvae that have high
50 dispersal potential might dampen population fluctuations (Eckert 2003). Thus, they would be advantageous
51 for species living in seasonal environments (Thorson 1950, Marshall and Burgess 2015) but also for
52 opportunistic species that rapidly colonize disturbed areas (McEdward 2000). However, the dispersal
53 potential of pelagic larvae does not always translate into higher connectivity among populations (Weersing
54 and Toonen 2009). Also the quality of the new colonizers determines their establishment and reproductive
55 success (Marshall 2010b, Burgess and Marshall 2011). Benthic larvae, with their predominantly local
56 recruitment, could be favored in temporally constant but spatially variable environments and when predation
57 in the plankton is high (Pechenik 1999).

58 The effect of developmental mode on population structure and dynamics can be investigated best in species
59 that express different developmental modes, as even between sibling species with different modes of
60 development speciation effects cannot be excluded (Knott and McHugh 2012). Variation in developmental
61 mode, also called poecilogony, was described for several spionid polychaetes (Blake and Kudenov 1981;
62 Duchêne 1984; Levin et al. 1991; MacKay and Gibson 1999) and sacoglossan sea slugs (Krug 2007; Krug
63 2009; Vendetti et al. 2012). The spionid *Pygospio elegans* (CLAPARÈDE 1863) is one of them (Morgan et al.
64 1999; Kesäniemi et al. 2012c). It is a common, small (10-15mm), tube-dwelling estuarine species with a
65 circum-boreal distribution that lives primarily on intertidal mud flats. It can form high density patches, or
66 tube-beds, with densities up to 600,000 individuals/m² (Morgan 1997). *P. elegans* has broad habitat
67 tolerances and is able to thrive in a wide range of temperatures and salinities (Hempel 1957; Armitage 1979;
68 Anger 1984; Morgan 1997). The average life span of *P. elegans* is about 9 months (Anger et al. 1986). The
69 time from hatching (as pelagic larvae) to first reproduction takes about 15-17 weeks (Anger et al. 1986).
70 Reproduction was reported to be seasonal with sexual reproduction that may consist of two broods occurring
71 in winter and asexual reproduction peaking afterwards in spring (Rasmussen 1973, Gudmundson 1985). The
72 versatile reproductive biology of *P. elegans* consisting of asexual and sexual reproduction and polymorphism

73 in larval developmental mode with both benthic and pelagic larvae (Rasmussen 1973) allows for different
74 life histories in this species. Within gravid females, two different kinds of eggs can be distinguished: nurse
75 eggs containing yolk and fertile eggs (true or genuine eggs *sensu* Rasmussen 1973) with a distinct nucleus.
76 These latter, fertile eggs develop into embryos that consume the nurse eggs while in egg capsules. The ratio
77 of nurse eggs to fertile eggs indicates the mode of development (Rasmussen 1973). Pelagic larvae are
78 expected from capsules containing a large number of fertile eggs (>10) and few nurse eggs. These larvae
79 emerge from the capsules at the 3 setiger stage, possess swimming setae, and feed and develop in the
80 plankton for about 4-5 weeks until they are 12-16 setigers in size, when they settle as juveniles (Hannerz
81 1956; Rasmussen 1973; Anger et al. 1986). In contrast, benthic larvae are expected from capsules with few
82 (1-3) fertile eggs and a large number of nurse eggs. They hatch when they are about 14-20 setigers in size
83 and immediately settle (Hannerz 1956; Hempel 1957; Anger et al. 1986). Intermediate types of larvae that
84 hatch at about 10 setigers and spend a short time in the plankton can also be found (Hannerz 1956;
85 Kesäniemi 2012). Mature individuals of *P. elegans* are usually larger than 35 setigers, in most cases around
86 45 (Gudmundsson 1985). Asexual reproduction occurs via fragmentation of the worm into 3-4 pieces that
87 subsequently remain in the tube and regenerate heads, tails or both (Rasmussen 1953).

88 It is not unusual for life history traits to differ among populations of the same species, particularly for
89 poecilogonous species (Levin 1984; Blanck and Lamouroux 2006; Lam and Calow 1989; Marshall and
90 Keough 2008). This is the case for *Pygospio elegans*, e.g. some populations rely solely/predominantly on
91 asexual reproduction (Kiel Bight (Germany), Anger (1977)), while others show no signs of it (Drum Sands
92 (North Sea), Bolam (2004)). Furthermore, the mode of development can differ, even among spatially close
93 populations (Isefjord Roskilde Fjord complex (Kattegat), Kesäniemi et al. (2014); English Channel, Morgan
94 et al. (1999)). For some populations the mode of development is expected to be fixed to either pelagic (e.g.
95 Drum Sands (Bolam 2004) and Somme Bay, English Channel (Morgan et al. 1999)) or benthic larvae (e.g.
96 Cullercoats (Gudmundsson 1985) and Ängsö, Finland (Kesäniemi et al. 2012b)), but also seasonal switches
97 from pelagic larvae in winter and benthic larvae in spring (Blyth estuary (Gudmundsson 1985) and Horsens
98 Fjord (Rasmussen 1973)) and simultaneous occurrence of multiple types of larvae have been observed

99 (Isefjord (Rasmussen 1973) and Schiermonnikoog, Netherlands (Kesäniemi et al. 2012b)). The basis for
100 variation in developmental mode could be a genetically based polymorphism (Levin et al. 1991), epigenetic
101 regulation of gene expression (Kesäniemi et al, 2016) or a plastic response to environmental cues (Krug
102 2009). Low genetic divergence among populations however, indicates that poecilogony in *P. elegans* is
103 probably not solely a genetically based polymorphism, but also influenced by the environment. So far,
104 variation in developmental mode of *P. elegans* has been observed in estuarine environments (Rasmussen
105 1973; Gudmundsson 1985). Hence, poecilogony in *P. elegans* might represent a bet-hedging strategy that is
106 favored in unpredictable, highly dynamic habitats (Chia et al. 1996; Collin 2012), while at more constant
107 sites mode of reproduction might be fixed.

108 Because developmental mode can have an impact on population persistence and connectivity (Jeffrey and
109 Emlet 2003), we wanted to document how populations and their developmental modes change over time and
110 relate how those changes are affected by environmental parameters. For that reason we surveyed population
111 and reproductive dynamics of the poecilogonous polychaete *Pygospio elegans* at four sites in the Isefjord
112 Roskilde Fjord estuary in Denmark, where it reproduces via multiple types of larvae, both seasonally and
113 simultaneously (Rasmussen 1973; Kesäniemi et al 2014).

114 METHODS

115 We monitored *Pygospio elegans* and several environmental parameters in the Danish Isefjord-Roskilde Fjord
116 complex from March 2014 until February 2015. Four sites, Lynæs, Lammefjord and Vellerup in Isefjord, and
117 Herslev in Roskilde Fjord (see Fig. 1), were sampled monthly at shallow areas along the shore (each
118 approx. 10 m² with 0.5-1 m water depth) (see Supplement Table 1 for coordinates and exact sampling dates).
119 These sites were chosen to cover genetically different populations of *P. elegans* and different habitats, as
120 described by Kesäniemi and others (2013). The Isefjord-Roskilde Fjord complex is the second largest estuary
121 in Denmark, located on the North of Zealand with an opening to the Kattegat. Isefjord has a surface area of
122 280 km² with mean depth of 7 m and salinities ranging from 18 to 30. Roskilde Fjord is connected to the
123 Kattegat via the Isefjord, has a surface area of 117 km² and lower salinities, ranging between 5 and 18. It is
124 divided into a long and narrow outer region and a shallow interior, which is not deeper than 6 m. The two
125 estuaries are similar in temperature, but not salinity patterns (Rasmussen 1973).

126

127 ***Population dynamics of Pygospio elegans***

128 *Pygospio elegans* were sampled monthly - excluding December - to determine size, gender, reproductive
129 activity and mode of development. Surface sediment was randomly sampled (using a shovel) and sieved on
130 site with a 1 mm mesh. Sand tubes of *P. elegans* were collected and transferred to the lab. In the lab, sand
131 tubes were spread evenly on a white photo tray marked with equal quadrants and worms were sampled as
132 they were leaving their sand tubes. By sampling all individuals from a certain quadrant we avoided biased
133 sampling, e.g. only the largest worms, and hence obtained a quantitative and representative subsample to
134 determine size and population structure.

135 At least 30 individuals were used to measure length in order to analyze the cohort structure of each
136 population. It is important to note that in this study the term cohort refers only to size classes and not
137 generations because asexual reproduction disrupts the relation between size and age. Hence, individuals of
138 the same size or assigned to the same cohort could be of different age. However, individuals clearly resulting

139 from asexual reproduction (those with small regenerated heads or tails, Supplement Fig. 1 F) constituted on
140 average 3% or less of the samples in all populations except Lynæs (ca. 9%). The worms were first narcotized
141 in seawater containing 10% sparkling water and then photographed with a Nikon camera mounted on a
142 dissecting microscope. Measurements were made using NIS BR software v. 4.2 (Nikon, RAMCON A/S
143 Birkerød, DK). The coefficient of variation for our size measurement was maximally 8% (obtained from
144 measuring ten individuals each ten times). Since many worms were damaged or regenerating, we decided to
145 measure the length from the eyespot to the start of the gills (see Supplement Fig. 1 A). Length frequency
146 plots were created using SPSS Statistics 22 (IBM, Armonk, New York) with automated binning to identify
147 the best grouping of the data. Cohort analysis was performed in FiSat II (FAO-ICLARM Stock Assessment
148 Tool) using Bhattacharya's method to identify the cohorts and NORMSEP to optimize the fit of a normal
149 distribution. The mean of the normal distribution is used as the mean size of the respective cohort. We aimed
150 for the identification of a maximal number of cohorts with minimum overlap ($S.I. > 2$) (Bhattacharya 1967).
151 Since we could not fit a von Bertalanffy growth curve through our data using the method implemented in
152 FiSat II, we followed a procedure similar to that of Bolam (1999, 2004). The progression of each cohort was
153 determined "by eye" and we obtained a growth rate via a regression analysis of the weighted mean size of
154 the cohorts using Systat 13 (Systat Software, Inc., San Jose, CA).

155 A subsample of at least 50 live specimens - including the 30 sized ones - was characterized according to
156 Table 1 and Supplement Fig. 1. The assessment of asexual reproduction was noted beginning in April. In
157 addition to the live specimens, all sand tubes were checked for the presence of egg strings and, if found, the
158 mode of development was determined (see Table 1 and Supplement Fig. 1). Due to seasonal variation in the
159 number of worms collected, the absolute number of egg strings was normalized to the total sample size (egg
160 strings per number of worms collected).

161 For determining density of *P. elegans*, benthic macrofauna were sampled in March, May, August and
162 November using a hand-held corer (15 cm diameter, 30 cm length). Three samples were taken randomly at
163 each sampling site, and each was sieved through a 1 mm mesh and fixed with 5% formaldehyde on site. In
164 the lab, formaldehyde was removed in several washing steps and samples were stored in 95% ethanol. To

165 better visualize the macrofauna, the samples were stained overnight by adding 5 ml of saturated Rose
166 Bengal. Afterwards, the Rose Bengal/ethanol solution was discarded and *P. elegans* retained on a 1 mm
167 sieve were identified and counted.

168

169 *Environmental dynamics*

170 At each site, a data logger (HOBO U24-002-C salinity logger, 100-55,000 μ S/cm, Onset Computer
171 Corporation, Bourne, MA) was deployed, which documented conductivity and temperature every ten
172 minutes during the survey period. Salinity was calculated according to the PSS-78 using the conductivity and
173 temperature measurements of the logger (UNESCO & SCOR 1981). The salinity of reference samples taken
174 monthly were measured with a salinometer (MS-310e Micro-salinometer, RBR-global, Kanata, Ontario,
175 Canada) and used to correct the logger for drift. Due to biofouling and frost, salinity data is not available for
176 Lammefjord from June until August, for Vellerup in August, and for Lynæs in January. Temperature and
177 salinity data were excluded when salinity dropped below 2 as these indicated exposure of the logger due to
178 low water levels.

179 Sediment characteristics were determined in March, May, August and November. For sediment
180 characteristics, three kajak cores (5 cm diameter, at least 15 cm length) were taken randomly at each
181 sampling site. These were sectioned into four layers (0-1 cm, 1-2 cm, 2-6 cm, 6-15 cm) and the respective
182 layers of each core were pooled and mixed. Wet weight and dry weight (24h at 105 °C) of 5 cm³ sediment
183 from each layer was determined for calculating porosity and water content.

184 Particle size was determined from 50 – 150 g of remaining wet sediment using a set of sieves corresponding
185 to the Wentworth size scale (8 mm, 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, 0.063 mm). The
186 weight percent of each size fraction was determined after 24h at 105 °C. Median grain size ($\Phi_{50\%}$) and
187 sorting were calculated via the inclusive graphic standard deviation coefficient IGSD, $(\Phi_{84\%} - \Phi_{16\%})/4 + (\Phi_{95\%} -$
188 $\Phi_{5\%})/6.6$ (McLusky and Elliott 2003). About 500 mg of the dried sediment from the samples were reserved

189 for C/N analysis, and the rest was used to determine organic content [%] via loss on ignition (LOI, 2h at 550°
190 C).

191 Carbon and nitrogen content of 30-50 mg ground sediment from the top layer (0-1 cm) were analyzed in
192 three analytical replicates using an element analyzer (Flash 2000 NCS- Analyzer, and FlashEA® 1112
193 CHNO Analyzer, Thermo Scientific). Due to a high quantity of shells in some samples, the difference in LOI
194 between dried and pre-combusted (to 500 °C) samples was used to calculate the carbonate free organic C
195 content.

196

197 *Relation of population & environmental dynamics*

198 Temporal and spatial differences in the population dynamics of *P. elegans* were determined using distance
199 based permutational multivariate analysis of variance (PERMANOVA) in PRIMER-E v.6 (Clarke and
200 Gorley 2006). The monthly data collected for *P. elegans* (size, proportion of males, females and non-
201 reproductive individuals, number and developmental mode of larvae, occurrence of asexual reproduction) at
202 each location was normalized and a resemblance matrix based on Euclidian distance was calculated
203 comparing all samples. A two-way (time, location) PERMANOVA design without interaction (due to lack of
204 replication) was performed using 9,999 permutations and default settings. Subsequently, pair-wise
205 comparisons among locations or among times were performed. The assumption of identical, independent
206 residuals was fulfilled. Residuals were distributed homogenously according to PERMDISP using distances to
207 median (location p-value 0.170, time p-value 0.098) and variances between different time points across sites
208 were equal according to Levene's test (p-value 0.989).

209 Furthermore, a distance-based linear model routine (DistLM) was used to analyze and model the relationship
210 between the population parameters of *P. elegans* (as was done for PERMANOVA, but also including worm
211 density) and the environmental data (mean temperature and SD, mean salinity and SD, sediment
212 characteristics as median grain size, sorting, porosity and water content, and organic content and C/N). For
213 that purpose we summarized the data into quartiles to account for the different sampling schemes: March

214 (consists of the data from January and February 2015 and March 2014), May (April – June 2014), August
215 (July - September 2014) and November (October - December 2014). For the DistLM procedure we used two
216 Euclidian resemblance matrices of the normalized data (*P. elegans* data and environmental data), 9,999
217 permutations and best selection procedure. The model (a subset of the environmental parameters) that best
218 explained the variation among the *P. elegans* data was determined according to the selection criteria BIC and
219 AICc. Subsequently, this best-fit model was entered in a distance-based redundancy analysis (dbRDA) to
220 visualize the variation in the *P. elegans* data that is explained by the selected model.

221

222 RESULTS

223 *Population dynamics of Pygospio elegans*

224 In general, worms were smallest at Lynæs (monthly means ranged from 1139 - 1731 μm) and Lammefjord
225 (1074 - 1648 μm), followed by Herslev (1343 - 1818 μm), with the largest worms at Vellerup (1496 - 1848
226 μm) (Fig. 4). The differences among populations were most noticeable during fall, when worms at Vellerup
227 remained a constant size while the average worm size at the other sites decreased. Worms were similar in
228 size across all populations at other times of the year.

229 Using our length measurements, we determined the number of cohorts present each month during the survey.
230 We distinguished one to four overlapping cohorts present at any one time (see Supplement Fig. 2 a-d). The
231 pattern at each site is summarized and simplified in Fig. 5, which shows the mean worm size of each
232 identified cohort and the fraction of the total population in that cohort. At Lynæs, two to three cohorts were
233 present at any one time and we observed four to five cohorts over the entire period that had growth rates
234 ranging from 3.31 – 6.41 $\mu\text{m}/\text{d}$. Small worms appeared in April, June, September and November. At
235 Lammefjord, mostly two cohorts were present at the same time and we could determine four to five
236 distinguishable cohorts during the whole period with growth rates ranging from 3.61 – 4.52 $\mu\text{m}/\text{d}$. Small
237 worms appeared in March, June, September and January. Likewise, mostly two cohorts were present at
238 Herslev at any one time, although three (to four) cohorts could be observed during summer, with growth
239 rates ranging from 1.52 – 4.20 $\mu\text{m}/\text{d}$. Small worms appeared in April and July. For the most part, only one
240 cohort was present at Vellerup during the whole period with a low overall growth rate of 0.88 $\mu\text{m}/\text{d}$, and
241 thus, almost stable worm size. Small worms appeared at Vellerup in April and November.

242 Sexual reproduction by *P. elegans* at our study sites was most prevalent during winter and spring (Fig 6A).
243 The percentage of gravid females and males carrying sperm was lowest at all sites during the summer (from
244 May to August). Two peaks of gravid females and males with sperm were observed in October and February
245 at Lynæs, Lammefjord, and Vellerup, whereas only one broad peak (November to March) was observed at
246 Herslev. The percentage of males carrying sperm was similar to or slightly higher than the percentage of

247 gravid females, and males either preceded gravid females or occurred simultaneously. The percentage of
248 gravid females was much lower at Lynæs (max. 10%) than in Lammefjord (max. 22%), Vellerup (max.
249 26%), and Herslev (max. 32%).

250 We observed egg strings in the tubes of *P. elegans* in winter and spring (Fig. 6B), which coincides for the
251 most part with the presence of gravid females. Gravid females were observed in October at Lynæs,
252 Lammefjord and Vellerup, but egg strings were not observed at these sites until November. Two peaks in the
253 number of egg strings, in accordance with the two peaks in gravid females, were noted only in Vellerup. At
254 Herslev, one major peak in number of egg strings resembles the single broad peak of gravid females.
255 Likewise, the lower normalized number of egg strings observed at Lynæs (max. 0.09) and Lammefjord
256 (0.12) compared to Vellerup (0.28) and Herslev (0.44) is in accordance with the observed lower number of
257 gravid females.

258 We observed a difference in the larval developmental mode between spring and winter as well as between
259 sites in winter (see Fig. 6B). In spring, multiple types of larvae (pelagic, benthic and intermediate) were
260 found at all sites, whereas in winter, pelagic larvae were predominant at Lynæs, Lammefjord and Vellerup
261 and benthic and intermediate larvae were predominant at Herslev. At Vellerup, the co-occurrence of the
262 second peak in gravid females and number of egg strings in February also coincides with a switch from only
263 pelagic larvae to a mixture of benthic, intermediate and pelagic larvae. At all sites, mainly in January and
264 February, we found females brooding egg capsules while also developing the next batch of eggs in their
265 coelom. At Herslev, the developmental mode of the brood in the egg capsules was benthic and the
266 developing eggs in the brooding mother were also likely to have a benthic developmental mode, since only a
267 few of the developing eggs were fertile eggs, containing a nucleus. At the other sites developmental mode of
268 the brood was pelagic, but the stage of the developing eggs in the mothers was too early to allow
269 determination of their developmental mode. Asexual reproduction occurs throughout the year, but peaks in
270 April when the frequency of sexual reproduction is in decline (Fig. 6A). The highest prevalence of asexual
271 reproduction was observed in Lynæs (up to 26%).

272 The mean density of *P. elegans* was lowest at Lynæs (means between sampling times ranged from 0 - 377
273 ind/m²), distinctly higher at Lammefjord (75 - 4357 ind/m²) and Herslev (189 - 4791 ind/m²) and highest at
274 Vellerup (132 - 7847 ind/m²) (see Fig. 3). While at three sites the population density was highest in May,
275 with a maximum of 7847 ± 6051 individuals per m² in Vellerup, it was generally low and constant at Lynæs.
276 Furthermore, the distribution of *P. elegans* was patchy, most noticeably during April and May at Herslev and
277 in October at Lynæs when the worms were associated with the presence of diatom mats (pers. obs.).

278

279 *Environmental dynamics*

280 The temperature and salinity data are illustrated in Fig. 2 and summarized in Table 2. Temperature patterns
281 at the sites were similar. Lowest weekly temperatures were observed from December through February, with
282 the minimum (-2.97 °C) in December at Lynæs. Highest weekly temperatures were observed in July and
283 August with the maximum (28.61 °C) in July at Lammefjord. There was more variation in temperature
284 during spring than in fall. In contrast to temperature, salinity patterns differed notably between the sites. In
285 Lammefjord there was more variation in salinity (SD = 4.0) in comparison to the other sites, and in Herslev
286 mean salinity was low (13.5).

287 Characteristics of the surface sediments (0-1 cm), which represents the habitat of *P. elegans*, are illustrated in
288 Supplement Figs. 3 and 4 and summarized in Table. 2. Median particle size was negatively correlated with
289 water content (Pearson correlation coefficient, $r = 0.775$, p-value 0.003, $n = 16$, $df = 6$), porosity ($r = 0.725$,
290 p-value 0.009) and sorting ($r = -0.818$, p-value 0.001). Hence, sediments at Lynæs and Lammefjord were
291 fine grained, had highest water content and porosity and were moderately to moderately well sorted.
292 Vellerup had poorly sorted coarse sediment with lowest water content and porosity, while sediment at
293 Herslev was medium in particle size, water content, porosity, and sorting. There were no major seasonal
294 changes in sediment characteristics. Sediment characteristics - except particle size - showed similar patterns
295 with depth at the different sites (data not shown).

296 Organic content of the sediments was generally higher in Lynæs and Lammefjord than in Vellerup and
297 Herslev (Supplement Fig. 4A). There was no difference between the sites when comparing organic content
298 depth profiles (data not shown). Seasonally, the percentage of organic content was variable in Lammefjord
299 and Vellerup, whereas it was stable in Lynæs and Herslev. The amount of organic matter in Lammefjord and
300 Herslev increased slightly during the year, while it decreased in Lynæs and Vellerup. Moreover, the C/N
301 ratio was lower in Lynæs, indicating more labile organic matter, compared to Lammefjord and Herslev. The
302 most refractory material was present in Vellerup, except for May (Supplement Fig. 4B). The C/N was nearly
303 constant at Lammefjord, decreased during the year at Lynæs and Herslev, and was quite variable at Vellerup.

304

305 ***Relation of population & environmental dynamics***

306 We found significant temporal (p-value 0.0006) and spatial (p-value 0.0001) patterns in the population
307 dynamics of *P. elegans*. Pair-wise comparisons revealed significant changes in the population dynamics (for
308 all locations) mostly between late spring until summer (May until August) and fall until beginning of spring
309 (October until April) (Supplement Table 2). Significant site differences (averaging over sampling times)
310 were found between Lynæs and all other sites (to Lammefjord p-value 0.033, to Vellerup p-value 0.001, to
311 Herslev p-value 0.011), and between Lammefjord and Vellerup (p-value 0.003) (Supplement Table 2). The
312 environmental parameters best correlating with the variation in the population dynamics, i.e. predicting 59%
313 of the total population variation, were mean temperature, sorting and mean salinity. Ordination of the *P.*
314 *elegans* samples fitted to the model is displayed in Fig. 7 where it is clear that it was warmer during May and
315 August, that Lynæs and Lammefjord had generally finer sediments and that Herslev had lower salinities.

316 DISCUSSION

317 We performed a field survey of four populations of *Pygospio elegans* in the Danish Isefjord Roskilde Fjord
318 estuary complex to gain further insight into the population dynamics of this poecilogenous polychaete. Our
319 specific focus was on its reproductive modes and whether its life history variation is related to environmental
320 conditions in the studied populations.

321 **Seasonal dynamics**

322 We observed a clear seasonality in the population and reproductive dynamics of *P. elegans*. New cohorts
323 appeared in spring and fall. Similar seasonal cohort structures have been observed in surveys of *P. elegans* at
324 other sites. For example, Gudmundsson (1985), Rasmussen (1973) and Bolam (2004) all observed a
325 continuous arrival of juveniles of *P. elegans* with 1-2 peaks in spring and/or fall. Larvae of *P. elegans* settle
326 when 14-20 setigers in size (Hannerz 1956; Hempel 1957; Anger et al. 1986) and reach sexual maturity
327 within a few months (Smidt 1951; Gudmundsson 1985; Anger et al. 1986; Bolam 2004). Accordingly, the
328 spring and fall cohorts at our sites corresponded to a mean size of 30 setigers and reached maturity after 5-6
329 months (spring cohort in September/October, fall cohort in February/March) with an estimated growth rate
330 of about 1.5 setigers per month. Bolam (2004) observed slightly higher growth rates of 4 setigers per month
331 for specimens of similar size.

332 Sexual reproduction occurred from winter until spring, indicated by the presence of gravid females and
333 males carrying sperm and egg strings. Similar patterns of seasonal sexual reproduction by *P. elegans* were
334 observed at other sites (e.g. Rasmussen 1973; Gudmundsson 1985; Bolam 2004), although there are
335 exceptions. For example, Morgan (1997) found gravid females peaking during spring/winter in 1990/91 and
336 during summer in 1992 as well as egg strings almost year round, but mostly during summer at Somme Bay.
337 We observed two peaks of gravid females and males with sperm at most sites. The two reproductive peaks
338 most likely reflect the maturity of different cohorts at different times. However, we also observed that some
339 individuals within a single cohort were able to produce two consecutive broods, making the peaks of
340 reproduction broad and the cohorts less distinct. Mainly during January and February we observed females

341 bearing eggs and brooding egg strings simultaneously. A similar finding was made by Gudmundson (1985)
342 for the population at Cullercoats.

343 Given that planktonic larvae of *P. elegans* are expected to spend 4-5 weeks in the plankton before settlement,
344 we expected to see new cohorts appearing with an approximate one month delay after the disappearance of
345 egg capsules. Although the planktonic larval development mode was prevalent at many of our study sites, we
346 only observed the expected one month delay between appearance of new cohorts and disappearance of egg
347 capsules at Vellerup. In contrast, when there is benthic development, juveniles are expected to settle
348 immediately after emerging from the capsules. Therefore, at Herslev, where we observed predominantly the
349 benthic developmental mode, we expected to see new cohorts coinciding with the disappearance of egg
350 capsules. Yet, this was not the case. The general lack of synchronization of reproduction and the combination
351 of different developmental modes in populations of *P. elegans* are possible reasons for the appearance of
352 new cohorts at different times. In addition, as mentioned in the introduction, the occurrence of asexual
353 reproduction disrupts clear definition of cohorts in this species. When sexual reproduction declined in April,
354 we observed an increase in asexual reproduction similar to what was observed by Rasmussen (1953),
355 Gudmundsson (1985), and Wilson (1985). Rasmussen (1953) proposed that asexual reproduction after
356 periods of low temperatures might help *P. elegans* populations recover from declines due to severe winter
357 conditions.

358 At the end of summer and during winter some cohorts disappeared. Accordingly, we observed many pale,
359 inactive and even degenerating individuals in July at Lynæs and Lammefjord and in January at Lammefjord.
360 Considering the short life span of *P. elegans* (Anger et al. 1986) , the appearance of new cohorts combined
361 with the disappearance of old ones slightly afterwards might have led to the drop in mean size we observed
362 after summer and spring, indicating that the population was partly substituted by smaller individuals. If so,
363 the highest densities might be present after new cohorts arrived but before old ones disappeared, i.e. end of
364 spring and beginning of winter. Indeed, we observed highest densities in May with about 4,000 - 8,000
365 individuals per m², but we did not measure density in December/January. In a previous study at Blyth
366 estuary, the highest densities were reached after the reproductive phase in May/June (Gudmundsson 1985)

367 and, at Drum Sands, highest densities (about 13,000 ind/m²) were reached in December and February (Bolam
368 2004). In contrast, the populations at Somme Bay had almost stable density levels of about 2,500 and 15,000
369 ind/m² (Morgan 1997). In general, the densities we observed were in the range of 200 - 8,000 ind/ m², similar
370 to what has been described for several locations in Denmark (Muus 1967) and in the English Channel (4,000
371 ind/m², Morgan et al. (1999)). Although our measurements exceed the densities of *P. elegans* observed by
372 Gudmundsson (1985) and Blomqvist and Bonsdorff (1986), they are far below the maximum densities of up
373 to 50,000 - 500,000 ind/ m² described at other sites (Linke 1939; Hempel 1957; Anger 1977; Armitage 1979;
374 Wilson 1985; Bolam 1999; Morgan et al. 1999).

375 To summarize, the population and reproductive dynamics of *P. elegans* were distinguished seasonally into an
376 non-reproductive phase lasting from May until August and a reproductive phase, characterized by the
377 presence of gravid females, egg strings and asexual reproduction, that lasted from September until April.
378 According to the dbRDA plot the seasonal dynamics of *P. elegans* correlated with temperature. These
379 observations support the previous work by Rasmussen (1973), who described the appearance of sexually
380 mature individuals when temperature dropped below 15 °C, and Anger et al. (1986), who detected a higher
381 rate of sexual reproduction at 5 °C and 12 °C compared to 18 °C. Moreover, male *P. elegans* exposed to a
382 temperature increase from 5 to 18 °C lost their soft appendages and sperm degenerated (Rasmussen 1973).
383 The influence of temperature on asexual reproduction is less clear. Rasmussen (1953) induced asexual
384 reproduction by exposing *P. elegans* to temperatures of 4-5 °C. However, we observed asexual reproduction
385 throughout the year (as did Rasmussen (1953)). Furthermore, asexual reproduction was prevalent at Lynæs
386 and less common at Vellerup and Herslev despite nearly identical water temperatures at all sites. Hence, in
387 addition to a strong seasonality in reproduction, there might be additional influences from other factors, such
388 as food availability and worm density (Branch 1975; Wilson 1985) that affect reproductive patterns.

389 In addition, there are some uncertainties in our cohort estimates of *P. elegans* due to the following issues.
390 Firstly, since we were interested in development mode, we focused on sexually mature individuals and we
391 used a 1 mm mesh for sampling, which might not have been sufficient for sampling juveniles. Using a 500
392 µm or 212 µm mesh would have been more appropriate for sampling and quantifying the smallest specimens

393 accurately (Gudmundsson 1985; Morgan 1997; Bolam 2004). Even though we identified new cohorts in
394 spring and fall with timing matching the results of previous studies (Gudmundsson 1985; Morgan 1997;
395 Bolam 2004), we likely underestimated the number of small individuals, especially at Vellerup and Herslev,
396 where coarse and poorly sorted sediment hindered the sampling. This might have led us to conclude that
397 small individuals appeared later than they actually did. In order to estimate the maximum delay in detection
398 of small individuals due to our sampling methods, we assumed a minimum juvenile growth rate similar to
399 adult growth rate (since growth rates seem to decrease with age (Anger 1986: 18 setigers a month for
400 planktonic larvae, Bolam 2004: 5 setigers a month for settled individuals)) and calculated that newly settling
401 *P. elegans* of 14 setigers would likely have needed a month to grow to a size large enough (> 20 setigers) for
402 our detection. The coarse heterogeneous sediment might have also contributed to a sampling artefact that can
403 explain the unrealistic high growth rates of 36 $\mu\text{m}/\text{d}$ and 22.5 $\mu\text{m}/\text{d}$ estimated for Vellerup. Here, new
404 cohorts appeared in April and November and seemed to merge instantaneously with the one cohort present
405 during the survey period. It is likely that we did not observe the true growth rate of cohorts at Vellerup given
406 our limitations for sampling small individuals.

407 Secondly, there could have been some inaccuracy in our size measurements. Instead of counting the total
408 number of setigers (Gudmundsson 1985; Morgan 1997) or measuring width of the 5th setiger (Bolam 2004),
409 we chose to assess worm size by measuring the length from the eyespot to the gills so that we could include
410 broken and regenerating individuals in the sample. In addition, because we wanted to save the specimens for
411 additional genetic analysis (to be reported a future contribution), we measured live animals that might have
412 moved slightly, despite being narcotized. To test the accuracy of our method, we compared the length from
413 eyespot to the gills and total number of segments for 62 individuals, collected from all sites from July to
414 October, and found only a moderate positive correlation ($r = 0.435$, $p\text{-value} < 0.001$) suggesting that the two
415 methods do not precisely agree. However, we believe that our measurements are adequate for comparisons
416 among times and stations presented in this study given that the same method is used for all samples.

417 **Site differences**

418 Besides a seasonal difference, we also observed consistent differences in the population dynamics of *P.*
419 *elegans* between the different sampling locations. Lynæs was unique due to its high fraction of asexual
420 reproduction and low worm density. Asexual reproduction might have led to the small mean size of worms
421 and the presence of many separate cohorts. Vellerup and Herslev differed from Lynæs and Lammefjord
422 because of their high number of egg strings, gravid females and males with sperm. Furthermore, Vellerup
423 and Herslev showed the highest densities and largest mean sizes. Herslev was characterized by a high
424 number of benthic larvae in winter.

425 DistLM and dbRDA indicated that sorting and mean salinity were the parameters that best explained the
426 observed site differences in population dynamics. In many ways, sorting describes the general sediment
427 characteristics well, as it correlated significantly with median grain size, porosity, and water content. In
428 general, sites with medium to coarse sediment, i.e. Herslev and Vellerup, had highest numbers of egg strings
429 but also highest densities, mean sizes and percentage of gravid females and males carrying sperm. *P. elegans*
430 populations performed better in sandy and heterogeneous sediments in our study as has been described
431 previously (Smidt 1951; Armitage 1979), despite the lower organic content and higher refractory fraction.

432 Although the fraction of asexual reproduction was higher at sites with low numbers of egg strings, no
433 correlation between output from sexual reproduction and asexual reproduction was found. However, Lynæs,
434 which had the highest amount of asexual reproduction, was distinguished by the most labile organic matter,
435 lowest densities, and best sorted sediment. Wilson (1985) observed that the asexual fission rate of *P. elegans*
436 is proportional to food availability and inversely proportional to density. In comparison to the study of
437 Wilson (1985), which tested densities of 12,000 to 50,000 ind/m², all of our locations would be considered to
438 have low density and thus should have high levels of asexual reproduction; but, this was not the case.
439 Therefore, low density and high organic content might not be the reasons for a high percentage of asexual
440 reproduction at Lynæs. Instead, the well sorted sediment might facilitate predation or other disturbances that
441 increase fission rates.

442 The lowest mean salinity was present at Herslev, which in turn was also the only site where no pelagic
443 larvae, but only benthic and intermediate ones, were found during winter. *P. elegans* is a euryhaline species
444 that occurs in salinities down to 5 (Hempel 1957) and all our sites are well within its tolerance range. Anger
445 (1984) showed that *P. elegans* has a higher reproductive rate at brackish sites compared to full marine sites,
446 however. Generally, benthic larvae have been found in brackish habitats such as Blyth estuary
447 (Gudmundsson 1985) or the Baltic Sea (Finland, Denmark, Kesäniemi et al. (2014) and Rasmussen (1973)),
448 whereas pelagic larvae are mostly described for full marine habitats (Drum Sands, Bolam (2004) and Somme
449 Bay, Morgan (1997)). Additionally, a previous study in the Isefjord Roskilde Fjord estuaries performed in
450 April 2010 found predominantly benthic and intermediate larvae in Roskilde Fjord and mainly pelagic larvae
451 or all three kinds of larvae in Isefjord (Kesäniemi et al. 2014). Although we could not test it statistically, the
452 fact that mode of development differs between sites only in winter suggest there may be an interaction of
453 temperature and salinity in determining the mode of development as described for other species (Schlieper
454 1929; Krug 2007). However, no combined effect of temperature and salinity on the mode of reproduction of
455 *P. elegans* was found in previous lab experiments (Anger 1984). We combined data from different years
456 (March 2014 and January, February 2015) in the March sample for the DistLM analyses in order to
457 summarize the seasonal patterns, but in doing so neglected any inter annual changes. Moreover, considering
458 that we monitored only four different sites, and that only one had lower mean salinity, it is difficult to draw
459 final conclusions from our results. Further manipulative lab experiments are needed to fully investigate the
460 effect of sediment and salinity on the degree and mode of reproduction. Furthermore, additional parameters
461 not monitored here, such as predation and disturbance might play a role in the population and reproductive
462 dynamics.

463 Although the mode of development of *P. elegans* was not fixed at our sites, we could not clearly relate the
464 presence of different developmental modes with the studied environmental parameters. The co-occurrence of
465 benthic and pelagic larvae might indicate that both exhibit a similar fitness, as otherwise one mode would
466 have been preferred via selection already (Levin and Huggett 1990). Indeed, Levin and Bridges (1995)
467 detected similar population dynamics between benthic and pelagic populations of the spionid polychaete

468 *Streblospio benedicti*. Likewise, we observed similar population dynamics at Herslev compared to
469 Lammefjord and Vellerup, despite a different larval development mode in winter. Furthermore,
470 heterogeneity of the environment might promote the coexistence of different modes of reproduction as a bet-
471 hedging strategy (Eckert 2003). Thus, the variance in fitness and risk of failure is reduced in the long run
472 (Collin 2012). *P. elegans* are common in shallow and estuarine habitats which are exposed to unpredictable
473 environmental fluctuations and its poecilogoneous character might support its survival in these
474 heterogeneous environments. Given that the genetic background of the populations may also affect the mode
475 of development (Levin et al. 1991), we will further investigate whether the different broods and larvae
476 observed in this study are produced by genetically different cohorts. At this point in time, we have not found
477 one clear factor determining the variable patterns of reproduction and population dynamics for *P. elegans* at
478 our study sites. It is likely that a combination of environmental, genetic and stochastic factors interact to
479 produce the dynamic and somewhat unpredictable population dynamics that we have observed.

480

481

482 CONCLUSION

483 The population dynamics of *P. elegans* in the Isefjord Roskilde Fjord estuary complex showed similar
484 seasonal dynamics as observed previously by Rasmussen (1973), Gudmundsson (1985) and Bolam (2004)
485 for other populations. Seasonality in sexual and asexual reproduction might be temperature induced. The
486 populations at the four study sites, however, also differed in some characteristics, such as proportion of
487 asexual reproduction and proportion of gravid females and males carrying sperm, as well as density and
488 mean sizes. These differences correlated with differences in environmental conditions at the sites, such as
489 sediment characteristics and salinity. We observed two reproductive peaks at three of the sites. At the same
490 sites also a switch in mode of development from spring to fall 2014 was found, whereas at one site
491 developmental mode remained constant. Consequently, we intend to use molecular tools to further
492 investigate whether the shift in larval developmental mode reflects reproduction of genetically differentiated
493 cohorts.

494

495

496 ACKNOWLEDGEMENT

497 This intensive field study would not have been possible without the great help of Anne Busk Faarborg, Rikke
498 Guttensen, Tiina Elina Salo, Swantje Enge, Louise Götterup, Bolette Lykke Holm Nielsen, Nicolai Lond
499 Frisk, Kristina Clement Diernæs, Lærke Bloch Noer, James Albino, Anastasija Martjanova, Laura Kehry,
500 Daniel Johansson, Siru Heiskanen, Farhan Khan, Cihan Süleyman Erdogan and Ole Brodnicke. We are
501 grateful to Christian Pedersen, Morten Frederiksen, Mejnert Rasmussen and Jörgen Winkel for the
502 permission to sample and deploy our logger at the different sites as well as for their assistance. Martin Mørk
503 Larsen and Peter Kofoed provided the salinometer and helped analysing the reference seawater samples.
504 Esbern Holmes created the map including sampling sites. Mark Wejlemann Holm assisted with FiStat II.
505 A.T. was financed by the Graduate School for Environmental Stress Studies of Roskilde University. This
506 research was supported by the Academy of Finland (Project 258365 to K.E.K) and Centre for Ocean Life
507 supported by The Villum Foundation to B.W.H. We declare no conflict of interest.

508

509

510 REFERENCES

- 511 Anger K 1977. Benthic invertebrates as indicators of organic pollution in the Western Baltic Sea. Intern.
512 Revue Hydrobiol. Hydrogr. 62: 245-254.
- 513 Anger K, Anger V & Hagmeier E 1986. Laboratory studies on larval growth of *Polydora ligni*, *Polydora*
514 *ciliata*, and *Pygospio elegans* (Polychaeta, Spionidae). Helgoländer Meeresuntersuch. 40: 377-395.
- 515 Anger V 1984. Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to
516 environmental conditions: A preliminary report. Forts. Zool. 29: 45-52.
- 517 Armitage DL 1979. The ecology and reproductive cycle of *Pygospio elegans* Claparède (Polychaeta:
518 Spionidae) from Tomales Bay, California. PhD thesis, University of the Pacific, Stockton,
519 California.
- 520 Bhattacharya CG 1967. A simple method of resolution of a distribution into Gaussian components.
521 Biometrics 23.1: 115-135.
- 522 Blake JA & Kudenov JD 1981. Larval development, larval nutrition and growth for two *Boccardia* species
523 (Polychaeta: Spionidae) from Victoria, Australia. Mar. Ecol. Prog. Ser 6: 175-182.
- 524 Blanck A & Lamouroux N 2007. Large-scale intraspecific variation in life-history traits of European
525 freshwater fish. J. Biogeogr. 34:862-875.
- 526 Blomqvist EM & Bonsdorff E 1986. Spatial and temporal variations of benthic macrofauna in a sandbottom
527 area on Åland, northern Baltic Sea. Ophelia, Suppl 4: 27-36.
- 528 Bolam SG 1999. An investigation into the processes responsible for the generation of the spatial pattern of
529 the spionid polychaete *Pygospio elegans* Claparède. Phd thesis, Edinburgh Napier University.
- 530 Bolam SG 2004. Population structure and reproductive biology of *Pygospio elegans* (Polychaeta: Spionidae)
531 on an intertidal sandflat, Firth of Forth, Scotland. Invertebr. Biol. 123: 260-268.
- 532 Branch G 1975. Intraspecific Competition in *Patella cochlear* Born. J. Anim. Ecol. 44.1: 263-281.
- 533 Burgess SC & Marshall DJ 2011. Are numbers enough? Colonizer phenotype and abundance interact to
534 affect population dynamics. J. Anim. Ecol. 80:681-687.

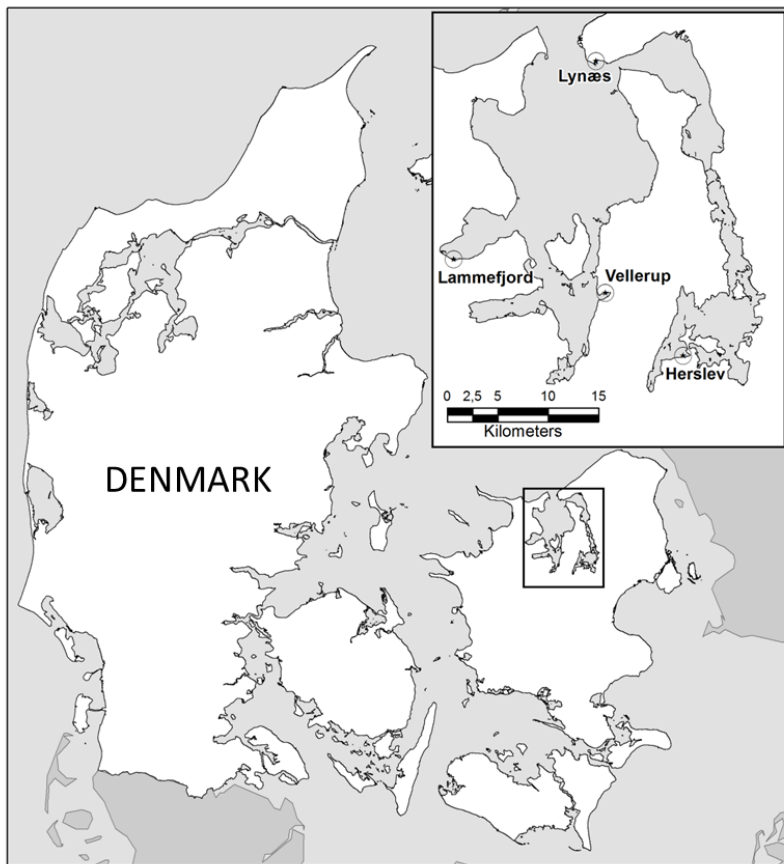
- 535 Chia F, Gibson G & Qian P 1996. Poecilogony as a reproductive strategy of marine invertebrates. *Oceanol.*
536 *Acta* 19: 203-208.
- 537 Clarke KR & Gorley RN 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth. 192pp.
- 538 Collin R 2012. Nontraditional life-history choices: what can “intermediates” tell us about evolutionary
539 transitions between modes of invertebrate development? *Integr. Comp. Biol.* 52: 128-137.
- 540 Dethier MN 2010. Variation in recruitment does not drive the cline in diversity along an estuarine gradient.
541 *Mar. Ecol. Prog. Ser.* 410: 43-54.
- 542 Duchêne J 1984. Reproductive biology of *Boccardia polybranchia* (Carazzi) in Kerguelen (Subantarctic
543 province). *Polar Biol.* 2: 251-257.
- 544 Eckert GL 2003. Effects of the planktonic period on marine population fluctuations. *Ecology* 84: 372-383.
- 545 Einum S & Fleming IA 2004. Environmental unpredictability and offspring size: conservative versus
546 diversified bet-hedging. *Evol. Ecol. Res.* 6:443-455.
- 547 Gaines S & Lafferty K 1995. Modeling the dynamics of marine species: the importance of incorporating
548 larval dispersal.
- 549 Gudmundsson H 1985. Life history patterns of polychaete species of the family Spionidae. *J. Mar. Biol.*
550 *Assoc. U.K.* 65: 93-111.
- 551 Hannerz DGL 1956. Larval Development of the Polychaete Families Spionidae Sars, Disomidae Mesnil, and
552 Poecilochetidae N. fam. in the Gullmar Fjord, Sweden. *Zool.Bidr. Upps.* 31: 1-204.
- 553 Hempel C 1957. Über den Röhrenbau und die Nahrungsaufnahme einiger Spioniden (Polychaeta sedentaria)
554 der deutschen Küsten. *Helgol. Mar. Res.* 6: 100-135.
- 555 Jeffery CH & Emler RB 2003. Macroevolutionary consequences of developmental mode in temnopleurid
556 echinoids from the Tertiary of southern Australia. *Evolution* 57: 1031-1048.
- 557 Kesäniemi JE 2012. Variation in developmental mode and its effects on divergence and maintenance of
558 populations. *Kehitysmuotojen variaatio ja sen populaatiogeneettiset seuraukset* 137. PhD thesis,
559 University of Jyväskylä, Finland.

- 560 Kesäniemi JE, Boström C & Knott KE 2012a. New genetic markers reveal population genetic structure at
561 different spatial scales in the opportunistic polychaete *Pygospio elegans*. *Hydrobiologia* 691: 213-
562 223.
- 563 Kesäniemi JE, Geuverink E & Knott KE 2012b. Polymorphism in developmental mode and its effect on
564 population genetic structure of a spionid polychaete, *Pygospio elegans*. *Integr. Comp. Biol.* 52: 181-
565 196.
- 566 Kesäniemi JE, Hansen BW, Banta GT & Knott KE 2014. Chaotic genetic patchiness and high relatedness of
567 a poecilogonous polychaete in a heterogeneous estuarine landscape. *Mar. Biol.* 161: 2631-2644.
- 568 Kesäniemi JE, Rawson PD, Lindsay SM & Knott KE 2012c. Phylogenetic analysis of cryptic speciation in
569 the polychaete *Pygospio elegans*. *Ecology and evolution* 2: 994-1007.
- 570 Kesäniemi JE, Heikkinen L & Knott KE 2016. DNA Methylation and Potential for Epigenetic Regulation in
571 *Pygospio elegans*. *PloS one* 11:e0151863.
- 572 Knott KE & McHugh D 2012. Introduction to Symposium: Poecilogony—A Window on Larval
573 Evolutionary Transitions in Marine Invertebrates. *Integr. Comp. Biol.* 52: 120-127.
- 574 Krug PJ 2007. Poecilogony and larval ecology in the gastropod genus *Alderia**. *Am. Malacol. Bull.* 23:99-
575 111.
- 576 Krug PJ 2009. Not my “type”: larval dispersal dimorphisms and bet-hedging in opisthobranch life histories.
577 *Biol. Bull.* 216: 355-372.
- 578 Lam P & Calow P 1989. Intraspecific life-history variation in *Lymnaea peregra* (Gastropoda: Pulmonata). I.
579 Field study. *The Journal of Animal Ecology*:571-588.
- 580 Levin L & Bridges T 1995. Pattern and diversity in reproduction and development. In: *Ecology of marine*
581 *invertebrate larvae*. McEdwardL (ed), pp. 1-48. CRC Press, Boca Raton Florida.
- 582 Levin LA 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three
583 coasts of North America. *Biol. Bull.* 166: 494-508.
- 584 Levin LA & Huggett DV 1990. Implications of alternative reproductive modes for seasonality and
585 demography in an estuarine polychaete. *Ecology* 71: 2191-2208.

- 586 Levin LA, Zhu J & Creed E 1991. The genetic basis of life-history characters in a polychaete exhibiting
587 planktotrophy and lecithotrophy. *Evolution* 45: 380-397.
- 588 Linke O 1939. Die Biota des Jadebusenwattes. *Helgoländer Meeresunters.* 1: 201-348.
- 589 MacKay J & Gibson G 1999. The influence of nurse eggs on variable larval development in *Polydora*
590 *cornuta* (Polychaeta: Spionidae). *Invertebr. Reprod. Dev.* 35: 167-176.
- 591 Marshall D, Monro K, Bode M, Keough M & Swearer S 2010. Phenotype–environment mismatches reduce
592 connectivity in the sea. *Ecol. Lett.* 13:128-140.
- 593 Marshall DJ, Bonduriansky R & Bussière LF 2008. Offspring size variation within broods as a bet-hedging
594 strategy in unpredictable environments. *Ecology* 89:2506-2517.
- 595 Marshall DJ & Burgess SC 2015. Deconstructing environmental predictability: seasonality, environmental
596 colour and the biogeography of marine life histories. *Ecol. Lett.* 18:174-181.
- 597 Marshall DJ & Keough MJ 2008. The relationship between offspring size and performance in the sea. *The*
598 *American Naturalist* 171:214-224.
- 599 Marshall DJ, Krug PJ, Kupriyanova EK, Byrne M & Emlet RB 2012. The biogeography of marine
600 invertebrate life histories. *Annual Review of Ecology, Evolution, and Systematics* 43:97.
- 601 McEdward LR 2000. Adaptive evolution of larvae and life cycles. In: *Semin. Cell Dev. Biol.* 11: 403-409.
- 602 McLusky DS & Elliott M 2003. *The Estuarine Ecosystem: ecology, threats and management* Oxford
603 *Biology: Amsterdam.*
- 604 Morgan TS 1997. The formation and dynamics of *Pygospio elegans* tube-beds in the Somme Bay, France.
605 PhD thesis, University of Southampton, France.
- 606 Morgan TS, Rogers AD, Paterson GLJ, Hawkins LE & Shearer M 1999. Evidence for poecilogony in
607 *Pygospio elegans* (Polychaeta: Spionidae). *Mar. Ecol. Prog. Ser.* 178: 121-132.
- 608 Muus BJ 1967. The fauna of Danish estuaries and lagoons: distribution and ecology of dominating species in
609 the shallow reaches of the mesohaline zone Høst, A. F.: København.
- 610 Pechenik JA 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life
611 cycles. *Mar. Ecol. Prog. Ser.* 177: 269-297.

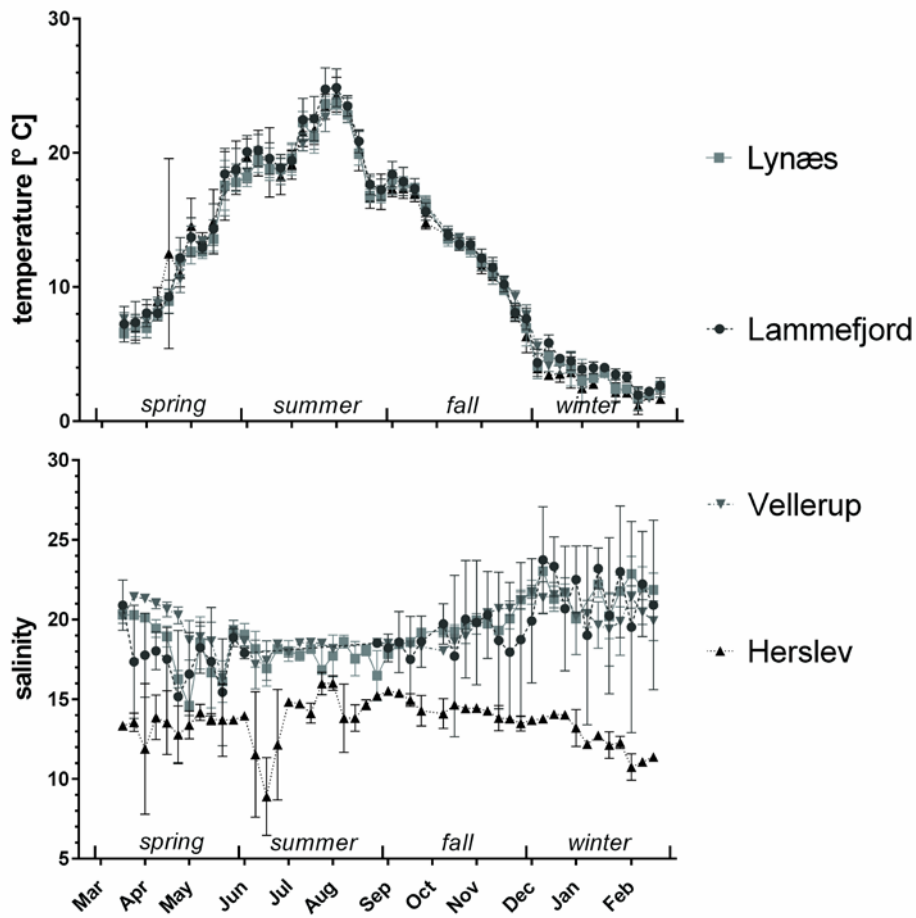
- 612 Rasmussen E 1953. Asexual reproduction in *Pygospio elegans* Claparede (Polychaeta sedentaria). Nature
613 171: 1161-1162.
- 614 Rasmussen E 1973. Systematics and ecology of the Isefjord marine fauna (Denmark) with a survey of the
615 eelgrass (*Zostera*) vegetation and its communities. *Ophelia* 11: 1-507.
- 616 Schlieper C 1929. Über die Einwirkung niederer Salzkonzentrationen auf marine Organismen. *J. Comp.*
617 *Physiol.* [A] 9: 478-514.
- 618 Smidt ELB 1951. Animal production in the Danish Wadden Sea. *Medd. Komm. Danm. Fisk. Havunders.* 11
619 (6):1-151.
- 620 Strathmann RR 1993. Hypotheses on the origins of marine larvae. *Annu. Rev. Ecol. Syst.*:89-117.
- 621 Strathmann RR, Hughes TP, Kuris AM, Lindeman KC, Morgan SG, Pandolfi JM & Warner RR 2002.
622 Evolution of local recruitment and its consequences for marine populations. *Bull. Mar. Sci.* 70: 377-
623 396.
- 624 UNESCO I & SCOR I. 1981. Tenth report of the joint panel on oceanographic tables and standards. pp. 24-
625 24.
- 626 Vendetti JE, Trowbridge CD & Krug PJ 2012. Poecilogony and population genetic structure in *Elysia pusilla*
627 (Heterobranchia: Sacoglossa), and reproductive data for five sacoglossans that express dimorphisms
628 in larval development. *Integr. Comp. Biol.* 52: 138-150.
- 629 Weersing KA 2007. Population genetics, larval dispersal, and demographic connectivity in marine systems.
- 630 Wilson WHJ 1985. Food limitation of asexual reproduction in a spionid polychaete. *Int. J. Invertebr. Repr.*
631 *Dev.* 8: 61-65.
- 632

633 FIGURES



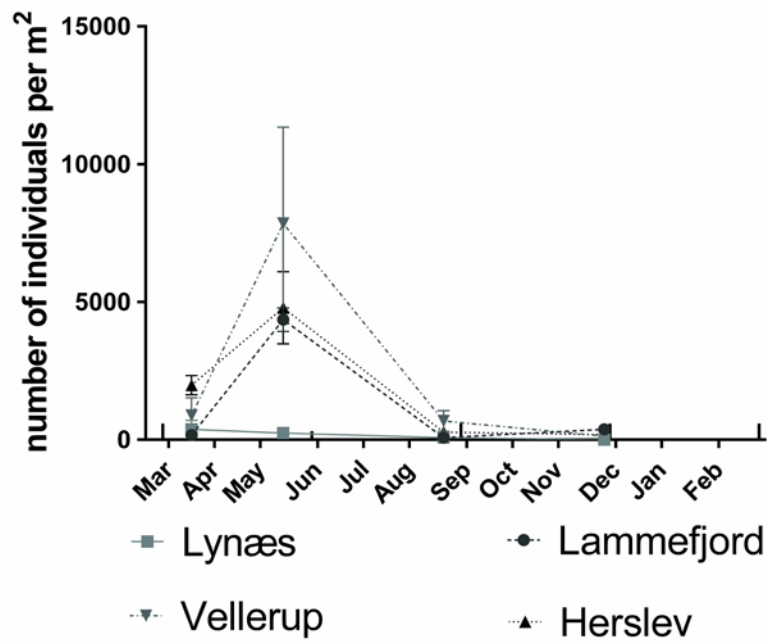
634

635 Fig. 1: Location of our four sampling sites in the Isefjord Roskilde Fjord estuary complex, Denmark.



636

637 Fig. 2: Temperature A) and salinity B) patterns at our study sites: weekly mean and standard deviation
 638 obtained from continuous logger data. Data is missing for one week in October and one week in January,
 639 when the loggers were taken in for maintenance. The logger at Lammefjord was deployed in the mouth of
 640 Lammefjords Søkanal, which likely contributed to the large salinity fluctuations observed there.

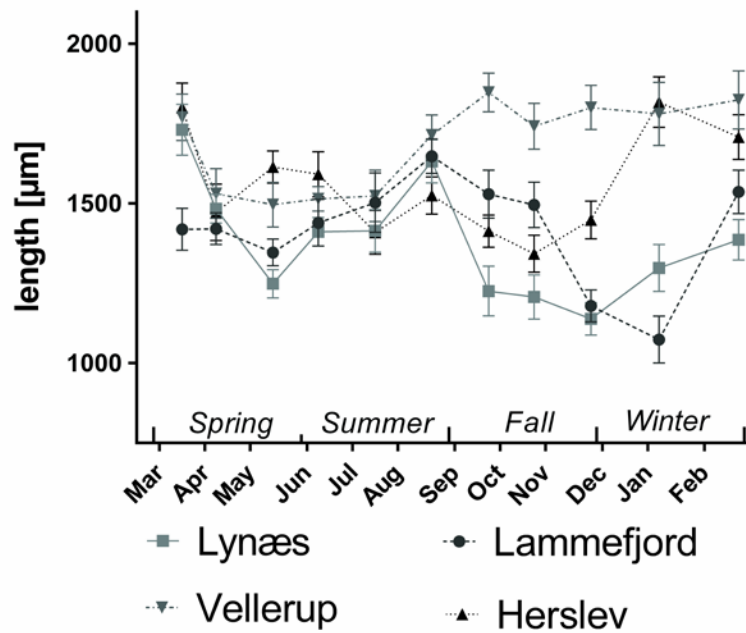


641

642 Fig. 3: Mean and standard error of density of *Pygospio elegans* [individuals/m²] in March, May, August and
643 November.

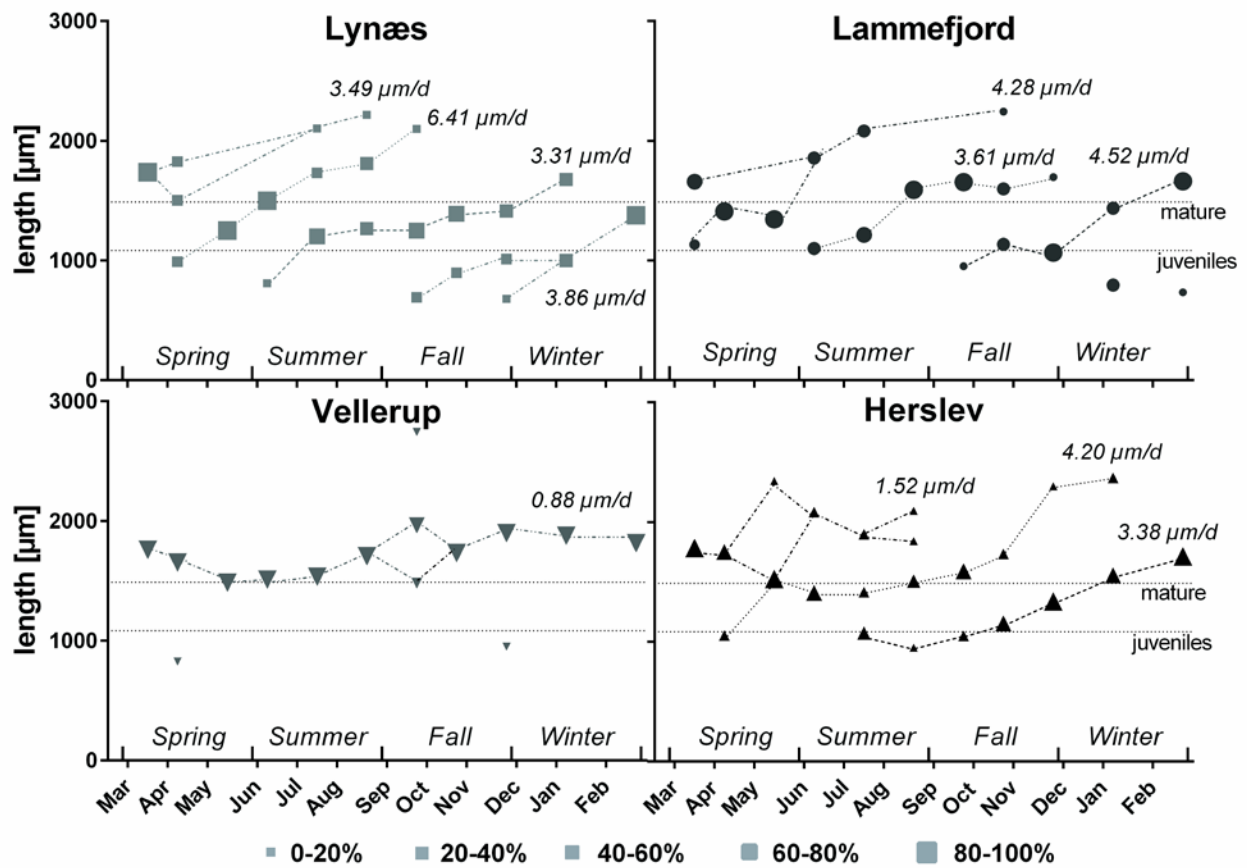
644

645



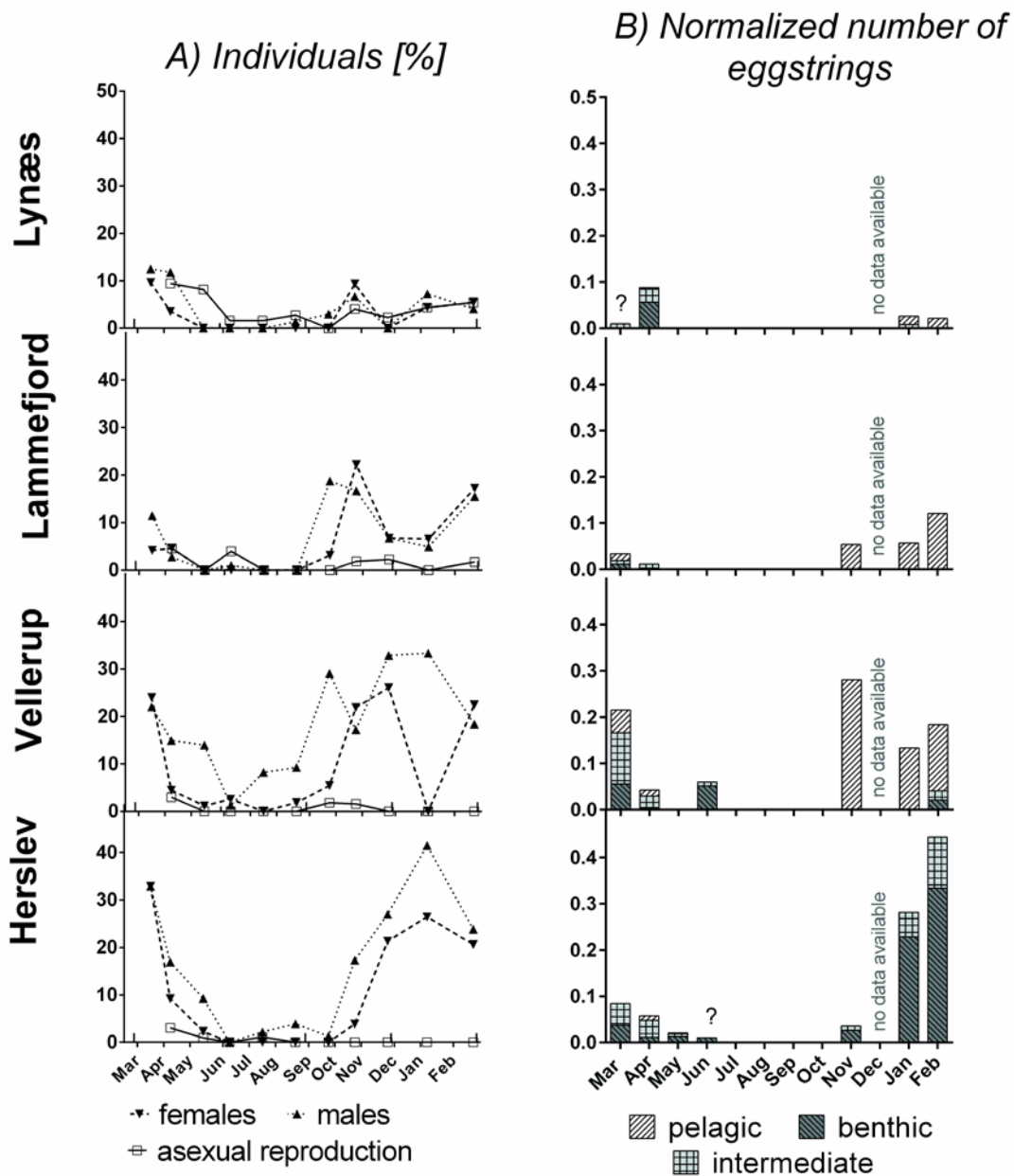
646

647 Fig. 4: Mean and standard error of the length from head until gills of at least 30 individuals of *Pygospio*
 648 *elegans* per month and site. There is no data available for December. Based on a regression (see details in
 649 discussion) between number of segments and our measurements (from head to the beginning of the gills),
 650 young individuals ready to settle having about 14 segments were expected to have a mean length from head
 651 until gills of 1085 µm, and mature individuals having 40 segments were estimated to have a mean length of
 652 1489 µm.



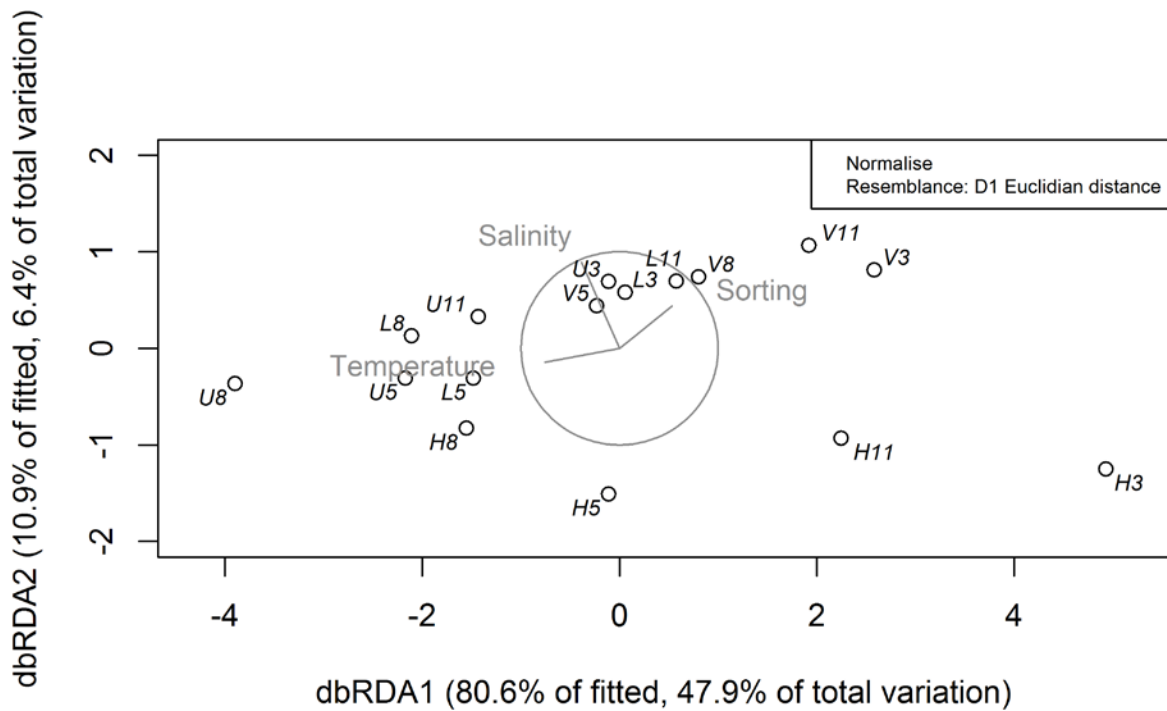
653

654 Fig. 5: Cohorts identified with FiSatII: mean of each size class as length from head until gills [µm] is
 655 illustrated per month and site. The size of each dot symbol correlates to the fraction of the total population in
 656 that cohort (<20%, <40%, <60%, <80%, <100%). Growth rates for each cohort calculated via linear
 657 regression, with normality being fulfilled in most cases. The size of small individuals of about 14 setigers
 658 (1085 µm) and minimum size of mature individuals (1489 µm) as described in the legend of Fig. 4 are
 659 indicated. Detailed length frequency histograms can be found in the Supplement Fig. 2.



660

661 Fig. 6: Reproductive activity A) Percentage of males (sperm and soft appendages at second setiger present),
 662 females (eggs or egg strings present) and individuals performing asexual reproduction (several worms
 663 sharing one tube and regenerating) per month and site. B) Number of egg strings normalized to the total
 664 number of individuals captured. The mode of development of the resulting larvae is indicated. '?' - due to
 665 missing data, the number of egg strings in March at Lynæs and number of individuals sampled in total in
 666 June at Herslev was estimated by interpolation. No sampling took place in December.



667

668 Fig. 7: Distance-based redundancy analysis (dbRDA): Ordination of the population dynamics data for
 669 *Pygospio elegans* (U – Lynæs, L – Lammefjord, V – Vellerup, H - Herslev, 3 – March, 5 – May, 8 – August,
 670 11 – November) fitted to the significant predictor environmental parameters temperature, sorting and
 671 salinity. The parameters explain 59% of the total variation in the population dynamics, with 54% explained
 672 by the first two axes as shown. Overlaid vectors indicate the loadings (importance) of the predictor
 673 parameters temperature, sorting and salinity on the two axes.

674

675 TABLES

676 Table 1: Characterization of *Pygospio elegans* and its developmental modes. For explanations, see
 677 introduction.

Non-reproductive	Individuals without gametes	
Male	Individuals with soft appendages at second setiger and sperm in coelom	
Female	Individuals with eggs in coelom	
Asexual reproduction	One individual fragmented architomically, hence more than one individual is occupying a given sand tube and specimens are regenerating	
Larvae	benthic	1-3 larvae per egg capsule
	intermediate	4-10 larvae per egg capsule
	pelagic	>10 larvae per egg capsule

678

679 Table 2: Annual mean and standard deviation of environmental parameters. Sediment characteristics refer to
 680 the top layer (0-1cm) of sediment only.

	Lynæs		Lammefjord		Vellerup		Herslev	
	mean	SD	mean	SD	mean	SD	mean	SD
Temperature [°C]	12.39	6.86	13.14	6.97	12.18	6.85	12.50	7.07
Salinity	19.07	2.07	19.27	4.00	19.55	1.63	13.53	2.00
Median grain size (Phi)	Fine 2.38	0.25	Fine 2.18	0.30	Coarse 0.95	0.24	Medium 1.68	0.22
Sorting (Phi)	Moderately well 0.54	0.06	Moderately 0.96	0.30	Poorly 1.66	0.38	Moderately 0.82	0.20
Water content [%]	19.91	1.43	19.94	0.67	16.68	1.42	18.50	1.38
Porosity [%]	0.40	0.06	0.39	0.01	0.32	0.02	0.35	0.02
Organic matter [%]	0.92	0.09	1.04	0.18	0.84	0.20	0.78	0.06
C/N [mol %]	8.28	1.43	8.83	0.27	9.53	1.40	8.74	0.80

681