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

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ABSTRACT

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

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

The effect of developmental mode on population structure and dynamics can be investigated best in species that express different developmental modes, as even between sibling species with different modes of development speciation effects cannot be excluded (Knott and McHugh 2012). Variation in developmental mode, also called poecilogony, was described for several spionid polychaetes (Blake and Kudenov 1981; Duchêne 1984; Levin et al. 1991; MacKay and Gibson 1999) and sacoglossan sea slugs (Krug 2007; Krug 2009; Vendetti et al. 2012). The spionid *Pygospio elegans* (CLAPARÈDE 1863) is one of them (Morgan et al. 1999; Kesäniemi et al. 2012c). It is a common, small (10-15mm), tube-dwelling estuarine species with a circum-boreal distribution that lives primarily on intertidal mud flats. It can form high density patches, or tube-beds, with densities up to 600,000 individuals/m^2 (Morgan 1997). *P. elegans* has broad habitat tolerances and is able to thrive in a wide range of temperatures and salinities (Hempel 1957; Armitage 1979; Anger 1984; Morgan 1997). The average life span of *P. elegans* is about 9 months (Anger et al. 1986). The time from hatching (as pelagic larvae) to first reproduction takes about 15-17 weeks (Anger et al. 1986). Reproduction was reported to be seasonal with sexual reproduction that may consist of two broods occurring in winter and asexual reproduction peaking afterwards in spring (Rasmussen 1973, Gudmundson 1985). The versatile reproductive biology of *P. elegans* consisting of asexual and sexual reproduction and polymorphism

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

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

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

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

METHODS

We monitored *Pygospio elegans* and several environmental parameters in the Danish Isefjord-Roskilde Fjord complex from March 2014 until February 2015. Four sites, Lynæs, Lammefjord and Vellerup in Isefjord, and Herslev in Roskilde Fjord (see Fig. 1), were sampled monthly at shallow areas along the shore (each approx.10 m^2 with 0.5-1 m water depth) (see Supplement Table 1 for coordinates and exact sampling dates). These sites were chosen to cover genetically different populations of *P. elegans* and different habitats, as described by Kesäniemi and others (2013). The Isefjord-Roskilde Fjord complex is the second largest estuary in Denmark, located on the North of Zealand with an opening to the Kattegat. Isefjord has a surface area of 280 km^2 with mean depth of 7 m and salinities ranging from 18 to 30. Roskilde Fjord is connected to the Kattegat via the Isefjord, has a surface area of 117 km^2 and lower salinities, ranging between 5 and 18. It is divided into a long and narrow outer region and a shallow interior, which is not deeper than 6 m. The two estuaries are similar in temperature, but not salinity patterns (Rasmussen 1973).

Population dynamics of Pygospio elegans

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

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

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

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

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

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

Environmental dynamics

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

Sediment characteristics were determined in March, May, August and November. For sediment characteristics, three kajak cores (5 cm diameter, at least 15 cm length) were taken randomly at each sampling site. These were sectioned into four layers (0-1 cm, 1-2 cm, 2-6 cm, 6-15 cm) and the respective layers of each core were pooled and mixed. Wet weight and dry weight (24h at 105 °C) of 5 cm^3 sediment from each layer was determined for calculating porosity and water content.

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

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

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

Relation of population & environmental dynamics

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

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

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

RESULTS

Population dynamics of Pygospio elegans

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

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

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

The mean density of *P. elegans* was lowest at Lynæs (means between sampling times ranged from 0 - 377 ind/m^2), distinctly higher at Lammefjord (75 - 4357 ind/m^2) and Herslev (189 - 4791 ind/m^2) and highest at Vellerup (132 - 7847 ind/m^2) (see Fig. 3). While at three sites the population density was highest in May, with a maximum of 7847 ± 6051 individuals per m^2 in Vellerup, it was generally low and constant at Lynæs. Furthermore, the distribution of *P. elegans* was patchy, most noticeably during April and May at Herslev and in October at Lynæs when the worms were associated with the presence of diatom mats (pers. obs.).

Environmental dynamics

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

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

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

Relation of population & environmental dynamics

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

DISCUSSION

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

Seasonal dynamics

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

Sexual reproduction occurred from winter until spring, indicated by the presence of gravid females and males carrying sperm and egg strings. Similar patterns of seasonal sexual reproduction by *P. elegans* were observed at other sites (e.g. Rasmussen 1973; Gudmundsson 1985; Bolam 2004), although there are exceptions. For example, Morgan (1997) found gravid females peaking during spring/winter in 1990/91 and during summer in 1992 as well as egg strings almost year round, but mostly during summer at Somme Bay. We observed two peaks of gravid females and males with sperm at most sites. The two reproductive peaks most likely reflect the maturity of different cohorts at different times. However, we also observed that some individuals within a single cohort were able to produce two consecutive broods, making the peaks of 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 m^2, 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)

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

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

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

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

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

Site differences

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

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

Although the fraction of asexual reproduction was higher at sites with low numbers of egg strings, no correlation between output from sexual reproduction and asexual reproduction was found. However, Lynæs, which had the highest amount of asexual reproduction, was distinguished by the most labile organic matter, lowest densities, and best sorted sediment. Wilson (1985) observed that the asexual fission rate of *P. elegans* is proportional to food availability and inversely proportional to density. In comparison to the study of Wilson (1985), which tested densities of 12,000 to 50,000 ind/m^2, all of our locations would be considered to have low density and thus should have high levels of asexual reproduction; but, this was not the case. Therefore, low density and high organic content might not be the reasons for a high percentage of asexual reproduction at Lynæs. Instead, the well sorted sediment might facilitate predation or other disturbances that 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

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

CONCLUSION

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

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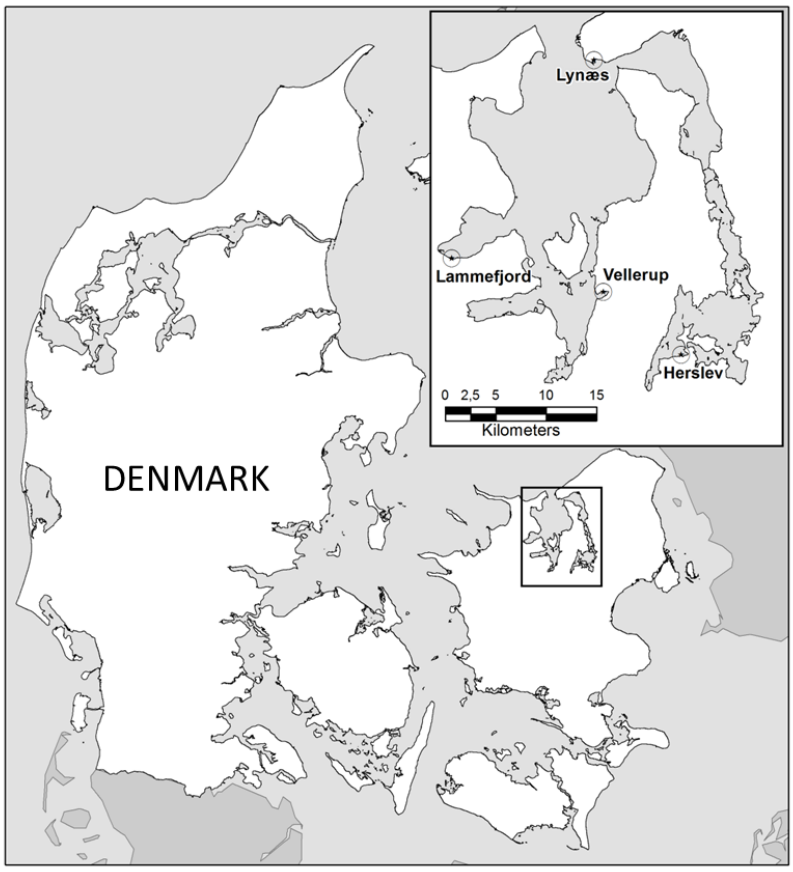
632

633 FIGURES



634

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

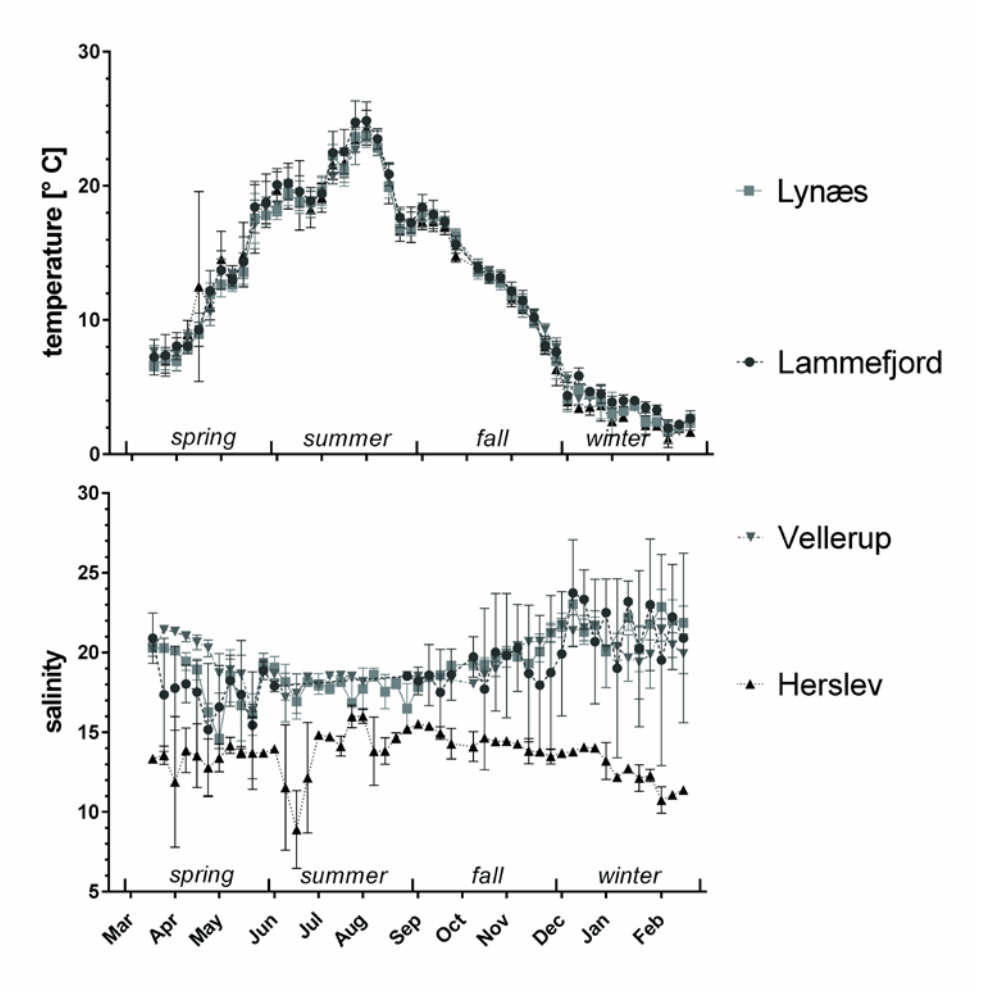
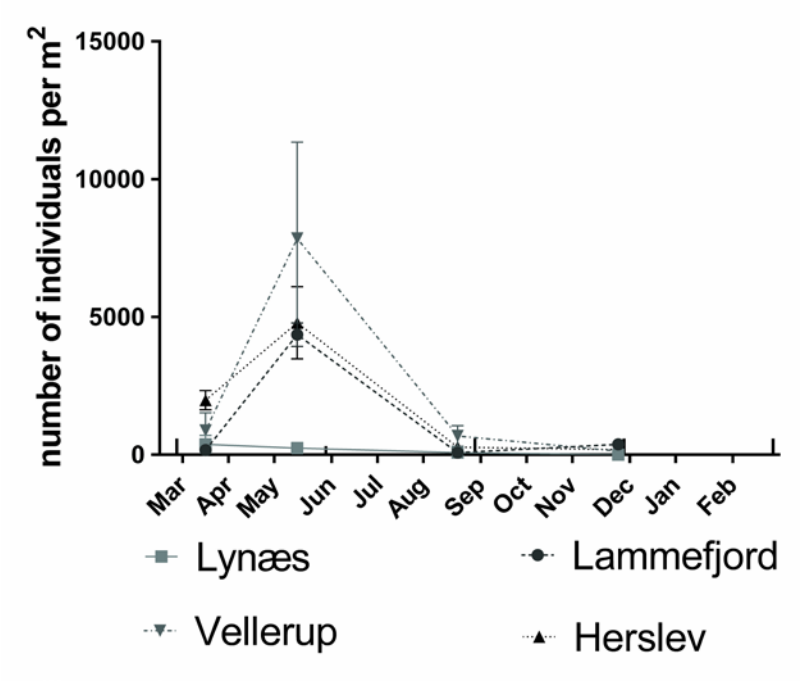


Fig. 2: Temperature A) and salinity B) patterns at our study sites: weekly mean and standard deviation obtained from continuous logger data. Data is missing for one week in October and one week in January, when the loggers were taken in for maintenance. The logger at Lammefjord was deployed in the mouth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



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642 Fig. 3: Mean and standard error of density of *Pygospio elegans* [individuals/m^2] in March, May, August and

643 November.

644

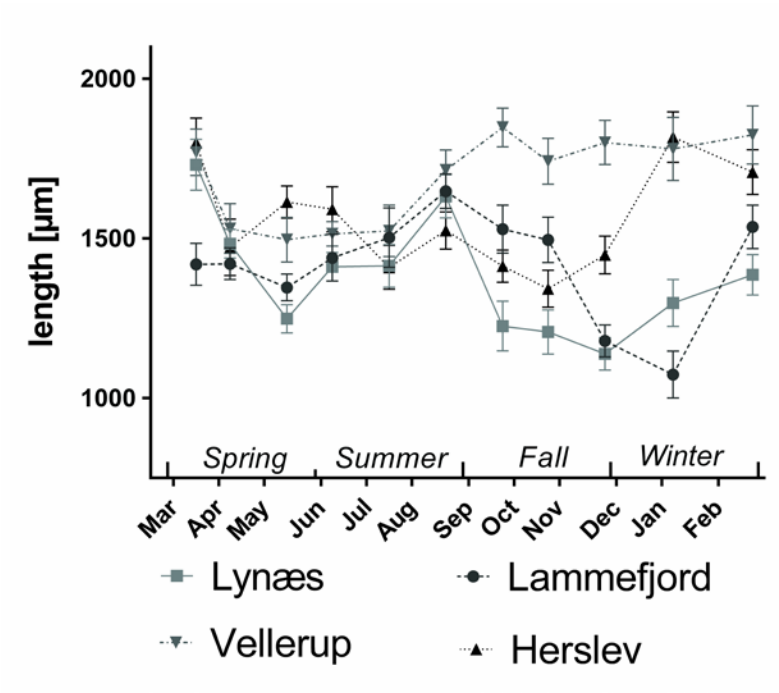
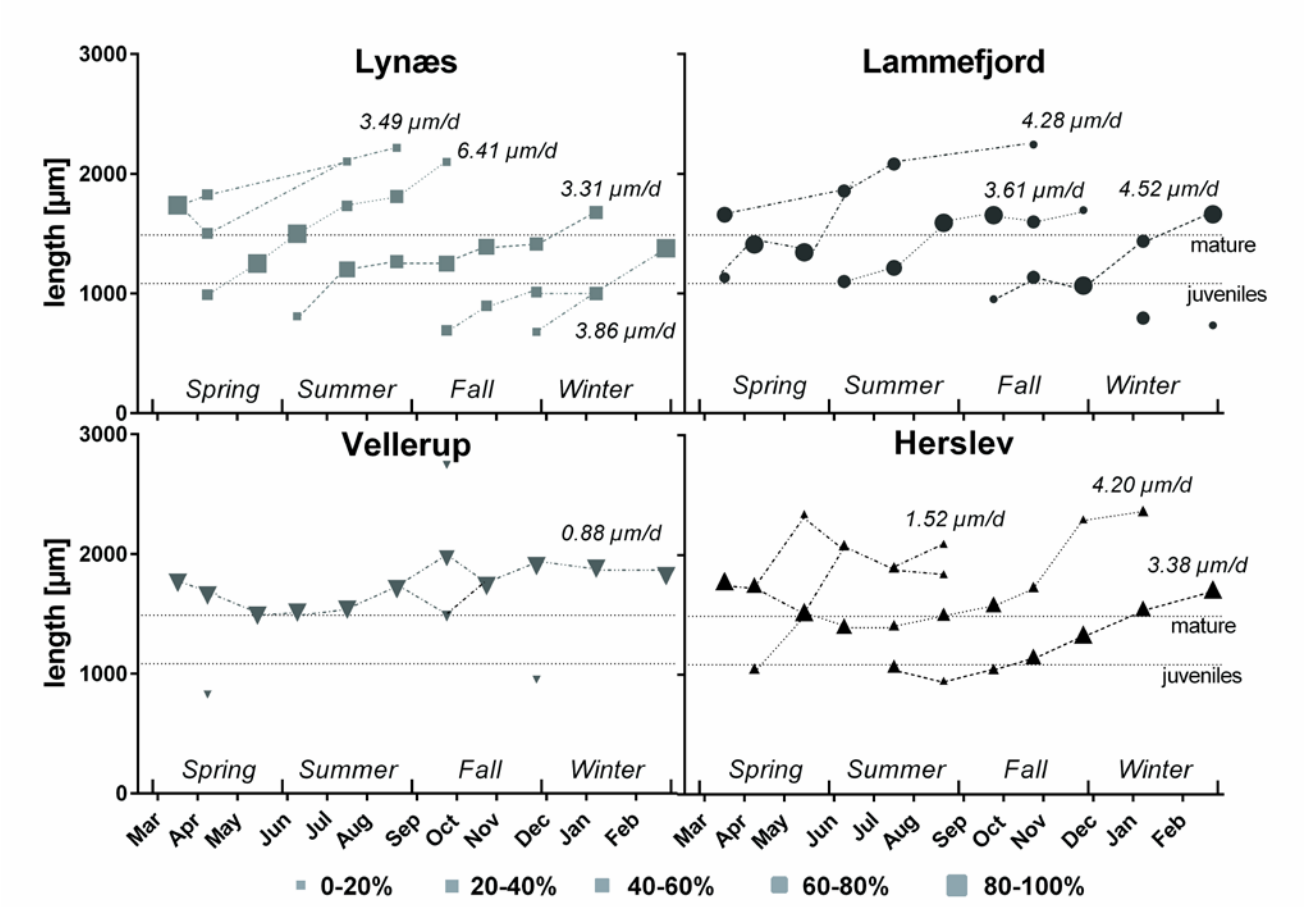
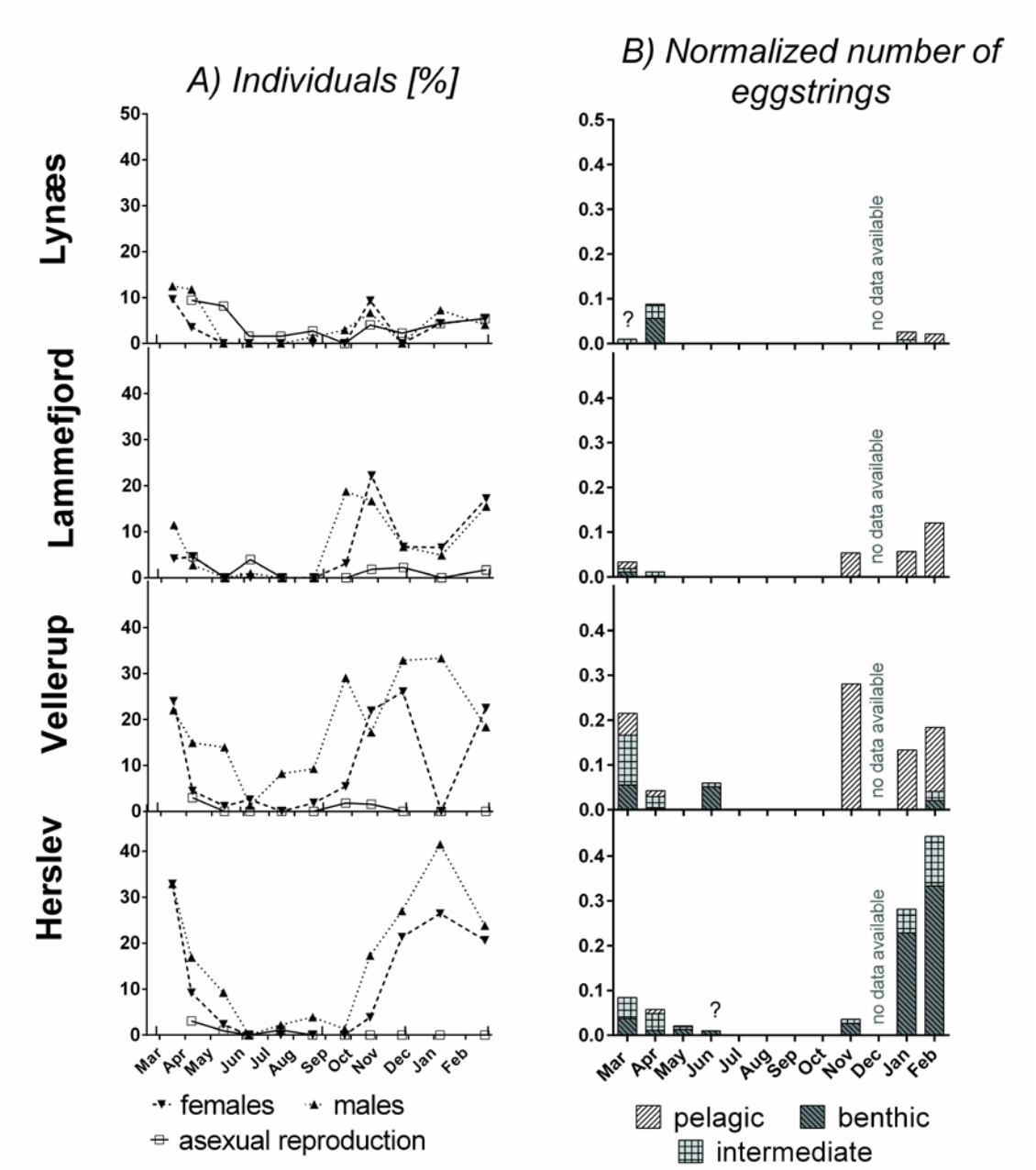


Fig. 4: Mean and standard error of the length from head until gills of at least 30 individuals of *Pygospio elegans* per month and site. There is no data available for December. Based on a regression (see details in discussion) between number of segments and our measurements (from head to the beginning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



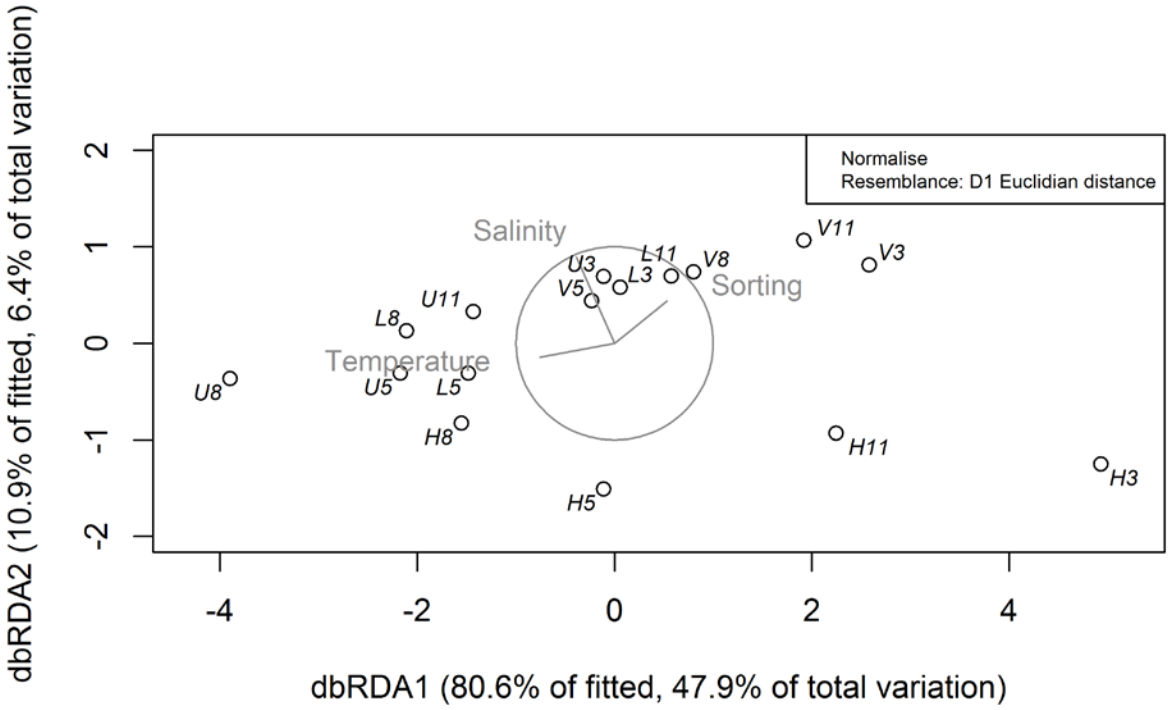
653

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

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