The Effect of Variation in Developmental Mode on the Population Dynamics of a Spionid Polychaete (Pygospio elegans) in a Heterogeneous Environment

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ABSTRACT

Thonig, Anne
The effect of variation in developmental mode on the population dynamics of a spionid polychaete (*Pygospio elegans*) in a heterogeneous environment.
Yhteenveto: Kehitysmuotojen variaatio ja sen vaikutus *Pygospio elegans* -monisukasmadon populaatiodynamiikkaan heterogeenisessä ympäristössä.
Oversigt: Effekten af variation I developmental mode på populations-dynamikken af en spionid børsteorm (*Pygospio elegans*) i et heterogent miljø.

There is a great diversity in larvae of marine invertebrates. To understand the causes and consequences of different modes of development on population dynamics, study of poecilogonous species that show a polymorphism in developmental mode might be more useful than are comparisons between species, since no confounding effects due to speciation arise. In this study, I documented the population ecology and genetics of the poecilogonous polychaete *P. elegans* and investigated the impact of abiotic and biotic variables on population dynamics. Four focal populations from the Isefjord-Roskilde-Fjord estuary complex, Denmark were sampled over one year. I observed highly dynamic population structure in both size cohort data and population genetic data that is possibly explained by the short life span of *P. elegans* and sweepstakes reproductive success. Additionally, stochastic events, such as rain storms, can lead to abrupt drops in salinity which can be detrimental for *P. elegans* and hence introduce further changes in population structure. Seasonal dynamics, including sexual reproduction, were correlated with temperature, whereas spatial differences in density, size and reproductive activity of *P. elegans* as well as species diversity of the benthic invertebrate community, were related to sediment structure. A positive correlation between species and allelic richness of *P. elegans* might indicate that environmental impacts are of greater importance in shaping population dynamics than are species interactions. Switches in developmental mode could reflect a strategy for coping with life in an unpredictable, heterogeneous habitat. Although switches in developmental mode were correlated with the appearance of genetically differentiated size cohorts, environmental or epigenetic effects cannot be ruled out.

Keywords: Benthic invertebrates; Isefjord-Roskilde-Fjord estuary complex; life history; poecilogony; population ecology and genetics; *Pygospio elegans*.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

I 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: 370–384.

II Thonig, A., Banta, G.T., Winding Hansen, B. & Knott, K.E. 2017. Seasonal genetic variation associated with population dynamics of a poecilogonous polychaete worm. *Ecology and Evolution, in press.*

III Knott, K.E., Thonig, A., Heiskanen, S., Winding Hansen, B. & Banta, G.T. 2017. Seasonal variation in diversity of marine benthic invertebrates leads to a positive species-genetic diversity correlation. Submitted manuscript.

IV Thonig, A., Banta, G.T., Winding Hansen, B. & Knott, K.E. 2017. Acute and chronic response to changes in salinity of the euryhaline polychaete *Pygospio elegans*. Manuscript.

The table shows the contributions to the original papers.

	I	II	III	IV
Original idea	KEK, JEK, GTB, BWH, AT	KEK, GTB, BWH, AT	KEK, GTB, BWH	AT, BWH, GTB
Data	AT, KEK, JEK, GTB, BWH	AT, SH, KEK	AT, SH	AT
Analyses	AT, GTB, BWH	AT, KEK	KEK, AT, SH, GTB	AT, KEK, GTB
Writing	AT, GTB, KEK, JEK, BWH	AT, KEK, GTB, BWH	KEK, AT, GTB, BWH	AT, KEK, GTB, BWH

AT = Anne Thonig, KEK = K. Emily Knott, GTB = Gary T. Banta, BWH = Benni Winding Hansen, JEK = Jenni E. Kesäniemi, SH = Siru Heiskanen

1 INTRODUCTION

1.1 Larvae of marine invertebrates

Marine invertebrates exhibit a wide diversity of reproductive strategies that can differ in gametogenesis, gamete release and, particularly, in the type of larvae they produce (Llodra 2002, Heyland *et al.* 2011, Henshaw *et al.* 2014). Larvae are an ancient characteristic of metazoans, and among marine invertebrates, larvae show a great variety with diverse structures to facilitate swimming, feeding, settlement and for defense against predators, which has raised the question of the evolution and the consequences of different larval forms (Strathmann 1985, Wray 1995). Just to name a few, veliger larvae are found in gastropods and bivalves, while some other molluscs have trochophora larvae, as do annelids and platyhelminths; nauplius and zoea larvae are characteristic for crustaceans; and echinoderms exhibit pluteus larvae, a complex version of the dipleurula larvae (Fig. 1, Levin and Bridges 1995).

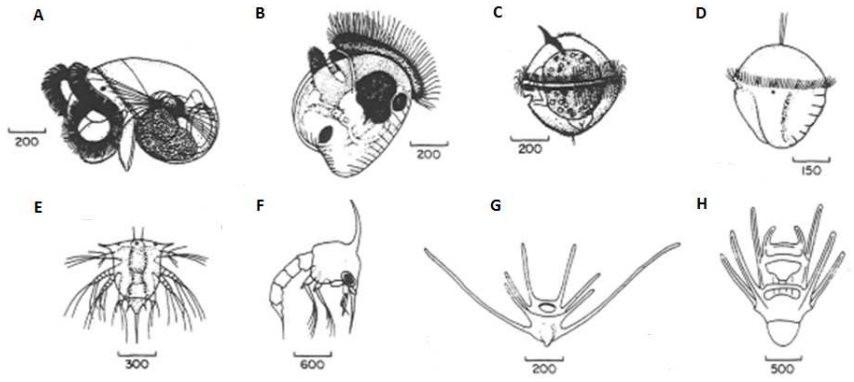

FIGURE 1 Different types of larvae of marine invertebrates (adapted from Levin and Bridges 1995). A) gastropod veliger, B) bivalve veliger, C) polychaete trochophora, D) polyplacophoran trochophora, E) crustacean nauplius, F) crustacean zoea, G) ophiuroid ophiopluteus, H) echinoid echinopluteus.

Ecologically, larvae can be categorized according to their site of development (planktonic, demersal, benthic) or their nutritional mode. Planktotrophic larvae swim and feed in the plankton and for that purpose exhibit efficient ciliary structures and a digestive system (Levin and Bridges 1995, Wray 1995). Larvae can also acquire their nutrients maternally via yolk deposited in the egg (lecithotrophy) or by feeding on other eggs or siblings within the brood (adelphophagy). Facultative planktotrophic larvae can complete their development without feeding (like lecithotrophic larvae), but have feeding structures and a planktonic phase like planktotrophic larvae and can feed if needed (McEdward 1997). Moreover, larval forms that gain nutrients from their mothers (translocation), uptake dissolved organic matter from the seawater (osmotrophy) or synthesize nutrients themselves (autotrophy) have also been described (Levin and Bridges 1995). Planktotrophy and lecithotrophy are present in most marine invertebrate phyla and within phyla, several evolutionary transitions from one mode to the other have occurred. In some taxonomic groups, planktotrophic larvae are thought to be the ancient form (e.g. echinoids, asteroids), while in other groups (e.g. polychaetes, gastropods) they are proposed to be the derived form (Strathmann 1993, Levin and Bridges 1995, Wray 1995, Rouse 2000, Collin *et al.* 2007).

Although transition in larval form is common among species, larval form is likely to be conserved within species. Only a few species of marine invertebrates are able to produce different types of larvae, resulting in a polymorphism in developmental mode called poecilogony. Many species that were initially thought to be poecilogonous, however, turned out to be cryptic species (Hoagland and Robertson 1988). So far, five poecilogonous species have been described among sacoglossan sea slugs, (*Costasiella ocellifera*, *Elysia chlorotica*, *Elysia zyleica*, *Elysia pusilla* [Vendetti *et al.* 2012], and *Alderia willowi* [Krug *et al.* 2012]); two species have been described among caenogastropods, (*Calyptrea lichen*[McDonald *et al.* 2014] and *Buccinum undatum* [Smith and Thatje 2013]); and seven species have been described among spionid polychaetes (*Streblospio benedicti* [Levin 1984b, Levin and Huggett 1990], *Pygospio elegans* [Söderström 1920, Hannerz 1956, Rasmussen 1973], *Boccardia proboscidea* [Blake and Kudenov 1981, Gibson *et al.* 1999], *Boccardia polybranchia* [Duchêne 1984], *Polydora cornuta* [Rice and Rice 2009], *Polydora hoplura* [David *et al.* 2014], and *Polydora cf websteri* [David *et al.* 2014]). Although all of these species are described as poecilogonous, their developmental mode can vary to different degrees (McDonald *et al.* 2014): between populations (e.g. in *Elysia chlorotica* and *Costasiella ocellifera* [Vendetti *et al.* 2012]), between females within the same population (e.g. *Streblospio benedicti* and *Pygospio elegans* [Söderström 1920, Hannerz 1956, Rasmussen 1973, Levin 1984b]), between broods of the same female (e.g. *Polydora cornuta* and *Calyptrea lichen* [Rice and Rice 2009, McDonald *et al.* 2014]) or even within broods (e.g. *Boccardia proboscidea* and *Buccinum undatum* [Blake and Kudenov 1981, Smith and Thatje 2013]).

1.2 Life history strategies

Size and number of larvae are important life history traits of marine invertebrates, as are characteristics of adult stages, such as maturity, age and size specific fecundity and mortality, and longevity. Therefore, larvae are major fitness components of the life history of an organism (Braendle *et al.* 2011). The life history of an organism is affected by environmental conditions and subject to natural selection that optimizes the reproductive value, i.e. the amount of expected future reproductive success of an individual (Fischer 1930, Edward and Chapman 2011). However, the investment into one fitness component can lead to reduced investment into another one, or trade-offs, leading to a negative correlation between them (Roff and Fairbairn 2007). Therefore, the combination of certain life history traits is restricted, due to genetic, physiological, developmental and phylogenetic limits. Developmental properties and historical contingencies can lead to constraints on certain traits. For example, among echinoderms feeding structures were lost many times, and once lost, feeding structures were not reacquired again (McEdward 2000). Genetic trade-offs can arise due to linkage disequilibrium and pleiotropy, whereas physiological trade-offs can arise due to the allocation of limited resources to competing functions, such as maintenance, growth and reproduction. Examples of physiological trade-offs are growth vs. reproduction, current reproduction vs. future reproduction and number vs. size of offspring (Levin and Bridges 1995, Llodra 2002, Braendle *et al.* 2011, Edward and Chapman 2011).

Different models have been developed to investigate the trade-offs between growth, reproduction and longevity (Cole 1954, Lewontin 1965, Charnov and Schaffer 1973, Grime 1977, Stearns 1992). A classic example is the model of r- vs. K-selection, describing strategies with high colonizing ability vs. high competitive ability (Wilson and MacArthur 1967, Pianka 1970). An extension of this, the pace-of-life syndrome hypothesis, suggests that certain physiological and behavioural traits co-evolved along with particular life history strategies in response to environmental conditions (Ricklefs and Wikelski 2002, Réale *et al.* 2010). In conclusion, several factors have been identified as important for shaping life history traits, namely abiotic factors (resource limitation and density-independent factors) and biotic factors (competition and predation), species specific factors (metabolic rates and mating systems), and whether variation in the environment is predictable or stochastic (Rockwood 2015).

Among marine invertebrates, the most commonly studied trade-off is the trade-off between fecundity and size of offspring. This trade-off might represent only a different way of packaging resources, not necessarily a difference in energy allocation, but it is important, since it affects other aspects of demography, such as developmental time, age-dependent survivorship and dispersal (Jaeckle 1995, Pechenik 1999, Llodra 2002). This trade-off is apparent when comparing marine benthic invertebrate species that either produce many

small eggs resulting in larvae that feed in the plankton (planktotrophic) versus few large eggs resulting in larvae that feed on yolk in the egg (lecithotrophic). Mathematical models that relate reproductive energetic efficiency to egg size in marine benthic invertebrates taking into account planktonic mortality, developmental time and egg number were derived by Vance (1973) and extended by Christiansen and Fenchel (1979) and McEdward (1997). They proposed that only the extremes of egg sizes are favoured. Hence, highest fitness was obtained when fecundity was maximized (planktotrophic larvae) or when developmental rate was maximized (lecithotrophic larvae). The former strategy is favoured when food is abundant and mortality is low in the plankton, while the latter strategy is favoured in opposite conditions (Vance 1973, McEdward 2000).

Yet, the presence of intermediate sized eggs of planktotrophic larvae, facultative planktrophic larvae and also frequent transitions between larval modes indicate that also other factors need to be taken into account (McEdward 1997, 2000, Allen and Pernet 2007, Collin 2012). For example, if conditions are unpredictable, variance in fitness can be reduced in the long term via bet-hedging strategies that buffer stochastic events. These include putting higher investment into offspring (conservative bet-hedging) or increasing offspring variation (diversified bet-hedging) (Collin 2012, Marshall and Burgess 2015). Furthermore, events in one life-stage can affect fitness in another one, hence provisioning of eggs needs to optimize performance in larval, juvenile and adult stages (Marshall and Keough 2006). Accordingly, larger eggs of lecithotrophic larvae might increase juvenile fitness, not larval fitness (Armstrong and Lessios 2015). Einum and Fleming (2004) propose that small egg size in salmon serves to increase maternal, not offspring fitness. For that reason Roughgarden (1989) integrated the larval phase in the entire life cycle, hence including pre- and post-settlement selection and proposed five stable life cycles based on his model, including a typical planktotrophic and a lecithotrophic one (Havenhand 1995).

However, the selection pressures favouring one life-history strategy over another in marine invertebrates are not yet fully understood (Marshall *et al.* 2012). According to Stearns (1992), to understand variation in a life history trait we need to know i) the phenotypic and genotypic variation, ii) the effects on a population and iii) the developmental and phylogenetic constraints. One study by Armstrong and Lessios (2015) used reciprocal hybrid crosses of two echinoderm species with different modes of development to disentangle the impacts of maternal investment and hormonal and genetic regulation on the mode of development. Another study by Collin (2001) investigated the consequences of different modes of development on population dynamics, i.e. gene flow, population structure and species distribution, in congeneric *Crepidula* species of the same geographical range using genetic markers. The advantage of using true poecilogonous species instead of differentiated or even sibling species to investigate variation in life-history traits is, however, that developmental and phylogenetic constraints can be disregarded (Knott and McHugh 2012).

1.3 Environmental impact and natural selection (CAUSES)

The evolution of phenotypic and genotypic variation of an organism and its life history is affected by the environment they experience, including abiotic (temperature, food, space, precipitation, etc.) and biotic factors (competition, predation, parasites, diseases). The performance of a population in an environmental gradient is described by its tolerance range, which is usually illustrated as a bell-shaped curve. Populations with broad ranges of medium performance are referred to as generalists, while populations with narrow ranges of high performance are specialists (Fig. 2). The tolerance range can be categorized according to the ecological performance of the organisms. At the optimum, organisms are able to grow and reproduce and hence maintain the population, while further to the edge of the tolerance range organisms are only able to survive, and even further they can only survive for a limited amount of time (Ricklefs 2001, Frederich and Pörtner 2000).

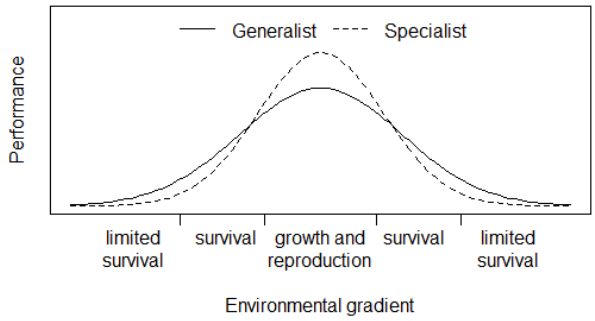

FIGURE 2 Tolerance curve displaying the performance of a population in a specific environmental gradient (according to Ricklefs 2001).

Provided that populations show variability in phenotypes and that phenotypes have a genetic basis, they are subject to natural selection that can change the tolerance range of a population (adaptation) to increase the mean individual fitness via changes in the genetic composition of the population. Depending on the environmental conditions, selection can be directional, balancing or disruptive; furthermore, it can be frequency- or density-dependent (Hamilton 2009). In species with complex life cycles, such as marine invertebrates with planktonic larvae, different life stages occupy different niches and are hence subject to different selection pressures, pre- and post-settlement selection. To optimize performance of both life stages, uncoupled evolution of both phases is necessary (Wray 1995).

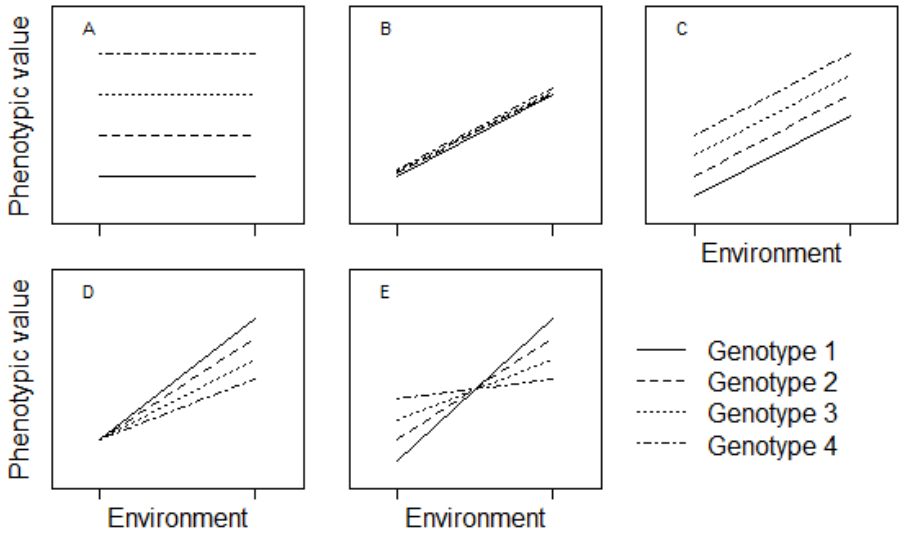

FIGURE 3 Reaction norms displaying the phenotype produced by a particular genotype in different environments. The different effects of genotype (A) and environment (B) as well as an additive effect of both (C) and their interactions (D, E) are illustrated according to Hamilton (2009).

Reaction norms describe the relationship between a phenotypic trait produced by a particular genotype and how it varies in response to variation in the environment. They are commonly used in quantitative genetics to illustrate the contribution of the environment and genotype to a particular phenotypic trait. Hereby, different genotypes can lead to different phenotypes regardless of environmental influences, which is called genetic polymorphism (Fig. 3A). In contrast, if the trait is environmentally controlled, different genotypes could lead to the same phenotype that shows similar changes according to the environment (Fig. 3B). More commonly, genotypic and environmental effects can be additive (Fig. 3C) or show interactions. In the latter case genotypes can differ in phenotypic variance (Fig. 3D) or the rank order of phenotypic values can change ranks across environments (Fig. 3E).

Genotype-by-environment interactions are also called phenotypic plasticity, or in case of discrete traits, polyphenism (Agrawal 2001, Hamilton 2009, Forsman 2015). Phenotypic plasticity can be divided into developmental plasticity and phenotypic flexibility. Developmental plasticity describes irreversible phenotypic variation due to developmental alterations, and is hence quite similar to genetic polymorphism. Phenotypic flexibility, in contrast, refers to reversible intra-individual plasticity in physiology, morphology or life history traits (Forsman 2015). Hence, mapping a genotype on a phenotype, i.e. disentangling genetic and environmental impacts, is difficult, since a single genotype can produce different phenotypes, and similarly, several genotypes can result in the same phenotype (Pigliucci 2010, Braendle *et al.* 2011). The interpretation of genotypic contribution is furthermore hampered because

reaction norms, although often displayed as such, are probably non-linear (DeWitt 2016). Furthermore, Pigliucci (2010) criticises that the genotype is only one of several factors determining the phenotype and proposes to also investigate RNA folding, protein functioning and gene networks.

Genetic polymorphism (local adaptation) or phenotypic plasticity can be responsible for variation in phenotypic traits. Which one will be favoured by selection depends on the benefits and costs of both, environmental heterogeneity, reliability of potential environmental cues, and a genetic basis of plasticity (Berrigan and Scheiner 2004). The advantage of plasticity is that an organism can thrive in a broader range of environments, yet there are probably costs to it, as a plastic organism rarely achieves the same performance level as a specialist at that range. Costs of plasticity can supposedly arise due to the maintenance of sensory structures to gather information about the environment and the process of plasticity itself as well as the genetic architecture (DeWitt *et al.* 1998). However, different studies propose that costs are rarely observed and limits to plasticity might rather be set by the unpredictability /unreliability of the environment, not enough time to adapt, or simply developmental constraints (Berrigan and Scheiner 2004). Also evolutionary transitions between monomorphism, polyphenism and genetic polymorphism could happen through multiple routes (Schwander and Leimar 2011). Plasticity could be the initial state, and only later be fixed as a genetic polymorphism. Likewise, a genetic cue for variants could be exchanged for an environmental one (Leimar and Schwander 2011).

In stable environments genetic polymorphism will be favoured (Leimar 2009). Costs of plasticity, environmental fluctuations that occur too quickly for an organism to detect or respond to, as well as environmental unpredictability decrease the probability that plasticity will evolve. For example, a model by Reed *et al.* (2010) predicted that populations with strong plasticity encounter higher extinction probabilities when no reliable cues were present, while population size could be buffered in variable environments when cues were reliable. Quantitative genetic models with continuous environmental variation proposed that spatial environmental heterogeneity in general leads to genetic polymorphism, while temporal fluctuations, within and between generations leads to plasticity. However, discrepancies between models exist. In a metapopulation, with high migration rates, spatial heterogeneity might be translated into temporal heterogeneity, and hence favour plasticity as well (Berrigan and Scheiner 2004). Predictability of environmental fluctuations favours plasticity due to the genetic load of fixed polymorphisms (Leimar 2009). However, there is an ongoing discussion about whether phenotypic plasticity has a genetic basis and is, hence, the target of selection or whether the different phenotypes are under selection and plasticity is just a by-product (Windig *et al.* 2004).

In respect to poecilogony, genetic polymorphisms as well as environmentally cued polyphenisms are known as underlying mechanisms resulting in the variation in developmental mode observed within species (Collin 2012, Knott and McHugh 2012). According to mating experiments

poecilogony in *Streblospio benedicti* is based on a genetic polymorphism (Levin *et al.* 1991, Levin and Bridges 1994). Comparative transcriptome analyses of *S. benedicti* with lecithotrophic and planktotrophic developmental modes, however, revealed recent gene flow between them and suggest that the genetic basis of poecilogony in *S. benedicti* is either due to differences at developmentally important loci or modest allele frequency differences at many loci, or alternatively, that recurrent ecological diversification has occurred (Zakas and Rockman 2015). A well-studied example of polyphenism as a mechanism of poecilogony is represented by the sea slug *Alderia willowi* (Krug *et al.* 2012). In this case, temperature and salinity are the cues that in some populations trigger the onset of planktotrophic clutches in winter by the same females that produce only lecithotrophic ones in summer. Since planktotrophic larvae lose their dispersal advantage in closed systems and the higher survival chances of lecithotrophic larvae prevail, this plasticity serves as an adaptation to the seasonal cycle of estuary opening and closing (Krug *et al.* 2012). Developmental mode can also be influenced by the phenotype or environment of the parents (parental effects) as shown for *Polydora cornuta* where sperm limitation leads to the production of lecithothrophic larvae (Badyaev and Uller 2009, Rice and Rice 2009). Diversified bet-hedging might be represented by the different types of larvae emerging from the same egg capsule in *B. proboscidea* (Gibson 1997, Oyarzun and Strathmann 2011).

1.4 Population ecology and population genetics (CONSEQUENCES)

The life history of an organism is directly linked to the dynamics of a population, since population growth is determined by the age-specific birth and mortality rates as well as the population´s age structure. Incorporating only these parameters would result into an exponential or geometric growth rate depending on whether reproduction occurs continuously or is restricted to certain periods in the year (Ricklefs 2001). The growth rate is, however, also subject to environmental impacts, both abiotic and biotic. These are categorized into density-dependent and density-independent factors. The availability of density-dependent resources decreases with increasing densities due to competition, e.g. food, area or hiding places. Moreover, at high densities diseases and parasites are easily spread and predators are attracted. Hence, population growth is self-limited resulting in logistic growth curves rather than exponential ones, and it approaches zero when the maximum number of individuals that can be supported under these environmental conditions is reached, i.e. the carrying capacity (Ricklefs 2001). Cyclic or chaotic oscillations in population size can also be observed as a result of time delays between the response of birth and mortality rates to changes in the environment. Density-independent factors including temperature, precipitation, or catastrophes such

as storms or sudden freeze do not regulate population growth but can influence it and decrease population size below its carrying capacity. Environmental conditions can vary temporally and spatially as well as be predictable or stochastic. For example, seasonal changes in temperature and food availability are predictable temporal fluctuations that have great impact on the timing of reproduction. Sudden rain storms or other catastrophes, on the other hand, are rather unpredictable (Ricklefs 2001).

Particularly important for populations of marine invertebrates is, however, that they resemble open populations. Due to the great dispersal potential of planktonic larvae and high connectivity of marine habitats, immigration and emigration also play an important role in local population dynamics (Gaines and Lafferty 1995). Consequently, marine invertebrate populations should rather be viewed as metapopulations that are composed of local subpopulations as done by Roughgarden and Iwasa (1986). The dynamics of metapopulations are determined by local extinction and recolonization of empty habitat patches (Levins 1970, Hanski 1998). Of further importance is the spatial heterogeneity of landscapes leading to habitat patches of different size, quality, and connectivity to other patches. Large, high quality patches can support large populations that in turn can serve as sources of individuals to smaller populations on poor quality patches and hence prevent them from potential extinction (Pulliam 1988, Ricklefs 2001). The concept and assessment of metapopulations, however, is more studied with terrestrial populations since there arise several problems when dealing with marine populations. Firstly, the large population sizes and high dispersal potential during the larval stage leads to weak genetic structure. Additionally, marine metapopulations occur on large spatial scales making it impossible to sample every subpopulation and they are subject to extreme temporal variability which can lead to stochastic migration patterns (Gaggiotti 2017). Gaggiotti (2017) suggested an integrated approach including genetic data, microchemical fingerprinting of larvae and biophysical modelling of larval dispersal using a Bayesian framework to solve these issues.

As mentioned earlier, complex life-cycles of marine invertebrates imply that different life stages are subject to different selection pressures, and that events in one life stage can affect performance in other life stages. Hence, population dynamics might be predicted more appropriately by models taking into account specific processes occurring in the benthos (competition during settlement, reproduction, benthic mortality) and the water column (dispersal via diffusion and advection, larval mortality) (Eckman 1996, Possingham and Roughgarden 1990). For example, considering that larval mortality is about 14 % higher than post-larval mortality for marine invertebrates in the inner Danish waters (Pedersen *et al.* 2008), a combinatorial approach might be needed.

Population structure and dynamics can be analysed via life tables that describe fecundity and survival rates at certain ages, but also via allele frequencies of genetic loci in the population. For example, with such data we can gain information about migration and differentiation between populations. High connectivity and migration rates between subpopulations that translate into gene flow will lead to homogeneity in allele frequencies between these

subpopulations. In contrast, limited gene flow due to a geographical, temporal or behavioural barrier can result into population structure, i.e. heterogeneity in allele frequencies between subpopulations. Such population differentiation can be caused by natural selection or random effects. Genetic drift describes the random change in allele frequency from one generation to the next and will eventually lead to the fixation/loss of one allele and a change in genotype frequency. Genetic drift resembles a sampling effect and is dependent on the number of individuals contributing gametes to the next generation, the effective population size. Hence, the effect of genetic drift is more pronounced in small populations. The founder effect, when a small number of individuals establish a new population, can lead to population differentiation for the same reason (Hamilton 2009).

Migration and subsequent gene flow between populations of marine invertebrates can occur via drifting of adult specimens but mainly occurs during the larval stage. The dispersal potential of larvae has been described as proportional to the time the larvae spend in the plankton (Weersing and Toonen 2009). Accordingly, subpopulations with planktotrophic larvae are expected to show high gene flow leading to homogenous allele frequencies between subpopulations and low fluctuations in population density, while subpopulations with lecithotrophic larvae are expected to be genetically differentiated due to lack of dispersal (Havenhand 1995, Eckert 2003). Based on their models, Palmer and Strathmann (1981) proposed that population growth increases with distance of larval dispersal, although the increase slows until reaching an asymptote with increasing dispersal distances. Moreover, using population genetics, a discrepancy between potential and realized dispersal was detected (Gaines and Lafferty 1995, Weersing and Toonen 2009). Weersing and Toonen (2009) suggested that there might not be a correlation between pelagic larval duration and population differentiation, because the time spent in the plankton can be a plastic trait and dispersal is also dependent on mesoscale oceanographic currents (Weersing and Toonen 2009). Moreover, larvae can detect environmental cues and actively position themselves in the water column, so that local recruitment to a favourable habitat might be preferred (Strathmann *et al.* 2002).

Population genetic models for metapopulations, additionally include the probability of subpopulations to go extinct and become re-colonized. Although there are different types of metapopulations, in general, depending on the mixture of larvae from different subpopulations re-colonization events can either decrease or increase differentiation between subpopulations (Harrison and Hastings 1996, Hamilton 2009). When larvae are not randomly mixed, i.e. when there is collective dispersal, a founder effect can be introduced, increasing differentiation between subpopulations (Broquet *et al.* 2013). Likewise, population structure can arise due to sweepstakes reproductive success (SRS), when variation of reproductive success in highly fecund organisms leads to only a small subsample of the population contributing to the next generation. This variation can be a result of stochastic oceanographic processes acting on fertilization, spawning, larval survival and settlement (Hedgecock 1994). Both

effects, collective dispersal and SRS, can lead to chaotic genetic patchiness (CGP), which describes temporal and spatial population structure and differentiation on small scales where dispersal should enable gene flow and homogenization (Johnson and Black 1982).

In respect to poecilogonous species, although different life history traits were observed, the population dynamics of the spionid *Streblospio benedicti* were similar regardless whether planktotrophic or lecithotrophic larvae were predominant (Levin *et al.* 1987, Levin and Huggett 1990). Higher larval and juvenile survivorship of lecithotrophic larvae was counterbalanced by higher fecundity of individuals with planktotrophic larvae. Moreover, sites dominated by lecithotrophic larvae had higher densities, while planktotrophic larvae had higher colonization abilities, and hence, were more common among disturbed areas (Levin *et al.* 1987, Levin and Huggett 1990). Planktotrophic larvae are typical for opportunistic species and might be advantageous in temporally varying environments to dampen population fluctuations and re-colonize empty habitat patches, but are also characterized by high mortality and poor competitive ability (Thorson 1950, McEdward 2000, Marshall and Burgess 2015). Lecithothrophic larvae, in contrast, might be advantageous in environments with high predation in the plankton and spatially heterogeneous habitat quality, to ensure larvae are recruited locally into favorable habitats (Pechenik 1999). The evolution of phenotypic polymorphisms might be promoted by environmental heterogeneity (Chia *et al.* 1996). Yet, a dispersal polymorphism, as observed in *S. benedicti*, might simply be the result of asymmetric dispersal commonly present in metapopulations due to their source-sink dynamics (Zakas and Hall 2012). Regardless of the underlying evolutionary and mechanistic basis, polymorphisms show similar effects on population dynamics such as increased niche breadth and colonization ability as well as decreased population fluctuations and vulnerability to environmental change. However, similar effects due to developmental plasticity, genetic polymorphism and bet-hedging are achieved via different mechanisms and based on certain assumptions, e.g. that plasticity has a genetic basis (Wennersten and Forsman 2012).

1.5 Aims of the thesis

The ultimate goal of this thesis was to contribute to a better understanding of the selective processes that favour a certain type of larvae in a certain environment, and consequently, lead to the evolution of larval diversity in marine invertebrates (Strathmann 1985). The type of larvae is a result of the trade-off between size and number of offspring and, as an important life history trait, has major consequences on the dynamics of a population. Advantages of one type of larvae or the other have been attributed to higher dispersal potential and avoidance of predation (Pechenik 1999), but are likely more complex since, for example, a greater dispersal potential of planktotrophic larvae is not always

realized (Weersing and Toonen 2009). Studies investigating the presence of different modes of development in poecilogonous species have the advantage that they are not confounded by developmental and phylogenetic constraints as are studies comparing developmental modes between different species (Knott and McHugh 2012). Therefore, the focus of this thesis were populations of the poecilogonous spionid polychaete *Pygospio elegans* in the Danish Isefjord-Roskilde Fjord estuary complex, since these populations are known to produce different types of larvae (Rasmussen 1973, Kesäniemi *et al.* 2014a).

In order to understand the evolution of a certain mode of development and the consequences of developmental mode on a population level, the ecology of the species in its particular environment must be known. Hence, the aim of study I in the thesis was to describe the structure and dynamics of four geographically close populations, particularly focusing on their reproduction, including larval type. Furthermore, certain abiotic environmental parameters were documented and their impact on the population dynamics of *P. elegans* investigated.

Population structure and dynamics might not only be the result of environmental conditions. The plasticity of an organism´s response to environmental conditions can be genetically determined. Hence, different degrees of plasticity might lead to different underlying mechanisms of poecilogony. Accordingly, in the sea slug *Alderia willowi* poecilogony was attributed to an environmentally cued polyphenism, while in the polycheate *Streblospio benedicti* the variation in developmental mode is based on a genetic polymorphism (Levin *et al.* 1991, Krug 2007). An interaction between environmental and genetic impacts on poecilogony could also occur via epigenetic effects. The aim of study II was to investigate the spatial and temporal genetic structure of populations characterized in study I and to relate genetic structure to the previously observed population dynamics and environmental parameters. The results of study I and II were expected to help clarify the mechanisms and consequences of poecilogony in *P. elegans*.

The genetic diversity within a species but also the species diversity of the whole community affects the dynamics of a population. Diversity at both of these levels can in turn be influenced in a similar way by ecological and evolutionary processes due to the carrying capacity of the habitat or environmental conditions (Vellend 2003). The aim of study III was to characterize the species diversity of the benthic fauna community at the same locations where the population dynamics and genetic diversity of *P. elegans* were assessed previously. Furthermore, patterns among locations and across seasons were compared to determine whether genetic diversity of *P. elegans* and species diversity correlate and, if so, which environmental parameters appear to affect diversity measures on both levels. While specific to these populations and locations, this study contributes to a general lack of study of such species-genetic diversity correlations (SGDC) in marine environments.

Heterogeneous environments promote metapopulations, which in turn could maintain dispersal polymorphisms, such as poecilogony, due to assymmetric dispersal (Chia *et al.* 1996, Dias 1996, Zakas and Rockman 2015).

Estuaries are generally described as heterogeneous environments due to temporal (tidal, seasonal and stochastic) and spatial fluctuations in salinity as well as other environmental parameters such as sediment structure, dissolved oxygen content, and temperature (Kaiser *et al.* 2011, Whitfield *et al.* 2012). *Pygospio elegans* is a euryhaline species and common in estuaries, and populations from estuaries show variation in developmental mode more often than do populations of *P. elegans* from more stable environments (Rasmussen 1973, Gudmundsson 1985, Morgan 1997, Bolam 2004, Kesäniemi *et al.* 2014b). The aim of study IV was to investigate how *P. elegans* copes with salinity changes at different time scales, measuring physiological and ecological responses, to clarify whether salinity fluctuations might be related to variation in developmental mode. Understanding the consequences of salinity stress might help explain why poecilogony is more common in estuaries and whether it can be related to metapopulation dynamics.

2 MATERIALS AND METHODS

2.1 Study species *Pygospio elegans*

Pygospio elegans is a small tube-dwelling spionid polychaete that commonly occurs on intertidal mudflats in the circumboreal region. *Pygospio elegans* is very versatile in many respects: it occurs in densities from several hundred up to 600,000 individuals per m2 (Linke 1939, Morgan 1997); it exhibits wide habitat tolerances, and for example, is present in salinities down to 5 (Anger 1984). It also exhibits a variety of feeding strategies and can act as a deposit-, suspension- as well as filter-feeder (Fauchald and Jumars 1979). Most interesting for this study, however, is its variable life history (Söderström 1920, Hannerz 1956, Gudmundsson 1985, Rasmussen 1973, Kesäniemi *et al.* 2012b). Specimens of *P. elegans* can reproduce asexually by fragmenting into three to four pieces that subsequently remain in the sand tube and regenerate (Rasmussen 1953). Moreover, it was confirmed as a truly poecilogonous species via morphological and genetic analysis (Morgan 1997, Kesäniemi *et al.* 2012c). Females lay one egg string that is composed of several egg capsules within their tube and ventilate it (Fig. 4B). One egg capsule contains the eggs produced in one segment (Söderström 1920). Depending on the ratio of fertile eggs to nurse eggs, larvae will emerge as planktotrophic/planktonic or adelphophagic/benthic larvae (Fig. 4A). Planktonic larvae hatch at a 3 setiger stage from egg capsules that contain more than 10 fertile eggs and no or only few nurse eggs. They possess swimming setae and remain in the plankton for 4–5 weeks to feed before they settle at a size of 12–16 setigers (Fig. 4C, D). Benthic larvae, on the other hand, develop within the egg capsules that contain up to three fertile eggs and many nurse eggs until they hatch at 14–20 setiger stage and are immediately ready to settle (Fig. 4E). Also intermediate types of larvae have been described that spend short time in the plankton before settling (Söderström 1920, Hannerz 1956, Rasmussen 1973).

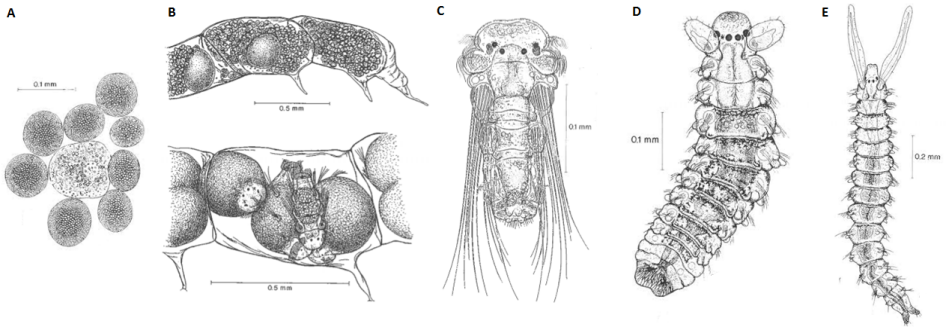

FIGURE 4 Drawings of egg capsules and larvae of *Pygospio elegans* by Rasmussen (1973): A) one fertile egg with nucleus and several nurse eggs. B) three egg capsules of an egg string showing benthic developmental mode with few fertile eggs and a typical hunchback shape of the embryos at a later stage, C) small pelagic larvae from plankton, D) pelagic larvae at the benthos after metamorphosis, E) benthic larvae shortly after hatching.

Other life history traits, such as maturity and longevity for populations of *P. elegans* with planktonic larvae were described by Anger *et al.* (1986). Larvae reproduce for the first time 15–17 weeks after hatching and mature individuals were usually larger than 35 setigers, and in most cases even larger than 45 setigers (Gudmundsson 1985, Anger *et al.* 1986). When specimens carry gametes, males and females are easily distinguished, but sex cannot be determined from live individuals at other times. In laboratory cultures the average life span of *P. elegans* was 9 months, however the oldest specimen survived for almost 2 years (Anger *et al.* 1986). Reproduction was described to be seasonal with sexual reproduction occurring from late autumn to early spring and asexual reproduction happening throughout the year but peaking in spring after sexual reproduction (Rasmussen 1973, Gudmundsson 1985, Bolam 2004). However, populations can differ in timing of reproduction with sexual reproduction also noted as occurring during summer (Söderström 1920, Hannerz 1956, Morgan 1997). Not only variation in timing but also differences in developmental mode could be observed even between geographically close populations (Morgan *et al.* 1999, Kesäniemi *et al.* 2014a). Accordingly, the population in Kiel Bight was solely maintained via asexual reproduction, while other populations showed no signs or only low degree of it (Anger 1977, Bolam 2004). Likewise, only benthic and intermediate larvae or only planktonic larvae could be observed in some populations, while others showed a seasonal switch from planktonic to benthic larvae or multiple types of larvae were present simultaneously (Anger 1977, Gudmundsson 1985, Morgan *et al.* 1999, Bolam 2004). Population genetic analyses of *P. elegans* revealed a pattern of isolation by distance from the Baltic to the North Sea and temporal variation (Kesäniemi *et al.* 2012c, Kesäniemi *et al.* 2014b).

2.2 Field survey

2.2.1 Isefjord-Roskilde Fjord complex and sampling scheme

The Isefjord-Roskilde Fjord estuary complex is the second largest estuary in Denmark (Fig. 5). Isefjord with its 280 km2 surface area and a mean depth of 7 m does not represent a typical estuary, as salinities are higher in the interior part than at the entrance or outside of the estuary because evaporation exceeds freshwater inflow. Its salinity is highest in winter and lowest in summer and it is mainly shaped by oceanic inflow, wind and river runoff. Roskilde Fjord, in contrast, shows the typical salinity gradient with higher salinities at the entrance compared to the interior part of the estuary and reaches its highest salinities during summer. It has a surface area of 117 km2 and consists of a shallow broad interior part (max. depth 6 m) and a long narrow upper part that connects it with Isefjord. Both estuaries are microtidal estuaries, with tidal ranges of < 20 cm, but wind-driven changes in water levels can be as large as 100 cm (Rasmussen 1973).

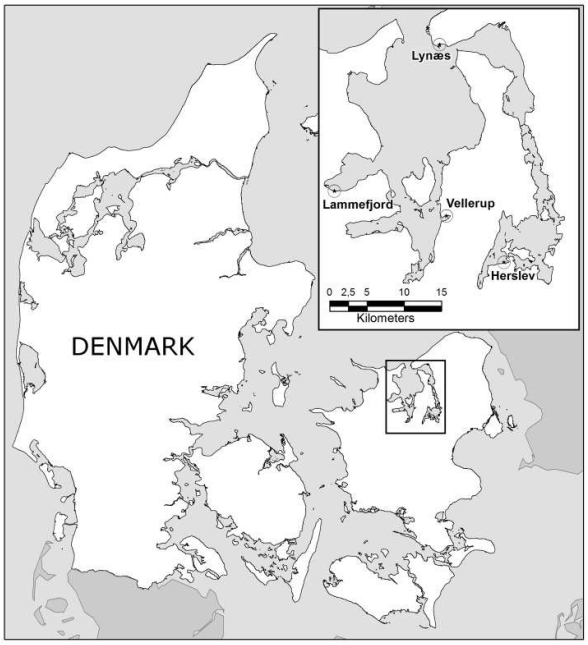

FIGURE 5 Location of the four sampling sites in Isefjord-Roskilde-Fjord estuary complex.

Pygospio elegans was described to reproduce asexually as well as produce benthic and planktonic larvae either seasonally or simultaneously in the Danish Isefjord-Roskilde Fjord estuary complex (Rasmussen 1973). Its population genetic structure in this estuary was described as patchy with temporal

variation (Kesäniemi *et al.* 2014a, b). For that reason, a field survey was conducted from March 2014 until February 2015 at four sites within the estuary complex including Lynæs located at the entrance, Lammefjord and Vellerup within Isefjord, and Herslev within Roskilde Fjord. Different sampling schemes were applied for the different parameters measured. Salinity and temperature were measured continuously, sediment characteristics and macrofauna were determined in March, May, August and November, population dynamics of *P. elegans* were monitored once per month and allele frequencies within *P. elegans* populations were analyzed from samples in March, May, August, October, November and February.

2.2.2 Abiotic environmental parameters (I+III)

The impacts of the environmental parameters salinity, temperature and sediment, including structure and nutrients were investigated, since these were described as important abiotic parameters that shape the distribution of species in estuaries (Kaiser *et al.* 2011). Salinity and temperature at all four sites were documented every 10 minutes via data loggers (HOBO U24-002-C salinity logger, 100–55,000µS cm-1). Reference seawater samples were taken monthly to determine salinity with a salinometer to account for potential drift of the loggers. Sediment characteristics, including water content and porosity, organic content, carbon and nitrogen content, and median particle size and sorting were determined from three replicate kajak cores (5 cm diameter, at least 15 cm length). For that purpose the top 1 cm of the cores was pooled and mixed and sediment characteristics were determined in three analytical replicates except for particle size. Water content and porosity were derived from the wet weight and dry weight (24h at 105° C) of 5 cm3 sediment. Organic content [%] was determined from the dried sediment via loss on ignition (2h at 550° C). Carbon and Nitrogen content was measured in dried and ground as well as pre-combusted and ground sediment samples (30–50 mg) to account for high amounts of shells using an element analyzer (Flash 2000 NCS-Analyzer and FlashEA® 1112 CHNO Analyzer, Thermo Scientific). Particle size was determined to calculate the median particle size and sorting of the sediment (Gray and Elliott 2009). For that purpose the proportion of the dry weight of each size fraction of the Wentworth size scale (8 mm, 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, 0.063 mm) was determined for 50–150 g wet sediment.

2.2.3 Species diversity (III)

Species distributions are furthermore affected by interactions with the biotic environment via intra- and interspecific competition, predation, parasites and diseases. The number of species, i.e. species richness, of a habitat can give valuable information in that respect. Species richness can hereby be measured at different spatial levels. Alpha diversity describes the number of species in a small homogeneous habitat, gamma diversity in all habitats of a geographic area and beta diversity describes the difference in species from one habitat to

another. However, comparisons of species richness can be problematic since species richness can be standardized to area or individuals (Gotelli and Colwell 2001). Standardizing species richness to area results in species density and somewhat assumes that species occur in similar densities, which is obviously not the case. When standardizing species richness to number of individuals more taxa will be detected the more individuals are sampled, with species richness eventually reaching an asymptote. To compare species richness among samples differing in total number of individuals sampled, rarefaction methods can be applied, which randomly draw equal sized subsamples from the total sample (Gotelli and Colwell 2001). However, species richness neglects the fact that also abundance of a species plays an important role for its function in the community (Ricklefs 2001). Different diversity indices, e.g. Shannon-Wiener or Simpson´s index, include both species richness and abundance, such that common species contribute to diversity more than rare ones. Hereby, evenness describes how equal the distribution of sampled individuals is among the different species, i.e. J´ = 1, when individuals are equally distributed among species and J´ will be close to zero when almost all individuals belong to one species.

For study III macrofauna were collected from three replicate sediment cores by sieving them with a 1 mm mesh. The material that remained on the sieve was fixed with 5 % buffered formaldehyde and sorted in the laboratory. Species were identified according to Barnes (1994) and Hayward and Ryland (1995). Subsequently, alpha-diversity was calculated to compare environmental influence on species and allelic richness. Furthermore, Shannon-Wiener index and Pielou´s evenness were calculated with PRIMER-E v. 6.1.13 according to the following formulas:

Shannon-Wiener index: $H' = -\sum P_i \log_e (P_i)$,
P_i - proportion of individuals of i^{th} species from total number

Pielou´s evenness: $J' = H' / \log_e S$,
S - species richness

From these same samples also the density of *Pygospio elegans* for study I was derived.

2.2.4 Population ecology (I+II)

Population dynamics of *P. elegans* were analyzed by determining their size, gender, reproductive activity as well as mode of development from samples collected monthly. For that purpose, surface sediment was collected randomly, sieved with a 1 mm mesh and sand tubes typical for *P. elegans* were collected. Gender and reproductive activity were determined from a subsample of at least 50 individuals. Males can be recognized by an additional pair of soft appendages at the second setiger and sperm in the coelom (Fig. 6A), while females carry eggs in the coelom (Fig. 6B). When several small individuals with

regenerating ends were found in the same tube it was noted as asexual reproduction (Fig. 6C). When egg strings were found in the sand tubes, the mode of development of larvae was categorized according to the number of larvae in the capsules into benthic (1–3) (Fig. 6D), intermediate (4–10) or planktonic (>10) (Fig. 6E) (Kesäniemi *et al.* 2012b). A size distribution of the population was obtained by measuring the length from the eye to the beginning of the gills for at least 30 individuals (Fig. 6F). This procedure was preferred because many individuals lost their tails during sampling. The measurement was performed on narcotized specimens using a camera mounted on a dissecting microscope and the software NIS BR v. 4.2 (Nikon, RAMCON A/S Birkerød, DK). The size distributions were used to subsequently identify size cohorts using Bhattacharya´s method implemented in FiSatII (FAO-ICLARM Stock Assessment Tools II User´s guide, Bhattacharya 1967, see Fig. 6G).

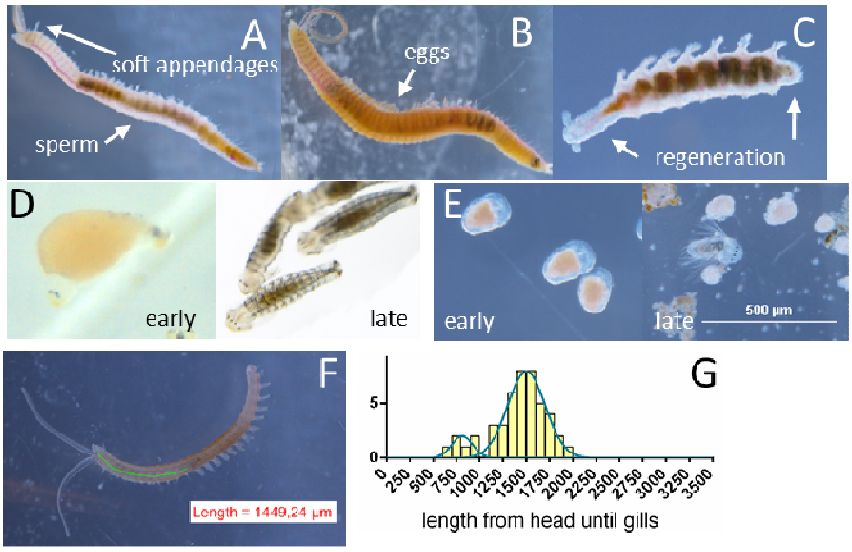

FIGURE 6 Specimens of *Pygospio elegans* were characterized according to the illustrated characteristics as male (A), female (B), performing asexual reproduction (C), producing benthic (D) or pelagic larvae (E). Size was determined from head until gills (F) and the size distributions used to determine average cohort size (G). Source of photographs: Anne Thonig.

2.2.5 Population genetics (II+III)

The genetic structure of *P. elegans* populations was investigated using microsatellite markers, which are useful to explore the most recent evolutionary changes in a population. DNA was extracted from complete specimens using the Qiagen DNeasy Blood & Tissue Kit. Subsequently, ten microsatellite loci were amplified using 1x Qiagen Multiplex PCR Master Mix. Fragments were separated on an ABI PRISM 3130xl and analysed with GeneMapper® v.5 Software (Applied Biosystems). Due to high percentage of missing data and null alleles in three of the loci, the population genetic analysis was continued with only seven loci Pe307, Pe385, Pe6, Pe19, Pe234, Pe294 and Pe369.

Descriptive measures of genetic diversity, such as observed and expected heterozygosity, gene diversity and FIS were calculated in Arlequin v.3.5.2 (Excoffier and Lischer 2010). Rarefied allelic richness was determined using Fstat v. 2.9.3.2 and HP-Rare v1.1 (Goudet 1995, Kalinowski 2005) and relatedness was calculated with the triadic likelihood estimator in Coancestry v.1 (Wang 2007, Wang 2011). The temporal and spatial population genetic structure was analysed using analysis of molecular variance (AMOVA) implemented in Arlequin v.3.5.2 (Excoffier and Lischer 2010). Furthermore, the differentiation between samples was calculated using the indices G´ST and Jost´s D with the R package diveRsity (Hedrick 2005, Jost 2008, Keenan *et al.* 2013). The number of genetic clusters present in the complete sample as well as assignment of individuals to the respective clusters was performed with three different programs: Structure v.2.3.4, InStruct and Flock (Pritchard *et al.* 2000, Gao *et al.* 2007, Duchesne and Turgeon 2012).

2.3 Salinity experiments (IV)

In order to investigate the effect of changes in salinity on *P. elegans* different physiological responses were measured after an acute exposure that more closely represents the fluctuating situation experienced in an estuary. Moreover, also ecological responses were examined after a long-term exposure to changed salinity that specimens occurring in the northern Baltic Sea might experience. Specimens for these experiments were collected in Herslev during summer 2015 and 2016. Hence, they originated from a salinity around 15 and were subsequently exposed to seawater of salinity 5 and 30. Acute exposures lasted for up to four hours and then changes in body volume, tissue water content and RNA expression of seven genes of interest in *P. elegans* were measured.

To document potential changes in body volume, a camera mounted on a dissection microscope combined with the respective NIS BR v. 4.2 software (Nikon, RAMCON A/S Birkerød, DK) was used to record a time lapse video of *P. elegans* after exposure. Additional measurements were taken once a day for the following week. For simplification, a cylindrical shape of the worms was assumed and hence the volume was calculated from measurements of length and width at the fifth setiger.

Tissue water content and RNA expression were measured 45 min and 4 hours after exposure. Tissue water content was determined as the percentage of weight loss between wet weight and dry weight (60°C for 2 hours) from a pool of about 30 specimens. For RNA expression RNA was extracted from whole specimens using Ambion RNaqueous Microkit and transcribed into cDNA using iScript cDNA synthesis Kit (BioRad). Primers for genes included in energy and amino acid metabolism, ion transport, cell signalling and construction of the cytoskeleton were designed based on transcriptome data (Heikkinen *et al.* 2017). Absolute RNA expression was measured using the

digital droplet PCR (ddPCR) technology, where the PCR mix is divided into an emulsion of several thousands of droplets that either contain no or a single or more copies of the template of interest. Amplification will lead to a positive fluorescence signal due to the incorporation of EVAGreen dye, so that the initial concentration of template of interest in the PCR mix can be derived from the ratio between positive and negative droplets.

For the long-term experiment, six groups of 30 specimens were exposed to salinity 5, 15, and 30. Three groups per treatment were sacrificed after 3 weeks, the rest was sampled after 6 weeks. Survival, mean length, and reproductive status including ripe individuals, presence of egg strings as well as signs of asexual reproduction were recorded.

2.4 Statistical analyses

2.4.1 Statistical techniques

Throughout these studies different statistical methods were applied including linear models and multivariate analyses. The basic concepts of both are explained in the following sections.

2.4.2 Linear models

Linear models (LM) are linear regressions with a single response variable and multiple predictor variables of the form:

$$y_i = a + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots + \beta_p x_{ip} + \varepsilon_i,$$

with y_i - value of Y of the i^{th} observation, x_{i1} - value of first predictor variable of the i^{th} observation, a and β - regression coefficients (intercept and slope), ε - residual error (Quinn and Keough 2002). The total variation is partitioned into variation explained by the regression coefficients (β's) and residual variation (ε). The significance of a regression coefficient can be evaluated by using F or t-statistics to test whether the coefficient is significantly different from zero. The importance of a predictor variable is then described by the p-value and the estimate of the respective regression coefficient. Hereby, predictor variables can be continuous or categorical. To include categorical predictor variables they need to be converted into numerical variables, so called indicator or dummy variables. For example, when including gender a dummy variable could encode all male individuals with 0 and all females with 1 or vice versa. Using dummy variables planned contrasts can be performed, which allow comparisons between groups that are of interest. Such comparison should be decided on (a priori) before data inspection.

Linear models assume normal distribution of the residual errors and therefore also of the response variable, as well as variance homogeneity

between treatments, and independence of observations (Quinn and Keough 2002). Whether a variable is normally distributed can be assessed via Shapiro or Kolmogorov-Smirnov tests or visually via QQ-plots. Variance homogeneity can be checked via Levene´s test or a residual plot, plotting fitted values against residuals. The probability distributions of certain types of data are, however, not expected to follow a normal distribution, such as count data or binary data. Count data, e.g. species abundances, are better described by a Poisson distribution where the mean equals the variance. If over-dispersion occurs and the variance is greater than the mean, e.g. when species distributions are patchy, then a negative binomial distribution describes count data best. For binary data, e.g. presence and absence data, the probability distribution resembles a logistic function. When the probability distribution of the response variable is not a normal distribution the models are termed generalized linear models (GLM). In GLMs the residual error will be modelled by the respective probability distribution. Moreover, a link function is needed that connects the response variable and the predictor variables. In the case of LMs this link function is an identity link function, for Poisson and negative binomial regressions it is a log link function and for logistic regressions a logit link function is needed.

Certain experimental designs do not allow for independence of observations, such as e.g. hierarchical sampling, time series or repeated measures. In generalized linear mixed models (GLMM) random factors can be included in addition to fixed factors to account for such lack of independence. The specific models used are described in more detail in the manuscripts.

2.4.3 Multivariate statistics

In contrast to linear models, multivariate statistics considers multiple response variables at the same time (Manly and Navarro Alberto 2016). Multivariate statistics distinguishes between R- and Q-mode, the former is based on the association between variables (covariances and correlations) including methods such as PCA. The latter is based on resemblance measure between objects including methods such as NMDS and cluster analysis. For this study, multivariate analyses based on resemblance matrices were used and were performed in PRIMER-E v.6 (Clarke and Gorley 2006). Resemblance matrices can describe similarities, dissimilarities or distances and are the basis for many analyses, however, since every response variable will be weighted equally, pre-treatment of the data is necessary in some cases. For example, variables that are on different scales, as is often the case for environmental parameters (temperature, organic content) need to be normalized (Clarke and Gorley 2006). Likewise, e.g. abundance data might need to be transformed to avoid the possibility that highly abundant taxa dominate the results (Clarke and Gorley 2006). Depending on the nature of the data different methods to create similarity or dissimilarity matrices exist. The most common ones are Euclidian distance, which describes a straight line between two points and is geometry based (e.g. environmental parameters, morphometric) and Bray-Curtis

dissimilarity, which is typically used for count data as it gives less meaning to zero abundances.

One way to visualize a resemblance matrix is by ordination using non-metric multi-dimensional scaling (NMDS). NMDS translates relative dissimilarities into relative distances between samples represented as points in a 2D space, i.e. similar samples are close together while different samples are far apart from each other (Clarke and Gorley 2006). To test whether predefined groups of samples differ from each other, the permutation based routine PERMANOVA can be applied. It performs an analysis of variance for several response variables using the resemblance matrix of the samples. For that purpose it partitions the total sums of squares into within and among group sums of squares to calculate the Pseudo F statistics. The distribution of the Pseudo F statistics is obtained via a permutation procedure and the p-value can be derived from it for a specific Null hypothesis (Clarke and Gorley 2006). Hence PERMANOVA does not assume normal distribution of the residuals, but independent and identical distribution of observations (the equivalent of the assumption of variance homogeneity in ANOVAs). While PERMANOVA tests the difference between groups, the routine SIMPER identifies which variables contribute to similarity within a defined group or dissimilarity between groups by decomposing the similarity/dissimilarity matrix (Clarke and Gorley 2006).

The focus of this study was to relate environmental parameters and population dynamics or species abundance. For that purpose the routine RELATE can be used, which measures the rank correlation (Spearman´s or Kendall) between all the elements of two resemblance matrices (one derived from the environmental parameters, the other one derived from the response variables, population dynamics in this case) (Clarke and Gorley 2006). This method is similar to a Mantel test that in contrast uses Pearson correlation. Note, RELATE gives a correlation not a cause-effect relationship between the two multivariate data sets. It is assumed, however, that the environment affects population dynamics and species abundances, hence, also a directional relationship can be investigated, e.g. using distance-based linear models (DistLM) and distance-based redundancy analysis (dbRDA). Similar to a regression, these routines aim to model the relationship between a matrix of predictor variables and a matrix of response variables via partitioning of variation (Clarke and Gorley 2006). Models including different predictor variables are tested and evaluated using various information criteria, such as Akaike or Bayesian information criteria (AIC, BIC). Constrained ordination via the dbRDA routine is used to visualize the variation explained by a specific model (scores) (Clarke and Gorley 2006). The overlaid vectors in a dbRDA plot indicate the importance (loadings) of the predictor variables (the longer the more important) and the relationship between predictor variables (rectangular vectors resemble independent predictor variables; vectors in opposite directions resemble predictor variables with opposite effects).

3 RESULTS AND DISCUSSION

3.1 Field survey

3.1.1 Aim of the field survey

Life history traits such as maturity, longevity, size and number of offspring, as well as number of broods can be genetically fixed due to selection or alternatively, be plastic. This study tried to disentangle the effects of genotype (3.1.2) and environment (3.1.3) on the population dynamics of the poecilogonous polychaete *P. elegans* (3.1.1) with special focus on developmental mode. For that purpose population ecology and genetics were correlated to several environmental parameters.

3.1.2 Population ecology (I)

Sexual reproduction of *P. elegans* occurred mainly from September until May in the Isefjord-Roskilde-Fjord estuary complex. During these times, the highest percentages of gravid females and ripe males were observed followed by the occurrence of egg strings one month later. In contrast, the lowest percentages of gravid females and ripe males were observed during summer, when egg strings were also absent. At Lynæs, Lammefjord and Vellerup two peaks of gravid females and ripe males were observed, while at Herslev only one plateau of sexual competent worms was detected. Additionally, at the three former mentioned sites a switch in developmental mode of the larvae within the egg strings was observed: mostly intermediate and benthic larvae were observed in spring 2014, whereas planktonic larvae were predominant in winter 2014/15. Additionally, benthic larvae occurred again at Vellerup in February 2015. At Herslev, in contrast, there was no switch in developmental mode and primarily benthic and intermediate larvae were found during the whole period. Asexual reproduction occurred throughout the year, but peaked slightly from April to June when sexual reproduction was declining. Similar patterns of sexual and asexual reproduction were observed by Rasmussen (1973, also in the same

estuary system), Gudmundsson (1985) and Bolam (2004), however, Söderström (1920) Gudmundsson (1985), and Morgan (1997), described sexual reproduction also occurring during summer. A seasonal switch in mode of development was described by Rasmussen (1973) in Horsens Fjord and Isefjord and by Gudmundsson (1985) in Blyth estuary (UK), whereas Armitage (1979), Morgan (1997), and Bolam (2004) observed only planktonic larvae year round and Gudmundsson (1985) only benthic ones at Cullercoats (UK). It is unclear whether *P. elegans* is truly iteroparous as suggested by Gudmundson (1985). During this study, gravid females were found within tubes that contained egg strings, however, it is possible that they switched tubes during the sampling and sorting procedure. Also, because the mode of development could be determined only from larvae within the egg strings, it remains uncertain whether a single female could switch the mode of development between broods.

Combining the density and cohort data of *P. elegans* observed in our study, recruitments of new individuals in spring and fall to the studied populations seems likely. At Lammefjord and Herslev new cohorts represented by smaller individuals appeared in spring and fall. At Lynæs the high percentage of asexual reproduction might have led to additional recruitments. In contrast, at Vellerup no cohorts could be distinguished; instead, small individuals seemed to reach adult sizes within a month. At Vellerup sampling of small specimens, however, might have been hampered due to the coarse sediment. Additionally, a mesh size of 1 mm was used, which was probably too coarse to sample the smallest specimens accurately (Gudmundsson 1985, Bolam 2004). Thus, according to the estimated growth rates, the arrival of new recruits might have been missed by about one month at every sampling site. Highest population densities were observed in May, except at Lynæs. No second density peak was observed in fall, which might be due to the fact that density was only quantified in March, May, August and November. The increases in density might be the result of overlapping cohorts: a new cohort arrived while the oldest cohort was still present (Beukema *et al.* 1999). Peaks in density in spring or fall were observed by Gudmundsson (1985) and Bolam (2004), but Morgan (1997) described a more stable population. In general, the densities observed in this study (75–7847 individuals m^{-2}) are in the range of other populations described in Denmark and the English Channel (Muus 1967, Morgan *et al.* 1999) but remain far below the values described at other sites, namely 50,000 to 500,000 individuals m^{-2} (Armitage 1979, Bolam 1999, Morgan *et al.* 1999).

Besides seasonal changes also differences between the sampling sites were observed. Vellerup and Herslev seemed to represent higher quality habitats, since at these sites large specimens (Vellerup: 1496–1848 µm, Herslev: 1343–1818 µm), relatively high densities (Vellerup: 132–7847 ind m^{-2}, Herslev: 189–4791 ind m^{-2}), high percentages of gravid females (maximum, Vellerup: 26 %, Herslev: 33 %) and ripe males (maximum, Vellerup: 33 %, Herslev: 42 %), and a high normalized number of egg strings (maximum, Vellerup: 0.28, Herslev: 0.44) were observed. In contrast, Lammefjord and Lynæs were characterized by small specimens (Lynæs: 1139–1731 µm , Lammefjord: 1074–1648 µm), lower

densities (Lynæs: 0–377 ind m^{-2}, Lammefjord: 75–4357 ind m^{-2}), and lower numbers of gravid females (Lynæs: 10 %, Lammefjord: 22 %), ripe males (Lynæs: 13 %, Lammefjord: 19 %) and egg strings (Lynæs: 0.09, Lammefjord: 0.12), hence, indicating poor habitat quality. This pattern was, however, not reflected in the growth rates of cohorts at the different sites: Lynæs: 3.31–6.41 µm d^{-1}, Lammefjord: 3.61–4.52 µm d^{-1}, Vellerup: 0.88 µm d^{-1}, Herslev: 1.52–4.20 µm d^{-1}. The discrepancy could be due to difficulties in distinguishing cohorts by size and different prevalences of asexual reproduction at the different sampling sites.

3.1.3 Population genetic structure (II)

The analysis of molecular variance (AMOVA) revealed that the population genetic structure of *P. elegans* shows spatial and temporal differentiation. Accordingly, the fixation indices GST and Jost´s D as well as the cluster analysis with the program Structure indicated that the populations at Lynæs and Lammefjord are genetically similar as well as the populations at Vellerup and Herslev, but that there is genetic structure between the two pairs of study sites. Moreover, seasonal genetic variation was observed in Lammefjord and Vellerup. Specimens sampled at Lammefjord in August and October differed genetically from specimens sampled during other months, and at Vellerup, worms differed in August, October and to some degree also in November. Likewise, highest allelic richness and expected heterozygosity as well as lowest relatedness were observed during August and October at all sampling sites, but these trends were most distinct at Lammefjord and Vellerup.

The change in the genetic composition of the populations at Lammefjord and Vellerup was associated with the arrival of small individuals in spring and fall noticed from the cohort data (see 3.1.1). When comparing genetic composition of the different cohorts identified at each site, it showed that cohorts differed genetically. At Herslev, cohort 2 and 3 differed significantly according to G_{ST}, at Lynæs cohort 3 differed significantly from all other cohorts, and at Lammefjord cohort 2 differed from the other cohorts (cohorts could not be defined based on size at Vellerup, see 3.1.1). These genetic differences are interesting, since they indicate a seasonal turnover of the populations due to the arrival of genetically differentiated cohorts and disappearance of older cohorts. Because there was little or no seasonal genetic differentiation at Lynæs and Herslev, recruitments at these sites might have been predominantly local or from genetically undifferentiated sites. At Lammefjord and Vellerup the arrival of new cohorts in fall 2014 (leading to genotyped adults sampled in November and February) could be the result of local recruitment from the egg strings laid in spring 2014 (by adults genotyped in March and May). Similarly, the spring recruitment 2014 (leading to genotyped adults sampled in August and October) might be the result from egg strings laid in winter 2013/14 (no samples or genetic data available). Since egg strings were observed from March until June at some sampling sites and new recruitments were registered from September on, this would result in a developmental time from egg capsule to juvenile of 2–

6 months. However, according to Anger *et al.* (1986), in the laboratory, planktonic larvae of *P. elegans* spend about 4–5 weeks in the plankton from hatching until settlement. Although not known with certainty, developmental time of benthic larvae is expected to be faster than that of planktonic larvae. Hence, the fall recruitment (leading to the genotyped adults sampled in November and February) more likely originate from egg strings laid in early fall 2014 (by adults genotyped in August and October). And the spring recruitment (leading to genotyped adults sampled in August and October) might emerge from egg strings laid in early spring 2014 (by adults genotyped in March and May). This would indicate that the spring and fall recruitment at Lammefjord and Vellerup occurred from one or more genetically differentiated sites located in Isefjord or even Kattegat since neither genetic cluster two persists through August and October nor cluster three persists through November and February. Interestingly, the genetically differentiated cohorts appear to be transient, but our sampling did not cover an additional recruitment event in spring 2015.

A pattern of chaotic genetic patchiness (CGP), when temporal and spatial variation is present even at small scales where dispersal should be able to homogenize the allele frequencies, was previously observed in this estuary complex by Kesäniemi *et al.* (2014a, b). In the present study, also seasonal variation in allele frequencies was observed that, furthermore, differed between sampling sites. Variance in reproductive success (Eldon *et al.* 2016) could be one explanation for the pattern of CGP observed for *P. elegans* in Isefjord-Roskilde-Fjord estuary complex. Hedgecock (1994) termed the process sweepstakes reproductive success (SRS), when only a small proportion of the population contributes to the next generation due to stochastic oceanic conditions. Indeed, at all study sites only 20–60 % of individuals in the populations produced gametes. Moreover, neither gravid females nor ripe males represent in equal proportions the genotypes present in the populations. For example, the genetic cluster dominating at Lynæs and Lammefjord in October and November, does not contribute to sexual reproduction. Such variance in reproductive success results in low effective population size and low genetic diversity of offspring per population. However, the effects of variation in reproductive success can be diminished if larvae from different populations are mixed during their dispersal phase. When larvae from the same population disperse together, a process termed collective dispersal, there can be reduced gene flow between occupied habitat patches and genetic bottlenecks when empty habitat patches are re-colonized, hence, leading to increased genetic diversity between populations (Broquet *et al.* 2013, Eldon *et al.* 2016).

Different cohorts were composed of different genotypes, which was very distinct at Lammefjord. Hence, not only different cohorts, but also different genotypes might explain the switch in mode of development observed at some sites. Indeed, the genetic composition of gravid females and ripe males showed a switch between March, October and February at some sites. Herslev, where predominantly benthic larvae were found, was dominated by the first genetic cluster. At Lynæs, Lammefjord, and Vellerup gravid females only or

predominantly belonged to the first and second genetic cluster from March until August, to the third genetic cluster in October and November, and from November on the number of gravid females belonging to the first or second genetic cluster increased again. Egg strings were dominated by benthic and intermediate larvae in spring and planktonic ones in fall and winter, while benthic ones reappeared in February at Vellerup. Although these genetic differences are suggestive, one has to be cautious when considering if there is a genetic basis of poecilogony of *P. elegans*. Firstly, the switch in developmental mode was observed mainly between breeding seasons (spring 2014 and fall 2015) and not between consecutive broods within a breeding season (fall 2015, spring 2016) since sampling was not conducted in spring 2016. Only a small proportion of benthic and intermediate larvae were detected in February 2015 at Vellerup. Secondly, developmental mode was not inferred directly from females genotyped but from all egg strings found in the sample and the genotypes of their parents are unknown. Unfortunately, as many individuals left their tubes during the sampling and sorting process, there were only a few instances when females were found together with their brood. Furthermore, Kesäniemi *et al.* (2012b) detected isolation by distance for *P. elegans* populations expressing different modes of development, rather than genetic differentiation among populations with different developmental modes, suggesting that different genotypes can produce the same phenotype and that gene flow exists among individuals with different developmental modes. In this study only seven microsatellite loci were used to genotype specimens, yet, a more elaborate study on *S. benedicti* using about 15,000 single nucleotide polymorphisms also revealed recent gene flow between populations with different modes of development (Zakas and Rockman 2015). Lastly, since the third genetic cluster arrived with a new cohort these specimens could have experienced different environmental conditions influencing their developmental mode directly or via epigenetic modifications (Kesäniemi *et al.* 2016).

Also other traits besides reproduction might differ among the three genetic clusters. Average size of specimens belonging to the second genetic cluster is generally small, even if the cluster is dominating the population. Additionally, this cluster shows a low number of gravid females, which in turn have smaller average sizes compared to individuals in the other clusters. Hence, maturity might occur earlier for these individuals and lead to lower total numbers of offspring produced, since number of egg capsules per egg string is related to the number of segments of the mother. Small average sizes and high percentage of asexual reproduction was observed among specimens at Lynæs and Lammefjord where the second genetic cluster predominated, perhaps indicating a prevalence of asexual reproduction in this cluster. Individuals assigned to the third genetic cluster on the other hand always exhibited large average sizes and numbers of gravid females. Specimens of the first genetic cluster showed generally large average sizes similar to those from the third genetic cluster, except at Lynæs and Lammefjord, where the second genetic

cluster dominated. Whether this is due to the abiotic conditions at the two sites or a result of competition between the two genetic clusters is unclear.

To conclude, high seasonal and spatial variation in allele frequencies were found, which are indicative of CGP. The seasonal genetic variation is in line with the turnover of genetically differentiated cohorts and could be a result of the short life span of *P. elegans* and SRS. Several traits such as density, size, reproductive activity and mode of development correlate with the identified genetic clusters. Whether this represents a correlation or causation should be investigated further using reciprocal transplant experiments or manipulative experiments.

3.1.4 Species diversity and macrofauna composition (III)

During the field survey, in total 51 benthic invertebrate taxa were observed. *Hydrobia* spp. was the most common taxon followed by Naididae (Tubifex), and the polychaetes *Hediste diversicolor* and *Pygospio elegans*, which were present in at least 38 out of 48 samples. In contrast, some species were very rare and found in only one sample (with only few individuals): *Sphaeroma serratum* (1), *Malacoceros fuliginosus* (1), *Pectinaria belgica* (1), *Idotea granulosa* (2), *Gibbula cineraria* (3), *Modiolula phaseolina* (4), *Glycera capitata* (4), *Parvicardium pinnulatum* (5), and *Gammarus locusta* (7). Of these rare species one was found at Herslev, one at Lammefjord, two at Lynæs, and six at Vellerup.

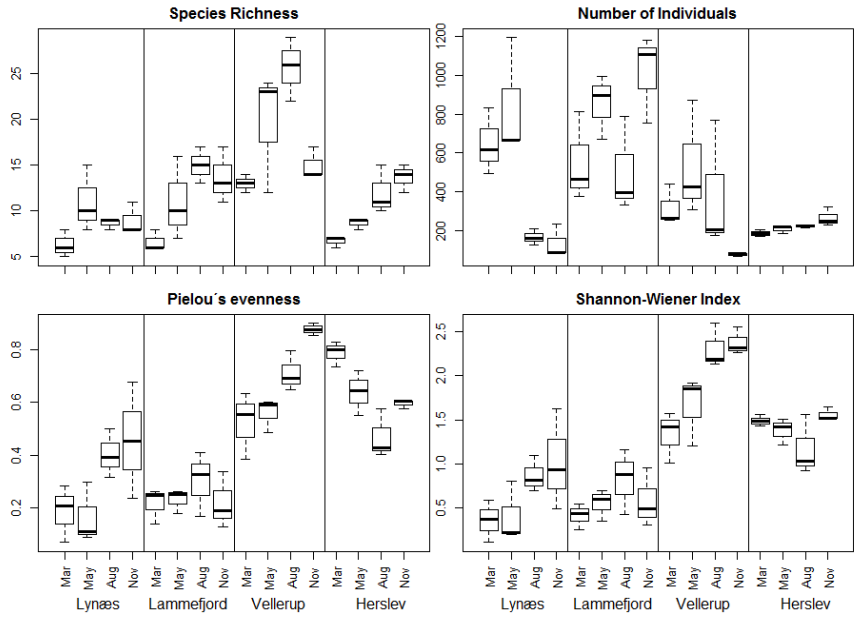

FIGURE 7 Different measures of species diversity averaged over three cores taken at four sites at times during the year 2014: Number of species (species richness), number of individuals, distribution of sampled individuals among the different species (Pielou's evenness), species diversity incorporating species richness and abundance (Shannon-Wiener Index).

Species richness was highest at Vellerup (Fig. 7), however, the functional importance of a species is not only defined by its presence but also by its abundance. The highest total number of individuals was observed at Lammefjord and in March and May at Lynæs (Fig. 7) due to the high abundance of *Hydrobia* spp. in those samples. When *Hydrobia* spp. is removed from the dataset, the highest total abundance was observed at Vellerup, followed by Herslev, Lammefjord and Lynæs. The high abundance of a single species, *Hydrobia* spp., leads to a low evenness and, thus, a low Shannon-Wiener index value at Lammefjord and in March and May at Lynæs (Fig. 7). In contrast, individuals were evenly distributed at Vellerup and Herslev, which resulted in a high Shannon-Wiener index value; higher for Vellerup than for Herslev due to the higher species richness. All diversity indices, species richness, abundance, evenness and Shannon-Wiener index, showed a significant interaction of site and time. Seasonally, the lowest species richness and Shannon-Wiener index was observed in March, while both increased during the year, peaking in August or November. An exception is Herslev, where evenness dropped from March until August and resulted in a decreasing Shannon-Wiener index. Most benthic invertebrates inhabiting mud flats in temperate climates are thought to reproduce in spring, with larvae subsequently settling in spring or summer. Thus, the number of newly arriving individuals would exceed the number of dying ones, leading to an increase in density in spring or sumer (Persson 1983, Beukema *et al.* 1999). Such a pattern was observed for *P. elegans* and for the species richness of the benthic community as a whole at Lynæs, Lammefjord and Vellerup. Note, however, that juveniles also can be transported via drift resulting in recruitments later on (Beukema *et al.* 1999).

In the present study species richness was standardized to area, making it a measure of species density (Gotelli and Colwell 2001). Using the sample-based and individual-scaled rarefaction method implemented in EstimateS v. 9.1.0, it was evaluated whether sufficient individuals were sampled per site and time point to determine species richness accurately. Accordingly, enough individuals were collected in half of our samples, since in these samples species richness reached the asymptotic phase. Five of our 16 samples nearly reached the asymptotic phase and three were still in the increasing phase of the rarefaction curves. Overall, sufficient individuals were collected for most of our samples to adequately estimate species richness. The samples in which not enough individuals were collected, leading to a poorer estimate of species richness originated mostly from Lynæs and Herslev.

Species diversity and abundance give information about the community, but to evaluate interactions between members of the community we need to know more about the biology and ecology of specific species and especially their life cycles. Species can have negative interactions due to predation, competition and parasitism but also positive interactions due to facilitation are possible (Gallagher *et al.* 1983, Thrush *et al.* 1992, Bruno *et al.* 2003). *Mytilus edulis*, for example, increases species richness by providing hard substrate and modifies species composition and abundance of the associated community

(Norling and Kautsky 2007). In respect to the composition of the total macrofauna, PERMANOVA revealed an interaction between site and time (p-value 0.001). Based on a NMDS plot, Herslev and Vellerup seemed to differ in macrofauna composition from each other while Lynæs and Lammefjord showed a composition intermediate to the previous sites and similar to each other. Furthermore, March and May look alike, while August differs slightly as does November, except for the sample from Lynæs. These apparent differences were, however, not significant based on pairwise comparisons between samples. SIMPER identified the taxa *Hydrobia* spp., Naididae, *Hediste diversicolor, Scoloplos armiger*, *Polydora* spp., Cardiidae, and *Pygospio elegans* to contribute mainly to the similarity within sites and time points. Lynæs and Lammefjord were thus characterized by high abundance of *Hydrobia* spp., Vellerup by high abundances of *S. armiger*, Naididae, as well as *Mytilus edulis* and Herslev by high abundance of *H. diversicolor*. The abundance of *Hydrobia* spp., Naididae and *P. elegans* was generally high in March and May, the abundance of Cardiidae was high in August, and in November high abundance of Cardiidae and *Polydora* spp. were observed. The focal species *P. elegans* showed highest abundance in May followed by March and lowest abundance in general at Lynæs.

Pygospio elegans lives at the sediment surface and can act as filter- or suspension feeder (Rasmussen 1973) and, as such might interact with bivalves and other polychaetes with similar habits. Bivalves can act as filter feeders, but also facultative surface deposit feeders such as *Macoma balthica* are known (Kube 1996). As such they act as competitors with *P. elegans* for food and space. The effects of filter feeders, including bivalves and epibenthic crustaceans, on larval stages of *P. elegans* are negative in a different way, namely they potentially prey on larvae (Kube and Powilleit 1997). In the present study the abundance of Cardiidae as well as *M. arenaria* and *M. balthica* increase during the year, and one could speculate, that the production of planktonic larvae by *P. elegans* might be a means to escape increased competition pressure, even though these species might feed also on the small larvae of *P. elegans*. *Mytilus edulis,* a suspension feeding bivalve, however, was also described as having a positive effect on *P. elegans* in areas with low suspended food supply by providing faeces that *P. elegans* can use as an extra food source (Kube and Powilleit 1997). In this study, *M. edulis* showed the highest abundance at Vellerup, where the highest species diversity and highest density of *P. elegans* were also observed. The total abundance of bivalves did not differ between sites, but gastropods were more abundant at Lynæs and Lammefjord due to *Hydrobia* spp. in particular. The high abundance of *Hydrobia* spp. is explained by their preference for fine sediment. They also represent deposit feeders that are grazing the sediment surface, and hence, might act as competitor for *P. elegans* at these sites (Newell 1965). Like bivalves, other polychaetes can act as competitors or predators on *P. elegans* adults and larvae. Due to their similar life style, other spionid polychaetes likely act as competitors for food. Furthermore, adult stages also may prey on larval stages (Dauer *et al.* 1981). In this study other small polychaetes or oligochaetes such as *Capitella* sp. and *S. armiger* or

Naididae did not seem to have a negative effect on *P. elegans*, since they were either present to a similar degree at all sites or showed highest abundance at the same site or time point as *P. elegans*. Larger polychaetes, such as Nereididae, and in particular *Alitta virens*, might also prey on *P. elegans* (Rasmussen 1973, Kube and Powilleit 1997). Yet, diversity and abundance of *P. elegans* was high at Herslev where *H. diversicolor* had its highest abundance. Predators of *P. elegans* also include shrimps and fish, which were not monitored during the survey. Muus (1967) described that plaice and flounder mainly feed on the tentacles and prostomia of *P. elegans*. The percentage of individuals regenerating prostomia, exluding the ones clearly performing asexual reproduction, was twice (6 %) as high at Lynæs than at the other sites (3 %). Yet, it remains difficult to draw any conclusions about the predation level at the different sites based on this data. Only large scale temporal and spatial changes were documented during the study while small scale fluctuations in macrofauna were not assessed. As an estimate for small scale spatial heterogeneity, i.e. patchiness, the coefficient of variance between the three replicates of each sample might be useful. Accordingly, no distinct differences in patchiness existed between sites or time points, and unfortunately, the assessment of short-term fluctuations was not possible.

It is unclear how much the dynamics of *P. elegans* are influenced by the species community due to competition and predation and how much the population dynamics of *P. elegans* and the other species in the benthic community respond similarly to common environmental impacts. For example, on the one hand, low density of *P. elegans* and low species diversity at Lynæs and Lammefjord could be a result of competition with *Hydrobia* spp.. On the other hand these two sites could have lower carrying capacities and/or higher predation levels or be more disturbed on a temporal scale, hence supporting lower diversities in general. Species-genetic diversity correlations (SGDC) investigate whether different levels of diversity (species diversity and genetic diversity) are affected by similar ecological and evolutionary processes (Vellend 2003). In the present study a positive correlation between species richness of the benthic community and allelic richness of *P. elegans* was found. Positive correlations are expected when the majority of the species in the community and the focal species exhibit similar life styles and hence probably are affected by the carrying capacity of the habitat and environmental conditions alike. Interactions between the focal species and the species community via facilitation or predation, in contrast, could lead to a negative correlation (Lamy *et al.* 2016). Both species and allelic richness were positively affected by temperature, whereas only species richness increased with coarser sediment structure (environmental parameters are described in detail in 3.1.3.2). The same seasonality of reproduction and larval recruitment common for temperate climates (Beukema *et al.* 1999) thus seems to underlie the diversity of *Pygospio elegans* and the benthic community in the Isefjord-Roskilde-Fjord estuary as well.

In summary, highest diversities were observed at Vellerup and Herslev, although a different composition of macrofauna was present. In contrast,

diversities were lower at Lynæs and Lammefjord exhibiting more similar species composition, most likely due to the presence of *Hydrobia* spp. An increase in diversity could be observed from March to August/November. Referring to the macrofauna composition, the samples in November differed from other time points, except at Lynæs, while the samples at other time points were similar. The biotic interactions between *P. elegans* and the benthic macrofauna are somewhat unclear, although according to the positive SGDC environmental parameters might have a greater impact on *P. elegans* dynamics than the benthic community.

3.1.5 Abiotic parameters and sediment characteristics (I)

Temperature

Temperature did not vary spatially, but showed a strong seasonal pattern. December until February were the coldest months, reaching a weekly minimum of -3 °C in December at Lynæs. Warmest months were July and August with a maximum weekly temperature of 28.6 °C in July at Lammefjord. Rasmussen (1973) described temperatures in Roskilde Fjord being higher than in Isefjord especially in stagnant water, but no such difference was apparent in this study.
Sexual reproduction occurred seasonally and might be induced at low temperatures. Ripe males and gravid females appeared at temperatures below 15 °C and sperm degenerated within males when temperatures rose from 5° to 18 °C (Rasmussen 1973). Likewise, Anger (1984) described higher rates of sexual reproduction at 5° and 12 °C compared to 18 °C. However, sexual reproduction in the field was also reported in summer (Söderström 1920, Gudmundsson 1985, Morgan 1997). According to observations of two peaks of ripe males and gravid females, sexual reproduction probably occurred in two batches at Lynæs, Lammefjord and Vellerup. Gudmundsson (1985) described *P. elegans* as iteroparous, reproducing more than once per lifetime. However, although several cohorts were present at the same time in the present study, usually only one cohort had an average size typical for mature individuals when sexual reproduction occurred. At Lynæs the first cohort that arrived (cohort 1) comprised gravid females only in March and cohort 3 in October, while no gravid females were present in cohort 2 and 4. At Lammefjord cohort 1 included gravid females in March and very few in October, cohort 2 included gravid females in October and cohort 3 in February. At Herslev gravid females were present in cohort 1 in March, in cohort 2 in low numbers in May, August, and November, and in cohort 3 in November and February. Hence, different cohorts might produce gametes at different times indicated by the two peaks of gravid females and ripe males. Yet, individuals were only assigned to cohorts for the months March, May, August, October, November and February, hence, no information about number of females in the different cohorts at other months is given here. Moreover, sexual reproduction might also be possible between cohorts, since females can store sperm in their receptacula seminis already at early ages, before they are sexually mature (Söderström 1920)

Salinity

Salinity showed both spatial and seasonal variation. The mean salinity at Lynæs, Lammefjord and Vellerup was 19–20, while it was distinctly lower at Herslev, about 14, due to its location in the innermost part of Roskilde Fjord. Seasonal fluctuations occurred as previously described by Rasmussen (1973), showing lower salinity in summer at the sites in Isefjord, while higher salinity at Herslev, in Roskilde Fjord. Similar trends were determined from the national monitoring program conducted in Roskilde Bredning and Ydrebredning in Isefjord (National Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment, NOVANA). Additionally, unpredictable short term fluctuations in salinity were observed at all sites, but fluctuations were twice as high at Lammefjord than at the other sites. This might be explained by the location of the data logger close to the entrance of Tuse Å, a larger freshwater input to Isefjord.

Although *P. elegans* represent a very euryhaline species, it was demonstrated that changes in salinity can affect fitness. Accordingly, time to maturity and production of egg strings might be delayed at very low (5) or full strength marine salinities (30) and could hence lead to fewer broods per season (see 3.2). Indeed, gravid females and ripe males occurred one month later at Herslev compared to the other sites (September instead of August), and this was the site with the lowest salinity. Additionally, only one plateau (from September to February) of gravid females and ripe males was present, and only benthic larvae were produced at Herslev. In contrast, two peaks of gravid females and ripe males (August/September and January/February) as well as a seasonal switch from planktonic larvae in winter to benthic ones in spring – although between different breeding seasons – was observed at the other sites. The production of benthic larvae might be more time consuming, since they remain longer in the egg capsules, and could possibly lead to an overlap of the two consecutive broods resulting in the observed plateau at Herslev. Populations only producing benthic larvae were described from low salinity habitats and populations producing only planktonic larvae were described from high salinity habitats, although exceptions also occurred (Gudmundsson 1985, Morgan 1997, Morgan *et al.* 1999, Bolam 2004, Kesäniemi *et al.* 2012b, 2014a, b). The production of benthic larvae that develop in the egg capsules until the size of 14 setigers might represent an adaptation to low salinities, since early life stages are described to be most sensitive to environmental stress in general (Kinne 1966). Similar adaptation to low salinity has been suggested for other polychaetes, for example, *Hediste limnicola*, a viviparous Nereididae that releases its larvae in the freshwater at a stage when larvae of *H. diversicolor* are already capable of osmoregulation (Oglesby 1965). However, it is unclear whether egg capsules prevent larvae from being exposed to low salinities, or rather serve to slow down water inflow, so that abrupt changes in the extra-capsular environment might take several hours to reach and impact the embryo (Pechenik 1983, Richmond and Woodin 1996). Reciprocal transplant

experiments would be necessary to confirm whether production of benthic larvae represents an adaptive response to low salinities. Since benthic larvae were observed during spring at all sampling sites regardless of their salinity, several different factors may act together to influence the developmental mode of *P. elegans*. Anger (1984) did not find a systematic change in developmental mode of *P. elegans* in response to temperature and salinity changes, and observed different types of larvae at only three instances. Dissolved oxygen content of the water could be another abiotic parameter of importance, since Kube and Powilleit (1997) found that *P. elegans* could survive moderate hypoxia but not severe anoxia.

Sediment structure

Spatial variation, but no clear seasonal trend, was observed in sediment structure. In general, Lynæs and Lammefjord had fine sediment, which correlated with high water content and porosity at these sites, and the sediment was moderately well or moderately sorted. At Vellerup and Herslev the sediment was coarse or medium grained, hence, water content and porosity were low, and it was only poor or moderately sorted. The medium grain size increased and water content decreased from May until November in Lynæs, Lammefjord, and Herslev, whereas at Vellerup medium grain size decreased and water content increased. The sediment changed somewhat over the year from March to November, becoming more poorly sorted at Lammefjord and Vellerup, and more moderately well sorted at Lynæs and Herslev. In the present survey, spatial and temporal fluctuations of sediment characteristics were analysed only on a large (site) scale. Sudden disturbances of the sediment that would represent unpredictable temporal changes were not registered. Furthermore, spatial differences within one site (patch scale) also were not analysed, since the different kajak cores were pooled.

Population ecology and genetics of *P. elegans* as well as species richness were affected by sediment structure, including median grain size, sorting, water content, and porosity. The medium to coarse grained sediments at Vellerup and Herslev supported the highest abundances of *P. elegans*, which additionally were largest and had highest numbers of gravid females, ripe males, and egg strings. Hempel (1957) and Armitage (1979) described that *P. elegans* prefers coarse sediment. Furthermore, Kube (1996) observed that high water content hampers the stability of unbranched burrows of the related, but much larger, polychaete *Marenzelleria viridis*, which might also be true for *P. elegans*. The coarser sediment at Vellerup and Herslev also supported higher species richness, species diversity, and when excluding *Hydrobia* spp., also total abundance of individuals. Coarse-grained sediment might provide more microhabitats, and hence allow for higher species diversities and abundance (Kaiser *et al.* 2011). Furthermore, the genetic structure of *P. elegans* correlated to some degree with sediment structure. While the first genetic cluster was more prevalent in coarse and poorly sorted sediment, as in Vellerup and Herslev, the

second genetic cluster was more common in fine and well sorted sediment, as found at Lynæs and Lammefjord.

Food supply

In general, the organic content of the sediment was highest at Lynæs, followed by Lammefjord, Vellerup and Herslev. However, the C/N content, which gives information about the bioavailability of carbon, indicated that the most nutritionally valuable material was present at Lynæs, followed by Herslev and Lammefjord, while very refractory material was found at Vellerup. Over the period of the survey, the organic content increased at Lammefjord and Herslev, but decreased at Lynæs and fluctuated at Vellerup. The C/N ratio followed the decreasing trend of water content at Lynæs, Lammefjord and Herslev, while it fluctuated at Vellerup similarly to the organic content.

In general, benthic invertebrates can be predatory, deposit feeders that feed on benthic diatoms and microorganisms in the sediment, or suspension feeders that consume the phytoplankton suspended in the water column. *Pygospio elegans* can thrive as both a deposit and suspension feeder (Fauchald and Jumars 1979). Since the highest densities and largest specimens of *P. elegans* were observed at sites with the lowest organic content and most refractory C/N ratio, it seems that suspension feeding may be preferred over deposit feeding or can support larger populations. A similar pattern was shown for other spionid polychaetes (Dauer *et al.* 1981, Kube 1996). Moreover, Kube and Powilleit (1997) detected a correlation of *P. elegans* abundance with chlorophyll concentration in the water column. According to the Danish national monitoring survey, a peak in chlorophyll content was observed in mid February 2014 in Isefjord and 3–4 weeks later in Roskilde Fjord. Only a small increase in chlorophyll content was observed in August at both locations simultaneously (National Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment, NOVANA). These typical spring and autumn phytoplankton blooms were not reflected in the trends of organic content of the sediment, since in the present study near shore sites were surveyed, which are affected less by sedimentation and more by local processes, including inputs of organic matter from benthic sources (benthic microalgae and macrophytes). Organic matter at deeper stations would likely be more reflective of the patterns in the water column chlorophyll monitored during the national survey. Other species at these sites might provide insights into the organic matter dynamics. Since *M. arenaria* is an obligatory filter feeder and *M. balthica* a facultative deposit feeder (Kamermans 1994), Kube (1996) suggested that the presence of *M. arenaria* might indicate the presence of phytoplankton sources while the presence of *M. balthica* indicates prevalence of benthic diatoms. In the present study *M. arenaria* had high abundance at Lynæs and Herslev, whereas *M. balthica* dominated at Vellerup. Hence, the high abundance and large size of *P. elegans* at Vellerup and Herslev cannot be related to one type of food supply in this study.

In general, the seasonal dynamics of *P. elegans* are largely impacted by temperature. In contrast, organic content and C/N did not affect population

dynamics, which might not be surprising since the differences in organic content and C/N were quite modest. Sediment structure correlated strongly with abundance, size and reproductive activity. The heterogeneity in habitat quality might support source and sink dynamics of a metapopulation. The impact of mean salinity and salinity fluctuations could not be evaluated clearly from the field study and physiological impacts of such will be discussed in more detail in the next section. This study, however, did not investigate small scale disturbances and the effects of, for example, chlorophyll or dissolved oxygen in the water column. These shallow water sites are not likely subjected to low oxygen levels very often, however.

3.2 Salinity tolerance (IV)

The population of *P. elegans* at Herslev reacts to abrupt decrease in salinity from 15 to 5 by increasing body volume and tissue water content due to inflowing water. The maximum body volume was about 2.7 fold larger than initial size and was reached about 30–120 minutes after exposure. Restoration of the initial volume was initiated but could not be fully achieved; within the next week, still a 1.7 fold increase was observed. Tissue water content increased by 9.5 % after 45 min, but decreased subsequently so that after four hours only an increase by 8.2 % was measured. In response to hyperosmotic medium (salinity 30), a decrease in tissue water content could be observed, whereas no change in body volume was apparent. The water content decreased by 11.5 % within 45 minutes and increased after four hours to a reduced tissue water content by 9 % compared to initial water content. The responses were very different depending on the individual, which might be a result of differences in size or condition of the worms. Similar responses with increasing body volume or weight in response to a hyposmotic environment and decreasing body volume or weight in response to a hyperosmotic environment were reported for other polychaetes or cells of polychaetes. The extent of the response differed hereby depending on the osmoregulatory abilities of the species (Oglesby 1965, Fletcher 1974, Costa *et al.* 1980, Dykens and Mangum 1984). The fact that initial body volume could not be restored after a transfer to salinity 5 and that half of the specimens of this treatment died indicates that an abrupt transfer from salinity 15 to 5 is more stressful than a transfer from 15 to 30. These observations suggest that *P. elegans* is a weak cell-volume regulator. This was also supported by the fact that no response in the RNA expression of alanine aminotransferase or tubulin in response to salinity changes could be observed although changes in cell volume affects the cytoskeleton structure (e.g. tubulin) and cell volume can be regulated by adjusting the osmolyte content (e.g. amino acids) in the cell.

Moreover, the RNA expression of genes involved in ionic and osmoregulation, by providing energy (ATP-Synthase), facilitating ion transport (Na^+K^+-ATPase, bicarbonate exchanger, carbonic anhydrase), and cell signalling (IGF) did not change in any clear pattern in response to changing salinities

suggesting that *P. elegans* does not regulate ions and osmotic concentration. However, the exposure time in the experiment might have been too short to elicit a strong response at the RNA level. Gene expression changes in response to salinity change have been described for several molluscs and crustaceans, although those exposures were not necessarily on the same time scale as studied here (Lovett *et al.* 2006, Lockwood and Somero 2011, Towle *et al.* 2011, Zhao *et al.* 2012, Havird *et al.* 2013, Lv *et al.* 2013, Li *et al.* 2014, Hu *et al.* 2015). Immediate response to salinity changes might be achieved via changes in protein concentration or changes in the functionality of existing proteins. To properly evaluate the osmoregulatory capacity of *P. elegans,* however, the osmolality of its body fluids would have to be determined. Measurement of ion concentration as well as osmolality throughuse of an ion chromatograph and nanoosmometer was pursued in this study, but the extraction of body fluid from these small polychaetes was not reliable and those results were questionable. Thus, they are not presented in this thesis.

Pygospio elegans from the Herslev population does not seem to cope well with sudden large decreases in salinity. At this site, natural fluctuations in salinity down to 5 were observed, however, they were not prolonged, and salinity was low for relatively short periods ranging from a few hours up to one day in duration. Longer periods of low salinity might be achieved at low water levels and during heavy rain storms. Such events could be detrimental for the population if the specimens do not have an avoidance mechanism, such as burrowing deeper in the sediment or closing their tubes.

Also during long-term exposure to different salinities, it became apparent that a transfer to salinity 5 is harsher for *P. elegans* than a transfer to salinity 30. At the control salinity 15 and at salinity 30, asexual reproduction took place, so that the number of individuals actually increased during the experiment, although the mean length of the specimens decreased. In general, the biomass in the two treatments was quite similar. In salinity 5 no asexual reproduction was observed and, in contrast to the other salinities, the number of individuals declined slightly to a minimum of 27. The mean length of these worms increased, however, both after 3 weeks and after 6 weeks. Overall biomass decreased over time at salinity 5. The number of specimens carrying gametes was highest at salinity 15, followed by 30, and 5. Egg strings were only observed at salinity 15 and 30, although in the latter treatment they were only observed after 6 weeks. Anger (1984) described that *P. elegans* had highest reproductive and survival rates in brackish water (salinity 10–16) even when populations originated from full strength marine habitats. Sublethal effects of polychaetes in response to salinity changes include slowed growth and development as well as delayed maturity (Kinne 1966, Qiu and Qian 1997, Pechenik *et al.* 2000). Salinity of 5 seems to be still within the tolerance range of this population of *P. elegans*, however, a decrease in fitness was observed. Populations of *P. elegans* are known to persist in the Northern Baltic Sea at salinities as low as 5 (Kesäniemi *et al.* 2012c). To investigate whether these populations have adapted to the low salinities or whether also *P. elegans* from Herslev could persist at such low salinities, reciprocal transplant experiments

would need to be performed. Moreover, experiments would need to last longer and include different life stages to study population persistence, since early life stages are usually the most vulnerable to salinity stress (Kinne 1966, Qiu and Qian 1997, Pechenik *et al.* 2000).

3.3 Metapopulation dynamics, dispersal and community structure

The population density of *P. elegans* fluctuated at three out of four sites, with the fourth site showing consistently low abundances. Fluctuations like this are characteristic for short-lived opportunistic species that colonize new habitats quickly and then overshoot their carrying capacity (Beukema *et al.* 1999). A high temporal turnover was also visible from the genetic structure of two of the studied populations, indicating CGP. Additionally, there were differences in allele frequencies among sites. Spatial heterogeneity was also apparent in sediment structure and according to abundance, size and reproductive activity of *P. elegans* seemed to affect the quality of the habitat. Kesäniemi and colleagues (2014a) suggested that *P. elegans* in the Isefjord-Roskilde-Fjord estuary complex represents a metapopulation consisting of several subpopulations, however, no real extinctions of any single subpopulation were observed during the present study. Nevertheless, due to the short life span of *P. elegans* with two overlapping size cohorts and low densities, changes in population allele frequencies were observed indicating immigration of individuals from genetically differentiated populations. In two of the study populations not all genotypes persisted, and these were replaced by others, possibly originating from other high quality habitats. Hence, these populations experienced dynamics similar to the metapopulation described for *Pectinaria koreni* in the Baie de Seine (Jolly *et al.* 2014). In addition to seasonal fluctuations, stochastic events such as rainstorms can quickly change the salinity in coastal areas and estuaries and challenge populations of *P. elegans*. As seen in the present study, an abrupt and prolonged decrease in salinity can lead to reduced fitness and ultimately death of *P. elegans*. Kube and Powilleit (1997) described extinctions of populations of three spionids after severe anoxia in the Baltic Sea. Although their numbers increased again quickly afterwards, the same abundances were reached only after about one year.

Considering the population dynamics of *P. elegans* in the Isefjord-Roskilde-Fjord estuary complex, different types of larvae of *P. elegans* might serve different purposes. Planktonic larvae with their high dispersal potential can disperse to or recolonize new habitat patches and thus dampen fluctuations in other subpopulations. Benthic larvae, in contrast, can be retained to maintain the local populations in high quality habitats (Pechenik 1999, Eckert 2003). In this study, local recruitment via benthic larvae resulted in no seasonal variation in allele frequencies at Herslev. Sites with planktonic larvae, namely

Lammefjord and Vellerup, in turn exhibited a turnover in genotypes. No clear seasonal genetic variation was observed at Lynæs, although planktonic larvae were produced in winter. One possible explanation for this could be the high percentage of asexual reproduction at Lynæs, which is another form of local recruitment. Unfortunately, the water circulation in the estuary complex is not known at the appropriate scale to allow an assessment of the impacts water circulation has on larval dispersal and population connectivity. Planktonic larvae could still be locally retained passively via oceanic currents or actively via habitat cues (Strathmann *et al.* 2002, Weersing and Toonen 2009). Likewise, benthic larvae or juvenile stages could disperse via drifting as described e.g. for *S. armiger* and *M. balthica* (Beukema *et al.* 1999). Although different types of larvae are advantageous under certain conditions, these conditions might not be predictable, which could lead to the stochastic production of one type of larvae as a bet-hedging strategy (Chia *et al.* 1996). However, the dispersal polymorphism in *P. elegans* in the Isefjord-Roskilde-Fjord estuary could also be maintained due to asymmetric dispersal between local populations acting as sources or sinks, as has been proposed for *S. benedicti* (Zakas and Hall 2012). Investigating the actual dispersal of larvae and the water circulation in the estuary could help to elucidate the consequences of larval type on population structure.

4 CONCLUSION

The present study confirmed the observations of Rasmussen (1973) that reproduction of *P. elegans* occurs from fall to spring in the Isefjord-Roskilde-Fjord estuary and that multiple types of larvae are present. Yet, in contrast to the results of Gudmundsson (1985), there is evidence that *P. elegans* is semelparous, i.e. produces only one brood per life-time. The two consecutive brood batches are instead produced by different cohorts and lead to the two recruitment events, one in spring and one in fall. Moreover, although *P. elegans* is a euryhaline species with broad distribution and salinity tolerances (Anger 1984), the results of the present study suggest that this species cannot cope with abrupt prolonged salinity changes and might need to adapt to enable persistence at low salinities.

TABLE 1 Summary of the population dynamics of *P. elegans* and the abiotic and biotic environmental conditions at the four sampling sites in the Isefjord-Roskilde-Fjord estuary in 2014/15.

		Lynæs	Lammefjord	Vellerup	Herslev
Environment	Sediment structure	fine	fine	coarse	medium
	Sediment sorting	moderately well	moderately	poorly	moderately
	Mean salinity	19	19	19	14
	Organic content	0.92	1.04	0.84	0.78
	C/N	8.28	8.83	9.53	8.74
	Species diversity	0.66	0.59	1.92	1.4
Pygospio elegans	Density (ind m-2)	0–337, no peak	75–4357	132–7847	189–4791
	Mean length (µm)	1139–1731	1074–1648	1496–1848	1343–1818
	Gravid females	10 %	22 %	32 %	26 %
	Developmental mode	benthic, planktonic	benthic, planktonic	benthic, planktonic	benthic
	Asexual reproduction	8.7 %	3.1 %	1.3 %	1.1 %
	Genetic cluster	2	2, 3	1, 2, 3	1

The main results of the field study relating the population ecology and genetics of the poecilogonous polychaete *P. elegans* in the Isefjord-Roskilde Fjord estuary to environmental parameters are summarized in Tab. 1. According to the size cohort and population genetic data, the populations were very dynamic with high seasonal and spatial turnover. The data supports that this is a result of the short life span of *P. elegans* and sweepstakes reproductive success as suggested by Kesäniemi *et al.* (2014a). Seasonal dynamics influenced timing of reproduction, which was correlated with temperature, while spatial variation was correlated with sediment structure. To properly document the dynamics and identify patterns, a longer field study would be needed. This should ideally include the assessment of larval dispersal via plankton samples, answering questions about the realized dispersal distances, the occurrence of collective dispersal and the water currents.

In respect to developmental mode it is still unclear whether poecilogony in *P. elegans* resembles i) a genetic polymorphism that is maintained via asymmetric dispersal or ii) a plastic response to certain environmental conditions or iii) a bet-hedging strategy in response to environmental unpredictability (Chia *et al.* 1996, Krug 2007, Zakas and Hall 2012). Although genetically differentiated cohorts might have resulted in different types of larvae, an environmental impact cannot be excluded. Reciprocal transplant experiments or mating experiments with populations exhibiting one or the other type of larvae could help to answer this question. Furthermore, manipulative experiments would need to be performed to identify potential selective pressures such as salinity fluctuations or level of predation on the mode of development. In these experiments specimens could be chosen according to genotype, and in addition to mode of development, also other life history traits such as age of maturity and longevity could be documented to get a better picture of their consequences on population dynamics.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Kehitysmuotojen variaatio ja sen vaikutus *Pygospio elegans* -monisukasmadon populaatiodynamiikkaan heterogeenisessä ympäristössä

Meressä elävillä selkärangattomilla pohjaeläinlajeilla on useita erilaisia lisääntymisstrategioita ja toukkamuotoja. Morfologiaerojen lisäksi toukkamuodot eroavat ravinnon lähteiden suhteen. Yleisin muoto on vedessä elävä ja ravintoa etsivä toukka, mutta toukat voivat saada ravintonsa myös maternaalisesti suoraan ravinteikkaasta munasolusta, tai naaraan tuottamista ylimääräisistä ravintomunista. Toukkamuotojen tuottamisessa on suuria energeettisiä eroja: veteen vapautettavia planktisia toukkia voidaan tuottaa pienistä munista suuria määriä, kun taas maternaalista lisäravintoa käyttäviä toukkia tuotetaan yleensä pienempi määrä, sillä isokokoisten munasolujen tai ylimääräisen ravinnon tuottaminen vie enemmän resursseja. Eri lisääntymisstrategioita, eli toukkien kehitysmuotoja suositaan eri ympäristöissä, ja toukkamuoto vaikuttaa myös lajien populaatiorakenteisiin. Esimerkiksi planktisten toukkien tuottamista voidaan suosia aikana jolloin vedessä on runsaasti ravintoa, eli kasviplanktonia. Planktisilla toukilla on korkea levittäytymispotentiaali, jolloin populaatioiden välinen migraatio ja geenivirta voi olla suurta. Planktisia toukkia tuotetaan usein runsaasti, mutta toisaalta niiden kuolleisuus on suuri verrattuna pohjasedimentissä tai suojaavissa rakenteissa eläviin toukkamuotoihin. Jos lajilla ei ole dispersoivaa planktista toukkavaihetta, populaatioiden välinen migraatio voi olla heikkoa ja populaatiot eriytyä geneettisesti. Vaikka eri ympäristöistä peräisin olevia eri toukkamuotoja tuottavia lajeja on vertailtu aikaisemmin, ei toukkamuotojen esiintymiseen liittyviä valintapaineita vielä täysin ymmärretä. Eri lajeja vertailtaessa tuloksiin vaikuttaa myös fylogeneettiset rajoitukset. Tutkimuksiin toukkien kehitysmuotoihin liittyvistä evolutiivisista valintapaineista onkin sopivampaa käyttää lajia, joka pystyy tuottamaan eri toukkamuotoa lajin sisällä (poecilogony).

Vain 14 lajin tiedetään omaavan lajinsisäistä variaatiota toukkamuodoissa. *Pygospio elegans* -hiekkaputkimato on yksi näistä. Nimessä mukaisesti tämä yleinen monisukasmatolaji elää rakentamissaan hiekkaputkissa vuorovesialueilla pohjoisella pallonpuoliskolla. Lajin eri populaatiot voivat tuottaa vain joko yhtä toukkamuotoa, vaihtaa toukkamuotoa vuodenajasta riippuen tai tuottaa erilaisia toukkamuotoja samanaikaisesti. Tässä väitöskirjassa tutkin *P. elegans* -hiekkaputkimatojen lajinsisäistä toukkamuotojen monimuotoisuutta, sen mahdollisia syitä ja seurauksia neljässä tanskalaisessa populaatiossa, joissa on havaittu sekä planktisia että naaraan hiekkaputkessa kasvavia ilman pelagista toukkavaihetta kehittyviä toukkia. Näitä Isefjord-Roskilde Fjord estuaarialueella olevia populaatioita seurattiin vuoden ajan keräten aineistoa populaatioiden rakenteen muutoksista ja populaatiogeneettisistä rakenteista, sekä useista bioottisista ja abioottisista muuttujista.

Tutkimuksessani selvisi, että kyseisissä *P. elegans* populaatioissa suvullista lisääntymistä tapahtui syyskuusta toukokuuhun, elinympäristön lämpötilan ollessa alimmillaan. Joissakin populaatioissa lisääntymisessä oli kaksi selkeää aktiivisuusaikaa vuodessa. Vuoden aikana yksilöiden kokojakaumassa nähtiin populaatiosta riippuen kolmesta neljään erillistä, mutta osittain päällekkäistä kohorttia. Populaatioiden geneettisessä rakenteessa havaittiin paikallista ja vuodenaikojen välistä vaihtelua, joka saattaa johtua *P. elegans* -matojen lyhyestä eliniästä ja suuresta variaatiosta yksilöiden välisessä lisääntymismenestyksessä. Joissakin populaatioissa vuodenaikojen väliset muutokset alleelifrekvensseissä ajoittuvat uusien kokoluokkien ilmestymisen kanssa yhtenäisesti. Lisäksi kolmessa populaatiossa havaittiin vuodenaikaiseroja toukkien lisääntymismuodoissa; vapaana elävät planktiset toukat vallitsivat talvella, kun taas hiekkaputkissa suojatut toukat olivat yleisiä keväällä. Eri toukkamuotoja tuottavat naaraat kuuluvat todennäköisesti geneettisesti erilaisiin kohortteihin. *Streblospio benedicti* monisukasmadon toukkamuotojen monimuotoisuudella on havaittu olevan geneettinen perusta, mutta tämän tutkimuksen tulosten perusteella ei kuitenkaan voida tehdä johtopäätöksiä *P. elegans* -lajin taustasta. Lisäksi myös ympäristön vaikutus ja epigenetiikan rooli olisi tutkittava käyttäen kokeellista näkökulmaa.

Sedimentin ominaisuudet vaikuttivat *P. elegans* -lajin populaatiodynamiikkaan neljässä tutkimuspopulaatiossani. Habitaateissa, joissa sedimentti oli karkeaa ja koostui erikokoisista partikkeleista oli suurin yksilötiheys, isokokoisimmat yksilöt sekä korkein lisääntymisfrekvenssi. Myös muun pohjaeläimistön lajirikkaus oli näillä alueilla suurin. *P. elegans* suosii karkeaa pohjasedimenttiä, ja erikokoisista partikkeleista koostuva sedimentti voi ylläpitävää elinympäristöjä monille eri pohjaeläinlajeille. Lisäksi *P. elegans* -madon geneettinen variaatio (alleelirikkaus) korreloi positiivisesti muun selkärangattomien pohjaeläinyhteisön lajirikkauden kanssa. Tämä viittaa siihen että ympäristömuuttujat, kuten esimerkiksi vuodenaikaisvaihtelut tai habitaatin kantokyky, vaikuttavat *P. elegans* populaatioihin samalla tavalla kuin muuhunkin yhteisöön, kun taas esimerkiksi vuorovaikutussuhteilla muiden pohjaeläinyhteisön lajien kanssa on pienempi vaikutus. Ympäristöolojen muutokset voivat olla ennustettavia, esimerkiksi vuodenajoista riippuvia, tai hyvinkin stokastisia. Vuorovesialueet, erityisesti murtovesialueet ja jokien estuaarit, ovat erittäin dynaamisia habitaatteja, joissa esimerkiksi veden suolapitoisuus saattaa vaihdella ajallisesti merkittävästi. Koska kehitysmuodoiltaan polymorfisia *P. elegans* -populaatioita tavataan erityisesti näillä alueilla, tutkin kokeellisesti näiden matojen fysiologisia ja ekologisia reaktioita muutoksiin veden suolapitoisuudessa. Kokeissani selvisi, että kun veden suolapitoisuutta alennetaan äkillisesti, *P. elegans* -matojen osmoregulaatiokyky heikkenee, joten voimakkaat stokastiset laskut meriveden suolapitoisuuksissa voivat olla lajille vahingollisia. Pitkäkestoisen kokeen mukaan alhainen suolapitoisuus (5 ppt) myös alentaa lajin yksilöiden suvullisen ja suvuttoman lisääntymisen frekvenssiä tai viivästyttää lisääntymistä.

Tutkimukseni antaa lisätietoa *P. elegans* -lajin populaatiodynamiikasta, populaatiogenetiikasta ja fysiologisen toleranssin rajoista. Populaatiotason erot viittaavat dynaamisen metapopulaation olemassaoloon tällä lajillaTanskan Isefjord-Roskilde Fjordin alueella. Toukkamuodoissa nähtävä polymorfia voi olla metapopulaatiota ylläpitävä tekijä tai sen seurausta. Ympäristön stokastisuus, sekä habitaatin ominaisuudet kuten lämpötila ja sedimentin laatu vaikuttavat lajin populaatiodynamiikkaan. Tutkimukseni tulokset viittaavat *P. elegans* -madon kehitysmuotopolymorfiaa ylläpitävään geneettiseen ja ympäristöstä johtuvaan taustaan, joskin lisätutkimuksia tarvitaan vielä. Koska tällä lajilla eri toukkamuotoja havaitaan usein juuri heterogeenisissä habitaateissa, lajinsisäinen variaatio toukkamuodoissa (poecilogony) saattaa olla strategia ajallisen kelpoisuusvaihtelun vähentämiseksi vaihtelevassa ennalta arvaamattomassa elinympäristössä.

OVERSIGT (RÉSUMÉ IN DANISH)

Effekten af variation I developmental mode på populationsdynamikken af en spionid børsteorm (*Pygospio elegans*) i et heterogent miljø.

Marine bundlevende invertebraters larver udviser stor variabilitet i form og funktion. Udover at variere i morfologi varierer de også i deres fødeoptagelse. De mest almindelige former er planktotrofe, der lever af partikelfiltrering i det pelagiske miljø og de lecitotrofe/ adelphofatiske, der lever i det bentiske miljø og får deres næring fra moderdyret enten i form af blommemasse eller nurse æg og søskendelarver i kuldet. En stor del af variationen kan ligge i at enten produceres der store mængder små æg, der udvikler planktotrofe larver eller færre store æg der udvikler lecitotrofe/ adelphofatiske larver. Forskellige larvestrategier, eller developmental modes favoriseres under forskellige forhold og resulterer i forskellige populationsstrukturer og dynamik; eksempelvis favoriseres planktotrofe larver i situationer med høj planteplankton forekomst og grundet disse larvers store spredningspotentiale resulterer dette i høj populations connectivitet. Omvendt vil høj larvedødelighed i planktonet favorisere udviklingen af lecitrotrofe larver, der udvikler sig i hav bundens miljø. Eftersom lecitotrofe larver oftest bundslår sig i udgangspopulationen bundmiljø fører bentisk developmental mode til populations differentiering. Men det er en kendsgerning, at selektionspresset der fører til den ene eller den anden larvestrategi ikke er fuldstændigt afklaret. Developmental modes mellem forskellige arter og habitater er sammenlignet i tidligere studier for netop at forsøge at afklare selektionspresset. Men disse studier lider under fylogenetiske uklarheder hvorfor anvendelsen af poecilogonous arter, der er kendetegnet ved indenfor en art at producere flere forskellige larvetyper, antageligt er en bedre strategi for at studere selektionspresset på developmental modes.

Til dato er blot 14 arter beskrevet som poecilogonous og en af disse er den lille spionide børsteorm *Pygospio elegans.* Denne er almindelig på lavvandede mudderflader i hele det circumpolare område. Forskellige populationer beskrives som enten fikseret i udviklingen af enten planktotrofe eller lecitotrofe larver eller at udvise sæsonvariation i developmental mode eller endelig at være i stand til at producere flere typer af larver samtidigt. Men meget få populationer er studeret gennem et helt år. I min thesis undersøgte jeg fænomenet poecilogony, dets potentielle årsager og konsekvenser i børsteormen *Pygospio elegans* på fire lokaliteter i det danske Isefjord-Roskilde-Fjord estuarie kompleks, eftersom både planktotorfi og lecitototrofi er beskrevet der. Jeg dokumenterede populationsdynamikken og populationsgenetikken på disse fire lokaliteter ved at indsamle individer gennem et helt år og relatere datamønstret til såvel biotiske som abiotiske variable i økosystemet.

Jeg opdagede at den kønnede formering blev igangsat af relativ lav temperatur og fandt sted fra september til maj samt at på nogle lokaliteter var dyrene reproduktiv aktive i to perioder om året. Jeg identificerede tre til fire forskellige men delvist overlappende størrelses-kohorter gennem året. Hver af

disse størrelses-kohorter varede cirka et halvt år og ny rekruttering fandt sted om foråret samt om efteråret. Den genetiske struktur af populationerne udviste såvel rumlig som tidslig variation som sandsynligvis skal tilskrives den korte generationstid af *P. elegans* samt fænomenet sweepstakes reproduktions succes. Ydermere foreslås at individerne i blot en størrelses-kohorte ad gangen er stor nok til at reproducere sig. Sæsonforskelle i allel frekvenser på nogle af lokaliteterne kunne associeres til fremkomsten af nye størrelses-kohorter. Der blev også fundet at mode of development på tre lokaliteter varierede over sæsonen med planktotrofe larver om vinteren og bundlevende om sommeren. Disse forskellige larvekuld er sandsynligvis produceret af hunner hidrørende fra forskellige størrelses-kohorter der udviser forskellige genetiske karakteristika. Men ud fra disse data kan jeg endnu ikke identificere en basis for poecilogony i *P. elegans* som beskrevet for en anden poecilogonous børsteorm, *Streblospio benedicti*. For at besvare dette spørgsmål skal manipulationsforsøg og/eller mating eksperimenter udføres hvor miljø og epigenetiske effekter kan udelukkes.

Rumlige forskelle i sedimentets grovhed og andre karakteristika mellem de fire lokaliteter blev korreleret med børsteormenes tætheder, kropsstørrelser og reproduktionsaktivitet samt med høj artsdiversitet i bunddyrssamfundet i almindelighed. Det var forventningen ud fra tidligere studier at *P. elegans* foretrækker groft sediment og at ringe sorteret sediment tilbyder forskellige nicher for mange invertebrat arter. Ved kombination af de tidslige og rumlige prøver korrellerede allel richness i *P. elegans* med arts rigdommen i bunddyrssamfundene. Dette indikerer at *P. elegans* udviser samme respons som det øvrige bunddyrssamfund og at alle bunddyrene i højere grad er under indflydelse af miljøvariable såsom sæsonvariation og bærekapaciteten i habitatet end af interaktioner mellem andre bunddyr. Miljøets indflydelse kan være forudsigelige, eks. sæsonvariation, men kan i høj grad også være stokastiske. I denne sammenhæng repræsenterer estuarier et meget udfordrende miljø karakteriseret ved store fluktuationer eksempelvis i saltholdighed forårsaget af kraftige regnvejrsepisoder. Jeg undersøgte fysiologiske og økologiske respons af *P. elegans* af akut og langtids ændringer i saltholdighed eftersom populationer af *P. elegans,* der ikke er fikseret i blot en developmental mode, netop er beskrevet fra estuarier. Jeg fandt at *P. elegans* er en svag volume ion- og osmoregulator ved lave saliniteter som respons på abrupte fald I salinitet. Derfor kan stokastiske salinitetsfald være uhyre skadelige på *P. elegans* populationer. Ifølge langtids eksperimentet var saliniteten 5 indenfor men på grænsen af toleranceområdet eftersom børsteormen voksede men udviste reduceret eller forsinket asexual og sexual reproduktion.

Mine studier tilvejebringer ny viden om *P. elegans* populationsdynamik og populationsgenetik samt fysiologisk salttolerance. Forskellene der blev observeret på *P. elegans* populationsniveau antyder en dynamisk metapopulationsstruktur i Isefjord-Roskilde Fjord estuarie komplekset der kan forklare både årsagen til og konsekvensen af variationen i developmental mode i arten der. Det er tydeligt at såvel temperaturen som sedimentstrukturen

spiller en rolle for populationsdynamikken samt at denne dynamik også er under indflydelse af stokastiske hændelser. Mine resultater antyder såvel genetisk som miljømæssig indflydelse på den observerede variation i developmental mode.i *P. elegans*. Disse opdagelser fortjener yderligere at blive forfulgt i fremtidige studier. Eftersom multible developmental modes oftere er udtrykt I estuarine miljøer kan poecilogony repræsentere en bet-hedging strategi som respons på miljøets ustabilitet.

ZUSAMMENFASSUNG (RÉSUMÉ IN GERMAN)

Der Wurm

von Raphaela Leonhard-Pfleger für Anne Thonig

Er ist nicht oft zulesen,
ein unbeachtet Wesen
und wahrlich auch kein Held,
was ist ein Wurm hier auf der Welt?

Doch ein Wurm hat mehr drauf als man denkt
dazu verschied'ne Wege er vermengt
denn er sich vielfältig selbst multipliziert
die Wissenschaft damit sehr verwirrt.

Zum einen Mal, wenn's ihn langweilt sehr
nimmt er sich selbst zu teilen her.
Schneidet sich entzwei in der Mitte
und wächst komplett nach, so ist's die Sitte.

Ein anderes Phänomen,
bei Würmern schon gar oft geseh'n,
sie folgen dem eingebauten Triebe
und machen heimliche Liebe.

Die Eier - Gott nur weiß warum
zerfallen teils zugrunde stumm.
Die übrige Geschwisterschar
frisst die Zerfall'nen mit Haut und Haar.

Wieviele so sterben ist unbekannt,
die Wissenschaft ist darum sehr gespannt,
wer zuerst steigt hinter all diese Zwänge,
wer als erstes beweist die Zusammenhänge.

Die Auswirkungen einer variablen Larvenentwicklung auf die Populationsdynamik eines Vertreters der Polychätenfamilie Spionidae (*Pygospio elegans*) in einem heterogenen Lebensraum.

Marine benthische Invertebraten besitzen eine groβe Vielfalt an Larven. Neben der Morphologie, unterscheiden sie sich auch anhand der Nahrungsaufnahme. Die häufigsten Formen sind hierbei planktotrophe Larven, die sich im Plankton ernähren, und lecithotrophe/adelphophage Larven, deren Nahrung von der Mutter in Form von Dotter, Nähreiern oder Geschwistern im selben Gelege bereitgestellt wird. Die Bildung von entweder vielen kleinen Eiern (Planktotrophie) oder wenigen groβen Eiern (Lecithotrophie/ Adelphophagie) stellt einen Konflikt zwischen Fertilität und Brutvorsorge dar, und unterliegt zumindest teilweise Schwankungen. Verschiedene Larventypen sind unter verschiedenen Bedingungen von Vorteil und können zu unterschiedlicher Struktur und Dynamik in der Population führen. Beispielsweise wären planktotrophe Larven von Vorteil, wenn der Phytoplanktongehalt im Wasser hoch ist. Diese würden aufgrund ihres hohen Verbreitungspotentials dazu führen, dass verschiedene Populationen im genetischen Austausch miteinander stehen. Im Gegensatz dazu würde hohe Sterblichkeit im Plankton lecithotrophe Larven bevorzugen, die sich im Benthos entwickeln. Da diese Larven typischerweise in ihrer Heimatpopulation siedeln, würden sie dazu führen, dass verschiedene Populationen voneinander isoliert sind. Es ist allerdings noch nicht völlig geklärt, welche Faktoren die eine oder andere Strategie selektieren. Um diese Faktoren zu bestimmen, wurde in vorangegangen Arbeiten untersucht, welcher Larventyp in welchen Lebensräumen vorhanden ist. Hierbei wurden jedoch verschiedene Arten miteinander verglichen, so dass der Einfluss verschiedener Lebensräume auf den Larventyp mit stammesgeschichtlichen Einschränkungen zwischen den Arten vermengt sein könnte. Daher könnten poecilogene Arten, das sind Arten die verschiedene Larventypen produzieren, besser geeignet sein, die Selektion für einen bestimmten Larventyp zu untersuchen.

Bisher sind lediglich 14 Arten bekannt, die tatsächlich poecilogen sind. Eine von ihnen ist *Pygospio elegans*. Dieser kleine röhrenbildende Polychät der Familie der Spioniden ist weit verbreitet im Schlick des Gezeitenbereichs der borealen Breiten. Es ist bekannt, dass einige *P. elegans* Populationen nur adelphophage oder planktotrophe Larven produzieren, während in anderen Populationen ein saisonaler Wechsel zwischen den Larventypen stattfindet oder verschiedene Larventypen gleichzeitig produziert werden. Allerdings beobachteten nur wenige Studien Populationen über einen längeren Zeitraum hinweg. In dieser Arbeit, habe ich das Phänomen der Poecilogonie, sowie seine möglichen Ursachen und Folgen in dem Polychaeten *P. elegans* an vier Standorten im Dänischen Isefjord-Roskilde-Fjord Ästuar untersucht, da hier sowohl plankotrophe als auch adelphophage Larven beschrieben worden sind. Über ein Jahr hinweg habe ich die Populationsdynamik und Populationsgenetik dokumentiert und diese mit den vorherrschenden biotischen und abiotischen Umweltbedingungen verglichen.

Ich habe festgestellt, dass die sexuelle Reproduktion durch niedrige Temperaturen eingeleitet wird und von September bis Mai stattfindet. Hierbei traten an einigen Standorten zwei Maxima sexueller Aktivität auf. Drei bis vier verschiedene, teilweise überlappende Größenkohorten konnten über das Jahr hinweg beobachtet werden; jede überdauerte etwa ein halbes Jahr und neue Kohorten siedelten im Frühling und Herbst. Die genetische Struktur der Populationen zeigte standortbedingte sowie saisonale Unterschiede, welche möglicherweise der kurzen Lebensdauer von *P. elegans* und dem Zufall einer erfolgreichen Fortpflanzung zuzuschreiben sind. Die Ergebnisse deuten darauf hin, dass möglicherweise zu jedem Zeitpunkt Individuen von nur einer Kohorte groß genug waren um sich fortzupflanzen. An einigen Standorten könnten die saisonalen Unterschiede in der genetischen Strukur der Population mit dem Auftreten einer neuen Kohorte übereinstimmen. Darüber hinaus wechselte der Larventyp saisonal an drei Standorten von planktotrophen Larven im Winter zu adelphophagen im Frühling. Diese verschiedenen Gelege werden vermutlich von Weibchen produziert, die verschiedenen Kohorten angehören, welche wiederum genetische Unterschiede aufweisen. Dennoch können wir von diesen Ergebnissen nicht ableiten, dass Poecilogony in *P. elegans* genetisch bedingt ist wie beispielsweise in dem Polychaeten *Streblospio benedicti*. Weitere manipulative Experimente und Paarungsstudien sind nötig um Umwelt- oder epigenetische Effekte auszuschließen.

Die unterschiedliche Sedimentstruktur an den verschiedenen Standorten beeinflusste die Populationen dahingehend, dass in grob körnigem Sediment, welches zusätzlich unterschiedliche Korngrößen aufwies, die Populationsdichte von *P. elegans* und der Anteil an reproduzierenden Individuen höher war und außerdem die Individuen größer waren. Dies war zu erwarten, da aus früheren Studien bekannt ist, dass *P. elegans* grob körniges Sediment bevorzugt. Weiterhin weiste die benthische Invertebratengemeinschaft in diesem Sediment eine höhere Artenvielfalt auf, was darauf beruhen könnte, dass verschiedene Nischen durch das heterogene Sediment vorhanden sind. Wenn man die Proben aller Standorte und Zeitpunkte zusammennimmt, korrelierte der Alleelreichtum von *P. elegans* mit dem Artenreichtum der benthischen Invertebratengemeinschaft. Dies weist daraufhin, dass *P. elegans* einen ähnlichen Lebensstil besitzt wie ein Großteil der Invertebratengemeinschaft und dass beide stärker durch Umweltbedingungen, wie saisonale Zyklen oder der Kapazität des Lebensraumes beeinflusst werden als durch die Interaktionen miteinander. Umweltbedingungen können vorhersehbar, beispielsweise saisonal sein, aber sie können auch unvorhersehbar sein. Dahingehend stellen Ästuare einen sehr anspruchsvollen Lebensraum dar, der starken Schwankungen, zum Beispiel im Salzgehalt aufgrund von plötzlichem Starkregen unterliegt. Da *P. elegans* nicht nur einen Larventyp, sondern meist verschiedene Larventypen in Ästuaren aufweist, habe ich die Reaktion von *P. elegans* sowohl auf akute als auch auf langwierige Veränderungen des Salzgehaltes auf physiologischer und ökologischer Ebene untersucht. Es stellte sich heraus, dass *P. elegans* das Zellvolumen sowie die Ionen- und osmotische Konzentration seiner Hämolymphe in niedrigen Salzgehalten vermutlich kaum

regulieren kann. Daher könnte ein plötzlicher Abfall im Salzgehalt verheerende Auswirkungen auf Populationen von *P. elegans* haben. Einem Langzeitexperiment zufolge befindet sich ein Salzgehalt von 5 zwar noch innerhalb - allerdings am Rande - des Toleranzbereiches von *P. elegans*, da es Individuen noch möglich ist zu wachsen jedoch die asexuelle und sexuelle Fortpflanzung verringert oder verzögert ist.

Im Allgemeinen liefert meine Arbeit neue Informationen über die Populationsdynamik und Populationsgenetik sowie die physiologische Toleranz von *P. elegans*. Unterschiede in der Dynamik der verschiedenen Populationen weisen darauf hin, dass eine Metapopulationsstruktur im Isefjord-Roskilde-Fjord vorherrschen könnte, welche sowohl Ursache für das Vorhandensein verschiedener Larventypen, als auch eine Konsequenz davon sein könnte. Temperatur und Sedimentstruktur spielen eine klare Rolle in der Populationsdynamik; zusätzlich sind beispielsweise auch unvorhersehbare Wetterereignissen von Bedeutung. Meine Ergebnisse lassen sowohl einen genetischen als auch umweltbedingten Einfluss auf den Larventyp in *P. elegans* vermuten, welcher allerdings weiter untersucht werden sollte. Da unterschiedliche Larventypen in *P. elegans* vorzugsweise in Ästuaren auftreten, könnte Poecilogonie eine Strategie darstellen um Fitnessschwankungen in unvorhersehbaren Lebensräumen auf längere Sicht zu verringern.

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