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ORIGINAL ARTICLE

Seasonal genotype dynamics of a marine dinoflagellate: Pelagic populations are homogeneous and as diverse as benthic seed banks

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Abstract
Genetic diversity is the basis for evolutionary adaptation and selection under changing environmental conditions. Phytoplankton populations are genotypically diverse, can become genetically differentiated within small spatiotemporal scales and many species form resting stages. Resting stage accumulations in sediments (seed banks) are expected to serve as reservoirs for genetic information, but so far their role in maintaining phytoplankton diversity and in evolution has remained unclear. In this study we used the toxic dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as a model organism to investigate if (i) the benthic seed bank is more diverse than the pelagic population and (ii) the pelagic population is seasonally differentiated. Resting stages (benthic) and plankton (pelagic) samples were collected at a coastal bloom site in the Baltic Sea, followed by cell isolation and genotyping using microsatellite markers (MS) and restriction site associated DNA sequencing (RAD). High clonal diversity (98%–100%) combined with intermediate to low gene diversity (0.58–0.03, depending on the marker) was found. Surprisingly, the benthic and pelagic fractions of the population were equally diverse, and the pelagic fraction was temporally homogeneous, despite seasonal fluctuation of environmental selection pressures. The results of this study suggest that continuous benthic–pelagic coupling, combined with frequent sexual reproduction, as indicated by persistent linkage equilibrium, prevent the dominance of single clonal lineages in a dynamic environment. Both processes harmonize the pelagic with the benthic population and thus prevent seasonal population differentiation. At the same time, frequent sexual reproduction and benthic–pelagic coupling maintain high clonal diversity in both habitats.

KEYWORDS
adaptation, *Alexandrium ostenfeldii*, evolution, phytoplankton, resting stage, selection

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1 | INTRODUCTION

Phytoplankton evolution and their response to global change are of growing research interest due to their pivotal role in global nutrient and gas fluxes, as well as in aquatic food webs (Field et al., 1998). Because of increasing toxic blooms in coastal waters with potential effects on ecosystems, there is a rising demand for studies explaining the role of genetic diversity in relation to environmental change (Bach et al., 2018; Chust et al., 2017; Collins et al., 2014; Lassus et al., 2016; Rabalais et al., 2009). Within the past few decades our understanding of phytoplankton evolution has changed fundamentally from the assumption that marine phytoplankton populations are panmictic—due to potentially high dispersibility and unlimited gene flow—to a more complex view of genetically differentiated populations. A growing body of literature suggests that phytoplankton populations can be temporally differentiated (Dia et al., 2014; Erdner et al., 2011; Gao et al., 2019; Godhe et al., 2016; Lebret et al., 2012; Richlen et al., 2012; Sassenhagen et al., 2015, 2018; Sefbom et al., 2018) and geographically structured due to physical barriers and/or environmental gradients (Godhe et al., 2016; Postel et al., 2020; Sjöqvist et al., 2015; Tahvanainen et al., 2012). Genetic structure developing within relatively short timescales or geographical distances implies that evolution may happen rapidly, for example within a few hundred generations (Collins et al., 2014). To become genetically differentiated, populations must be affected by one or several processes, such as natural selection, mutation or genetic drift, in combination with limited gene flow (Rengefors et al., 2017).

Phytoplankton species are currently facing multifaceted and simultaneous climate-change-mediated alterations of their oceanic environment, in addition to existing natural environmental variability (Reusch & Boyd, 2013). These environmental changes require phytoplankton communities to respond by complex eco-evolutionary processes, including short-term cellular acclimation, and long-term adaptation to selection based on standing genetic variation (Collins & Gardner, 2009). Genetic diversity is the basis for these processes and in the last decade numerous studies have shown that phytoplankton populations are genetically and phenotypically diverse (Rengefors et al., 2017). Diverse phytoplankton communities and populations are expected to contain a variety of phenotypic traits, facilitating successful adaptation to changing environmental conditions (Litchman et al., 2010) and resistance to perturbations (Ptacnik et al., 2008; Sjöqvist & Kremp, 2016). Recently, the importance of large intraspecific diversity has been emphasized, potentially allowing rapid population-level adaptation to climate change (Godhe & Rynearson, 2017; Wolf et al., 2019).

The relationship between diversity and evolution of phytoplankton is fundamentally affected by their ability to form resting stages. Resting stages (such as resting cysts of dinoflagellates) are cells with strongly reduced metabolic activity, formed by many phytoplankton groups to survive adverse environmental conditions (Fryxell, 1983). They may play different roles in the life cycle, are crucial for seasonal survival (Anderson & Rengefors, 2006; McQuoid & Hobson, 1996; Rengefors & Anderson, 1998; Reynolds, 1984) and might aid expansion, for example by migratory water birds (Rengefors et al., 2021). Resting stages accumulating at the sea floor after consecutive growth periods can be considered reservoirs and—when deposited over time in stratified layers—archives of genetic information.

Seed banks are an important eco-evolutionary strategy, increasing the chance to persist through environmental variation in several ways: they facilitate increased genetic diversity and population differentiation in the presence of gene flow (Sundqvist et al., 2018), but can also conserve genetic structure of populations in the long run (Härnström et al., 2011; Ribeiro et al., 2011). Thus, seed banks may both slow down and enhance evolution by adaptation, depending on whether the fraction of emerging genotypes is a random or nonrandom sample of the total gene pool (Hairston Jr & De Stasio Jr, 1988). The evolutionary role of seed banks also depends on the mode of resting stage formation. Sexual resting stage formation is generally considered to contribute to genetic diversity of eukaryotic phytoplankton populations (von Dassow & Montresor, 2011; Rengefors et al., 2017) and accumulation of sexually produced resting stages could increase the genetic diversity of the seed bank, compared to a clonally growing seasonal pelagic population. To our knowledge, only one study has so far compared the intraspecific diversity of a phytoplankton seed bank with the actively growing part of the population and could not confirm a higher diversity in the seed bank of asexually produced resting stages of the diatom *Skeletonema marinoi* (Godhe & Härnström, 2010). Resting stage production of dinoflagellates does not always require genetic recombination either, as demonstrated for some species (Figueroa et al., 2008; Jerney et al., 2019; Kremp & Parrow, 2006). Thus, sedimentation of resting stages produced without genetic recombination could reduce the genetic diversity of the seed bank or balance it with a sexually reproducing pelagic part of the population.

Besides sexual reproduction, several other mechanisms may sustain high genetic diversity of phytoplankton populations. Environmental heterogeneity can, for example, promote evolutionary diversification, even more with temporal fluctuation (Cooper & Lenski, 2010), which is a prevailing explanation for maintenance of genetic diversity (Godhe et al., 2016; Gsell et al., 2012). In regions that experience seasonal climate variation, phytoplankton blooms are subject to varying environmental factors throughout the year, potentially selecting for different geno- and phenotypes (Reusch & Boyd, 2013). Clonally reproducing genotypes or lineages, thriving under certain conditions, could outnumber less competitive genotypes and thus lead to seasonal genetic population structure. A large set of coexisting subpopulations was, for example, found in the globally abundant marine cyanobacterium *Prochlorococcus* and differentiation was linked to seasonal selection (Kashtan et al., 2014). Alternatively, continuous germination of resting stages over time might result in a loss of differentiation and increase of gene diversity within a single clonally growing phytoplankton bloom (Lebret et al., 2012). Once successfully growing subpopulations form resting stages, they may increase the genetic diversity of the seed bank.

Strong seasonality characterizes coastal waters of the Northern Baltic Sea, where high geno- and phenotypic diversity was reported

for the dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech and Tangen (1985), which grows from May to September and occasionally forms dense toxic blooms (Kremp et al., 2016; Tahvanainen et al., 2012). These are associated with local accumulations of benthic resting stages (Hakanen et al., 2012; Kremp et al., 2009; Suikkanen et al., 2013), which makes *A. ostenfeldii* an excellent study organism to investigate if (i) the benthic seed bank is more diverse than the pelagic population and (ii) the pelagic population is seasonally differentiated. Prerequisites for increased seed bank diversity are clonal growth and genetic differentiation of subpopulations, without sexual recombination between them. Once selected genotypes of differentiated subpopulations form resting stages, they can accumulate in the sediment and generate a seed bank with large diversity. If different subpopulations are part of the seed bank and germinate or become more abundant at different times of the season, the pelagic population could be genetically structured. Based on these assumptions we hypothesize that: (i) the genetic diversity of the seed bank is higher, compared to the pelagic population, and (ii) the pelagic population displays temporal genetic differentiation. To investigate the genotype dynamics and diversity of *A. ostenfeldii*, a sampling campaign was carried out in 2015, followed by cell isolation and genotyping using microsatellite markers (MS) and restriction site-associated DNA sequencing (RAD).

2 | MATERIALS AND METHODS

2.1 | Sampling

The sampling site (60°05′45.4″N 20°31′26.6″E) is located in a shallow bay (water depth <3 m) in the Northern Baltic Sea (Föglö archipelago, Åland Islands), which is an extensively studied bloom site of *Alexandrium ostenfeldii* (Hakanen et al., 2012; Jerney et al., 2019; Kremp et al., 2009). The soft, muddy bottom is densely vegetated and summer salinity (SP) ranges typically between 5 and 6. From December to April the bay is usually ice-covered and in summer water temperature can rise to +24°C. Between March and October 2015, seven sampling campaigns were carried out (Table 1). At all dates water samples were taken to measure salinity and analyse the concentration of nutrients and chlorophyll *a* (Chl *a*) as well as the abundance of *A. ostenfeldii* as described in the following sections. Seawater temperature was measured *in situ*. At the end of March and in October sediment was sampled for isolation of *A. ostenfeldii* resting stages (referred to as seed bank populations S0 and S6, Table 1) and between June and August water samples were taken to isolate motile *A. ostenfeldii* cells (referred to as pelagic populations P1–P5). Between June and August replicate water samples were taken at each date to characterize algal toxins and the phyto- and zooplankton community (details below). Water chemistry, and phyto- and zooplankton community data were visualized with SIGMA PLOT version 10.0 (Systat Software).

2.2 | Water chemistry

For nutrient analysis water samples (250 ml) were taken at 0.5 m water depth with a Ruttner sampler (1 L; Hydro-Bios), transferred to hypochloric acid-washed 250-ml bottles and stored cool and dark until further processing. Nutrients were analysed within 24 h, at the Marine Research Center of the Finnish Environment Institute (SYKE MRC) according to an in-house protocol (accredited by the Finnish Accreditation Service). Total nutrients (total phosphorus [TP] and total nitrogen [TN]) and dissolved inorganic nutrients (NH_4, NO_3, NO_2, PO_4, SiO_4) were measured spectrophotometrically (Grasshoff et al., 2009; Koroleff, 1979) and molecular masses of N and P as a percentage of inorganic nutrients were used to calculate N:P ratios. Temperature was measured in the field with a thermometer integrated in the Ruttner sampler and salinity was measured with a salinometer (Guildline's Autosal 8400B Laboratory Salinometer by OSIL) at SYKE MRC. Chl *a* was measured spectrophotometrically, according to standard protocols (HELCOM, 1988, 2017a).

For sampling of hydrophilic paralytic shellfish poisoning (PSP) toxins, 10–20 L of seawater was taken with a 2-L measuring beaker, filtered through a 100-µm net, onto a 25-µm sieve and material

TABLE 1 Culture establishment and microsatellite-based population metrics for temporal parts of the population

Sampling date	Population	Cells isolated (*n*)	Cultures established (%)	Genotyped isolates (*n*)	MLG	H_E	$1 - D$	I_A	$\bar{r}_d$	Mean allelic richness
31-Mar	S0	100	50	46	45	0.58	0.98	−0.11	−0.01	4.01
02-Jun	P1	132	37	45	45	0.54	0.98	0.05	0.01	3.85
30-Jun	P2	84	35	24	24	0.55	0.96	0.11	0.01	4.15
26-Jul	P3	138	36	44	44	0.56	0.98	0.07	0.01	3.59
11-Aug	P4	92	45	36	34	0.53	0.97	0.07	0.01	3.64
25-Aug	P5	108	28	29	29	0.53	0.97	−0.17	−0.02	3.81
19-Sep	S6	100	34	33	32	0.53	0.97	−0.11	−0.01	3.75

Note: S = seed bank, P = pelagic parts; MLG = number of multilocus genotypes; H_E = Nei's (1978) gene diversity; $1 - D$ = Simpson index of diversity; I_A = index of association; *p*-value obtained after 999 permutations; $\bar{r}_d = I_A$ adjusted for the number of loci; *p*-value obtained after 999 permutations, I_A and $\bar{r}_d$ calculations were based on randomly subsampled populations ($n = 20$).

retained on the sieve was washed into a 50-ml centrifugation tube. The concentrated sample was filtered onto a Whatman GF/F glass fibre filter (Ø 25 mm), which was stored at −20°C temporarily. For the analysis of PSP toxins, filters were placed in cryovials and approximately 0.9 g lysing matrix D was added to each vial followed by 1 ml 0.03 M acetic acid. Sample homogenization and toxin extraction was performed at maximum speed (6.5 m s^{-1}) for 45 s using a Bio101 FastPrep (Thermo Savant) instrument. Homogenized samples were centrifuged at 16,100 *g* at 4°C for 15 min, after which the supernatants were transferred to 0.45-µm pore size spin filters (Millipore Ultrafree) and centrifuged for 30 s at 6000 *g*. Supernatants were transferred to high-performance liquid chromatography (HPLC) vials for toxin analysis. PSP toxins were determined by ion pair liquid chromatography coupled to post-column derivatization and fluorescence detection, as described in Krock et al. (2007) and Van de Waal et al. (2015). PST toxins (C1/2, dcGTX2/3, GTX2/3, GTX1/4, B1, dcSTX, STX and NEO) were quantified by external calibration curves with standard solutions ranging from 5 pg μl^{-1} to 6 ng μl^{-1}. The total amount of toxins is expressed as the sum of detectable toxins per date in pg per cell, presumably produced by *A. ostenfeldii* (STX + GTX2/3). Detection limits for each toxin are given in Table S1.

2.3 | Biotic environment

Water samples for phytoplankton analysis were taken at 0.5 m depth with a Ruttner sampler (1 L; Hydro-Bios) and fixed with Lugol's solution. Depending on cell densities, sample volumes of 3–50 ml were settled in Utermöhl counting chambers for 3–24 h (Utermöhl, 1958) and counted at 200× and 400× magnification using an inverted microscope (Leica DMI3000 B). At least 500 cells were counted per sample. Cell abundances were transformed into biomasses according to HELCOM (2017b). For isolation of *A. ostenfeldii* cells, plankton samples were collected with a 20-µm mesh plankton net and transferred to 200-ml culture flasks. Water samples (10 L) for zooplankton analysis were taken from the surface with a measuring beaker, filtered through a 63-µm sieve, conserved in 70% EtOH and counted with an inverted microscope (Leica DMI LED) at 50× magnification according to HELCOM (2017c).

2.4 | Isolation and culturing of *A. ostenfeldii*

For *A. ostenfeldii* resting stage isolation, four replicate sediment cores were sampled in April and October 2015. Sediment sampling and processing were carried out as described in detail by Jerney, Suikkanen, et al. (2019). Approximately 100 single resting stages were isolated after both samplings and clonal cultures were established and maintained at 16°C, as explained in Jerney, Ahonen, et al. (2019) and Jerney, Suikkanen, et al. (2019). Similarly, 84–138 motile cells were isolated randomly from plankton samples after each sampling date from June to September and clonal cultures were established as described above, but incubated at 20°C (instead of 16°C), 14:10-h light–dark cycle and ~100 µmol photons m^{-2} s^{-1} to simulate summer growth conditions.

2.5 | Genotyping

2.5.1 | Microsatellite marker analysis

Fifty exponentially growing clones were randomly selected from each sampling date, 0.5–10 ml of each culture was harvested and centrifuged at 2,000 *g* for 10 min. The obtained cell pellet was stored at −20°C until further processing and DNA was extracted in 5% Chelex solution as described by Nagai et al. (2012). Primer pairs and the PCR (polymerase chain reaction) conditions were described by Nagai et al. (2014) and nine primer pairs (excluding primers for locus *Aosten144*) were used in this study. PCR products were electrophoresed on an ABI 3730xl DNA Analyzer (Applied Biosystems) and allele sizes were determined using a 600 LIZ size standard (Applied Biosystems) and GENEMAPPER version 4.0 (ABI). Allele numbers at the 10 loci ranged from two to 12 (average of 5.3), and estimates of gene diversity (Nei, 1978) from 0.10 to 0.92, suggesting that the microsatellites are suitable to characterize the genetic structure of *A. ostenfeldii* at the population level (Sildever et al., 2019).

2.5.2 | Restriction site-associated DNA sequencing

Four sampling dates were included for RAD (S0, P1, P3, P5; Table 2) with 19–20 isolates per date. The seed bank sampling, and three pelagic samplings with maximal temporal distance were chosen to increase the chance of capturing emerging population structure during the season. Exponentially growing cultures (~10,000 cells ml^{-1}) were harvested and concentrated by centrifugation. After addition of 100 µl cetyltrimethylammonium (CTAB) buffer, the cell pellets were stored at −80°C. A freeze–thaw cycle was repeated three times to break down cell walls and ease DNA extraction: samples were thawed at 65°C for 5 min with a bench heating block and immediately frozen again at −80°C for 24 h before starting the next cycle. DNA of each strain was extracted using a CTAB-based protocol (Dempster et al., 1999) modified as described by Sassenhagen et al. (2015).

2.5.3 | RAD library preparations

A pilot test was first carried out with three strains (AOB325, AOB413, AOB504). From this data set a minimum number of reads and number of samples that can be multiplexed was determined. Genomic DNA was digested with high-fidelity *Sbf*I (New England Biolabs), applying a RAD library preparation protocol modified from Amores et al. (2011) and Etter et al. (2011). Modifications (according to Rengefors et al., 2021) included an increased amount of ligase

TABLE 2 Basic population metrics of RAD loci present in 80% of the individuals

Dataset	Population	N	Private alleles (n)	Polymorphic loci (%)	π	H_E	I_A	$\bar{r}_d$	Mean allelic richness
p4	S0	19	117	0.06	0.03	0.03	0.32	0.00	1.13
	P1	20	105	0.05	0.03	0.03	**0.96**	**0.01**	1.13
	P3	19	104	0.05	0.03	0.03	**0.85**	**0.01**	1.13
	P5	20	132	0.06	0.04	0.04	−0.12	0.00	1.16
p2	S	19	—	—	—	0.03	0.72	0.00	1.24
	P	19	—	—	—	0.03	0.27	0.00	1.26

Note: N = number genotyped isolates. H_E = Nei's (1978) gene diversity, π = estimate of nucleotide diversity of variant sites; I_A = index of association; p-value obtained after 999 permutations; $\bar{r}_d = I_A$ adjusted for the number of loci; p-value obtained after 999 permutations; I_A, $\bar{r}_d$, H_E and allelic richness were calculated based on rarefaction analysis (n = 19 for S, P, S0, P1, P3 and P5). Bold numbers indicate significant deviation from a random distribution ($p < .01$).

(2000 U µl^{-1} T4 ligase) and decreased volume of NEB2 buffer (1 µl). In addition, AMPure XP beads were used to remove redundant P1 adapters and elution was done in three steps to increase the DNA yield, following the repair end and overhang addition step. AMPure XP beads were also used instead of column purification after the P2 adapter ligation. The final full amplification was performed with 16 µl template and 16 PCR cycles. After P1 adapter ligation, 20 uniquely barcoded (6 bp) strains were multiplexed per lane. Samples were subsequently sequenced on a HiSeq2500 system (Illumina), using paired-end 125-bp read length and v4 sequencing chemistry. Raw sequence reads are deposited in the European Nucleotide Archive (BioProject PRJEB41677).

2.5.4 | RAD—single nucleotide polymorphism identification

Quality control was done with FASTQC version 0.11.6 (Babraham Bioinformatics), followed by demultiplexing, and processing with STACKS software (Catchen et al., 2011, 2013) version 2.41. The STACKS pipeline was run manually. Prior to the main analysis, parameter settings were tested with a subset of 12 strains (three from each population) with high coverage, as suggested by Paris et al. (2017) and Rochette and Catchen (2017). Two samples (B376 and C115) with fewer than one million reads were excluded from the final analysis. Overall, the average sequencing coverage was very low (<30×) and therefore parameters were optimized to maintain a coverage of at least 10× per sample and maximize the number of utilized reads and polymorphic SNPs, by varying mismatch (M) and depth of stack (m). The final STACKS parameters were set to -m 5 -M 1 (ustacks) and all other parameters were set to default. Raw counts and sequencing coverage were tracked at all possible stages (after process_radtags, ustacks, gstacks and populations) as summarized in Table S2. All genotypes were assigned to belong to a single population in STACKS populations to maximize the number of loci. Furthermore, only loci present in at least 80% of the individuals were utilized to investigate the population genetic structure and metrics. Only the first SNP of each RAD-locus was included, and all data files were created directly with STACKS populations for downstream analyses. Potential human or bacterial contaminant sequences were identified using the taxonomic sequence classifier KRAKEN2 (Wood et al., 2019).

2.6 | Population genetic metrics and statistical analysis

Genetic diversity (π) for RAD data was calculated directly in STACKS based on all SNPs utilized by the STACKS population program. Data on number of RAD sites, variant alleles and polymorphic sites were also obtained from STACKS. Data exploration and all other statistical analysis for RAD and MS data were carried out with R version 3.6.1 (R Core Team, 2018) and R studio version 1.2.5019 (RSTUDIO Team, 2019) focusing on analysis suitable for nonmodel populations including clonal or partially clonal organisms (Grünwald et al., 2017). RAD data were analysed following two approaches. To investigate if the seed bank is more diverse than the pelagic part of the population, a subset of 19 genotypes from each group (seed bank and pelagic part) were randomly selected and pooled again into a new data set (2p). To investigate seasonal differentiation, genotypes of all four temporal subpopulations (S0, P1, P3 and P5) were utilized (4p). For both RAD data configurations (2p and 4p) genotypes had less than 70% missing values and loci less than 50% missing values. Basic population metrics of MS data were extracted using the package POPPR (Kamvar et al., 2014, 2015) and genotypes with more than 25% missing data were excluded. Population differentiation (F_{ST}) for both markers was calculated in R, package HIERFSTAT (Goudet & Jombart, 2015; Nei, 1973), and p-values were obtained after 999 permutations followed by a Monte Carlo Test (package APE4, Bougeard & Dray, 2018). In addition, pairwise genetic distances D (Jost) and G_{ST} (Hedrick) were calculated with the package MMOD (Winter, 2012). Allelic richness was calculated with the package POPGENREPORT (Adamack & Gruber, 2014). Analysis of molecular analysis (AMOVA) was performed with the package POPPR (Kamvar et al., 2014, 2015) followed by a Monte Carlo test. Package GGPLOT2 (Wickham, 2016) was used for plotting the results and the packages MAGRITTR (Bache & Wickham, 2014) and DPLYR (Wickham et al., 2019) were used for data manipulation. The mode of reproduction was assessed for all data sets by evaluating observed linkage

among loci against expected distributions from permutation (999 permutations) using the index of association (I_A) described by Brown et al. (1980) and index $\bar{r}_d$, which accounts for the number of loci sampled and is less biased (Agapow & Burt, 2001). I_A and $\bar{r}_d$ were assessed with the package POPPR (Kamvar et al., 2014, 2015).

Population structure was investigated using principal components analysis (PCA) in the R package (Jombart & Ahmed, 2011) and remaining missing values were ignored. In addition, a *k*-means clustering approach, which identifies clusters using successive *k*-means, was used from the same package. These functions implement a clustering procedure of running successive *k*-means with an increasing number of clusters (*k*), after transforming data using a PCA. For each model, a statistical measure of goodness of fit (Bayesian information criterion [BIC]) was computed to choose the optimal *k*. Furthermore, a discriminant analysis of principal components (DAPC) was carried out, followed by DAPC cross-validation to choose the number of principal components retained.

3 | RESULTS

3.1 | Sequencing and basic metrics

Between 84 and 138 single cells were isolated after each sampling, of which 28%–50% were culturable (Table 1). With MS, 261 strains were successfully characterized in total, based on nine loci and 54 alleles. A genotype accumulation curve showed that at least nine loci are required to discriminate between unique individuals. Four genotypes with more than 25% missing values were excluded from the MS analysis. Of the remaining 257 genotypes, the number of multilocus genotypes (MLGs) was between 24 (P2) and 45 (S0 and P1) and four clones were detected (Table 1). Reads of the RAD data set were 113 bp in length, the mean number of RAD reads per sample was 17,032,952 (± 839,864 *SE*) and the mean per-locus coverage across all samples was 26.8× (Table S2). Sequence classification of the resulting loci using KRAKEN2 identified 17.16% (± 7.74 *SD*) of sequences as of potential bacterial origin and 4.78% (± 1.78 *SD*) of sequences as of potential human origin. Only unclassified sequences (i.e., originating from the dinoflagellate) were included in downstream analysis. The number of retained loci was compared for different STACKS populations settings using (i) all sequences and (ii) only unclassified sequences (Table S3). After filtering loci found in at least 80% of the individuals, 666 RAD loci remained, of which 646 were variant (1,292 alleles). The mean length of the RAD loci was 584.52 (± 2.64) and all genotyped individuals were unique.

3.2 | Diversity measures

High genotype (or clonal) diversity, which is the ratio of the number of unique genotypes (G) to the total number of isolates genotyped (N), was calculated with MS data (G:N = 0.98) and RAD data (G:N = 1.00). Gene diversity (Nei, 1978) varied from ~0.53 (P4, P5, S6) to 0.58 (S0) based on MS data. The number of MS alleles per locus ranged from three to nine with an average of 5.4 (Table S4) and the mean allelic richness after rarefaction was between 3.64 (P3) and 4.15 (P2) (Table 1). In both RAD data configurations (2p and 4p) the seed bank and pelagic fraction had a similar mean gene diversity (H_E) of 0.03–0.04 (p4) and 0.03 (p2), (Table 2), which equals the nucleotide diversity (π), as we only utilized the first SNP of each locus. RAD-locus allelic richness of the temporal population parts ranged from 1.13 (S0, P1, P3) to 1.16 (P5) and was very similar in the seed bank (1.24, S), compared to the pelagic part of the population (1.26, P). The highest number of private alleles occurred in the P5 (132) and the lowest number in the P3 (104) population. The level of polymorphism was generally very low; based on RAD data, 0.05%–0.06% of all sites were polymorphic.

3.3 | Linkage equilibrium

Based on MS data the index of association (I_A) and the index of association adjusted for the number of loci ($\bar{r}_d$) of each temporal subpopulation ranged from −0.17 to 0.11 and −0.02 to 0.01 (Table 1). The *p*-values obtained for both indices indicated no significance. For the 2p RAD data configuration, I_A values of 0.72 (S) and 0.27 (P), corresponding to $\bar{r}_d$ values below 0.00, were calculated (Table 2). For the 4p RAD configuration, the I_A was between −0.12 (P5) and 0.96 (P1), corresponding to $\bar{r}_d < 0.002$ (S0, P5) and 0.01 (P1, P3). The I_A and $\bar{r}_d$ obtained for P1 and P3 were very low, but significantly different from a random distribution ($p < .01$, Table 2, Figure 1).

3.4 | Genetic differentiation and population metrics

The analysis of microsatellite and SNP data showed that there was no significant differentiation between all pairwise population comparisons (Tables 3 and 4). Based on MS data pairwise F_{ST} values were between 0.009 (P4–S6) and 0.027 (P2–P5) and based on RAD data between 0.028 (P1–P5) to 0.032 (S0–P5) (Table 3). MS-based F_{ST} values between the spring seed bank population (S0) and temporal pelagic populations increased marginally towards the end of the season (0.010–0.018, P1–S6), but there was no significant differentiation. The pairwise distances were higher at P1–P3 (0.025) and P1–P4 (0.023), but none of the F_{ST} values were significant. The overall F_{ST} value between pelagic and seed bank isolates was even lower after rarefaction (0.011). Pairwise G_{ST} (Hedrick) values ranged from 0.002 (S6–P4) to 0.017 (P1–P3 and P2–P5) based on MS data and displayed almost no variation for the 4p RAD data (−0.008 or −0.009, between most pairs). *D* (Jost) ranged from 0.003 (P4–S6) to 0.041 (P1–P3) for the MS data and was 0.024 or 0.025 between all population pairs based on 4p RAD data. The AMOVA results indicated no population differentiation across the season nor between the benthic and pelagic population (Phi = −0.007). One hundred per cent variation was found at the population level, with no significant differences between populations ($p = .08$ for p4 and $p = .87$ for p2, Table S5).

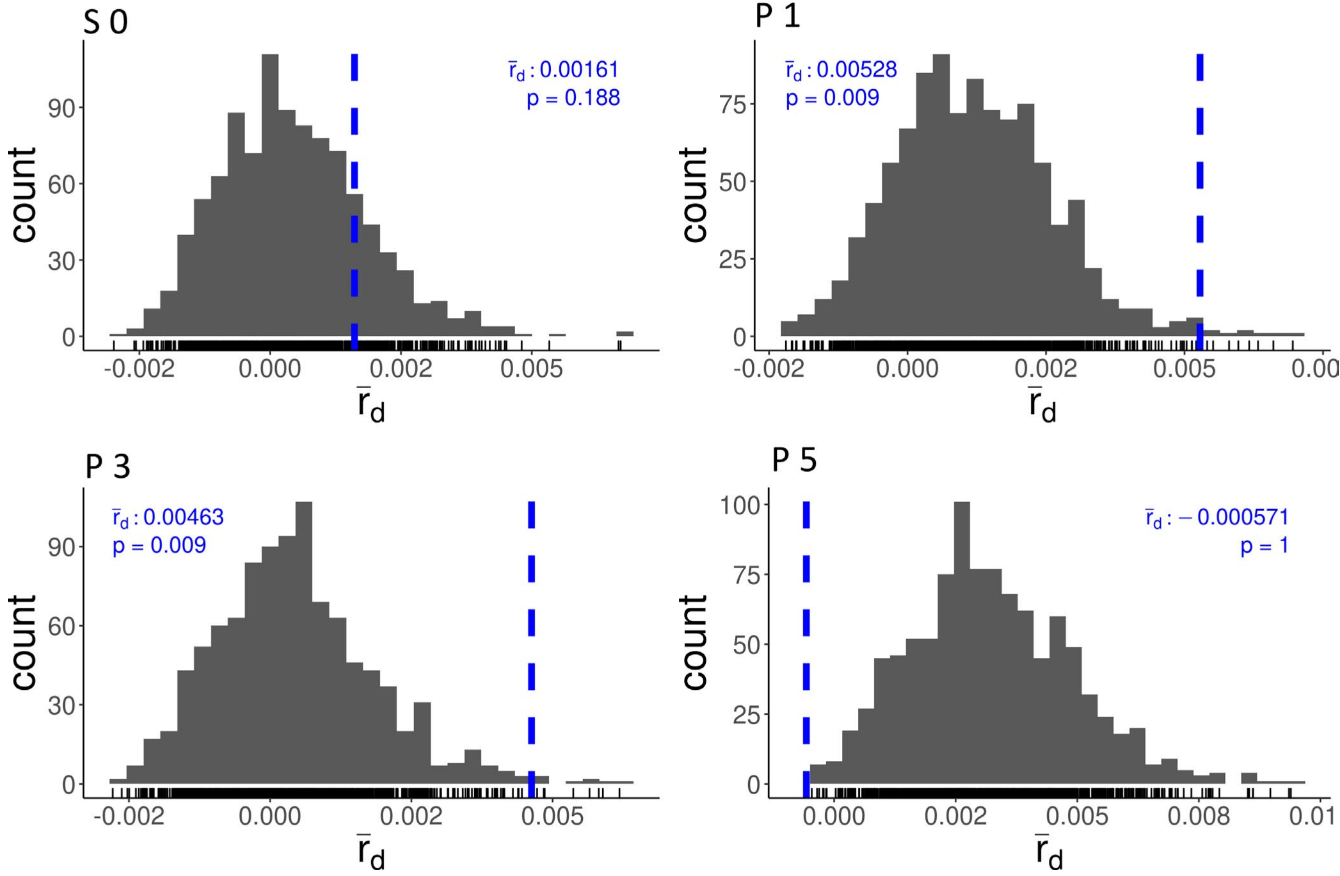

FIGURE 1 Indices of association adjusted for the number of loci ($\bar{r}_d$), based on RAD data for temporal population parts. $\bar{r}_d$ values are plotted as a dashed line. The grey area represents the resampled distribution of unlinked loci. Based on $\bar{r}_d$ there is no evidence of linkage disequilibrium among loci for S0 and P5 ($p > .05$), consistent with a sexually recombining population. For P1 and P3 the hypothesis of no linkage among markers is rejected ($p < .01$), indicating a higher degree of clonality (p-value obtained after 999 permutations, $n = 19$)

TABLE 3 Pairwise genetic distances for MS and RAD

	S0	P1	P2	P3	P4	P5
S0		0.032		0.032		0.028
P1	0.010			0.031		0.028
P2	0.014	0.015				
P3	0.015	0.025	0.017			0.028
P4	0.016	0.023	0.019	0.014		
P5	0.016	0.016	0.027	0.017	0.013	
S6	0.018	0.017	0.015	0.018	0.009	0.012

Note: P = pelagic part of the population, S = seed bank: F_{ST} for MS data below the diagonal, and RAD data above the diagonal (Nei, 1973); p-values obtained with a Monte-Carlo test after 999 permutations indicated no significant difference for all pairwise comparisons.

3.5 | Population genetic structure

PCA showed that the first three principal components explained less than 15% of the total variance in the MS and the 4p RAD data set. With increasing number of principal components, eigenvalues decreased gradually (without a sharp decrease). Thus, plotting the first two principal components was not meaningful to display the variation of the data sets and PCA plots are not shown. A K-means cluster analysis, based on allele frequencies, showed that there was no support for more than one cluster ($K = 1$) based on BIC. BIC values increased steadily with the number of clusters, indicating that one cluster explained the variation of the data set best. Posterior membership probability plots, based on DAPC, showed that all individuals belong to one group, and therefore no plots are presented. DAPC cross-validation indicated that the proportion of successful outcome prediction reached 0%–60%

TABLE 4 Pairwise genetic distances for MS (left side) and RAD data (right side): *D* (Jost) above/G_{ST} (Hedrick) below the diagonal

MS	S0	P1	P2	P3	P4	P5	S6	RAD	S0	P1	P3	P5
S0		0.010	0.022	0.023	0.026	0.023	0.029	S0		0.024	0.024	0.025
P1	0.004		0.016	0.041	0.037	0.021	0.024	P1	−0.008		0.024	0.025
P2	0.009	0.007		0.025	0.024	0.039	0.013					
P3	0.009	0.017	0.010		0.019	0.023	0.026	P3	−0.008	−0.008		0.025
P4	0.011	0.017	0.010	0.008		0.012	0.003					
P5	0.010	0.009	0.017	0.010	0.005		0.008	P5	−0.009	−0.009	−0.009	
S6	0.012	0.011	0.006	0.011	0.002	0.004						

for any number of principal components retained (1–78) but was highest for 47 principal components (90%).

3.6 | Selection regimes

Abiotic and biotic environmental data displayed a pronounced seasonal variation (Figure 2), resulting in distinct selection regimes for each temporal population, as indicated in Figure 3. The major differences were that earlier time points of the season were characterized by higher grazing pressure, together with highest toxin levels of STX (P1) and GTX2/3 (P2), whereas a higher phytoplankton biomass and higher nutrient levels occurred later in the season. The environmental conditions at the first seed bank sampling S0 (end of March) were characterized by a low temperature (3°C) and low nutrient levels (TN and TP below 1 μmol L^{-1}), combined with a low Chl *a* level (11.6 μg L^{-1}), and no *Alexandrium ostenfeldii* cells in the water column (Figure 2d). At the beginning of June (P1) the temperature had increased to 14.4°C and a low number of *A. ostenfeldii* (360 cells L^{-1}) was found together with high total toxin cell quota of 8.3 pg per cell. At the same time a low phytoplankton biomass (~1 mg L^{-1}, Figure 2a), a high zooplankton biomass (1,800 individuals L^{-1}, Figure 2c) and elevated nutrient concentrations (TN and TP, Figure 2e) were measured. A different situation was observed at the end of June (P2), when the temperature had reached 20°C. An increase of the *A. ostenfeldii* abundance (~1000 cells L^{-1}) and toxin cell quota (12.0 pg PSP toxins per cell) co-occurred with the highest zooplankton abundance (2300 individuals L^{-1}), but the overall phytoplankton biomass and Chl *a* remained low. A prolonged period of elevated temperatures (above 15°C) occurred in July and August (P3–P5), with decreasing zooplankton abundance (below 360 individuals L^{-1}) and increased growth of *A. ostenfeldii* (above 6,000 cells L^{-1}, Figure 2d) and other phytoplankton (in total 14 mg L^{-1}, Figure 2a), corresponding to a Chl *a* peak of ~110 μg L^{-1} (P5). During this period, we measured the second highest total toxin cell quota of the season (8.9 pg per cell, P4 sampling, Figure 2b) and a maximum concentration of TN and TP (~80 and 3 μmol L^{-1}, Figure 2e). During the P5 sampling the ratio of inorganic N:P was ~50, whereas N:P ratios were below 5 during all other pelagic samplings. Towards the end of the season (sampling S6 in September) temperature and the Chl *a* level had declined to 16°C and 65 μg L^{-1}, but total phytoplankton biomass was still high (~12 mg L^{-1}) and the cell density of *A. ostenfeldii* reached its maximum of 11,844 cells L^{-1}.

4 | DISCUSSION

Algal blooms can have a duration of several weeks to months, despite short generation times and substantial changes in the aquatic environment during the bloom period. Consequently, it has been suggested that the blooms are exposed to shifting selection regimes, which could result in separate subpopulations that are favoured at different times during the growth season. Here, we tested the

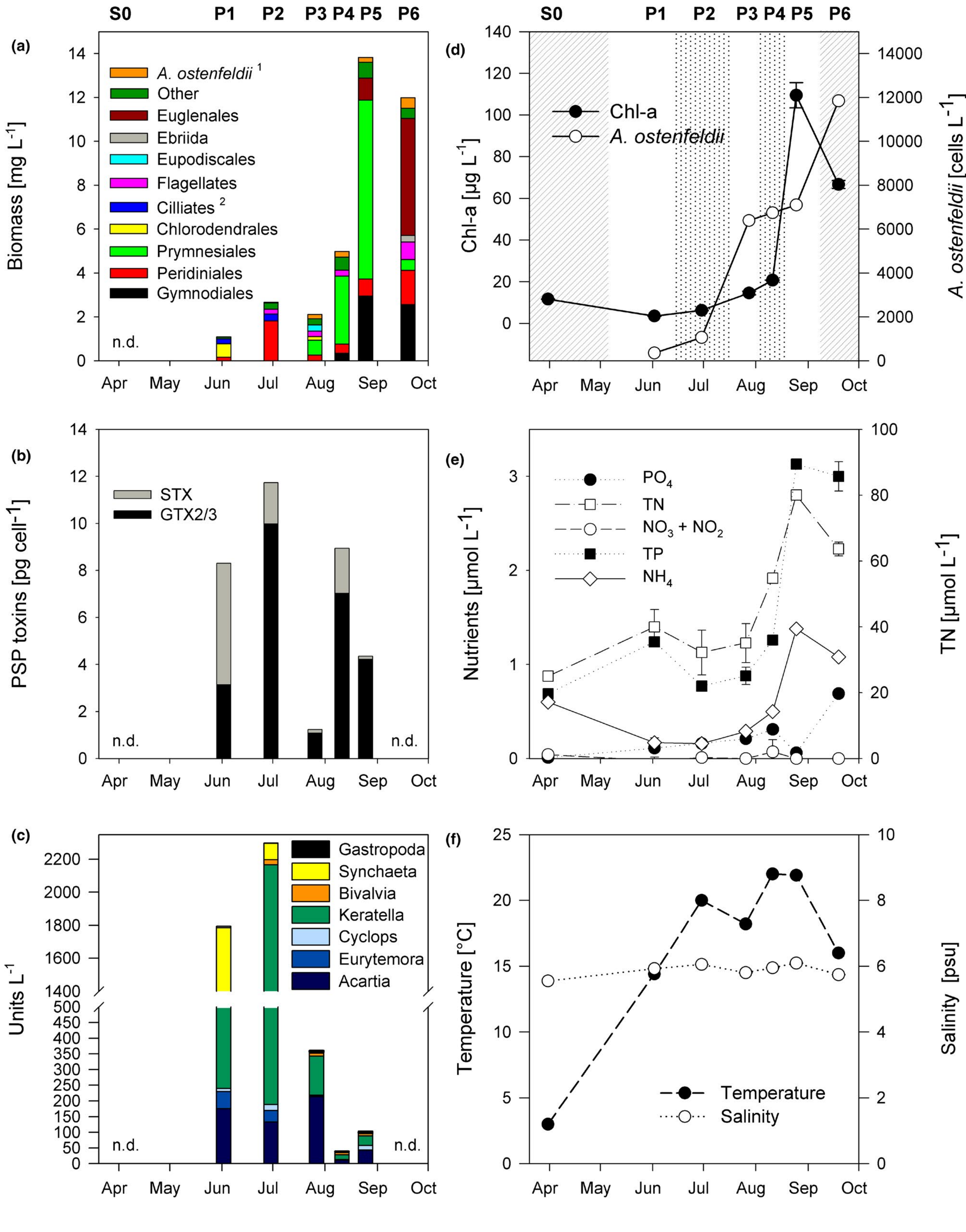

FIGURE 2 Biotic and abiotic environmental parameters (mean values, $n = 4$). (a) Phytoplankton taxa contributing to more than 80% of the total biomass: [1]Gonyaulacaceae, [2]with endosymbiotic algae; (b) *Alexandrium ostenfeldii* toxins; (c) zooplankton community; (d) total Chl *a* and cell concentration of *A.ostenfeldii*; (e) nutrient concentrations: left side *x*-axis—PO_4 (filled circles), total phosphorus (TP, filled squares) and dissolved inorganic nitrogen ($NO_3 + NO_2$, empty circles and NH_4, empty diamonds); secondary *x*-axis—total nitrogen (TN, empty squares); (f) water temperature and salinity; n.d., not determined

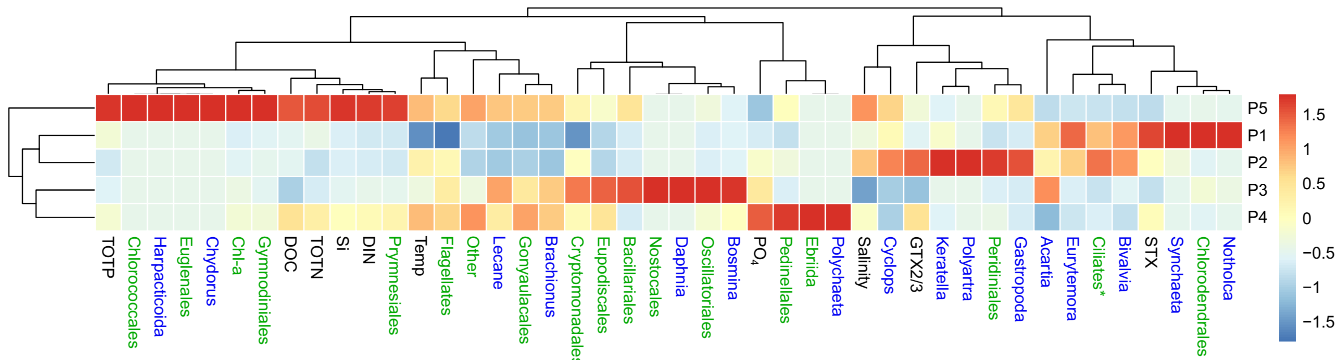

FIGURE 3 Heatmap of environmental variables at different times of the season (P1–P5). Vertical black labels = abiotic variables, green labels = phytoplankton and blue labels = zooplankton; values have been centred and scaled variable-wise; that is, 0 is the variable mean and, for example, 1.5 denotes a value that is 1.5 standard deviations higher than the mean for that variable

hypotheses that the pelagic part of the population consists of separate subpopulations, and that the resting stage "seed bank" serves as a genetic reservoir with high genetic diversity. Contrary to our expectations, we found little evidence of population differentiation within the pelagic population of the dinoflagellate *Alexandrium ostenfeldii*. Moreover, genetic diversity was equally high in the pelagic part of the population and the seed bank.

4.1 | Seed bank diversity– unravelling the reservoir function

Both types of genetic marker applied in this study revealed similar diversity levels in the benthic and the pelagic phase of a Baltic *A. ostenfeldii* population, which contrasts with the common expectation of increased diversity of seed banks (Rengefors et al., 2017) or zooplankton egg banks (Brendonck & De Meester, 2003). The results of this study were similar to those found in the diatom *Skeletonema marinoi*, which produces asexual resting stages (Godhe & Härnström, 2010). Circumstances that would facilitate an increased diversity in the seed bank, compared to the actively growing part of the population in the water column, are accumulation of sexually produced resting stages in the sediment, combined with germination and growth of a smaller subset of the entire population, and limited exchange between the benthic and pelagic habitat. For most dinoflagellates with a known life cycle, resting cysts are a result of sexual reproduction, whereas asexual proliferation and selection occur in the pelagic phase of the life cycle (Rengefors et al., 2017). Thus, a "typical" dinoflagellate life cycle could promote increased diversity of the benthic seed bank, compared to a mainly clonally growing subset of the pelagic population, if genotypes with certain traits are selected and germinate at the start of the season. On the other hand, successive recruitment of diverse genotypes has been considered as a factor explaining high genetic diversity in prolonged phytoplankton blooms (Lebret et al., 2012). We can assume that the shallow water at the Föglö sampling site facilitates constant exchange between the seed bank and the plankton through resting stage formation and germination. *A. ostenfeldii* has a generalist life cycle strategy, forms diverse resting stage types, including sexual heterothallic and asexual (and/or sexual homothallic) ones, under a variety of conditions (Jerney, Ahonen, et al., 2019; Jerney, Suikkanen, et al., 2019) and can germinate within a wide temperature range throughout the season (Figueroa et al., 2008; Jerney, Ahonen, et al., 2019). Frequent resting stage formation and germination throughout the season would ensure strong coupling between the seed bank and the pelagic part of the population, which will harmonize the diversity between the two phases.

Our results for clonal diversity (0.97 MS and 1.00 RAD) and gene diversity H_E (0.53–0.58 MS) fall within the range of other studies on *Alexandrium*, reporting clonal diversity of 0.47–1.0 and gene diversity of 0.54–0.88 (Collins et al., 2014 and references herein). Nagai et al. (2004) reported a gene diversity of up to 0.97 for *A. catenella* (reported as *A. tamarense*), but relatively low gene diversity of 0.27 was reported for *A. tamiyavanichii* (Nishitani et al., 2009). Gene diversity values were lower (H_E, equal to Pi, between 0.03 and 0.04) based on RAD data, compared to MS data in this study and previously reported values (0.07–0.11) derived from amplified fragment length polymorphism (AFLP) markers (Tahvanainen et al., 2012). Likewise, different markers revealed diverging results for *A. catenella* (reported as *A. tamarense*): gene diversity of North Sea populations ranged from 0.03 to 0.51 based on AFLP loci and from 0.31 to 0.83 based on microsatellite loci (Alpermann et al., 2010). Inconsistent gene diversity results obtained with different markers for the same species probably reflect marker-specific characteristics (Fischer et al., 2017), as discussed later.

4.2 | Frequency of sexual reproduction

To determine if populations are in linkage equilibrium or not, the linkage between loci was tested by calculating the index of association (I_A) and the index of association, adjusted for the number of loci sampled ($\bar{r}_d$) (Agapow & Burt, 2001). For clonal growth significant disequilibrium (LD) is expected due to linkage among loci,

whereas for sexual growth linkage among loci is not expected. The null hypothesis tested is that alleles observed at different loci are not linked if populations are sexual, while alleles recombine freely into new genotypes during the process of sexual reproduction. Values close to zero indicate linkage equilibrium, while deviations from zero indicate disequilibrium. In this study low and nonsignificant $\bar{r}_d$ values were calculated for the entire season based on MS. Thus, we fail to reject the null hypothesis of no linkage among markers and conclude that sexual reproduction occurs not only at the end of the growth season, but more frequently in the pelagic phase. Based on RAD data, subpopulations P1 and P3 exhibited weak significant LD (Table 2, Figure 1), pointing at increased clonal reproduction at the beginning of June and end of July. During this time high clonal growth and increased intra- and interspecific competition could have selected for the most competitive clonal lineages. Weak, but significant LD has been reported earlier for other Baltic *A. ostenfeldii* populations, but not for the Föglö population (Tahvanainen et al., 2012), which is partly in line with results of this study. Other studies that have addressed reproduction patterns in population genetic analyses reported weak or no LD (Alpermann et al., 2009; Dia et al., 2014; Sassenhagen et al., 2015; Wyngaert et al., 2015), indicating that regular recombination events occur in phytoplankton populations despite their primarily asexual mode of growth. Sexuality indicators may be present in some and absent in other populations or subpopulations (Tahvanainen et al., 2012; Wyngaert et al., 2015), which suggests that the level of sexual reproduction, and consequently genetic diversity, is influenced by, for example, habitat conditions, life-cycle strategies or population history (Rengefors et al., 2017). The frequency of sexual reproduction has a strong impact on short-term evolution, as it may erase genetic structure emerging during a bloom event (Dia et al., 2014).

4.3 | Seasonal population structure and selection

Pairwise genetic distances calculated for MS and RAD data (Tables 3 and 4) suggest that the *A. ostenfeldii* bloom populations were not temporally differentiated. Although increased pairwise genetic distances between S0 and P1–P5 (MS, Tables 3 and 4) suggested weak diversification throughout the season, this trend was not significant. No significant population structure or variation of diversity was detected, based on both markers, despite seasonal variation of potential selection pressures (Figure 3). In order for populations to become genetically differentiated, one or more of the following processes are involved: natural selection, mutation or genetic drift, in combination with limited gene flow (Rengefors et al., 2017). Some probable explanations for the lack of genetically differentiated subpopulations here are the following: first, no single clone(s) dominate(s) for long enough to form a separate subpopulation. This can be due to frequent sexual reproduction and resulting recombination, as indicated by the index of association. It could also be explained by a dynamic environment that never allows dominance of one or a few clones, analogous to the Intermediate Disturbance Hypothesis (Connell, 1978), or as Rynearson and Armbrust (2005) hypothesized: individual clonal lineages could be prevented from becoming numerically dominant by extensive clonal diversity, generated through past sexual reproduction events, coupled with frequent environmental changes. Second, population differentiation may be prevented by high phenotypic plasticity, allowing lineages to survive environmental changes, which counteracts selection for different ecotypes/subpopulations.

The investigated *A. ostenfeldii* bloom site represents a dynamic environment with seasonal variation of potential selection pressures (Figure 3). For example, at the end of June (P2), when zooplankton abundance peaked, predation presumably represented a strong selection pressure, triggering high toxin production, which was suggested to be a defence mechanism against grazing, for example, of *A. catenella* (Griffin et al., 2019). Later, relaxation of selection pressures allowed for an increase of *A. ostenfeldii* cell numbers (Figure 2d) until the end of July (P3), which marked the end of an exponential growth phase, associated with a maximum clonal relative abundance, as evidenced by low diversity (allelic richness, Table 1) and significant LD (Figure 1). Thereafter, increasing competition may be selected for the most competitive clonal lineages, as diversity remained low (P4, allelic richness, Table 1) and the second highest PSP toxin cell quotas were measured (Figure 2b), equalling highest toxin concentration per litre of sea water (Figure S1). At the end of August (P5) phosphorus limitation and even stronger competition could have selected for competitive lineages when phytoplankton abundance peaked. The inorganic N:P ratio was ~50 during P5 sampling, which is above 16 and suggests phosphorus limitation based on Redfield (1958), whereas inorganic N:P ratios below 5 indicate nitrogen limitation during the rest of the season (Redfield, 1958). None of the aforementioned selection pressures lead to measurable differentiation or population structure, which could only occur with limited gene flow.

Previous studies on other *Alexandrium* species reported temporal (Alpermann et al., 2009; Richlen et al., 2012) and spatiotemporal (Dia et al., 2014; Gao et al., 2019) genetic intrabloom differentiation. These were probably driven by natural selection through environmental conditions, such as nutrient concentrations (Gao et al., 2019), but solid evidence for this hypothesis is lacking. Similarly, Rynearson et al. (2006) speculate that environmental conditions (solar irradiance and silicic acid concentrations) regulate bloom dynamics of distinct diatom populations via selection. A dominant diatom population of *S. marinoi*, encountered in the plankton and the sediment of a Swedish Fjord, showed weak genetic differentiation of the plankton population in two consecutive years, but the shifts could not be attributed to specific selection regimes (Godhe & Härnström, 2010). Tammilehto et al. (2016) reported significant progressive loss of genetic differentiation during an arctic bloom of the diatom *Fragilariopsis cylindrus* within a few weeks, coupled with stable genetic diversity levels, but predominantly asexual growth is assumed for this species and resting stages are not known. Similarly, a loss of genetic differentiation over time (coupled with an increase in genetic diversity) was observed within a single bloom of the raphidophyte *Gonyostomum semen* in freshwater lakes (Lebret et al., 2012). The authors conclude that those observations may reflect

the continuous germination of resting stages from the sediment, which highlights the importance of taking life cycle dynamics into account when estimating intraspecific genetic diversity of microbial organisms. Another example demonstrating the impact of different life cycle phases on the genetic structure of microalgal populations is the diatom *Pseudo-nitzschia multistriata*, where intermittent periods of weak and strong diversification were related to the temporary predominance of clonal expansions over sexual recombination (Ruggiero et al., 2018). Other diatom studies (e.g., Postel et al., 2020) reported robust genetic divergence along environmental gradients with marked differences in environmental conditions, but focused on much larger geographical distances, compared to this study. Mechanisms that are probably responsible for the observed patterns may vary between environments or systems, which should be considered when comparing them. For example, different dynamics can be expected for blooms that cover a much wider geographical extent or occur in systems that are deeper (fjords) or stratified. Benthic–pelagic coupling may be less intense in such systems, which probably affects reproduction strategies. Similarly, not all taxa have such frequent or continuous sexual reproduction, so the mechanisms shaping population dynamics of those taxa may be different.

4.4 | MS vs. RAD

Genome-wide analyses of SNP diversity were suggested to be more powerful, compared to MS markers, to study genetic diversity within and among populations, because a limited number of microsatellite markers, marker ascertainment bias and a high variance in microsatellite-derived diversity estimates may not adequately reflect genome-wide genetic diversity (Fischer et al., 2017). MS mutation rates are orders of magnitude higher and much more variable than those of single SNPs and almost certainly do not reflect those of the genome as a whole (Hodel et al., 2017). Thus, the unusually high mutational properties, which makes MS excellent for distinguishing different individuals, may inflate statistics such as F_{ST} and heterozygosity relative to the rest of the genome (Hodel et al., 2017). F_{ST} values calculated for both markers were similar in this study, although slightly higher based on the RAD data. Since F_{ST} values are typically underestimated when based on microsatellites, the SNP-based F_{ST} is likely to be more reliable. Estimates of F_{ST} may become inflated as missing data increase (Hodel et al., 2017), and thus strict filtering (SNPs present in 80% of individuals or exclusion of MS genotypes with more than 25% missing data) was applied in this study to avoid F_{ST} inflation. However, a more liberal filtering of loci could retain loci valuable for population differentiation inference, since inflation of population genetic statistics might not happen as quickly, as implied in simulation studies when loci with missing data are added (Hodel et al., 2017). Thus, more information about population structure might have been lost due to the strict filtering of loci applied in this study. Furthermore, the low SNP-based gene diversity of the present study might be an underestimation potentially arising from a bias in allele frequency estimation due to nonrandom haplotype sampling when using restriction digestion (Arnold et al., 2013). This could be because some recognition sequences themselves will be polymorphic, resulting in missing data for some chromosomes, and nonrandom sampling of lineages in a sample (Arnold et al., 2013). Additionally, a high degree of repetitive sequences characterizing the *A. ostenfeldii* genome (Jaeckisch et al., 2011) could contribute to the relatively low RAD diversity estimates. The vast majority of the *A. ostenfeldii* genome appears to consist of large tandem arrays, and together they comprise at least 58% of the total sequence (Jaeckisch et al., 2011), which may be captured with RAD, but not with highly variable MS. An additional issue could be the size of the *A. ostenfeldii* genome. Because the genome is very large — around100 Gb; based on the general formula genome size (bp) = (0.921 × 109) × DNA content (pg) (Jaeckisch et al., 2011) and a DNA content of 115 pg per cell, measured by flow cytometry (Figueroa et al., 2010) — many loci that are unique were perhaps not covered in the analyses because they were excluded by the strict filtering settings. Thus, it is possible that preferentially repeated or conservative regions were included in the analysis leading to an underestimation of gene diversity and F_{ST}. Despite minor deviations, the results for both markers are in line in this study and highlight the importance of comparing alternative genotyping methods to study nonmodel organisms.

4.5 | Limitations of this study

The relatively low success of culture establishment from benthic and pelagic samples in this study (28%–50%, Table 1) means that 50% or more of the genetic diversity and variation were potentially not captured. This limitation is common for many culture-based genotyping studies, but culturing success is rarely reported. The survival rate in a previous *A. ostenfeldii* study ranged from 35% to 71% in different Baltic populations (mean survival rate was 51.6%) (Tahvanainen et al., 2012) and is similar to the survival rate of, for example, *A. minutum* (42.7%) (Dia et al., 2014) or *A. fundyense* (38%) (Erdner et al., 2011) and this study.

4.6 | Evolution and adaptation

Depending on whether the fraction of emerging genotypes is a random (well mixed) or nonrandom (e.g., only individuals from the previous season germinate) sample of the total gene pool, seed banks may both slow down and enhance evolution by adaptation (Hairston Jr & De Stasio Jr, 1988). It can be assumed that the *A. ostenfeldii* seed bank has a stabilizing effect that slows down evolution, because the uppermost flocculent sediment layer is well mixed, allowing a random set of genotypes, formed in different years and seasons, to germinate. If a random set of genotypes germinates, individuals which are maladapted to present-day conditions could pass their genes on to the next generation and thereby slow down adaptation. Furthermore, a shallow water seed bank

allows for rapid switching between the benthic and pelagic habitat, supporting high phenotypic plasticity, which can weaken the effect of natural selection on individuals and their associated traits (Chevin et al., 2010). High plasticity of growth rates was reported from transplant experiments with this species (Jerney, Suikkanen, et al., 2019) and is supported by flexible resting stage formation and germination (Jerney, Ahonen, et al., 2019). Thus, the population is buffered against seasonal selection and rapid differentiation, which can slow down evolution.

Simultaneously, the large clonal diversity can facilitate evolutionary adaptation if selection pressures last longer, become more intense, or benthic–pelagic coupling is prevented or strongly reduced. Moreover, new genotypes with highly variable competitive abilities may evolve at timescales significantly shorter than climate change (Bach et al., 2018). Thus, climate change-related environmental variation will probably not compromise the occurrence of this species in the Baltic Sea, as large phenotypic plasticity and a generalist life cycle were reported (Jerney, Ahonen, et al., 2019; Jerney, Suikkanen, et al., 2019), in addition to high clonal diversity.

5 | CONCLUSIONS

Similar diversity levels of benthic and pelagic *Alexandrium ostenfeldii* population parts and a lack of seasonal structure, despite pronounced selection pressures, probably result from several processes. Tight benthic–pelagic coupling combined with sexual and asexual growth and cyst formation maintain gene flow throughout the season and harmonize the diversity in both zones. A lack of seasonal population structure could be associated with unlimited gene flow, high phenotypic plasticity, intermediate disturbance or too weak selection by environmental factors. Local seed banks provide a buffer against short-term environmental variability and potentially slow down evolution, if individuals maladapted to present-day conditions germinate and contribute to the gene pool. Simultaneously, long-term adaptation to future environmental conditions is possible because genetic diversity is stored, and new genotypes arise from frequent recombination.

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CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

The study was planned by A.K. and K.R. The samples were collected by J.J., A.K. and S.S. The cultures were initiated and maintained by J.J. The data were collected and analysed by J.J., supported by all authors. The paper was drafted by J.J., A.K. and S.S. J.J. wrote the paper with contributions of all authors.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making the digitally-shareable data, required to reproduce the reported results, publicly available at the European Nucleotide Archive (BioProject PRJEB41677) and the Dryad Digital Repository (https://doi.org/10.5061/dryad.d51c5b041).

DATA ACCESSIBILITY AND BENEFIT-SHARING STATEMENT

RAD sequence data have been submitted to the European Nucleotide Archive (BioProject PRJEB41677 – https://www.ebi.ac.uk/ena/browser/view/PRJEB41677). Scripts used for RAD data analysis with the STACKS pipeline, R scripts for MS and RAD data analysis, MS raw data and supporting information can be accessed via Dryad DOI https://doi.org/10.5061/dryad.d51c5b041.

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SUPPORTING INFORMATION

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