

LETTER

Intraspecific trait variation and trade-offs within and across populations of a toxic dinoflagellate

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Abstract

Intraspecific trait diversity can promote the success of a species, as complementarity of functional traits within populations may enhance its competitive success and facilitates resilience to changing environmental conditions. Here, we experimentally determined the variation and relationships between traits in 15 strains of the toxic dinoflagellate *Alexandrium ostenfeldii* derived from two populations. Measured traits included growth rate, cell size, elemental composition, nitrogen uptake kinetics, toxin production and allelochemical potency. Our results demonstrate substantial variation in all analysed traits both within and across populations, particularly in nitrogen affinity, which was even comparable to interspecific variation across phytoplankton species. We found distinct trade-offs between maximum nitrogen uptake rate and affinity, and between defensive and competitive traits. Furthermore, we identified differences in trait variation between the genetically similar populations. The observed high trait variation may facilitate development and resilience of harmful algal blooms under dynamic environmental conditions.

Keywords

Eco-evolutionary dynamics, biodiversity, population ecology, harmful algal blooms, *Alexandrium ostenfeldii*.

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INTRODUCTION

Ecosystem functioning is often linked to biodiversity, with enhanced productivity and stability in systems with higher species richness (Cardinale *et al.* 2006; Ptasnik *et al.* 2008; Cardinale 2011; Schindler *et al.* 2015). By increasing the number of species in the community, complementarity of functional traits within the system may increase, resulting in higher resource use efficiencies, enhanced resistance to consumers and resilience to environmental changes (MacArthur 1955; Hooper *et al.* 2005; Bolnick *et al.* 2011; Cardinale 2011). Similarly, a high intraspecific trait diversity, as well as variation within traits may facilitate population growth by enhanced complementarity (Violle *et al.* 2012). Moreover, high standing genetic and trait variation within populations allows species to adapt more quickly to changes in the environment (Barrett & Schluter 2008). This variation is ultimately responsible for the diversification and evolutionary adaptation of species, which can even happen on ecological time scales (Carroll *et al.* 2007; Pelletier *et al.* 2009). Especially with respect to climate change, intraspecific trait variation could modulate species' responses to changing environments and buffer biodiversity losses (Reusch *et al.* 2005; Albert *et al.* 2010; Sjöqvist & Kremp 2016).

Phytoplankton populations can possess a very high genetic diversity with substantial intraspecific trait variation (Maranda *et al.* 1985; Medlin *et al.* 2000; Burkholder & Glibert

2006; Alpermann *et al.* 2010; Godhe *et al.* 2016). Specifically, harmful algal bloom (HAB) populations have been shown to exhibit a high variation in the expression of traits (Tillmann *et al.* 2009; Hakanen *et al.* 2014; John *et al.* 2015). HAB species are known to possess specialized functional traits, including toxin production, as well as N₂ fixation and mixotrophy (Fogg 1969; Hallegraeff 1993; Burkholder *et al.* 2008), which are expected to contribute to the success of HAB populations (Paerl & Huisman 2009; Litchman *et al.* 2010; Carey *et al.* 2012). Among common HAB-forming phytoplankton groups are dinoflagellates, a ubiquitous group of protists that particularly contributes to primary production in coastal waters (Falkowski & Knoll 2007). The toxic dinoflagellate genus *Alexandrium* is among the most common HAB species, imposing an eminent threat to human health and ecosystem functioning by causing shellfish poisoning and fish kills (Cembella *et al.* 2002; Durbin *et al.* 2002; Anderson *et al.* 2012). Like most HAB species, *Alexandrium* populations possess a high functional trait diversity (Ogata *et al.* 1987; Cembella *et al.* 2002; Masseret *et al.* 2009; Tillmann *et al.* 2009; Alpermann *et al.* 2010). Indeed, strains can substantially differ in the production of paralytic shellfish poisoning (PSP) toxins, with some strains even lacking the ability to produce these toxins (John *et al.* 2014; Murray *et al.* 2015). Furthermore, some *Alexandrium* species and strains can produce two additional types of neurotoxins; gymnodimines (GYM) and spirolides (SPX; Cembella *et al.* 2000; Kremp *et al.* 2014; Van de Waal

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et al. 2015), as well as allelopathic compounds that are excreted from the cell and can lyse competing phytoplankton species and small protozoan grazers (Tillmann & John 2002; Tillmann *et al.* 2007).

The high functional trait variation within *Alexandrium* populations can partly be attributed to their life histories. In addition to vegetative growth, *Alexandrium* reproduces sexually and forms resting cysts, allowing recombination of genetic material (Anderson *et al.* 2012). Cysts may hatch continuously throughout the vegetative growth season and thereby supply novel genotypes to the water column (Genovesi *et al.* 2009; Angles *et al.* 2012; Kremp *et al.* 2015). This mechanism may contribute to a high genotypic diversity, and support a high variation in traits within a population (Alpermann *et al.* 2009). High trait variation is further enhanced by shifting selection pressures that can occur during toxic algal blooms (Driscoll *et al.* 2015). For instance, before the onset of a bloom, interactions occur mainly interspecifically, and competition with other phytoplankton species together with a high grazing pressure may select for defensive traits, such as toxin and allelochemical production. During the development of a bloom, however, when population densities increase and nutrients become depleted (Brandenburg *et al.* 2017), intraspecific interactions become more important, and selection may shift towards higher resource use efficiencies. Such low nutrient conditions may support the success of non-toxic or non-allelopathic strains, as production of secondary metabolites involves biochemical and energetic costs (Pohnert *et al.* 2007). Consequently, populations may exhibit intraspecific trade-offs, for instance between defensive and competitive traits. Trade-offs between functional traits stand at the base of eco-evolutionary dynamics, and are key drivers underlying phenotypic diversity and variation, as they constrain trait evolution and thereby prevent selection for a 'super-clone'. Trade-offs furthermore facilitate coexistence of species and strains, and are important for maintaining diversity (Tilman 1982; Grover 1997; Kneitel & Chase 2004; Blows & Hoffmann 2005; Van de Waal *et al.* 2011). The variation in selection pressures during a bloom can concede and maintain a high genetic and functional diversity within a population, and result in genetic differentiation over very short time scales (Richlen *et al.* 2012). However, empirical data on intraspecific trade-offs in HAB populations is still lacking.

Here, we assessed the variation in eight traits in 15 isolates from two geographically distinct toxic *Alexandrium ostenfeldii* populations. *A. ostenfeldii* is a globally widespread dinoflagellate species that can produce several types of neurotoxins (PSP, GYM and SPX), as well as allelochemicals (Kremp *et al.* 2009; Van de Waal *et al.* 2015). It generally occurs in background levels, but may also cause dense harmful blooms in brackish waters including embayments, creeks and coastal marine ecosystems (Kremp *et al.* 2009). One of the studied populations is relatively large and already established between 8000 and 3000 BP in the Baltic Sea (Tahvanainen *et al.* 2012). The other population is much smaller and only established recently in a small creek in the Netherlands (Burson *et al.* 2014; Van de Waal *et al.* 2015). For all isolates, we first determined general traits such as growth rate, cell size and stoichiometry; nitrogen (N) acquisition traits including the

maximum N uptake rate and affinity (since N is the limiting nutrient during summer blooms at both locations); and defensive traits including production of toxins (i.e. PSP toxins, GYM and SPX), as well as allelopathic potency, and afterwards tested for putative trade-offs. We aim to assess how different environments affect diversification of traits, and how physiological constraints lead to intraspecific trade-offs. Specifically, we expect a higher trait variation in the longer established Baltic Sea population as this population had more time to diversify, and we predict selection of defensive traits (i.e. toxin quota and allelochemical potency) in the recently established Dutch population as this would support its colonisation. Moreover, we anticipate distinctive trade-offs between traits that scale with size, such as growth rate and cell volume, as well as between defensive and competitive traits. Lastly, we expect a positive relationship between relative phosphorus (P) content and growth rate following the growth rate hypothesis, which predicts that cells require more P in order to grow faster (Elser *et al.* 1996, 2003; Sterner & Elser 2002).

MATERIAL AND METHODS

Culturing

Alexandrium ostenfeldii strains from the Netherlands were isolated in summer blooms from the Ouwerkerkse Kreek (51°62'N, 3°99'E) in 2015 and 2016. *A. ostenfeldii* strains from the Föglö Archipelago (Åland) in the Northern Baltic Sea (60°05'N, 20°31'E) were isolated from a late summer bloom in 2015. Both geographic populations are phylogenetically closely related (Van de Waal *et al.* 2015) representing a distinct group of estuarine *A. ostenfeldii* (group I; Kremp *et al.* 2014). Clonal isolates were genotyped using six microsatellite markers (Nagai *et al.* 2014), which confirmed that strains represented different haplotypes (Table S1). We randomly selected 15 *A. ostenfeldii* strains (five from the Netherlands in 2015, five from the Netherlands in 2016, and five from the Baltic Sea), which we cultured in ½K – medium (after Keller *et al.* 1987) with 0.2 µm sterilized North Sea water adjusted to a salinity of 10. Cultures were grown at a temperature of 18 °C and an incident light intensity of 85 µmol photons m⁻² s⁻¹, with a light:dark cycle of 16:8 h. First, the cultures were acclimated to experimental conditions for 2 weeks (approximately five generations), after which they were transferred to 500 mL Erlenmeyer flasks using four biological replicates. Cultures were grown as batch starting at a cell density of 400 cells mL⁻¹. Each replicate was grown for three to five generations, and cultures were harvested for the assessment of traits while in exponential growth phase at densities of approximately 5.0 × 10³ cells mL⁻¹.

Growth rate and cell size

To estimate cell densities, 5 mL samples were taken every second or third day and fixed with neutral Lugol's iodine solution (Lugol) to a final concentration of 1%. Samples were stored in the dark at 4 °C until analysis. *A. ostenfeldii* cells were counted on an inverted microscope (DMI 4000B; Leica Microsystems CMS GmbH, Mannheim, Germany). Maximum

growth rates (μ) were calculated for each replicate by fitting an exponential function through cell counts over time (excluding potential lag phases; see also Fig. S1 and Table S2), following:

$$N_t = N_0 \exp^{\mu t}$$

where N_t refers to the cell concentrations at time t , and N_0 to the cell concentrations at the start of each experiment (or exponential growth phase).

To establish cell size data, Lugol fixed samples from the last day were analysed using a FlowCam (Fluid Imaging Technologies, Yarmouth, ME, USA), where surface area was measured and used to calculate cell volume based on a sphere. For each replicate, at least 100 cells were measured.

Elemental composition

Cellular carbon (C), nitrogen (N) and phosphorus (P) were determined at the end of the experiment in 20 mL of culture material collected on a prewashed (100 mL distilled water) glass microfiber filter (Whatman GF/F, Maidstone, UK). Filters were dried overnight at 60 °C and stored in the dark until further analyses. For C and N analyses, a subsample (14%) of each filter was folded into a tin cup and analysed on a FLASH 2000 organic elemental analyser (Brechtbueler Incorporated, Interscience B.V., Breda, the Netherlands). Cellular P was analysed by combusting the remainder of the filter (86%) for 30 min at 550 °C in Pyrex glass tubes, followed by a digestion step with 5 mL persulfate (2.5%) for 30 min at 120 °C. This digested solution was measured for PO_4^{3-} on a QuAAtro39 AutoAnalyzer (SEAL Analytical Ltd., Southampton, UK).

Allelopathic potency

The allelopathic potency of the *A. ostenfeldii* cultures was determined after Tillmann *et al.* (2009), using a short-term bioassay with the cryptophyte *Rhodomonas salina* as target species. Lytic effects were quantified by adding *A. ostenfeldii* filtrate to an *R. salina* culture at concentrations reflecting different cell densities, and subsequently assess the *A. ostenfeldii* cell densities required to reduce the *R. salina* abundance by 50%.

To collect the filtrate, 200 mL of *A. ostenfeldii* culture from the end of the experiment was centrifuged at 168 *g* for 10 min (Thermo Scientific SL16, Thermo Fisher Scientific, Langensfeldbold, Germany). Hereafter, the cell pellet was used for the N uptake bioassays (see below) and the filtrate was filtered over a sterile 0.2 μm cellulose acetate filter and stored at -20 °C until further analyses.

Cell counts from the end of the experiment were used to estimate the cell densities of *A. ostenfeldii* associated with the filtrate. From the filtrate, eight dilutions were made corresponding to a range of 0.3–8 $\times 10^3$ cells mL^{-1} in vials of 4 mL. Medium without filtrate was used during each run as a control.

For the bioassays, 0.1 mL of *R. salina* culture was added to each vial containing *A. ostenfeldii* filtrate reaching a final cell density of 10×10^3 cells mL^{-1} . The samples were incubated for 24 h under the same culture conditions as the experiment.

At the end of the incubations, *R. salina* cells were counted using an automated cell counter (Beckman Coulter Multisizer 3, Indianapolis, IN, USA).

In order to estimate the EC_{50} for each strain (i.e. the *A. ostenfeldii* cell density leading to a 50% decline in *R. salina* cell densities), the following function was fitted through *R. salina* counts as a function of log-transformed *A. ostenfeldii* cell concentrations associated with the filtrate (Fig. S2):

$$N_{\text{final}} = \frac{N_{\text{control}}}{1 + \left(\frac{x}{\text{EC}_{50}}\right)^h}$$

where N_{final} is the final target cell density of *R. salina* using *A. ostenfeldii* filtrates, N_{control} is the final target cell density of *R. salina* in control samples, x is the log-transformed *A. ostenfeldii* cell density, and EC_{50} and h are fitted parameters, where h is the Hill coefficient.

Nitrogen uptake kinetics

Determination of the N uptake kinetics of the different *A. ostenfeldii* strains was done using ^{15}N bioassays, after Moschonas (2015). Centrifuged cell pellets of *A. ostenfeldii* were re-suspended in medium lacking N and incubated under experimental culture conditions for 24 h.

Hereafter, samples for cell counts were taken and the *A. ostenfeldii* culture was divided and transferred to 7 \times 50 mL tubes. Afterwards, ^{15}N labelled NO_3^- (Sigma Aldrich, Steinheim am Albuch, Germany, 10 atom % ^{15}N) was added to yield the following concentrations: 0.1, 0.3, 0.6, 1, 3, 10 $\mu\text{mol L}^{-1}$. For every *A. ostenfeldii* strain, a control without addition of ^{15}N labelled NO_3^- was taken in order to estimate background ^{15}N values. The samples were incubated for 1 h under the same culture conditions, and afterwards filtered through glass microfiber filters (GF/F; Whatman, Maidstone, UK). Filters were dried overnight at 60 °C and stored in the dark until further analyses.

A subsample (14%) of every filter was folded in a tin cup and analysed on an elemental analyser (Flash 2000, Thermo Scientific, Langensfeldbold, Germany) coupled to an isotope ratio mass spectrometer (IRMS, Thermo Scientific), and $\delta^{15}\text{N}$ values were calculated as the difference in ^{15}N abundance between the sample and a reference gas (with 0.366 atom% ^{15}N).

The $\delta^{15}\text{N}$ values were subsequently used to calculate N uptake rates using the equation from Dugdale & Wilkerson (1986):

$$\rho = \frac{(^{15}\text{N}(t)_{\text{sink}} - ^{15}\text{N}(t_0)_{\text{sink}})}{(^{15}\text{N}(t_0)_{\text{source}} - ^{15}\text{N}(t_0)_{\text{sink}}) \times t} \times \text{PON}$$

where ρ is the absolute N uptake rate (in $\mu\text{mol N L}^{-1} \text{h}^{-1}$), $^{15}\text{N}(t_0)_{\text{source}}$ is the isotopic ratio of the enriched dissolved N pool (in atom%), $^{15}\text{N}(t_0)_{\text{sink}}$ is the natural abundance of ^{15}N (in atom%), $^{15}\text{N}(t)_{\text{sink}}$ is the abundance of ^{15}N in the sample after the incubation (in atom%), t is the incubation time (in hours) and PON, the particulate organic nitrogen concentration (in $\mu\text{mol N L}^{-1}$).

The absolute N uptake rates calculated for each concentration of NO_3^- can then be used to estimate the N uptake

kinetics for each *A. ostenfeldii* strain. To this end, a Michaelis-Menten function was fitted through all data points within the bioassays for each replicate of each strain:

$$\rho = \frac{(V_{\max} \times \text{NO}_3^-)}{(K_{1/2} + \text{NO}_3^-)}$$

where ρ (in $\mu\text{mol N L}^{-1} \text{h}^{-1}$) is the N uptake rate, V_{\max} the maximum uptake rate (in $\mu\text{mol N L}^{-1} \text{h}^{-1}$), $K_{1/2}$ the half saturation constant (in $\mu\text{mol N L}^{-1}$) and NO_3^- the nitrate concentration. Subsequently, V_{\max} was divided by the cell densities at the end of each experiment and units were converted to $\text{pmol N cell}^{-1} \text{day}^{-1}$.

Toxin sampling and analyses

Samples for toxin analyses (PSP toxins and cyclic imines) were taken by filtration of 20 mL of *A. ostenfeldii* culture over glass microfiber filters (GF/F, Whatman, Maidstone, UK), which were stored at -20°C until further 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). The cyclic imine toxin measurements (SPX and GYM) were performed on an Agilent 1100 LC liquid chromatograph (Waldbronn, Germany) coupled to a 4000 Q Trap triple-quadrupole mass spectrometer (AB-Sciex, Darmstadt, Germany) with a Turbo V ion source. Toxins were quantified by external calibration curves of SPX-1 and GYM A with standard solutions ranging from 10 to 1000 $\text{pg } \mu\text{L}^{-1}$, each. Other SPXs and GYMs for which no standards are available were calibrated against the SPX-1 and GYM A calibration curve, respectively, and expressed as SPX-1 or GYM A equivalents. For further details, also see Van de Waal *et al.* (2015).

Statistical analyses

Statistical analyses were performed in R version 3.2.2 (R Core Team 2015). For the measured traits across all strains, coefficients of variation (CV) were calculated as the ratio of the standard deviation to the mean. Significant differences in the expression of traits between the three groups of *A. ostenfeldii* strains were determined using a one-way ANOVA, including all replicates, followed by post hoc comparison of the means using Tukey's HSD. Trade-offs and relationships were recognized using a linear regression between the log-transformed trait values, including all replicates. Differentiation of traits within the distinct populations was assessed with the R package 'vegan' (Oksanen *et al.* 2017), where the multivariate homogeneity of group dispersions was analysed. Significant differences between the groups were determined using a permutation test.

RESULTS

We observed substantial variation of the analysed traits among the 15 studied *A. ostenfeldii* strains within and among their corresponding populations. Maximum growth rates ranged from 0.12 to 0.39 d^{-1} in different strains (Figs 1 and 2a), and cell volumes from 7.5 to $32.4 \times 10^3 \mu\text{m}^3$ (Fig. 2d), with CVs of 0.32 and 0.38, respectively. PSP toxin quotas varied from 1.1 to

$81.7 \text{ pg cell}^{-1}$ (CV 0.73) and cyclic imine toxin quotas from 18.8 to 443 pg cell^{-1} (CV 0.66; Fig. 2g–h; Fig. S3). Allelopathic potencies also varied strongly between the strains, with estimated EC_{50} values from 0.9 to $33.5 \times 10^3 \text{ A. ostenfeldii cells mL}^{-1}$ (CV 1.09; Fig. 2i). For N uptake kinetics, $K_{1/2}$ values ranged from 0.3 to $11.9 \mu\text{mol N L}^{-1}$ (CV 0.8), and V_{\max} values from 3.6 to $5.4 \text{ pmol N cell}^{-1} \text{day}^{-1}$ (CV 0.74; Fig. 2e–f). Moreover, we also observed variation in the elemental stoichiometry of the different strains, ranging from 5.4 to 7.2 for cellular C:N ratios (CV 0.08; data not shown), from 50.9 to 154.6 for cellular C:P ratios (CV 0.21) and from 7.6 to 18.5 for cellular N:P ratios (CV 0.23; Fig. 2b–c).

Significant differences in the expression of traits between the Dutch and Baltic Sea *A. ostenfeldii* populations were found for growth rate ($P < 0.001$), cell size ($P < 0.001$), C:P ratio ($P < 0.001$), N:P ratio ($P < 0.001$), PSP toxin quotas ($P < 0.001$), cyclic imine toxin quotas ($P < 0.001$) and allelopathic potencies ($P < 0.01$; Fig. 2). Differences were less pronounced for N uptake kinetics, with significant differences only between the Baltic Sea population and the 2016 Dutch population for $K_{1/2}$ ($P < 0.01$; Fig. 2). Strains from the Dutch population isolated in different years were generally similar in their trait expression. Significant differences were only found for PSP toxin quotas ($P < 0.01$), cyclic imine toxin quotas ($P < 0.05$) and $K_{1/2}$ ($P < 0.05$; Fig. 2).

Using non-metric multidimensional scaling in an ordination plot we could define two distinct clusters that clearly separated the two geographically distinct *A. ostenfeldii* populations (Fig. 3a). There was more variation in the expression of traits within the Baltic Sea population as compared to the Dutch population from both years together (Fig. 3b; $P < 0.001$).

The analysed selection of traits showed various distinct relationships. For instance, we observed strongest positive correlations between cell size and toxin quota and between PSP toxins and cyclic imines, while we observed negative

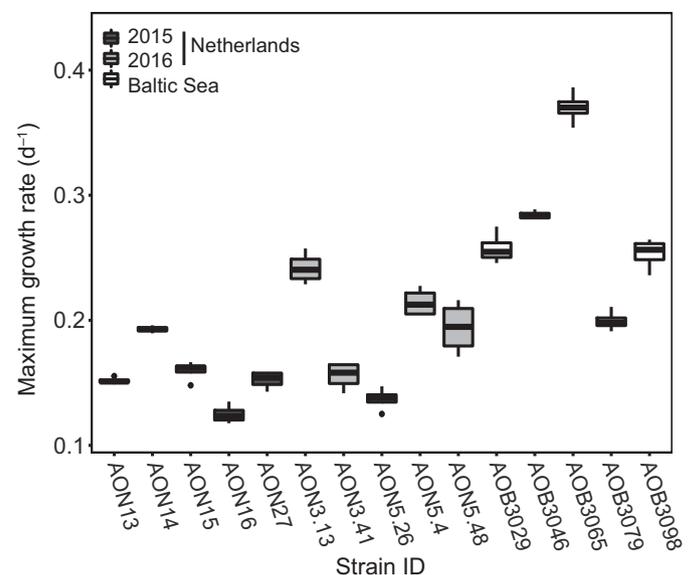


Figure 1 Maximum growth rates of the different *Alexandrium ostenfeldii* strains ($n = 4$), isolated from the Netherlands (2015, light grey; 2016, dark grey) and the Baltic Sea (white).

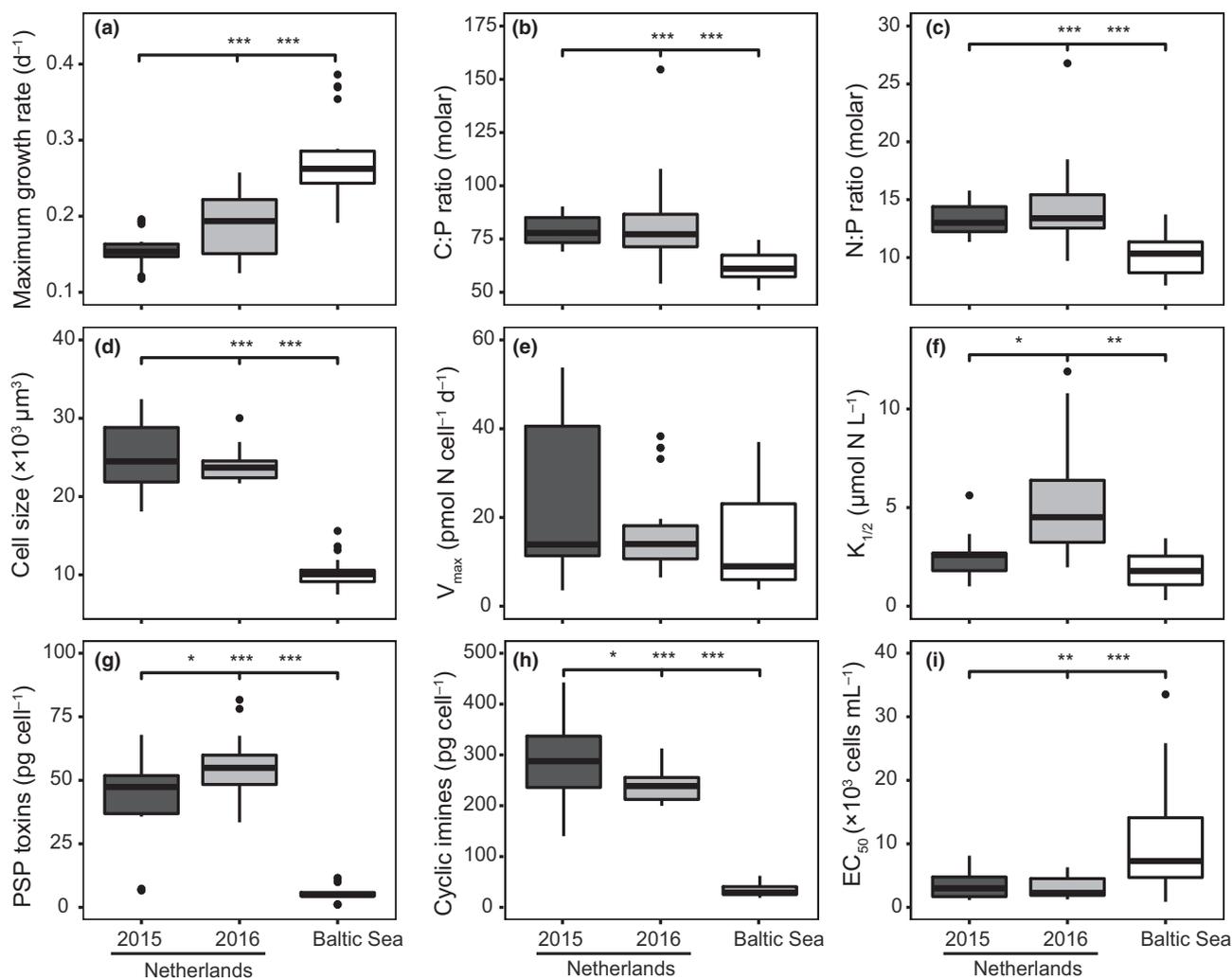


Figure 2 Overview of traits assessed for the different *Alexandrium ostenfeldii* populations ($n = 20$), showing variation in (a) maximum growth rates, (b) C:P ratios, (c) N:P ratios, (d) cell sizes, (e) V_{\max} , (f) $K_{1/2}$, (g) PSP toxin quotas, (h) cyclic imine toxin quotas and (i) EC_{50} , isolated from the Netherlands (2015, light grey; 2016, dark grey) and the Baltic Sea (white). Significance differences between the populations are indicated by the asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

correlations between growth rate and cell size as well as between growth rate and toxin quota, which may represent putative trade-offs (Table 1). We note that these trade-offs were mainly driven by clustering of the two distinct *A. ostenfeldii* populations (Fig. S4). Interestingly, we observed a very distinct trade-off between V_{\max} and $K_{1/2}$, both within and across populations (Fig. 4).

DISCUSSION

Our results reveal large intraspecific variation in trait expression within one phytoplankton species at two different levels, namely across and within two distinct populations. In fact, even within the recently established Dutch population, we found substantial variation in trait expression, which may point towards rapid diversification. We also observed distinct differences in the analysed traits between the two populations. Overall, the Baltic Sea strains grew faster, were smaller, contained relatively more P and produced less toxins and

allelopathic compounds than the Dutch strains (Fig. 2). These particular traits are closely linked, and exhibit fundamental relationships and trade-offs.

The observed intraspecific trade-off between growth rate and cell size is in line with earlier findings on interspecific trade-offs, where smaller phytoplankton cells typically grew faster than larger cells (Banse 1976; Finkel *et al.* 2010). Furthermore, in order to grow faster, the growth rate hypothesis predicts that organisms should contain relatively more P, as more P-rich rRNA is required (Elser *et al.* 1996, 2003; Sterner & Elser 2002). The applicability of the growth rate hypothesis for phytoplankton, however, has been debated (Flynn *et al.* 2010). Phytoplankton may accumulate P under non-limiting growth conditions, as well as at limitations other than P (Elrifli & Turpin 1985; Hillebrand *et al.* 2013). Thus, the relationship between growth rate and the relative cellular P contents will strongly depend on the growth-controlling factors. Our results show intrinsic differences in growth rate between strains cultured under the same non-limiting conditions, with

a close association with the relative P contents (i.e. C:P ratio, Table 1). This was further confirmed by a positive correlation between growth rate and cellular P contents corrected to cell volume ($R^2 = 0.16$, $P < 0.01$). Our results thus revealed an ideal test for the growth rate hypothesis, showing that the

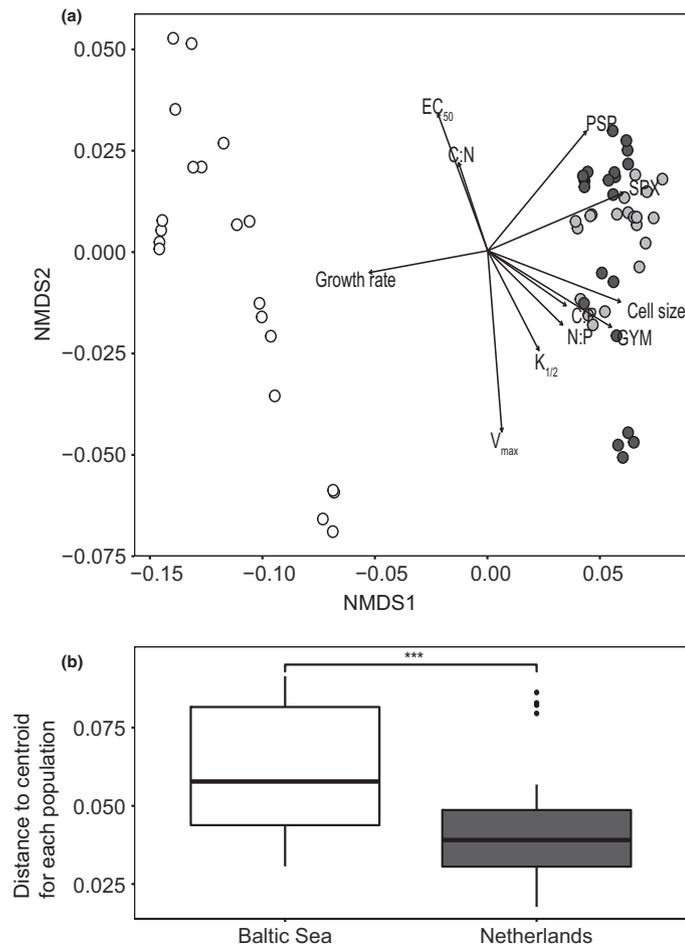


Figure 3 Observed variation between all analysed traits of *Alexandrium ostenfeldii* populations illustrated by (a) non-metric multidimensional scaling ordination, where symbols denote the Dutch (2015, light grey; 2016, dark grey) and Baltic Sea (white) populations for each replicate, and the arrows indicate which traits vary along the axes, and (b) differences in total variation between the Dutch and Baltic Sea populations.

Table 1 R^2 values derived from linear regressions between log-transformed trait values of *A. ostenfeldii*

	Growth rate	Cell size	N:P	C:P	C:N	V_{max}	$K_{1/2}$	Cyclic imine toxins	PSP toxins
Cell size	-0.49***								
N:P	-0.13*	0.41***							
C:P	-0.20***	0.40***	0.91***						
C:N		-0.10**	-0.36***	-0.10**					
V_{max}		0.17***	0.20***	0.17***					
$K_{1/2}$	-0.14*	0.27*	0.11*			-0.43***			
Cyclic imine toxins	-0.56***	0.86***	0.45***	0.46***	-0.082*	0.15**	0.30**		
PSP toxins	-0.36***	0.59***	0.29***	0.23***	-0.14**		0.41***	0.64***	
EC_{50}	0.051*	-0.22***	-0.30***	-0.23***	-0.20***	-0.064*		-0.29***	-0.34***

Only significant regressions are presented and significance levels are indicated by the asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

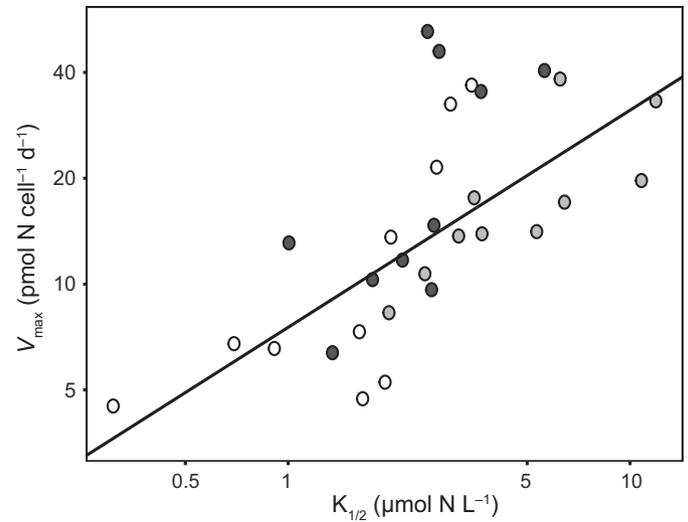


Figure 4 Trade-off between V_{max} and $K_{1/2}$ across the different *Alexandrium ostenfeldii* strains. Symbols indicate strains isolated from the Netherlands (2015, light grey; 2016, dark grey) and Baltic Sea (white).

Baltic Sea *A. ostenfeldii* cells contained relatively more P, and could indeed grow faster than Dutch isolates.

Faster growth and smaller cells were also associated with lower toxin quota in *A. ostenfeldii* (Table 1). Interestingly, the negative relationships between growth rate and PSP toxins ($R^2 = 0.21$, $P < 0.001$) as well as cyclic imines ($R^2 = 0.52$, $P < 0.001$) remained significant after correcting for cell size. This may represent a trade-off between toxin synthesis and growth, as the production of these secondary metabolites has certain biochemical and energetic costs (Pohnert *et al.* 2007). Cellular toxin content also positively correlated with C:P and N:P ratios (Table 1), suggesting that a relatively lower P content is associated with higher toxin quota. This relationship, however, might as well be caused by differences in cell size, where larger cells contain relatively less P and more toxins.

Toxin production may have developed in dinoflagellates as an adaptation to offset the ecological disadvantage of low nutrient uptake affinities by reducing grazing pressure (Smayda 1997; Guisande *et al.* 2002; Frangopoulos *et al.* 2004). Consequently, there may be a trade-off between toxin production and species' competitive ability for nutrients. Indeed, a higher PSP toxin content was associated with a lower affinity

for P for several *Alexandrium* species (Frangopulos *et al.* 2004). Similarly, we observed a higher toxin content (PSP toxins and cyclic imines) with a lower N affinity (i.e. a higher $K_{1/2}$, Table 1), also after correcting for cell size (PSP: $R^2 = 0.36$, $P < 0.001$; cyclic imines: $R^2 = 0.27$, $P < 0.01$). PSP toxins have been associated with grazer defence (Teegarden 1999; Colin & Dam 2003; Wohlrab *et al.* 2010, 2017), however their exact function is still under debate. Allelochemicals have been shown to act as a defence against competing phytoplankton species as well as grazers (Tillmann & John 2002; Tillmann *et al.* 2007; John *et al.* 2015). Yet, the putative trade-off between allelopathic potency and affinity for N is very weak ($R^2 = 0.062$, $P = 0.099$). Excretion of allelochemicals can potentially play a role in heterotrophic feeding, as lysis of other algal species results in a release of organic compounds (Place *et al.* 2012). Thus, allelochemicals may not only act as a defensive trait, but possibly also as an offensive trait, particularly in mixotrophic algae such as *A. ostenfeldii* (Jacobson & Anderson 1996; Gribble *et al.* 2005).

Nutrient uptake in phytoplankton is based on fundamental relationships between cell surface to cell volume scaling, and enzyme kinetics (Aksnes & Egge 1991; Litchman *et al.* 2007). Only a small area of the cell surface is available for nutrient uptake, and here the uptake sites with a small active area per site (resulting in a higher V_{\max} and higher $K_{1/2}$) compete with uptake sites with a relatively large active area (resulting in a lower V_{\max} and lower $K_{1/2}$; Raven 1981). This trade-off between faster rates (i.e. higher V_{\max}) and higher affinities (i.e. lower $K_{1/2}$; note the inverse correlation) for N uptake was observed earlier for different phytoplankton species (Litchman *et al.* 2007). Here, we demonstrate that this trade-off occurs intraspecifically, both within and across the tested *A. ostenfeldii* populations (Fig. 4).

Most of the analysed traits were relatively conserved within *A. ostenfeldii*. Specifically, the observed intraspecific variation in growth rate, cell size, V_{\max} for nitrogen and elemental

composition across the tested *A. ostenfeldii* strains was lower as compared to the interspecific variation across phytoplankton species (Fig. S5). This is presumably caused by the vast range in cell size across species, that affects growth rate, nitrogen acquisition and elemental composition (Finkel *et al.* 2010; Edwards *et al.* 2015a, b; Garcia *et al.* 2018). The intraspecific variation in $K_{1/2}$ values for nitrogen, however, was comparable to the earlier reported interspecific variation across marine phytoplankton species (Fig. 5; Edwards *et al.* 2015a). These ranged from high $K_{1/2}$ values (i.e. low affinities) observed for larger phytoplankton groups such as dinoflagellates, down to low values (i.e. high affinities) observed for small phytoplankton groups such as coccolithophores. This emphasises that trait variation occurring in a single phytoplankton species can be substantial, and may have important implications for its ecological functioning. Indeed, $K_{1/2}$ largely determines the competitive ability for a resource (Tilman 1982; Litchman *et al.* 2007; Edwards *et al.* 2011). Consequently, low $K_{1/2}$ values may support strains under low nutrient conditions, while high $K_{1/2}$ values associated with high nutrient uptake rates (Fig. 4; Litchman *et al.* 2007; Edwards *et al.* 2011) may increase the competitive ability of strains under nutrient replete conditions.

Both of the studied *A. ostenfeldii* populations are phylogenetically closely related, belonging to the same ribotype, but live in different habitats (Kremp *et al.* 2014; Van de Waal *et al.* 2015). The Baltic Sea isolates showed more pronounced trait variation than the Dutch isolates (Fig. 3). Besides habitat constraints, this might be explained by the presumably different histories of the populations in their respective habitats. Colonisation of the Baltic Sea by marine organisms is estimated to have occurred after the postglacial opening of the North Sea, approximately 8000–3000 BP. The population genetic structure of *A. ostenfeldii* in the Baltic Sea, together with long plankton records, supports this early colonisation scenario (Björck 1995; Tahvanainen *et al.* 2012). In contrast, the *A. ostenfeldii* population in the Netherlands was first reported in 2012 (Burson *et al.* 2014). The Dutch population has presumably colonised the small creek very recently, and may have only started with a small inoculum through the bottleneck effect selecting for functional traits supporting the establishment of an invasive species in a new environment (Carson 1959; Nei *et al.* 1975; Olden *et al.* 2004). For instance, the Dutch population exhibited a stronger expression of defensive traits such as the production of toxins and allelopathic compounds, which might be more important in novel environments with high interspecific competition. The recent establishment of the Dutch population is supported by genetic analysis of the *A. ostenfeldii* isolates using microsatellites. The applied markers could not differentiate between all the different Dutch genotypes, suggesting that the tested strains were genetically very similar while showing a high variation in phenotypic trait expression, which is consistent with rapid evolution. The same microsatellite markers clearly differentiated the Baltic Sea isolates and thus indicate larger genetic differences (Table S1). The observed difference in intraspecific variation between the Baltic Sea and Dutch population might thus represent an example of established and founder populations, respectively (Carson 1959; Dlugosch & Parker 2008).

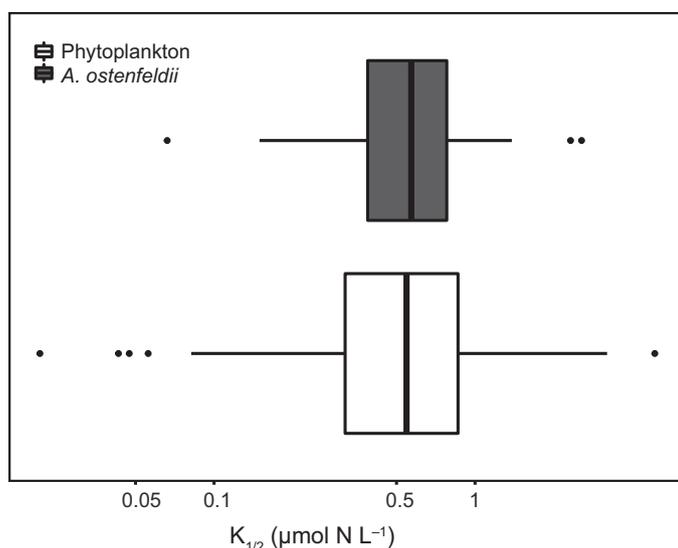


Figure 5 Variation in $K_{1/2}$ across marine phytoplankton species (white; Edwards *et al.* 2015a) and *Alexandrium ostenfeldii* strains (grey; this study).

The differences in trait expression between both populations may not only derive from different evolutionary histories, but also from distinct environmental conditions. Both populations occur in relatively calm and shallow water bodies, with high nutrient inputs from the surrounding land. Yet, the environmental conditions in the Föglö area of the Baltic Sea are likely more stable than in the creek where the Dutch *A. ostensfeldii* population resides, which is strongly influenced by nutrient and salinity fluctuations caused by rainfall (Brandenburg *et al.* 2017). In contrast to the Dutch creek, nutrient conditions quickly become limiting at the Föglö bloom site in the Baltic Sea (Hakanen *et al.* 2012). The cell density of competing phytoplankton species or abundance of grazers during the blooms in the Dutch creek appears higher than during blooms in the Föglö area (Fig. S6). The more stable conditions in the Föglö area together with a relatively weaker competition with other algal species, lower grazing pressure and stronger nutrient limitation may have selected for smaller and faster growing, and thereby less toxic genotypes. In contrast, the more intensive competition, the higher grazing pressure and the higher availability of nutrients in the Dutch creek may have selected for more toxic and allelopathic, and thereby larger but slower growing genotypes. In addition, a higher genetic diversity and trait variation within populations can be attained through gene flow (Slatkin 1987; Sexton *et al.* 2014). In the northern Baltic Sea bloom areas, multiple differentiated local *A. ostensfeldii* populations have established in close proximity due to a highly fragmented coast line (Tahvanainen *et al.* 2012). Gene flow between these different populations allows for genetic exchange, which is in a strong contrast to the isolated bottleneck population from the Dutch creek. This may explain the higher degree of differentiation in the Baltic Sea isolates. We note that the described traits were derived from cultures grown under a constant salinity and temperature, while both factors can vary substantially during a bloom and may affect the expression of traits.

Overall, we found considerable intraspecific trait variation within and across populations of *A. ostensfeldii*, which was for one trait even comparable to the interspecific trait variation across marine phytoplankton species. Such large trait variation can be achieved and maintained through shifting selection pressures during toxic algal bloom periods, which can possibly contribute to their prevalence and success (Driscoll *et al.* 2015). Varying ecological pressures may thus result in high functional trait variation within populations, and lead to intraspecific trade-offs between, for instance, defensive and competitive traits. Moreover, our results suggest the importance of evolutionary history that may have led to the higher trait variation in the Baltic Sea population. We here report a high functional trait variation within *A. ostensfeldii* populations that allows for rapid diversification, as was observed for the Dutch population. This intraspecific trait variation may facilitate bloom development under dynamic environmental conditions and promote the success of HABs under global environmental change.

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AUTHORSHIP

KB, UJ, SW and DvdW designed the experiment; KB, SW, AK and JJ supplied the analysed strains; and KB performed the experiment. BK helped with the toxin analyses. KB and DvdW wrote a first draft of the manuscript, and all authors contributed to the writing process.

DATA ACCESSIBILITY STATEMENT

All data supporting this study are stored in the Dryad Digital Repository under <https://doi.org/10.5061/dryad.6502mg2>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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