Bioluminescence of Toxic Dinoflagellates in the Baltic Sea – From Genes to Models

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Academic Dissertation

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List of original publications and author's contribution

This thesis is based on the following papers, which are referred to in the text by their Roman numerals. In addition, some previously unpublished results are presented.

I **Le Tortorec A.H.**, Tahvanainen P., Kremp A. and Simis S.G.H. (2016) Diversity of luciferase sequences and bioluminescence production in Baltic Sea *Alexandrium ostenfeldii*. European Journal of Phycology, 51: 317-327.

AL, SGHS and AK planned the study. AL and PT performed the laboratory analyses. AL and SGHS collected field data. AL analysed the data and prepared the manuscript with contributions from all co-authors.

II **Le Tortorec A.H.**, Hakanen P., Kremp A., Olsson J., Suikkanen S. and Simis S.G.H. (2014) Stimulated bioluminescence as an early indicator of bloom development of the toxic dinoflagellate *Alexandrium ostenfeldii*. Journal of Plankton Research, 36: 412-423.

SGHS and AK planned the study. AL, SGHS and JO designed the measuring equipment. AL, SGHS, JO and PH conducted the field work. AL performed laboratory analyses, except toxin analysis performed by SS and phytoplankton counts performed by PH. AL analysed the data and prepared the manuscript with contributions from all co-authors.

III Kremp A., Oja J., **Le Tortorec A.H.**, Hakanen P., Tahvanainen P., Tuimala J. and Suikkanen S. (2015) Diverse seed banks favour adaptation of microalgal populations to future climate conditions. Environmental Microbiology and Environmental Microbiology Reports, 18: 679-691.

AK and SS planned the study. AL helped in conducting the experiments. AL planned and conducted the bioluminescence measurements, analysed the bioluminescence data and wrote the bioluminescence part for the method section. AK prepared the manuscript with contributions from all co-authors.

IV **Le Tortorec A.H.**, Lindehoff E., Virtanen E., Kremp A., Le Tortorec E. and Simis S.G.H. Modelled distribution of the toxin-producing dinoflagellate *Alexandrium ostenfeldii* in the Baltic Sea under current and future climates. Manuscript.

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Abbreviations

ASP	Amnesic shellfish poisoning
CFP	Ciguatera fish poisoning
Chl-a	Chlorophyll-a
DSP	Diarrhetic shellfish poisoning
GYM	Gymnodimines
HA	Harmful algae
HAB	Harmful algal bloom
LBP	Luciferin-binding protein
Lbp	Luciferin-binding protein gene
LCF	Luciferase enzyme
Lcf	Luciferase gene
PCR	Polymerase chain reaction
PSP	Paralytic shellfish poisoning
PST	Paralytic shellfish toxin
PSU	Practical salinity unit
SDM	Species distribution model
SOOP	Ship-of-opportunity platform

Abstract

Phytoplankton constitute the basis of food webs and are responsible for almost all photosynthesis in the open sea. Occasionally, given suitable conditions, one phytoplankton species can increase in abundance and form a mass occurrence known as a bloom. Harmful Algal Blooms (HABs), in turn, can have adverse effects on the ecosystem, for example causing oxygen depletion, clogging of fish gills, or toxicity. HABs are common in shallow and stratified coastal waters and the number, frequency and coverage of areas affected by HABs are increasing globally mainly due to eutrophication, changes in nutrient ratios and global climate change. One of the main phytoplankton groups forming HABs are dinoflagellates. Among dinoflagellates the genus *Alexandrium* is particularly notorious with many species in this genus producing a variety of potent neurotoxins. Dinoflagellates are the only known photosynthetic organisms that can produce bioluminescence and the majority of bioluminescence in marine surface waters is produced by dinoflagellates. Bioluminescence in dinoflagellates is considered a defensive mechanism against grazing and many harmful dinoflagellate taxa produce bioluminescence.

The dinoflagellate *Alexandrium ostenfeldii* forms dense bioluminescent blooms in shallow and sheltered areas of the Baltic Sea during the late summer. These blooms pose a potential threat to humans and ecosystems in the region due to observed production of toxins. The distribution and abundance of *A. ostenfeldii* is poorly known in the coastal waters of the Baltic Sea. The aim of this thesis was to study the variability of *A. ostenfeldii* bioluminescence production to evaluate whether bioluminescence can be used as an early warning signal of harmful dinoflagellate blooms in the Baltic Sea. More precisely, the aim was to study the expression of *A. ostenfeldii* bioluminescence to learn how much genetic, phenotypic and environmentally induced variability there is in bioluminescence production in the species. Studies conducted on luciferase genes and bioluminescence emission showed that bioluminescence is a prominent feature in Baltic *A. ostenfeldii*. Measurements on individual cell cultures and natural populations revealed large daily and seasonal variation in bioluminescence emission relating to the circadian rhythm in bioluminescence expression and seasonal changes in *A. ostenfeldii* abundance. Large intraspecific variation in bioluminescence production was observed in response to changes in temperature and salinity. *A. ostenfeldii* abundance, toxin concentrations and bioluminescence intensity were positively correlated in our field data. All tested *A. ostenfeldii* strains produced both bioluminescence and toxins and it can be assumed that concurrent production of both is a predominant property in Baltic *A. ostenfeldii*.

The obtained results show that it is possible to relate bioluminescence to *A. ostenfeldii* bloom succession using automated measurements. However, the highly-localized distribution of blooms may require advance knowledge of potential bloom locations to be able to target the monitoring efforts. Species distribution modelling showed promise to address this problem by identifying potentially suitable habitats for *A. ostenfeldii*.

Tiivistelmä

Kasviplankton muodostaa ravintoketjun perustan ja on vastuussa lähes kaikesta perustuotannosta avoimilla merialueilla. Toisinaan suotuisissa olosuhteissa yksi kasviplanktonlaji saattaa runsastua huomattavasti ja muodostaa massaesiintymän eli kukinnan. Haitallisiksi leväkukinnoiksi kutsutaan sellaisia kukintoja, jotka aiheuttavat haittaa ympäristölle, muille vesieliöille tai ihmisille esimerkiksi vähentämällä hapen määrää vedessä, tukkimalla kalojen kiduksia tai erittämällä myrkkyjä. Haitallisia leväkukintoja esiintyy yleisesti matalissa ja suojaisissa rannikkovesissä ja niiden määrät ovat lisääntyneet maailmanlaajuisesti rehevöitymisen, ravinteiden suhteiden muuttumisen ja ilmastonmuutoksen seurauksena. Yksi tärkeimmistä haitallisia kukintoja muodostavista ryhmistä on panssarisiimalevät. Erityisesti monet *Alexandrium*-sukuun kuuluvat panssarisiimalevälajit tuottavat useita erilaisia hermomyrkkyjä. Monet panssarisiimalevälajit tuottavat lisäksi bioluminesenssia eli sinistä valoa, joka on parhaiten nähtävissä veden pinnalla yöaikaan. Ilmiö tunnetaan myös nimellä merituli.

Alexandrium ostenfeldii –panssarisiimalevä muodostaa tiheitä valoa tuottavia kukintoja Itämeren rannikkoalueilla erityisesti loppukesästä. *A. ostenfeldiin* tuottamien myrkkyjen on osoitettu kertyvän simpukoihin ja kaloihin, ja lisäksi myrkyillä on haitallisia vaikutuksia kasvi- ja eläinplanktoniin. Lajin levinneisyys ja siihen vaikuttavat ympäristötekijät Itämeren rannikkoalueilla tunnetaan huonosti. Tämän väitöskirjan tavoitteena oli tutkia kuinka paljon geneettistä, phenotyyppistä ja ympäristön aiheuttamaa vaihtelua *A. ostenfeldii* -lajin bioluminesenssin tuotannossa on ja voitaisiinko bioluminesenssin havainnointia käyttää ennakkovaroituksena myrkyllisestä panssarisiimaleväkukinnasta. Tulokset osoittavat, että kaikki Itämeren *A. ostenfeldii* -populaatiot tuottavat bioluminesenssia ja, että bioluminesenssin tuotossa on selvä vuorokausirytmi. Lisäksi tuotetun valon määrä vaihtelee populaation koon ja kehitysvaiheen mukaan. Lajin sisällä havaittiin suurta vaihtelua tuotetun bioluminesenssin määrässä suhteessa muutoksiin lämpötilassa ja suolapitoisuudessa. Luonnosta kerätyssä aineistossa havaittiin selvä positiivinen yhteys *A. ostenfeldiin* runsauden, tuotettujen myrkkyjen ja bioluminesenssin välillä. Kaikki testatut *A. ostenfeldii* -kannat tuottivat sekä bioluminesenssia että myrkkyjä, mikä viittaa siihen, että nämä kaksi ominaisuutta esiintyvät rinnakkain Itämeren populaatioissa.

Saadut tulokset osoittavat, että on mahdollista yhdistää tuotetun valon määrä *A. ostenfeldii* -populaation kehittymiseen käyttäen automaattisia mittausmenetelmiä. Kukintojen hyvin paikallinen esiintyminen hankaloittaa kuitenkin niiden havaitsemista, minkä takia etukäteistieto mahdollisista kukintapaikoista olisi tärkeää. Habitaattimallien avulla voidaan kartoittaa ne alueet, jotka ovat erityisen alttiita *A. ostenfeldii* -kukinnoille. Lisäksi habitaattimalleja voidaan käyttää apuna ennustettaessa, miten etenevä ilmastonmuutos vaikuttaa *A. ostenfeldii* -kukintoihin Itämerellä.

1 Introduction

1.1 Harmful algal blooms

The term harmful algal bloom (HAB) is used in a broad sense in this thesis to refer to blooms formed by phytoplankton that can cause adverse effects. These include negative aesthetic effects such as beach fouling, overgrowth and shading of seaweed and seagrass, oxygen depletion due to algal respiration or decay of algal biomass, suffocation of fish from stimulation of gill mucus production, mechanical interference with filter feeding by fish and bivalve molluscs, negative effects on other benthic species and direct toxic effects for example on fish, shellfish, seabirds, marine mammals and humans (Landsberg 2002; Granéli and Turner 2006; Glibert and Burford 2017). Various potent algal toxins can be produced during HABs, which can cause poisoning in humans who consume contaminated seafood. The best known and widespread examples are paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP) and ciguatera fish poisoning (CFP) (Granéli and Turner 2006). The number, frequency and coverage of areas affected by HABs have been increasing globally mainly due to eutrophication, global climate change and changes in nutrient ratios (Anderson *et al.* 2002; Heisler *et al.* 2008; Glibert and Burford 2017; Gobler *et al.* 2017). Commonly, HABs occur in shallow and stratified waters in estuaries and coastal marine areas, although some blooms are initiated offshore before being transported into near-shore waters (Granéli and Turner 2006; Hallegraeff 2010; Anderson *et al.* 2012; Berdalet *et al.* 2017). HABs are also encountered in upwelling systems, generally when upwelling relaxes (Pitcher *et al.* 2017), and offshore areas (Townsend *et al.* 2001; Granéli and Turner 2006).

The term harmful algae (HA) refers to a phytoplankton species that cause HABs. Of the approximately 5000 known phytoplankton species only around 300 species form HABs that are deleterious to aquatic ecosystems, and of these approximately 80 species produce toxins (Smayda 1997; Granéli and Turner 2006). The main groups forming HABs include cyanobacteria, diatoms, haptophytes, raphidophytes, and dinoflagellates (Granéli and Turner 2006). Dinoflagellates account for an estimated 75% of species that form HABs (Smayda 1997). Dinoflagellates have many unique properties that have been suggested to provide an advantage when forming blooms (Smayda 1997; Jeong *et al.* 2015; Murray *et al.* 2015). As a nutrient-retrieval strategy, dinoflagellates migrate through the water column. During the day, they are at the surface layer to capture photosynthetic irradiation and during the night they migrate into nutrient rich bottom waters to replenish their nutrient storages (Smayda 1997). Dinoflagellates utilise a variety of feeding strategies, including photoautotrophy, mixotrophy and obligate heterotrophy (Burkholder *et al.* 2008; Jeong *et al.* 2010; Hansen 2011; Stoecker *et al.* 2017). It has been shown that many harmful dinoflagellates previously thought to be photosynthetic are actually mixotrophic, using a combination of phototrophy and phagotrophy (Jacobson and

Anderson 1996; Jeong *et al.* 2005, 2010; Hansen 2011). For most species, the relative importance of photosynthesis, dissolved organic nutrients and feeding is not known (Burkholder *et al.* 2008; Hansen 2011; Stoecker *et al.* 2017). In general, mixotrophic species have higher growth rates when prey is available than without prey (Jeong *et al.* 2005, 2010; Adolf *et al.* 2006). Obligate heterotrophic harmful dinoflagellate species also exist (Granéli and Turner 2006; Jeong *et al.* 2010). Several dinoflagellate species produce allelochemicals to enhance interspecific competition and to serve as antipredatory survival mechanisms (Tillmann *et al.* 2008; John *et al.* 2015; Kim *et al.* 2016; Schwartz *et al.* 2016; Xu *et al.* 2017). As another survival mechanism, various dinoflagellates can rapidly form thin-walled pellicle cysts in response to sudden stress conditions e.g. allelopathic compounds, parasitic- or viral attacks, or an unfavourable environment, which can help them to survive in unfavourable situations (*Fistarol et al.* 2004; Bravo *et al.* 2010; Kremp 2013). Thick-walled dormant resting cysts are produced by some species either as part of sexual reproduction in the dinoflagellate lifecycle or asexually (Anderson *et al.* 2012; Kremp 2013; Dhib *et al.* 2016). For some cyst-forming species in shallow areas, the location of cyst beds is closely coupled with bloom areas and cyst beds are suggested to provide an inoculum for blooms (Anderson *et al.* 2012; Hakanen *et al.* 2012).

The dinoflagellate genus *Alexandrium* is one of the major HAB genera in terms of diversity, severity and distribution of bloom impacts (Anderson *et al.* 2012). Species in this genus produce a variety of different types of toxins including paralytic shellfish toxins (PSTs, see Lim & Ogata 2005; Stüken *et al.* 2011; Savela *et al.* 2016), spirolides (Gribble *et al.* 2005; Ciminiello *et al.* 2007; Tillmann *et al.* 2014), gymnodimines (Van Wagoner *et al.* 2011; Van de Waal *et al.* 2015; Harju *et al.* 2016; Martens *et al.* 2017), goniodomins (Murakami *et al.* 1988; Hsia *et al.* 2006; Triki *et al.* 2016) and in addition allelochemicals (Tillmann *et al.* 2008; John *et al.* 2015). Of these toxins, PSTs, to which saxitoxins belong, are responsible for PSP, which is the most widespread of the HAB-related shellfish poisoning syndromes and can cause human intoxication or death through contaminated shellfish or fish (Anderson *et al.* 2012; Cusick and Sayler 2013). Associated impacts include losses to aquaculture, accumulation of toxins into benthic and littoral food webs, mortalities of fish, seabirds and marine mammals and losses to environmental and recreational value of affected areas (Jester *et al.* 2009; Anderson *et al.* 2012; Cusick and Sayler 2013). Both spirolides and gymnodimine are fast-acting macrocyclic imines with high toxicity to laboratory mice when injected intraperitoneally (Munday *et al.* 2004, 2012). There are no reported negative human health effects related to spirolides but they are known to accumulate in shellfish (Aasen *et al.* 2005; González *et al.* 2006; Rundberget *et al.* 2011; Ciminiello *et al.* 2014). Gymnodimines also accumulate in shellfish (Stirling 2001; Biré *et al.* 2002) but are regarded as low risk to humans because of low oral toxicity (Munday *et al.* 2004). The goniodomins cause paralysis and mortality in finfish, shellfish and gastropods but are not linked to human illness (Gates and Wilson 1960; Harding *et al.* 2009; May *et al.* 2010; Anderson *et al.* 2012).

1.2 The Baltic Sea

The Baltic Sea is situated in northern Europe between latitudes 54 °N and 66 °N. It is a geologically young sea that evolved into its current form after the withdrawal of ice at the end of the last glaciation approximately 10 000 years ago (Björck 1995). It is a semi-enclosed brackish water estuary with saline water input from the Skagerrak and Kattegat area and freshwater input from rivers (Feistel *et al.* 2009). The Baltic Sea has a naturally low biological diversity (Johannesson and André 2006), and flora and fauna in the area are a mixture of marine, brackish water and fresh water species (Leppäkoski and Olenin 2001; Leppäkoski *et al.* 2002). Many species have a foreign origin (Leppäkoski *et al.* 2002). Strong salinity and temperature gradients shape the environment and affect species diversity (Leppäkoski *et al.* 2002). Surface salinity ranges from 20 - 25 practical salinity unit (PSU) in the Kattegat area to 2 - 3 PSU in the innermost parts of the Bothnian Bay and Gulf of Finland (Feistel *et al.* 2009). Surface water temperature varies from approximately 14 – 22 °C in late summer to close to freezing in winter (Leppäkoski *et al.* 2002). Northern areas normally have ice cover during winter months. The coastline is highly complex and Finland alone has 39 000 km of coastline (Leppäkoski *et al.* 2002). Areas belonging to the Baltic Sea are defined according to ICES where the Kattegat and Skagerrak are defined as part of the North Sea and the border between the Baltic Sea and the North Sea is on the Baltic Sea side of the Kattegat (ICES 2004, see Figure 3).

1.2.1 Harmful dinoflagellate blooms in the Baltic Sea

Approximately 60 potentially harmful species are encountered in the Baltic Sea, according to the ICES checklist in 2007 (ICES 2007). Extensive cyanobacteria blooms formed during summer by *Nodularia spumigena*, *Aphanizomenon flos-aquae* and different *Anabaena* and *Microcystis* species are currently the biggest problem (Luckas *et al.* 2005; Lilover and Stips 2008; Svedén *et al.* 2016). The ICES checklist also lists 26 potentially harmful dinoflagellates. Most species occur in the Kattegat and Belt Sea area, but *Akashiwo sanguinea, Alexandrium ostenfeldii, Dinophysis acuminata, Dinophysis acuta, Dinophysis norvegica, Dinophysis rotundata, Gonyaulax spinifera, Heterocapsa triquetra, Lingulodinium polyedrum, Peridiniopsis polonicum, Protoceratium reticulatum, Prorocentrum minimum* and *Scrippsiella trochoidea* are encountered in the central and/or northern Baltic Sea (ICES 2007). *Dinophysis spp.* commonly occurs in the area and toxins produced by *Dinophysis spp.* have been detected in small concentrations in plankton communities, blue mussels (*Mytilus edulis*) and common flounder (*Platichthys flesus*, see Pimiä *et al.* 1997; Sipia *et al.* 2000; Kuuppo *et al.* 2006; Hällfors *et al.* 2011; Setälä *et al.* 2011). Yessotoxin, likely produced by *P. reticulatum* or *G. spinifera*, has also been detected from the central and northern Baltic Sea plankton communities (Setälä *et al.* 2011). The potentially toxic invasive species *P. minimum* has become a regular component of the phytoplankton community in the

central and northern Baltic Sea, but has not been reported to produce toxins in this region (Hajdu *et al.* 2005; Pertola *et al.* 2005).

A relatively new phenomenon in the central and northern Baltic Sea is the yearly occurrence of toxin-producing *A. ostenfeldii blooms* (Figure 2 A, see Larsson *et al.* 1997; Hajdu *et al.* 2006; Kremp *et al.* 2009). PSTs produced by these blooms are shown to accumulate in natural bivalve communities and perch (*Perca fluviatilis*, see Setälä *et al.* 2014). There are no reported negative effects to humans caused by harmful dinoflagellate species in the region, likely because there is no commercial shellfish industry in the Baltic Sea and the naturally occurring shellfish are not generally eaten by humans in this region. However, systematic large-scale studies on the distribution and bioaccumulation of algal toxins should be performed to be better able to assess the threat caused by these toxins to humans and ecosystems in the Baltic Sea.

1.3 Bioluminescence in dinoflagellates

Bioluminescence is the emission of visible light produced in a chemical reaction by an organism (reviewed by Wilson & Hastings 1998; Haddock *et al.* 2010; Widder 2010). The majority of bioluminescent organisms occur in oceans in a range of different habitats from tropical to polar waters and from the deep sea to surface waters (Haddock *et al.* 2010; Widder 2010; Berge *et al.* 2012; Cronin *et al.* 2016). A wide range of marine organisms, from bacteria, unicellular algae and zooplankton to squids and fish, are able to produce light in this manner (Wilson and Hastings 1998; Haddock *et al.* 2010; Widder 2010; Johnsen *et al.* 2014). In oceans, bioluminescence is mainly of blue colour, corresponding to the waveband with lowest light attenuation by water (Widder 2010). Bioluminescence serves a range of functions from self-defence and camouflage to reproduction and intra-species communication (Haddock *et al.* 2010; Widder 2010). Bioluminescence has independently evolved at least 40 times and genes responsible for bioluminescence are unrelated between different types of organisms (Wilson and Hastings 1998; Haddock *et al.* 2010). However, production of bioluminescence always involves the reaction of molecular oxygen with a light-emitting molecule (luciferin) together with a catalysing enzyme (luciferase or photoprotein) and results in photons of visible light (Wilson and Hastings 1998; Haddock *et al.* 2010).

The majority of bioluminescence in the surface waters of oceans is produced by dinoflagellates (Swift *et al.* 1995; Marcinko *et al.* 2013; Cronin *et al.* 2016). Dinoflagellates are responsible for sparkling lights that can be seen in the surface of the sea during the night (Figure 1.) and bright blue glow as e.g. seen in the famous bioluminescent bays of Puerto Rico (Phlips *et al.* 2006; Reidhaar *et al.* 2016). Dinoflagellates are the only known photosynthetic organisms that can produce bioluminescence (Sweeney 1987). Bioluminescence in dinoflagellates is considered a defensive mechanism against grazing, possibly attracting the predators of predators (Buskey *et al.* 1983; Abrahams and Townsend 1993; Fleisher and Case 1995) or functioning as an aposematic signal (Cusick and Widder 2014). Bioluminescence

expression in dinoflagellates exhibits a clear circadian rhythm with the brightest signal during the night and a barely discernible signal during the day (Hastings 1989; Knaust *et al.* 1998; Marcinko *et al.* 2013). Of 117 described dinoflagellates genera 17 have bioluminescent members (Valiadi and Iglesias-Rodriguez 2013) and many of the harmful dinoflagellate taxa produce bioluminescence (Valiadi *et al.* 2012; Cusick and Widder 2014).

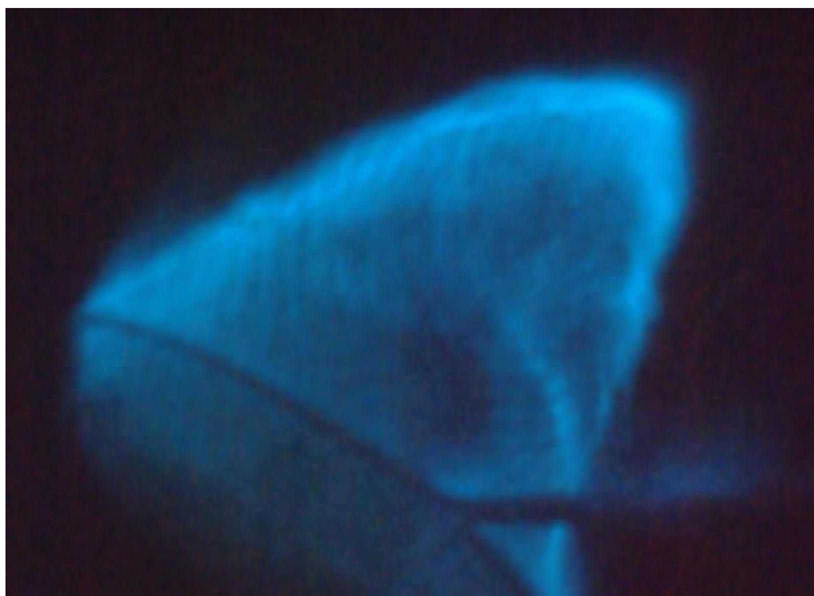

Figure 1. Bioluminescence produced by *Alexandrium ostenfeldii* in response to a net being moved through the water. Photo: Stefan Simis.

Dinoflagellates produce bioluminescence in specific cellular organelles named scintillons (DeSa and Hastings 1968), which contain a light-emitting luciferin substrate, a luciferase enzyme (LCF) and, in some species, a luciferin-binding protein (LBP, see Knaust *et al.* 1998; Akimoto *et al.* 2004; Valiadi and Iglesias-Rodriguez 2014). Bioluminescence is produced in response to mechanical stimulation creating pressure on the cell wall due to water movement, for example by breaking waves or contact with grazers (Sweeney 1987). This results in an action potential across the vacuole membrane of the cell, leading to a proton influx from the acidic vacuole into the scintillons decreasing the pH from 8 to 6 and causing the LCF to take on its active form. In species that have LBP, it binds the luciferin protein, protecting it from autoxidation at physiological pH and releasing it at pH below 7, when LCF also takes it active form. Luciferin is oxidised by LCF, which results in the emission of photons as a brief flash of blue light at a wavelength of approximately 475 nm (Wilson and Hastings 1998; Haddock *et al.* 2010). All dinoflagellate species are assumed to have the same luciferin, since luciferin characterised from *Pyrocystis lunula* has been shown to cross- react with the LCF of other bioluminescent dinoflagellate species (Schmitter *et al.* 1976; Knaust *et al.* 1998). The luciferase gene (*lcf*) that controls the production of LCF is the most studied compound in the bioluminescence system of dinoflagellates. It has been fully sequenced from eight dinoflagellate species (Li and Hastings 1998; Liu *et al.*

2004; Liu and Hastings 2007; Valiadi and Iglesias-Rodriguez 2013) and partial sequences are available from a wider range of species (Baker *et al.* 2008; Valiadi *et al.* 2012, 2014). In photosynthetic dinoflagellate species, *lcf* consists of three tandemly repeated domains, with a highly conserved central region that is bordered by more variable N- and C- terminal regions (Li *et al.* 1997; Li and Hastings 1998; Okamoto *et al.* 2001; Liu *et al.* 2004). In general, within-species differences between *lcf* domains are larger than between-species differences of the same domain (Okamoto *et al.* 2001; Liu *et al.* 2004). There are also differences in the untranslated region sequences and in the length of these regions between species (Okamoto *et al.* 2001; Liu *et al.* 2004). In dinoflagellates the bioluminescence system using LBP is likely to be more common than the system that does not use LBP (Valiadi and Iglesias-Rodriguez 2014). The LBP gene (*lbp*) has been found in three different dinoflagellate genera and in several bioluminescent species in the genus *Alexandrium* (Fogel and Hastings 1971; Liu and Hastings 2007; Valiadi and Iglesias-Rodriguez 2014). *Pyrocystis* is the only genus known to lack the LBP (Knaust *et al.* 1998).

Dinoflagellate bioluminescence can be stimulated in several ways. Laboratory studies have used, for example, bubbling, stirring and flow to mechanically stimulate bioluminescence in dinoflagellates (Biggley *et al.* 1969; von Dassow *et al.* 2005; Cussatlegras and Le Gal 2007; Deane *et al.* 2016). Chemicals can be also used to stimulate bioluminescence. These include chemicals that decrease the pH e.g. acids and different ions e.g. Ca2+, K+, H+ (Hamman and Seliger 1972; Widder and Case 1982). Experiments have shown that circadian inhibition of bioluminescence during the day can be by-passed using chemical stimulation (Hamman and Seliger 1972; Widder and Case 1982). In the open ocean and coastal zone, bioluminescence intensities have been studied using bathyphotometers, which generally include a measuring chamber with photometer and a pump or impeller to keep water flow constant and excite the organism (Aiken and Kelly 1984; Marra 1995; Herren *et al.* 2005; Latz and Rohr 2013; Cronin *et al.* 2016).

1.3.1 Bioluminescent dinoflagellates in the Baltic Sea

Many bioluminescent dinoflagellate species occur in the Kattegat and Belt Sea area, but only three bioluminescent species are known to occur in the central and northern Baltic Sea (Hällfors 2004). *P. reticulatum* is generally found in low abundances throughout the open Baltic Sea (Hällfors 2004; Mertens *et al.* 2012) and *L. polyedrum* is encountered in the Southern Baltic Sea (Hällfors 2004). During the last decade, *A. ostenfeldii* has been increasingly observed in shallow waters and bays of the Baltic Sea up to the Archipelago Sea, with reports from Åland, the Gulf of Gdansk, Gotland and Kalmar (Larsson *et al.* 1998; Hajdu *et al.* 2006; Kremp *et al.* 2009). *A. ostenfeldii* is currently the only bioluminescent dinoflagellate known to form dense blooms in the coastal areas of the central and northern Baltic Sea (Kremp *et al.* 2009; Hakanen *et al.* 2012).

1.4 *Alexandrium ostenfeldii*

A. ostenfeldii is encountered in low abundances in the oceans from temperate to arctic waters (Moestrup and Hansen 1988; Mackenzie *et al.* 1996; John *et al.* 2003; Gribble *et al.* 2005; Tillmann *et al.* 2014). As a relatively new phenomenon, dense blooms of the species are increasingly observed from coastal areas worldwide, including the east coast of the USA (Borkman *et al.* 2012; Tomas *et al.* 2012), South America (Almandoz *et al.* 2014), Italy (Ciminiello *et al.* 2006), the Netherlands (Burson *et al.* 2014) and the Baltic Sea (Kremp *et al.* 2009). These blooms pose a risk to aquatic organisms and humans in the affected areas as *A. ostenfeldii* are known to produce three different types of neurotoxins (Suikkanen *et al.* 2013; Salgado *et al.* 2015). Strains from the Atlantic Ocean and Mediterranean Sea are reported to produce spirolides (Gribble *et al.* 2005; Touzet *et al.* 2008; Ciminiello *et al.* 2010). Strains from Malaysia and the Baltic Sea are known to produce PSTs, and Baltic Sea strains also produce gymnodimines (Lim *et al.* 2005; Suikkanen *et al.* 2013; Harju *et al.* 2016; Savela *et al.* 2016), whereas strains from the USA and the Netherlands produce all three types of toxins (Van Wagoner *et al.* 2011; Borkman *et al.* 2012; Van de Waal *et al.* 2015; Martens *et al.* 2017). It is not known why strains from different areas have different toxins profiles. It has been shown that toxin profiles are genetically determined and that salinity affects the concentration and type of toxin analogs produced, while it does not change the main toxin profile (Suikkanen *et al.* 2013). *A. ostenfeldii* produces allelochemicals with negative effects on heterotrophic and autotrophic phytoplankton (Tillmann *et al.* 2007). The species is mixotrophic and has been shown to feed on ciliates and other dinoflagellates (Jacobson and Anderson 1996; Gribble *et al.* 2005). As many other species in the genus, *A. ostenfeldii* produces bioluminescence (Kremp *et al.* 2009).

A. ostenfeldii and *A. peruvianum* have been recently combined into the *A. ostenfeldii* species complex (Kremp *et al.* 2014). This consists of six distinct genetics groups with differences in morphology, toxin composition, ecophysiological properties and geographic distribution patterns (Kremp *et al.* 2014). Baltic Sea *A. ostenfeldii* belongs to group 1 together with strains from the USA east coast estuaries and the Netherlands (Kremp *et al.* 2014; Van de Waal *et al.* 2015). Typically, strains in this group occur in shallow productive coastal embayments or river estuaries with brackish water (Kremp *et al.* 2014). All strains in group 1 produce PSTs, including saxitoxin analog (Kremp *et al.* 2014) and strains from the USA and the Netherlands also produce spirolides (Tomas *et al.* 2012; Van de Waal *et al.* 2015). It has recently been show that strains from all locations in group 1 produce gymnodimines (Van de Waal *et al.* 2015; Harju *et al.* 2016; Martens *et al.* 2017).

A. ostenfeldii is regularly encountered in low abundance in the Kattegat and Belt Sea (ICES 2007), but its distribution in general in the Baltic Sea is not well known. In the main Baltic Sea *A. ostenfeldii* is known to form blooms in the Puck Bay, Gulf of Dansk, Poland in Kalmar, East coast of Sweden, Valleviken in Gotland and in Föglö and Kökar in the Åland archipelago (Figure 3; Tahvanainen *et al.* 2012). In the northern Baltic Sea, bioluminescent blooms of *A. ostenfeldii* occur regularly

during July and August in the Åland archipelago in shallow sheltered areas with salinities around 6 - 7 PSU (Figure 2; Kremp *et al.* 2009; Hakanen *et al.* 2012). The magnitude of the bloom changes from year to year with a maximum observed abundance of 6.3 x 10^6 cells L^{-1} in 2014 (Savela *et al.* 2016). The blooms are not monospecific, but *A. ostenfeldii* occurs as a part of a diverse phytoplankton community (Hakanen *et al.* 2012). *A. ostenfeldii* blooms generally start to form at water temperatures around 20 °C (Hakanen *et al.* 2012). Baltic *A. ostenfeldii* strains produce PSTs and gymnodimines (Suikkanen *et al.* 2013; Harju *et al.* 2016) in which PSTs have been shown to accumulate in natural bivalve communities and fish (Setälä *et al.* 2014). Laboratory experiments have shown allelopathic effects on co-occurring dinoflagellates (Hakanen *et al.* 2014) and copepods (Sopanen *et al.* 2011). Baltic *A. ostenfeldii* forms both thin-walled pellicle cysts, in response to unfavourable conditions, and thick walled resting cyst (Figure 2 C), and blooms occurring during summer are closely coupled to benthic cyst beds (Hakanen *et al.* 2012).

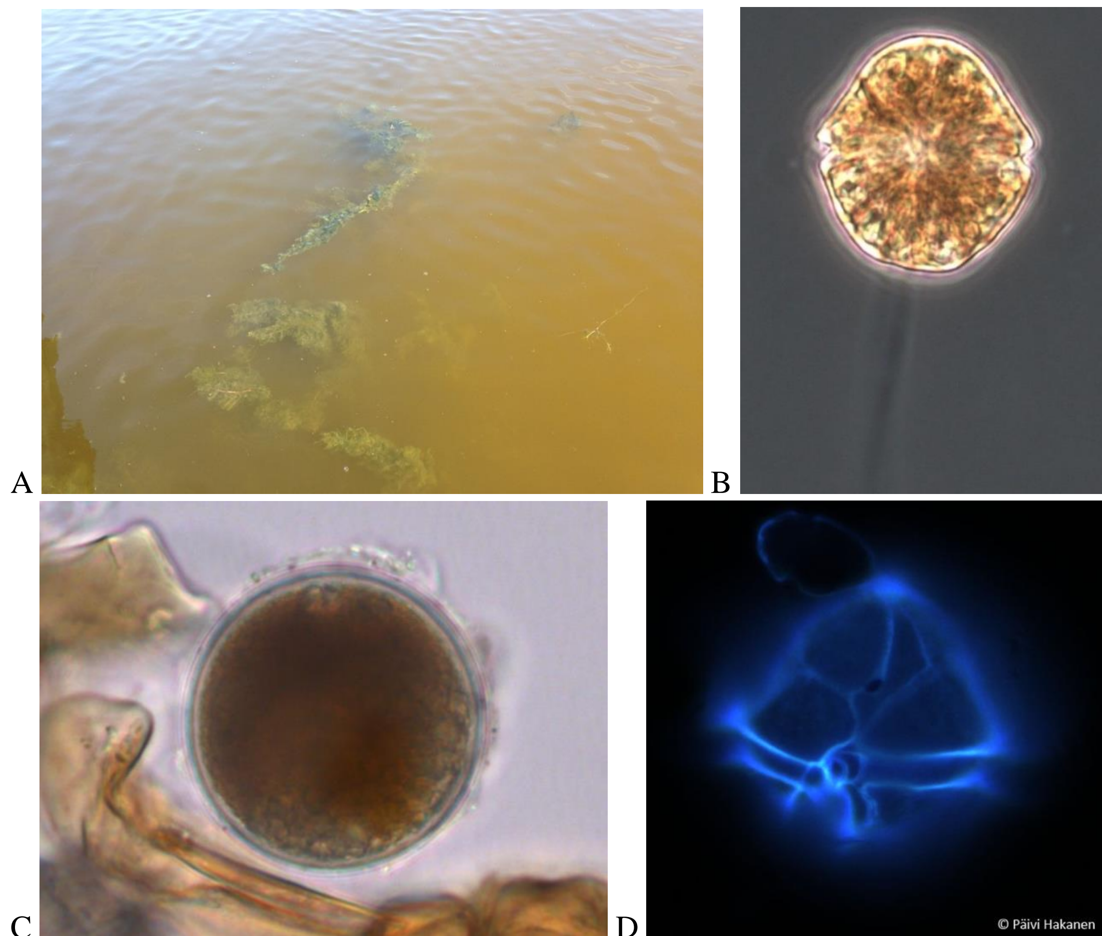

Figure 2 A) Dense *Alexandrium ostenfeldii* bloom in Föglö, Åland during the summer 2014. Photo: Elin Lindehoff. B) Vegetative cell of *A. ostenfeldii*. Photo: Päivi Hakanen. C) Cyst of *A. ostenfeldii*. Photo: Anke Kremp. D) Vegetative cell of *A. ostenfeldii* stained with fluorescence brightener. Photo: Päivi Hakanen

1.5 Monitoring harmful algal blooms

Phytoplankton monitoring in the Baltic Sea relies on the systematic collection of water samples from a pre-determined network of stations at certain times of the year. This system has produced long time-series revealing phytoplankton composition in monitored areas and changes in phytoplankton dynamics throughout the years (Kononen and Niemi 1984; Suikkanen *et al.* 2007). Unfortunately, the spatial and temporal coverage of such monitoring efforts remains limited and has been found insufficient in providing representative details of phytoplankton short term variability (Rantajärvi *et al.* 1998). The Alg@line –project was started in 1993 to improve the coverage and resolution of the existing monitoring network. The ship-of-opportunity platform (SOOP) is an automated sampling method that can be placed on board a passenger ferry or a merchant ship. This technique has provided a cost-effective way to observe the dynamics in physical-, chemical and biological properties in the upper water layer with high spatial and temporal coverage (Leppänen *et al.* 1995; Rantajärvi *et al.* 1998; Rantajärvi 2003). One of the main objectives of the Alg@line network was to monitor the phytoplankton community and harmful algal blooms (Leppänen *et al.* 1995). Currently the near real-time chlorophyll-a (Chl-a) and phycocyanin fluorescence data combined with automatically collected water samples from the Alg@line system together with satellite images, and observations from the Finnish Environment Institute and regional environmental centres are used to report the cyanobacteria bloom situation a near-daily intervals, during the intensive phytoplankton growth period (Rantajärvi 2003; SYKE 2017). Water samples collected in the Alg@line network have also been used to study the harmful algal species present in the Baltic Sea and the long-term and seasonal distribution of dinoflagellates in the open waters of the Baltic Sea (Leppänen *et al.* 1995; Hällfors *et al.* 2013). The relevance of using the Alg@line system to study the temporal and spatial distribution of *A. ostenfeldii* is not known since based on current observations *A. ostenfeldii* blooms occur in the shallow coastal areas and the Alg@line samples are collected along shipping routes. The Alg@line ferry *Silja Serenade* travels regularly past a prominent *A. ostenfeldii* bloom site in Föglö in the Åland archipelago. One option to decrease the sampling costs and reduce the sampling effort is to perform targeted sampling in high-risk areas. Unfortunately, most areas that have a high risk for *A. ostenfeldii* blooms in the Baltic Sea are not currently known, but different modelling methods such as species distribution modelling could be used to define potential risk areas.

A specific problem in using the routine monitoring data is that many dinoflagellate species, particularly in genus *Alexandrium*, cannot be identified to species level by routine methods such as light microscopy of lugol-preserved samples, even by a skilled taxonomist. The discrimination of thecate dinoflagellates to the species level is difficult since many species are morphologically uniform and small details such as the size and shape of individual thecal plates need to be examined to ensure identification (Murray *et al.* 2015). Study of thecal plates typically requires staining of cells (with Calcofluor white or other staining

techniques, see Figure 2 D) and subsequent fluorescence microscopy (Fritz and Triemer 1985). This is not generally done as part of routine monitoring, which is why *A. ostenfeldii* cells found in routine monitoring would likely be grouped at a higher taxonomic level than the species. Microscopy is also time consuming and can rarely be done at the temporal resolution necessary for early detection of HAB. New automated sampling devises like FlowCAM and flow cytometers have been developed to help with phytoplankton enumeration and characterisation (Alvarez *et al.* 2014). These methods could be useful in detecting harmful dinoflagellates but are often limited to taxonomic level of genus or above (Alvarez *et al.* 2014). Molecular methods, for example species-specific DNA-based molecular probes, DNA barcoding or microarrays (McCoy *et al.* 2013; Taylor *et al.* 2014; Comtet *et al.* 2015) have been used with good results in recognition of harmful dinoflagellate species and provide an effective tool for relatively cheap and precise monitoring. Many toxin-producing dinoflagellates produce bioluminescence (Valiadi *et al.* 2012; Cusick and Widder 2014), and it has been suggested that bioluminescence could be used as an indicator of HABs (Kim *et al.* 2006; Haddock *et al.* 2010).

In summary, a clear need for an early warning system of *A. ostenfeldii* presence exists in the coastal areas of the Baltic Sea during summer. Due to the complex coastline with many isolated and remote bays, this demand cannot be met with traditional sampling and analysis of water samples. Therefore, new methods are needed to assess the distribution and abundance of *A. ostenfeldii* in the Baltic Sea. When exploring new methods, it should be taken into account that suitable monitoring methods should be easy to deploy, be well targeted and cost-efficient, as accurate but expensive methods are less likely to be used.

2 Aims of the thesis

The overall aim of this thesis is to study *Alexandrium ostenfeldii* bioluminescence to evaluate whether bioluminescence can be used as an early warning signal of harmful dinoflagellate blooms in the Baltic Sea. More precisely, I aim to study the expression of *A. ostenfeldii* bioluminescence to learn how much genetic, phenotypic and environmentally induced variability there is in bioluminescence production in the species.

More specific aims are:

1) To study the distribution and consistency of bioluminescence expression in different bloom populations of Baltic *A. ostenfeldii* to determine if bioluminescence can be reliably used in monitoring.
2) To study daily and seasonal variation in bioluminescence emission of *A. ostenfeldii* to evaluate how bioluminescence can be optimally detected throughout bloom development.
3) To determine if there is a relationship between bioluminescence, cell numbers and toxicity, to establish which combinations of observation methods can be used effectively in routine HAB risk monitoring.

4) To find out how different environmental variables affect bioluminescence expression to predict how their variability will affect the monitoring of bioluminescence.
5) To increase the knowledge of the poorly known distribution of *A. ostenfeldii* in the Baltic Sea by using species distribution modelling.
6) To evaluate how any of the novel methods based on bioluminescence could help in early detection of potentially toxic *A. ostenfeldii* blooms in the Baltic Sea.

In **paper I** I study the distribution and consistency of the luciferase gene (*lcf*) in different bloom populations of Baltic *A. ostenfeldii*, and determine if the presence of *lcf* is consistently paired with bioluminescence production. This is of interest to evaluate usefulness of applications for early detection of toxic blooms based on bioluminescence. Information from paper I is used to evaluate the results from **paper II** where I investigate the spatiotemporal patterns of bioluminescence produced during different stages of an *A. ostenfeldii* bloom and relate this to Chl-a concentrations, cell numbers and toxin concentrations. This information is used to develop an autonomous monitoring and early warning strategy based on bioluminescence. In **paper III**, effects of different salinities and temperatures on growth, bioluminescence emission and toxin production are studied to gather information on genetic diversity and phenotypic variation in *A. ostenfeldii* seed banks, in order to estimate the adaptive potential of *A. ostenfeldii* to projected climate change. In **paper IV**, data on *A. ostenfeldii* distribution collected during previous studies and based on literature searches are used to model the potential distribution of *A. ostenfeldii* in the coastal waters of Finland under current and future climate conditions.

3 Material and methods

3.1 Sample collection

Clonal cultures of Baltic *A. ostenfeldii* used in laboratory experiments were established from sediment samples containing resting cysts. Sediments were collected from six bloom sites in different areas of the Baltic Sea: Föglö, Sandviken and Kökar in Åland (Finland), Valleviken (Gotland, Sweden), Kalmar Sound (Sweden) and Puck Bay (Gulf of Gdansk, Poland). The locations of these sites are shown in Figure 3. The sampling procedure is described in detail in Tahvanainen *et al.* (2012). The *A. ostenfeldii* strains from the North Sea, Canada, China and Spain obtained from culture collections were grown from cells collected from the water column (**Paper I**). A detailed list of *A. ostenfeldii* strains used in experiments is given in Table 1 (**Papers I** and **III**).

The Föglö study area (Figure 4), where semi-continuous bioluminescence, Chl-a and temperature measurements, and collection of phytoplankton, toxin and *lcf* samples took place (**Papers I** and **II**), is situated in the Åland archipelago between Finland and Sweden (Figure 3). Phytoplankton and toxin samples were collected and processed every second week between mid-July and the end of September 2011 according to protocols described in **Paper II** and Hakanen *et al*. (2012). Phytoplankton samples and seawater samples for *lcf* detection were collected at 10 locations along a 7 km transect through a known *A. ostenfeldii* bloom site (Figure 3). Stimulated bioluminescence was recorded from the same locations during the night prior to water sampling by lowering the submersible light sensor to a depth of 0.5–1.0 m at each location for approximately 2 min. Additionally, *lcf* samples were collected around the Föglö and Kökar island group in the Åland archipelago, around islands close to the Tvärminne Zoological Station (University of Helsinki, in Gulf of Finland), on-board *R/V Aranda* and with the Alg@line ferry *Silja Serenade*, which travels regularly through the Archipelago Sea. The protocol for collecting the *lcf* samples is described in **Paper I.**

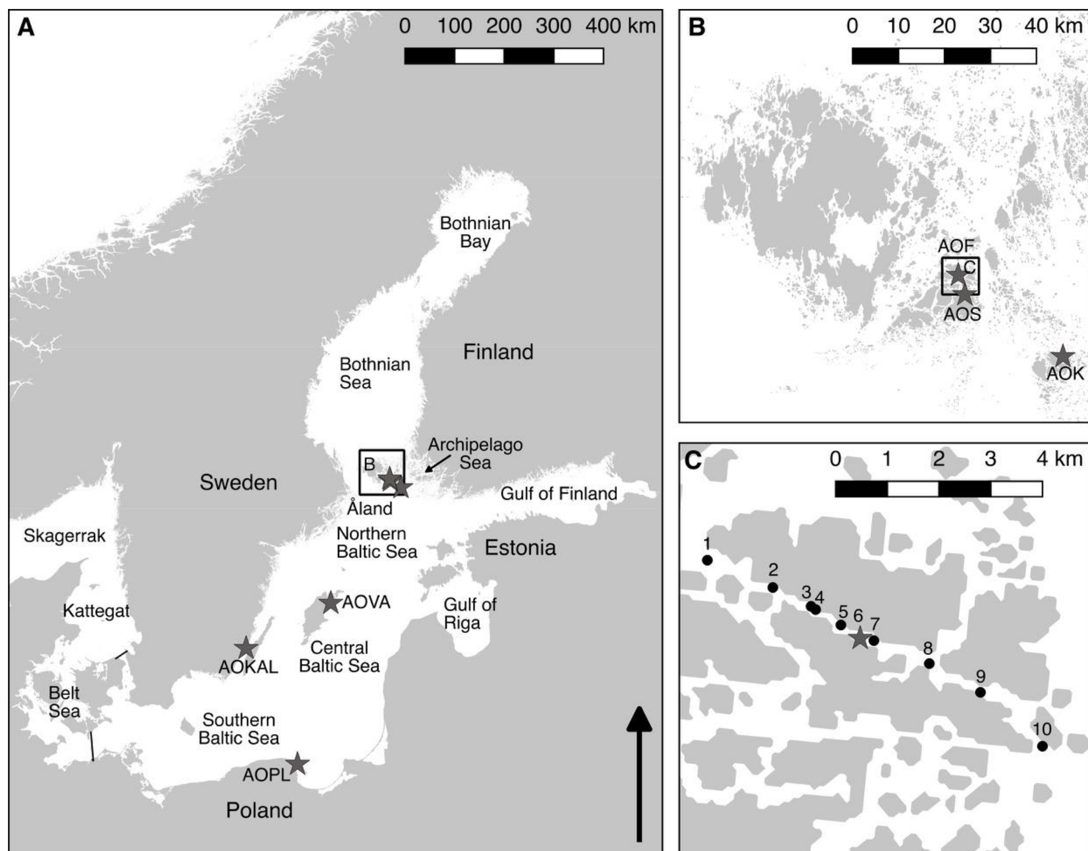

Figure 3. A) Map of the Baltic Sea, showing different sea areas and the location of the *Alexandrium ostenfeldii* populations used in articles I and III (grey stars). Black lines show the border between the Baltic Sea and North Sea. B) Detailed map of the Åland islands showing the location of three *A. ostenfeldii* bloom populations. C) Details of the islands around the Föglö study site showing station numbers along the sampled transect used in articles I and II and location of the Föglö high-frequency measurement station in a known bloom area (grey star), where field sampling took place for article II.

3.2 DNA extraction, PCR and sequencing

DNA was extracted from clonal cultures in their exponential growth stage using a Plant Mini Kit (Qiagen). The procedure is described in detail in **Paper I**. Extracted DNA was purified with a PCR Template Purification Kit (Roche) according to manufacturer instructions. DNA samples were amplified using previously developed primers LcfUniCHF3 and LcfUniCHR4 for dinoflagellate *lcf* (Baker *et al.* 2008). PCR was performed according to protocol described in Baker *et al.* (2008). The expected size of the PCR product was between 500–550 base pairs. Strains that produced a clear PCR product on the gel were sequenced to confirm that the correct product had been amplified and to allow further phylogenetic analysis. For the *lcf* detection from seawater samples, DNA was extracted from filters using a Chelex procedure described in **Paper I.** PCR was carried out as described above. PCR products from field samples were not sequenced.

Figure 4. A photo of the Föglö study site where regular *Alexandrium ostenfeldii* blooms are encountered.

Table 1. Information on Alexandrium ostenfeldii isolates used in papers I and III, indicating strain identification, origin, information on luciferase gene presence, bioluminescence production and known toxin production. Information on toxin production is also based on article Kremp et al. (2014). The symbol – indicates that the information is not known.

Strain	Origin	Presence of the *lcf*	Biolum. production	Toxin production	Used in papers
AOF0901	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0905	Föglö, Åland, Finland	KT732837	Yes	PSP	I and III
AOF0909	Föglö, Åland, Finland	KT732838	Yes	-	I
AOF0915	Föglö, Åland, Finland	KT732839	Yes	-	I
AOF0919	Föglö, Åland, Finland	KT732840	Yes	PSP	I and III
AOF0922	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0923	Föglö, Åland, Finland	-	Yes	-	I

Strain	Origin	Presence of the *lcf*	Biolum. production	Toxin production	Used in papers
AOF0926	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0927	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0930	Föglö, Åland, Finland	KT732841	Yes	-	I
AOF0935	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0936	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0938	Föglö, Åland, Finland	KT732842	Yes	-	I
AOF0939	Föglö, Åland, Finland	-	Yes	PSP	III
AOF0940	Föglö, Åland, Finland	-	Yes	PSP	I and III
AOF0957	Föglö, Åland, Finland	KT732843	Yes	-	I
AOTVA4	Föglö, Åland, Finland	KT732881	Yes	-	I
AOK1006	Kökar, Åland, Finland	KT732844	Yes	-	I
AOK1007	Kökar, Åland, Finland	KT732845	Yes	-	I
AOK1009	Kökar, Åland, Finland	KT732846	Yes	-	I
AOK1013	Kökar, Åland, Finland	KT732847	Yes	-	I
AOK1014	Kökar, Åland, Finland	KT732848	Yes	-	I
AOK1020	Kökar, Åland, Finland	KT732849	Yes	-	I
AOK1028	Kökar, Åland, Finland	KT732850	Yes	-	I
AOK1032	Kökar, Åland, Finland	KT732851	Yes	-	I
AOK1037	Kökar, Åland, Finland	KT732852	Yes	-	I
AOK1038	Kökar, Åland, Finland	-	Yes	-	I
AOK1045	Kökar, Åland, Finland	KT732853	Yes	-	I
AOS1001	Sandviken, Åland, Finland	KT732874	Yes	-	I
AOS1002	Sandviken, Åland, Finland	KT732875	Yes	-	I
AOS1004	Sandviken, Åland, Finland	KT732876	Yes	-	I
AOS1006	Sandviken, Åland, Finland	KT732877	Yes	-	I
AOS1011	Sandviken, Åland, Finland	-	Yes	-	I
AOS1013	Sandviken, Åland, Finland	KT732878	Yes	-	I
AOS1014	Sandviken, Åland, Finland	KT732879	Yes	-	I
AOS1017	Sandviken, Åland, Finland	-	Yes	-	I
AOS1020	Sandviken, Åland, Finland	KT732880	Yes	-	I
AOPL0902	Hel, Poland	KT732864	Yes	PSP	I and III
AOPL0906	Hel, Poland	KT732865	Yes	PSP	I and III
AOPL0909	Hel, Poland	KT732866	Yes	-	I
AOPL0913	Hel, Poland	-	Yes	PSP	III
AOPL0914	Hel, Poland	KT732867	Yes	-	I
AOPL0917	Hel, Poland	-	Yes	PSP	III
AOPL0918	Hel, Poland	KT732868	Yes	-	I
AOPL0924	Hel, Poland	KT732869	Yes	-	I
AOPL0925	Hel, Poland	-	Yes	PSP	III
AOPL0930	Hel, Poland	KT732870	Yes	PSP	I and III
AOPL0945	Hel, Poland	KT732871	Yes	-	I
AOPL0961	Hel, Poland	KT732872	Yes	-	I
AOPL0967	Hel, Poland	KT732873	Yes	-	I

Strain	Origin	Presence of the *lcf*	Biolum. production	Toxin production	Used in papers
AOVA0901	Gotland, Sweden	KT732882	Yes	-	I
AOVA0903	Gotland, Sweden	KT732883	Yes	PSP	I and III
AOVA0904	Gotland, Sweden	KT732884	Yes	-	I
AOVA0905	Gotland, Sweden	-	Yes	PSP	III
AOVA0906	Gotland, Sweden	KT732885	Yes	PSP	I and III
AOVA0907	Gotland, Sweden	-	Yes	PSP	I and III
AOVA0910	Gotland, Sweden	KT732886	Yes	-	I
AOVA0915	Gotland, Sweden	-	Yes	PSP	III
AOVA0917	Gotland, Sweden	-	Yes	PSP	III
AOVA0923	Gotland, Sweden	KT732887	Yes	-	I
AOVA0924	Gotland, Sweden	KT732888	**No**	PSP	I and III
AOVA0928	Gotland, Sweden	-	Yes	PSP	III
AOVA0929	Gotland, Sweden	KT732889	Yes	-	I
AOVA0930	Gotland, Sweden	-	Yes	PSP, GYM	III
AOVA0931	Gotland, Sweden	KT732890	Yes	PSP	I and III
AOKAL0902	Kalmar, Sweden	KT732854	Yes	-	I
AOKAL0909	Kalmar, Sweden	KT732855	Yes	PSP	I and III
AOKAL0913	Kalmar, Sweden	KT732856	Yes	PSP	I and III
AOKAL0916	Kalmar, Sweden	KT732857	Yes	-	I
AOKAL0918	Kalmar, Sweden	-	Yes	PSP	I and III
AOKAL0919	Kalmar, Sweden	KT732858	Yes	PSP	I and III
AOKAL0923	Kalmar, Sweden	KT732859	Yes	-	I
AOKAL0924	Kalmar, Sweden	-	Yes	PSP	III
AOKAL0925	Kalmar, Sweden	-	Yes	PSP	I and III
AOKAL0927	Kalmar, Sweden	KT732860	Yes	PSP	I and III
AOKAL0928	Kalmar, Sweden	KT732861	Yes	PSP	I and III
AOKAL0933	Kalmar, Sweden	-	Yes	PSP	III
K1354	Öresund, Denmark	KT732892	Yes	PSP	I
CCAP1119/45	North Sea, Scotland	KT732835	Yes	Spirolides	I
CCAP1119/47	North Sea, Scotland	KT732836	Yes	Spirolides	I
S6_P12_E11	North Sea, Scotland	KT732863	Yes	Spirolides	I
NCH85	North Sea, Norway	KT732893	Yes	Spirolides	I
AONOR4	Oslofjord, Norway	KT732862	Yes	Spirolides	I
LSA06	Lough Swilly, Ireland	No	No	Spirolides	I
LSE05	Lough Swilly, Ireland	No	No	Spirolides	I
WW516	Fal River, UK	No	No	Spirolides	I
WW517	Fal River, UK	No	No	Spirolides	I
IEO-VGOAMD12	Palamos, Spain	KT732891	No	Spirolides	I
IOE-VGOAM10C	Palamos, Spain	Yes	No	Spirolides	I
ASBHO1	Bohai Sea, China	Yes	Yes	PSP	I
AOPC1	Saanich, Canada	Yes	Yes	-	I

3.3 Bioluminescence measurements

For **paper I**, dense *A. ostenfeldii* cultures in their late exponential to early stationary phase were visually tested for bioluminescence emission by shaking the culture bottles in a dark room during the scotophase. If no visual bioluminescence occurred the culture was chemically stimulated by adding acid and recording the bioluminescence signal with a Varian Cary Eclipse spectrofluorometer. Bioluminescence in **Paper III** was recorded using the same spectrofluorometer while bioluminescence was stimulated by the addition of acid. The detailed procedure for these bioluminescence measurements is described in **Papers I** and **III**. For **Paper III**, the bioluminescence measurements were zeroed using the signal recorded at the start of the measurements, before acid addition. The onset of bioluminescence was detected as the first peak in the second derivative of the recorded signal. The duration of bioluminescence following the acid addition was up to 18 s in the majority of samples. Bioluminescence intensity (BL, in instrument units) was therefore normalised to the cell number and period of bioluminescence in seconds.

Daily rhythms of *A. ostenfeldii* bioluminescence were studied experimentally with strain AOF-0930 from Föglö, Åland, which based on previous studies was known to produce bioluminescence. Experiments were conducted with cultures in their exponential growth phase and growth dynamics were monitored by in vivo Chl-a fluorescence measurements. At the beginning of each experiment, the cell density was approximately 500 cells mL^{-1}. For bioluminescence measurements 150 mL of culture was transferred to a 250 mL plastic measurement bottle containing a 30 mm wide stirring cross (Figure 5A). The bottle was placed inside a dark measuring chamber containing a stirring platform, a sensitive light sensor (GlowTracka, Chelsea Technologies Group, UK) and a lamp to illuminate the culture during its day phase (Figure 5A). Stimulated bioluminescence was induced for 1 minute every 30 minutes by stirring (225 rpm) during the 24 hour study period. During the day phase the light was switched off during the 1-min measurement period. The bioluminescence data were averaged over each recorded 1-min period.

The results from the bioluminescence rhythm experiments in the laboratory were used to plan the field measurement strategy. The same light sensor (GlowTracka) was used to measure bioluminescence in situ but it was attached to a pump to let water flow through the sensor chamber at a constant rate of 6 L min^{-1}. The bioluminescence meter was mounted on a submerged frame together with a temperature logger and a Chl-a fluoroprobe (Figure 5B). Daily patterns of stimulated bioluminescence and Chl-a fluorescence were recorded continuously from 27 July to 7 September 2011, covering the start, peak and decline of an *A. ostenfeldii* bloom. The sensor frame was moored circa 10 m from the shore at 0.5 – 1.0 m depth (Figure 3). Water depth at the site varied between 1.5 - 2 m. The sensors were connected to a data logger on the shore and powered from a solar panel. The exact description of the measurement procedure is given in **Paper II**. Data was recorded for 1 min every 10 minutes. The raw bioluminescence and fluorescence data were averaged over each recorded 1-min period. Chl-a fluorescence values were converted to pigment concentrations using a laboratory calibration.

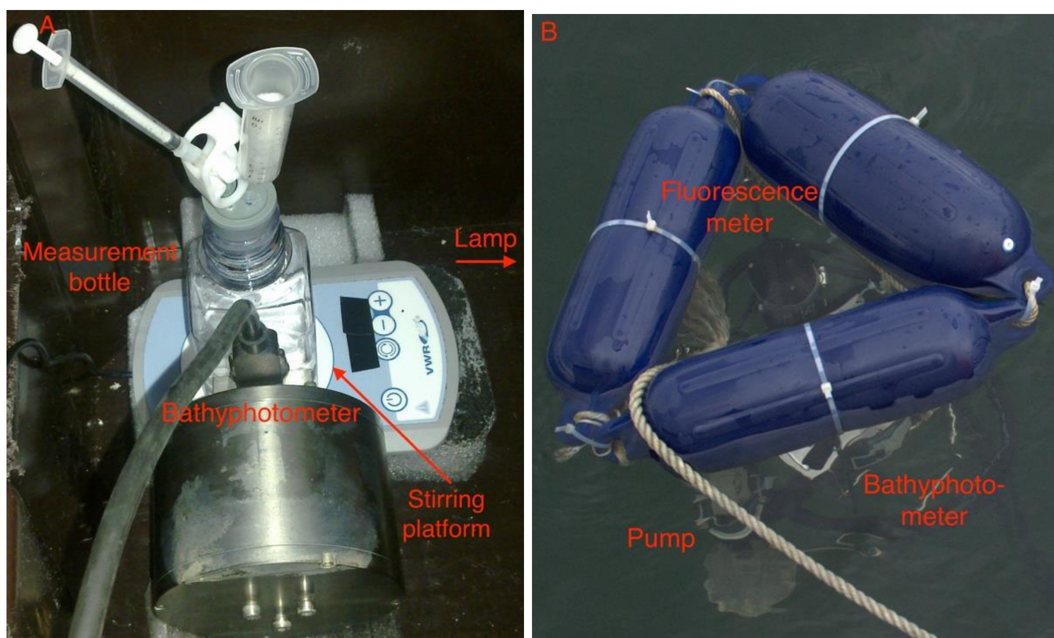

Figure 5 A) Measurement setup to study the daily rhythm of stimulated bioluminescence emission in the laboratory. B) Setup of instruments to measure bioluminescence, temperature and Chl-a fluorescence underwater at the Föglö field site.

3.4 Experiments

For experiments in **Paper III**, six to ten strains from clonal cultures originating from each of the four Baltic Sea bloom sites (AOF, AOKAL, AOVA, AOPL, Figure 3) were individually exposed to seven different temperature and salinity combinations in 4-week batch culture experiments (Table 2). Altogether, 34 clonal strains were used. The growth conditions were selected to represent the natural range in the native Baltic Sea habitats and under predicted future climate change. Temperature effects were tested at salinity 6 PSU and salinity effects at temperature 20 °C. The 20 °C and 6 PSU treatment was defined as the baseline treatment as it represents typical bloom conditions at most bloom sites. During the 4-week experiment, growth dynamics were monitored by in vivo fluorescence measurements. At the end of the experiment, samples for cell counts and cellular toxin content were taken, and bioluminescence was measured from each culture.

Table 2. Experimental treatments for studying salinity and temperature effects on growth, bioluminescence emission and toxin production.

Temperature & Salinity	2 PSU	4 PSU	6 PSU	8 PSU
16 °C				
20 °C			Control	
24 °C				
28 °C				

3.5 Species distribution modelling

Observations of *A. ostenfeldii* were collected from around the Baltic Sea by contacting the local authorities responsible for phytoplankton monitoring in each country. ICES and Hertta databases for phytoplankton, as well as reports in scientific literature, were searched for *A. ostenfeldii* observations. In addition, in Finnish territorial waters data from field campaigns documenting *A. ostenfeldii* cells and cysts, the presence of the luciferase gene (*lcf*), presence of the paralytic shellfish poisoning toxins (PSTs), bioluminescence measurements, and reports of bioluminescence by citizens were used.

A. ostenfeldii observations from Finnish marine waters were used to build a species distribution model (SDM) for *A. ostenfeldii* in the coastal waters of Finland (**Paper IV**). The principle of SDMs is to relate known species occurrences to environmental predictors like landscape, climate and habitat variables to explain and predict species distribution (Elith *et al.* 2006; Phillips *et al.* 2006). In this study, high resolution environmental information layers developed by the Finnish underwater inventory program (Velmu) were used to train the SDM with environmental parameters. Presence-only modelling was conducted with MaxEnt (version 3.4.1), available from https://biodiversityinformatics.amnh.org/open_source/maxent/. The detailed modelling procedure is described in **Paper IV**. The predictive performance of the model was evaluated using the area under the curve (AUC) of the receiver-operating characteristic plot (Phillips *et al.* 2006). AUC provides a measure of the predictive capability ranging between 0.5 (no predictive power) and 1 (a perfect model) (Hanley and McNeil 1982). Jackknife cross-evaluation was used to determine the relative contribution of each environmental variable to predicting *A. ostenfeldii* distribution.

3.6 Statistical analyses

Linear least-squares regression analysis in SAS software was used to assess the relations between bioluminescence intensity and Chl-a concentration, *A. ostenfeldii* cell density, and toxicity in the semi-continuous monitoring study in **Paper II**. The same method was used to relate cell density to bioluminescence intensity in the transect study. The linear mixed effects models used in **Paper III** were fitted separately to each response variable (growth rate, toxin concentration and bioluminescence), using the R package 'nlme' (Pinheiro *et al.*, 2012) in R 2.15.2 (R core team, 2012). The final model was selected based on the model's Akaike information criterion value. Statistical significance of the random effect for strain was assessed by comparing a generalized least squares model without any random effects, but with the same main and interaction effects as described above, with the final LME model. The comparison was made using a likelihood ratio test (LRT). Normality and homoscedasticity of residuals were checked for each response variable. Tukey's pairwise post-hoc comparisons (R package 'lsmeans'; Lenth, 2013) were used to determine which of the effects of explanatory factors and their

interactions significantly differed from each other. Student's t-test was used to detect if the 'effect size' of the different temperatures and salinities for growth rate, toxin concentration and bioluminescence of each strain significantly differed from the baseline condition. In **Paper IV**, the covariance of the environmental layers was checked based on 30,000 points randomly chosen from the study area using R package 'sp 1.2.4' in R 3.3.1 (R core team 2017).

4 Results and discussion

4.1 Presence of the luciferase gene and bioluminescence production

The results of **paper I** provide the first detailed overview of the presence and diversity of the *lcf* and bioluminescence production in six Baltic Sea *A. ostenfeldii* bloom populations. The PCR amplification and sequencing revealed that *lcf* was consistently present in all *A. ostenfeldii* strains isolated from the Baltic Sea. Bioluminescence emission was observed in 60 out of 61 strains (Table 1) with AOVA0924 from Gotland Sweden being the only Baltic strain that did not produce bioluminescence even though it had the *lcf*. The close coupling between *lcf* presence and bioluminescence production has been previously shown for many dinoflagellate species (Baker *et al.* 2008; Valiadi *et al.* 2012). More variation between these properties was observed from *A. ostenfeldii* strains from other locations. The *lcf* was detected in all North Sea strains except two strains from Ireland (LSA06 and LSE05) and the UK (WW515 and WW517), and it was present in strains from Spain (IEO-VGOAMD12, IOE-VGOAM10C), China (ASBHO1) and Canada (AOPC1). *A. ostenfeldii* strains from China, Canada, Norway and Scotland produced bioluminescence but no bioluminescence production was observed in strains from Ireland, the UK and Spain (Table 1). Intraspecific variation in bioluminescence production has been found in some dinoflagellate species, e.g. *C. horrida* and *A. tamarense*, both bioluminescent and non-bioluminescent strains co-occur but also the non-bioluminescent strains always have *lcf* (Valiadi *et al.* 2012). According to rDNA sequences, Baltic *A. ostenfeldii* strains belong to phylogenetic group 1 in the *A. ostenfeldii* species complex, whereas strains from the UK and Ireland where neither *lcf* nor bioluminescence was detected belong to group 2 (Kremp *et al.* 2014). Strains from Spain where the *lcf* was found, but no bioluminescence was observed, also belong to group 2 in the *A. ostenfeldii* species complex. This suggests that the strains in group 2 have either lost the *lcf* or the gene has mutated so that it is no longer functional. The primers used in **Paper I** targeted the most variable region of the *lcf* (Baker *et al.* 2008). It has been shown that primers designed for this variable region may not always give a positive signal for the *lcf* even if it is present, likely due to too many nucleotide differences at the primer binding sites (Valiadi *et al.* 2012). Based on this result from **Paper I** it is not possible to say if the *lcf* is modified or truly absent in the respective strains.

Field sampling in the Föglö study area (**Paper I**) showed that it was possible to amplify the *lcf* sequences from natural water samples. Sampling along the transect that passed through a known *A. ostenfeldii* bloom site showed that presence of the *lcf*, *A. ostenfeldii* cells and bioluminescence emission were closely coupled (Table 2 in Paper I). Sensitivity of *lcf* detection was considered sufficient for detection of non-bloom abundances of *A. ostenfeldii* as it was possible to amplify the *lcf* from water sample that contained between 40 - 120 *A. ostenfeldii* cell L^{-1}. The water samples collected with the Alg@line ferry *Silja Serenade* did not reveal any positive signal for the *lcf* and therefore a different water sampling strategy closer to the coast is needed. Other field studies have found that bioluminescence measurements comparatively underestimated the presence of bioluminescent dinoflagellates compared to detection of the *lcf* (Valiadi *et al.* 2014). The result by Valiadi *et al.* (2014) could have been due to the presence of different dinoflagellate species with different bioluminescent properties and co-occurrence of bioluminescent zooplankton (Valiadi *et al.* 2014). In the coastal waters of the Baltic Sea where the bioluminescent plankton community consists entirely of *A. ostenfeldii*, bioluminescence detection can be considered as reliable an indicator of the presence of *A. ostenfeldii* as detecting the *lcf* directly. The obtained results indicate that, from the perspective of environmental monitoring, the consistent presence of *lcf* in the Baltic *A. ostenfeldii* strains and close coupling between *lcf* and bioluminescence production suggests that bioluminescence can be used to reliably monitor the presence of *A. ostenfeldii* in the Baltic Sea.

4.2 Daily and seasonal variation in bioluminescence signal

This section refers to the unpublished results of Le Tortorec A., Kauko H. and Simis S.G.H.

Experimental studies on the daily rhythm of *A. ostenfeldii* bioluminescence showed that the studied culture exhibits a clear rhythm in stimulated bioluminescence emission. Bioluminescence started to increase approximately 1 hour before the lights went off, peaked circa 1 hour after the dark period started, stayed around the same level for 1.5-2 hours and declined towards the morning with some bioluminescence still visible 1 hour after the lights went on (Figure 6). This result is in line with other laboratory studies showing a clear circadian rhythm in bioluminescence emission of different dinoflagellate species (Widder and Case 1982; Fritz *et al.* 1990; Knaust *et al.* 1998). It has been shown that the circadian rhythm in dinoflagellates can be controlled in two different ways. In *L. polyedrum* (former *Gonyaulax polyedra*) the scintillons, the luciferase enzyme and the LBP are all synthesised and broken down on daily bases (Fritz *et al.* 1990; Knaust *et al.* 1998) whereas in *Pyrocystis* species, which do not have the LBP, the amount of luciferase stays the same but its location inside the cell changes (Widder and Case 1982; Knaust *et al.* 1998). It is not known which one of these systems controls the circadian regulation in *A. ostenfeldii*. However, since *lbp* has been found in four other *Alexandrium* species (Valiadi and Iglesias-Rodriguez 2014) it is likely that the

circadian rhythm of *A. ostenfeldii* bioluminescence is controlled through daily synthesis and breakdown of scintillons and relevant proteins. As it has been shown that the stimulation method greatly affects the amount of bioluminescence produced by dinoflagellates, the exact bioluminescence values measured in different studies cannot be compared but bioluminescence patterns are still comparable (Cussatlegras and Le Gal, 2007; Kauko, 2013; Latz and Rohr, 2013).

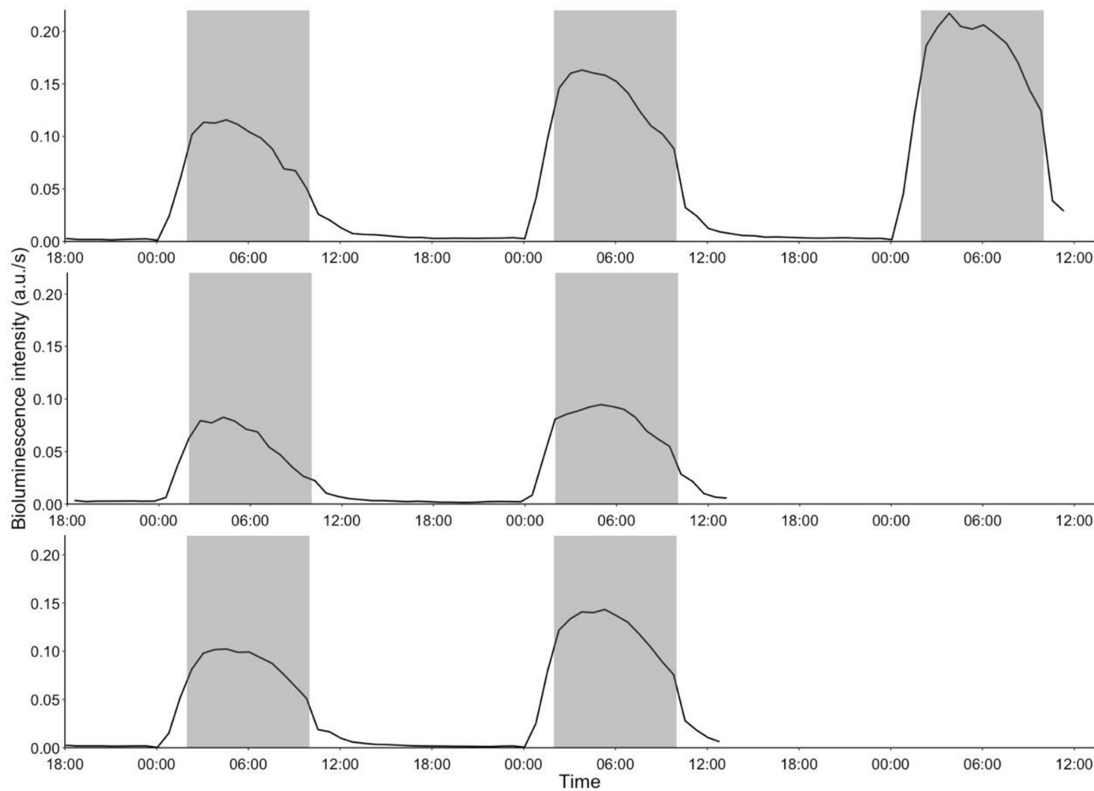

Figure 6. Stimulated bioluminescence of *Alexandrium ostenfeldii* under 16:8 light dark rhythm. Grey bars represent dark periods.

Studies at the Föglö field site (Figure 3) showed that it is possible to measure bioluminescence *in situ* in optically attenuating Baltic Sea waters. In **Paper II** stimulated bioluminescence produced by *A. ostenfeldii* was recorded in the field between 27 July and 7 September 2011 (Figure 7). During this period, intense (>2nW cm-2) bioluminescence, indicating the bloom of *A. ostenfeldii*, started from mid-August and lasted until the beginning of September (circa 15 days). The maximum intensity of bioluminescence (1-min average) was 14.46nW cm-2 recorded on 26 August 2011 (**Paper II**). Compared to laboratory experiments the field measurements showed more variation in the daily pattern of bioluminescence production, but large day-to-night variation in the intensity of bioluminescence was still observed during the bloom period, with bioluminescence starting to rise shortly after sunset, peaking around midnight and declining towards the morning (Figure 3 in Paper II). During the peak bloom period, some bioluminescence could be recorded even during the day. The results of **Paper II** show that bioluminescence is

already detectable when *A. ostenfeldii* is present at low cell concentrations in the water column (Figure 8). The primary source of variation in the recorded signal both in laboratory studies and in the field measurements was the circadian rhythm of bioluminescence. The diurnal variation in bioluminescence emission has been repeatedly observed in nature (Utyushev *et al.* 1999; Geistdoerfer and Cussatlegras 2001; Marcinko *et al.* 2013) as well as in dinoflagellate cultures as shown above. The observed maximum ratio in bioluminescence intensity between night and day was 32.90 ± 22.20 during the period of high bioluminescence (**Paper II**), which is similar to ratios found in other natural populations e.g. maximum ratio of 23 in Marcinko *et al.* (2013).

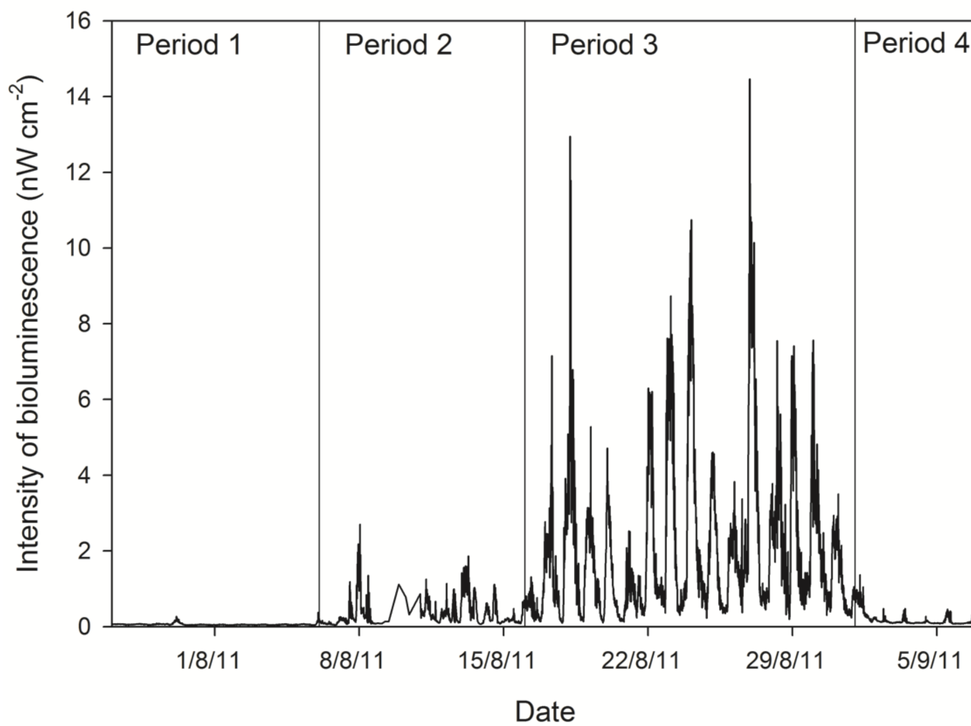

Figure 7. Intensity of *Alexandrium ostenfeldii* bioluminescence, measured at 10-min intervals. (**Paper II**).

The second source of variation in the bioluminescence signal found in **paper II** was the seasonal change in the bioluminescence intensity likely caused by fluctuations in *A. ostenfeldii* abundance. Studies conducted in previous years show that *A. ostenfeldii* abundance peaked after mid-July and declined at the end of August. Outside the peak period, the species persisted in the water column at moderate to low abundance (Hakanen *et al.* 2012). In **Paper II** *A. ostenfeldii* cells were present in the water column throughout the study period, but high abundances were only observed in late August simultaneously with high bioluminescence intensities (Figure 8). A positive relation between night-time bioluminescence and cell numbers was observed. Night-time bioluminescence intensity was also positively correlated with night-time Chl-a concentration, although there was

variability in this correlation depending on the state of the bloom (initiation, peak, termination) (Figure 5 in Paper II). During the bloom period from mid-August to end of August 60% of the variability in mean night-time bioluminescence could be attributed to changes in mean night-time Chl-a (**Paper II**). Seasonal changes in bioluminescence intensity related to the abundance of *Pyrodinium bahamense* has also been reported by Soler-Figueroa and Otero (2016). Studies conducted in the open ocean have also related changes in bioluminescence intensities to changes in total dinoflagellate abundance (Lapota *et al.* 1989; Marra 1995).

The bioluminescence measurements conducted in **Paper II** along a ~7km transect showed that bioluminescence was highly localized prior to the bloom peak (Figure 6 in Paper II). The strongest signal was measured in the inner areas of the sound and it decreased towards the open sea. This observation suggests that the bloom spread out from a single location, supporting the earlier findings showing that the distribution of *A. ostenfeldii* is highly localized in the Baltic Sea (Larsson *et al.* 1998; Hajdu *et al.* 2006; Hakanen *et al.* 2012). It also supports the previous finding showing that the *A. ostenfeldii* blooms in Föglö area are closely coupled with benthic cystbeds (Hakanen *et al.* 2012). These results confirm that it is possible to use bioluminescence as an early indicator of toxin-producing dinoflagellate blooms in the Baltic Sea. The measurement system used in **Paper II** was sensitive enough for the early detection of *A. ostenfeldii* blooms, since induced bioluminescence could be measured during periods when *A. ostenfeldii* was not yet abundant.

4.3 Bioluminescence as a proxy of toxicity

Many bioluminescent dinoflagellates produce toxins (Valiadi *et al.* 2012; Cusick and Widder 2014) but the relationship between bioluminescence intensity and cellular toxin concentration has not been studied before. The experiments in **Paper III** revealed a high phenotypic diversity in genetically diverse seed banks of *A. ostenfeldii*. Results showed that the examined 34 clonal *A. ostenfeldii* isolates had varying growth, toxin and bioluminescence properties and responded differently to temperature and salinity changes (Figure 9). At baseline conditions (temperature 20°C and salinity 6 PSU) high growth rates were associated with high cellular toxicity in strains from Föglö (AOF) and Poland (AOPL), but this was not the case with strains from Kalmar (AOKAL) and Valleviken (AOVA) (Figure 2 in paper III). An increase in temperature to 24 °C and 28 °C affected toxin production, but responses were strain specific and both negative and positive changes occurred (Figure 9). A decrease in temperature to 16 °C rarely affected toxin production. Changes in salinity only affected the toxin production in approximately half of the strains, and most of the observed significant changes (positive or negative) occurred at salinity 2 PSU (Figure 9). No clear trend was observed between growth rate or toxicity and intensity of bioluminescence emission in *A. ostenfeldii*, and strains that produced high amounts of toxins or bioluminescence were generally different (**Paper III**).

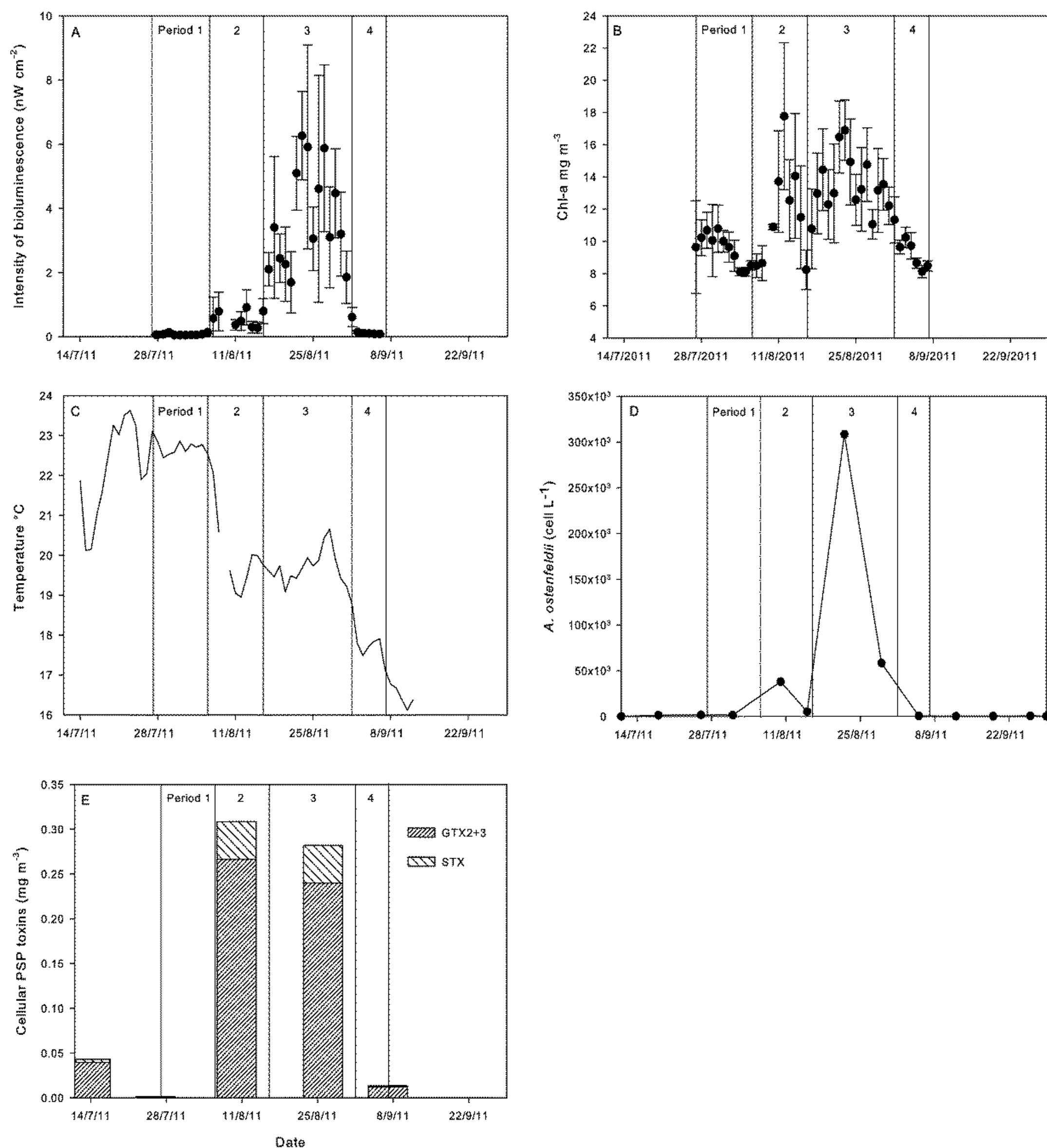

Figure 8. Dynamics of A) night-time bioluminescence (nightly mean, standard deviation as error bars), B) night-time chlorophyll-a (nightly mean, standard deviation as error bars), C) water temperature, D) *A. ostenfeldii* cell abundance (one replicate per sampling) and E) total cellular PSP toxin concentration (mean of two replicates) (* = traces of GTX toxins found on 29 September) at station A from 11 July to 29 September. (**Paper II**).

High variability in toxin content of *A. ostenfeldii* strains in response to elevated temperatures has previously been observed by Kremp *et al.* (2012). The latter study additionally showed that an increase in temperature to 24 °C alters the toxin composition of *A. ostenfeldii*, resulting in higher saxitoxin production, which was also observed in **Paper III** among the strains growing best at 24 °C. Strain-specific responses of increased salinity to total cellular toxin concentration and toxin composition have also been previously observed in *A. ostenfeldii* (Suikkanen *et al.* 2013). These results highlight that there is likely no uniform response in toxin

production of *A. ostenfeldii* to different salinities and temperatures, except for the increased proportion of saxitoxin produced in response to elevated temperatures. All strains that were tested produced both bioluminescence and PSTs (Table 1). Therefore, it can be assumed that production of both bioluminescence and PST toxins is predominant property in Baltic *A. ostenfeldii*.

The field measurements in **Paper II** showed that low concentrations of cellular PSP toxins were detected through the sampling period, while higher toxin concentration occurred when night-time intensity of bioluminescence was >1 $nWcm^{-2}$ and could easily be detected by eye (Figure 8). In **Paper II** a positive correlation between toxicity and *A. ostenfeldii* cell numbers was observed. This result is in line with studies by Hakanen *et al.* (2012) who showed that PST toxin dynamics correlated with *A. ostenfeldii* cell numbers and that toxin peaks mirrored *A. ostenfeldii* abundance peaks. More studies are needed on the relation between bioluminescence intensity and cellular toxin concentration, both in cultures and in natural communities.

4.4 Effects of salinity and temperature on *A. ostenfeldii* bioluminescence

The physiological status of the cell and environmental factors affect the intensity of bioluminescence in dinoflagellates (Valiadi and Iglesias-Rodriguez 2013). Bioluminescence emission has been shown to decrease when heterotrophic dinoflagellates are starved (Buskey *et al.* 1992; Li *et al.* 1996). In autotrophic dinoflagellates bioluminescence may diminish when cultures become nutrient depleted (Esaias *et al.* 1973). In **Paper III** large differences in bioluminescence emission between strains were observed in all temperature and salinity treatments (Figure 9). Swift *et al.* (1973) showed intraspecific variation in bioluminescence emission between four to five clones of two species of *Pyrocystis* in response to mechanical stimulation. However, at this magnitude intraspecific variation in dinoflagellate bioluminescence emission has not been shown before, although it is likely widespread since intra-population differences are common in other traits involved in defence e.g. toxins and allelochemicals production (Tillmann *et al.* 2009, 2014).

The experiments in **paper III** showed that salinity and temperature clearly affected *A. ostenfeldii* bioluminescence production. Low salinity (2 PSU) decreased bioluminescence emission per cell. An increase in salinity to 8 PSU, and a decrease to 4 PSU only affected bioluminescence emission in one third of the strains, and a decrease in bioluminescence emission was observed more often than an increase. High temperatures (24°C and 28°C) significantly decreased bioluminescence emission per cell (Figure 9), whereas a slight increase in *A. ostenfeldii* bioluminescence emission was observed at 16 °C. The effects of environmental factors on dinoflagellates bioluminescence have remained poorly studied (Valiadi and Iglesias-Rodriguez 2013). Experiments with the dinoflagellate *P. lunula* have

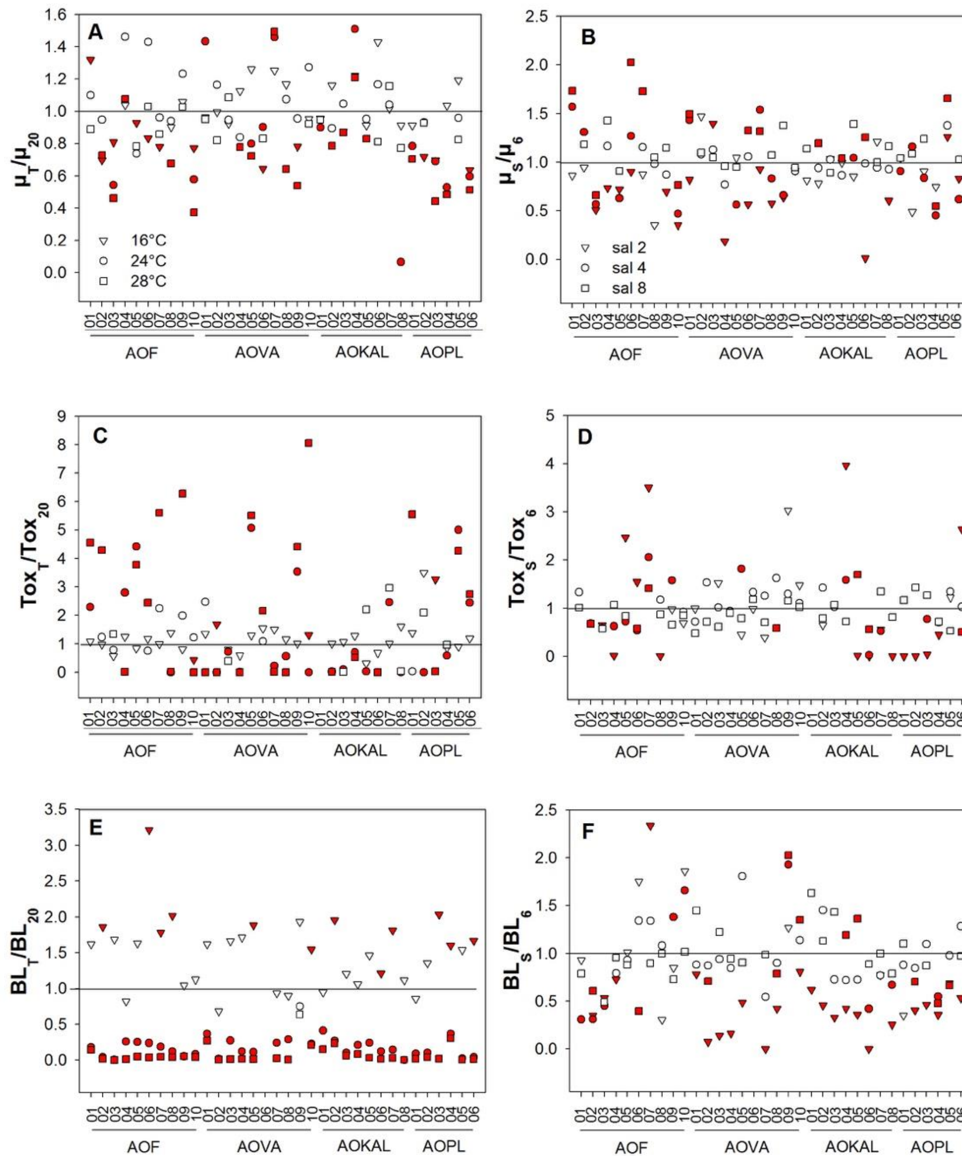

Figure 9. Effects of experimental temperatures and salinities on single strains of *Alexandrium ostenfeldii*. Effect sizes are calculated as the ratio of maximum growth rate at treatment conditions to maximum growth rate at control conditions (20ºC and salinity 6 PSU). 1 = no effect, >1 = positive effect, <1 = negative effect. Significant effects ($p < 0.05$. Tukey) are marked red. (**Paper III**).

shown that lowering the salinity from its optimum range negatively affected the re-establishment of bioluminescence, whereas an increase in salinity resulted only in small decline (Craig *et al.* 2003). This finding, together with the results of **Paper III**, suggests that salinity likely has a regulatory function in bioluminescence of dinoflagellates. Regarding to temperature, Ueda *et al.* (1994) showed a temperature optimum at 22.5 °C for the enzyme kinetics of firefly luciferase, and a sudden decrease in bioluminescence at temperatures above was observed. There are likely some differences in bioluminescence responses to temperature changes between

species, as re-establishment of bioluminescence in *P. lunula* was positively correlated with increasing temperature between 10 - 30 °C (Craig *et al.* 2003) and the tropical bioluminescent dinoflagellate Pyrodinium bahamense regularly forms bioluminescent blooms in water temperature above 25 °C (Phlips *et al.* 2006).

Field measurements from the Föglö study site (**Paper II**) showed that *A. ostenfeldii* bloom was initiated at temperatures above 22°C (Figure 8). During the bloom period, which was characterised by intense bioluminescence, the water temperature remained above 19°C, and a drop in water temperatures to below 19°C coincided with a decrease in the intensity of bioluminescence (Figure 8). This result is in line with earlier studies showing that *A. ostenfeldii* blooms in Föglö formed at water temperatures around 20 °C (Hakanen *et al.* 2012). The results from **Paper II**, together with earlier studies, support the idea suggested by Hakanen *et al.* (2012) that proliferation of *A. ostenfeldii* to bloom concentrations in the Baltic Sea is connected to warm water. Brandenburg *et al.* (2017) showed that *A. ostenfeldii* abundance in a brackish water creek in the Netherlands belonging to the same phylogenetic group 1 as Baltic *A. ostenfeldii* was also positively correlated to temperature and blooms occurred at temperatures between 15 and 25 °C. In a study conducted in a natural plankton assemblage of the Mediterranean Sea no direct correlation was found between bioluminescence and temperature or salinity (Cussatlegras *et al.* 2001). Altogether it is likely that large variation, both between and within species, exists in bioluminescence emission of dinoflagellates in response to environmental parameters. However, more studies are needed regarding the effects of environmental factors on bioluminescence of dinoflagellates to be able to formulate a general response pattern.

4.5 Distributions of *A. ostenfeldii* in the Baltic Sea

A map of all locations where *A. ostenfeldii* has thus far been observed in the Baltic Sea is presented in Figure 10. Data displayed on the Figure 10 are from the author and research group, Hertta database, ICES database, existing literature, citizen observations and Department of Ecology, Environment and Plant Sciences, Stockholm University, Sweden (per. comm. Helena Höglander), Institute of Oceanography, University of Gdansk, Poland (per. comm. Justyna Kobos) and Marine Systems Institute, Tallinn University of Technology, Estonia (pers. comm. Inga Lips). Most observations are from shallow coastal areas, and observations from the open sea are often close to a known bloom site. This is in line with previous studies from Baltic Sea reporting *A. ostenfeldii* observations from shallow coastal areas (Larsson *et al.* 1998; Hajdu *et al.* 2006; Kremp *et al.* 2009; Hakanen *et al.* 2012). Worldwide, *A. ostenfeldii* observations from coastal areas are increasingly reported (Ciminiello *et al.* 2006; Borkman *et al.* 2012; Tomas *et al.* 2012; Almandoz *et al.* 2014; Burson *et al.* 2014). The reasons behind this increase are not known but could be related to eutrophication, changes in nutrient ratios or global climate change (Heisler *et al.* 2008; Glibert and Burford 2017; Gobler *et al.* 2017).

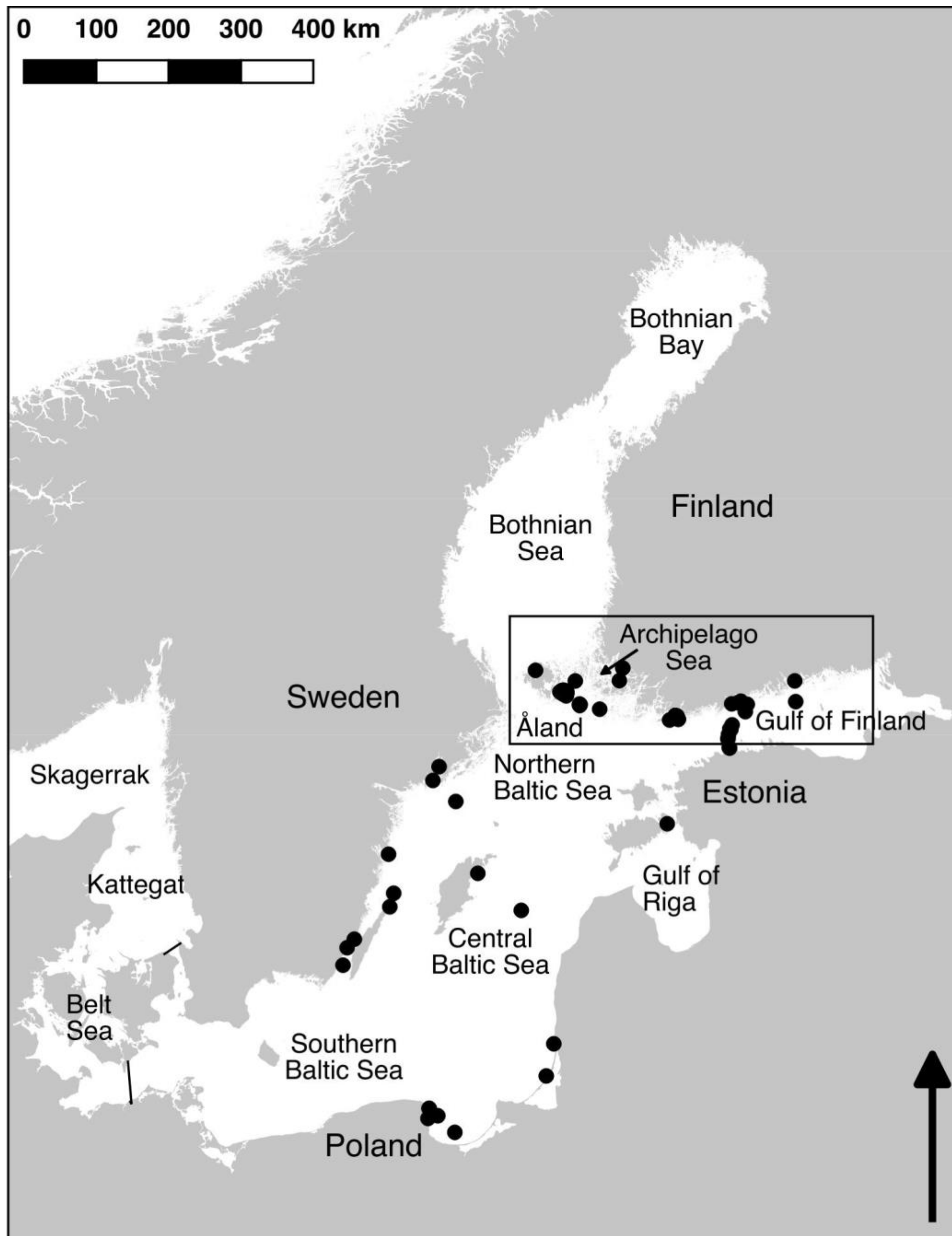

Figure 10. Map of locations where *Alexandrium ostenfeldii* has been observed in the Baltic Sea area. Observations inside the black box were used to build the distributions model for Finnish coastal waters. (Unpublished).

The *A. ostenfeldii* SDM for Finnish coastal waters presented in **Paper IV** showed that the environmental variables that were most strongly associated with *A. ostenfeldii* occurrence were total phosphorus divided by inorganic phosphorus, depth, *Fucus spp.*, depth exposure, minimum bottom temperature and total nitrogen divided by inorganic nitrogen (Table 2 in paper IV). *A. ostenfeldii* occurrence was weakly associated with distance to shore and salinity. A map of the potentially suitable habitats for *A. ostenfeldii* is shown in Figure 11, with values of relative habitat suitability ranging from 0 (low suitability) to 1 (high suitability). The model indicated previously unknown but potentially suitable habitats for *A.*

ostenfeldii mainly around the Åland islands, around the Hanko Peninsula in the Gulf of Finland and, interestingly, around the islands in the Vaasa area in the northern part of the Bothnian Sea. New potential habitats around the Åland and Hanko peninsula were expected since *A. ostenfeldii* has been observed in other locations in these areas before. This model is the first indicator that *A. ostenfeldii* could potentially spread to the Bothnian Sea, which was previously thought to have too low salinity to support the growth of *A. ostenfeldii*. Indeed, in **Paper III** we showed that *A. ostenfeldii* can grow in salinities as low as 2 PSU. Other factors like dispersal barriers such as land masses or currents may also prevent or delay expansion of *A. ostenfeldii* distribution (Palumbi 1994; Casabianca *et al.* 2012; Sjöqvist *et al.* 2015). To validate the model results further sampling from these newly identified areas is advised.

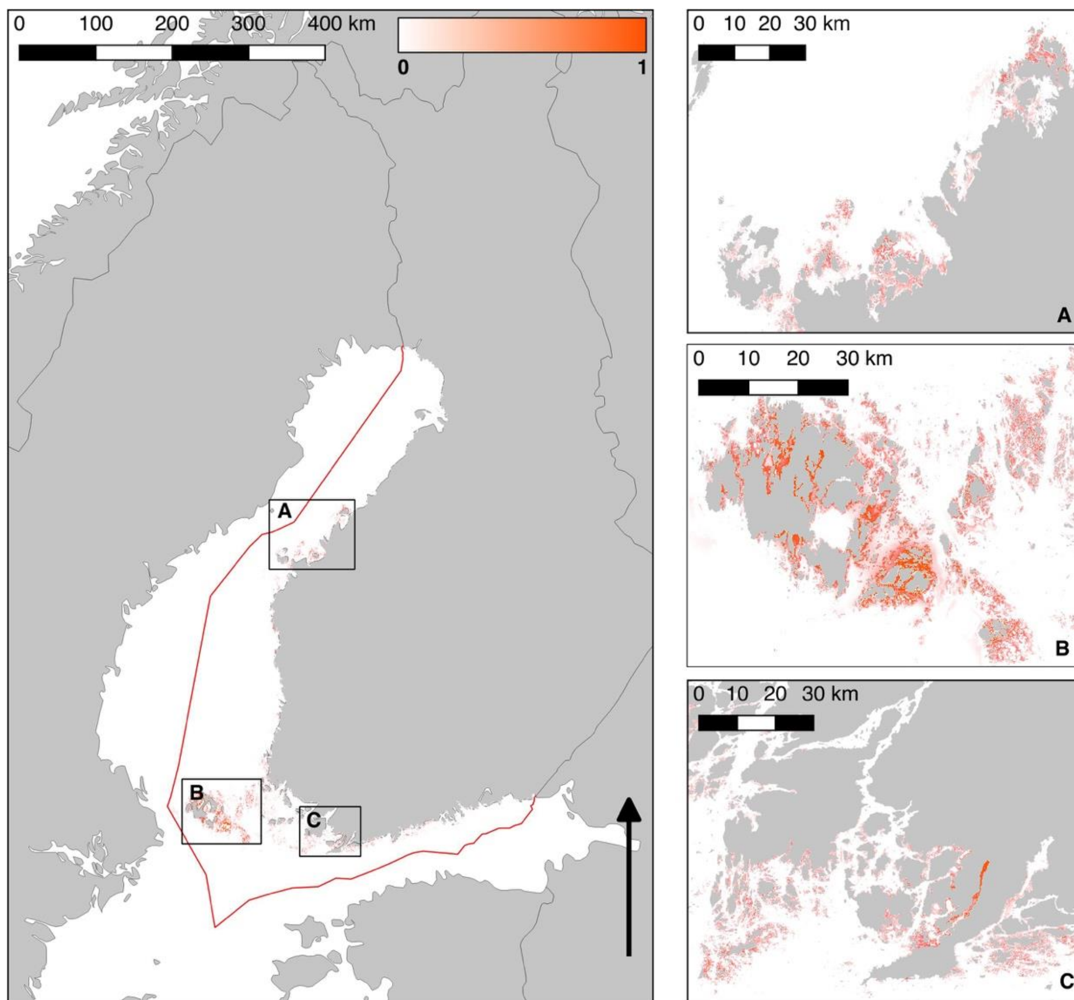

Figure 11. Potential distribution of *Alexandrium ostenfeldii* in the coastal waters of Finland. Red colour indicates the relative habitat suitability ranging from 0 (low suitability) to 1 (high suitability).

5 Conclusions and future perspectives

In this thesis, I have shown that bioluminescence is a prominent feature in Baltic Sea *A. ostenfeldii* both in cultures and in natural communities. *Lcf* was present in all studied Baltic *A. ostenfeldii* strains and its presence was closely coupled with bioluminescence production. Many different factors starting from the nutritional status of the cells to salinity, temperature and light exposure affect the bioluminescence emission in dinoflagellates. In this thesis, large previously unknown intraspecific variation was observed in *A. ostenfeldii* bioluminescence emission in relation to salinity and temperature changes. Bioluminescence emission of *A. ostenfeldii* was found to follow a circadian rhythm with bioluminescence observable during night and barely discernible during the day. Observed bioluminescence also showed seasonal variation related to fluctuations in *A. ostenfeldii* abundance. The abundance of *A. ostenfeldii* and toxin concentrations were positively correlated in our data set. All *A. ostenfeldii* strains tested during this thesis produced both bioluminescence and PST toxins. Therefore, it can be assumed that concurrent production of bioluminescence and PST toxins is a predominant property in Baltic *A. ostenfeldii*.

The main aim of this thesis was to evaluate whether bioluminescence can be used as an early warning signal of harmful dinoflagellate blooms in the Baltic Sea. The results obtained showed a close coupling between *lcf* and bioluminescence production in Baltic *A. ostenfeldii*, which suggests that bioluminescence could be used to reliably monitor the presence of *A. ostenfeldii* in the Baltic Sea. The results from the field studies showed that bioluminescence was already detectable when *A. ostenfeldii* was present in the water column at low cell concentrations, which indicates that the measurement system was sensitive enough for the early detection of *A. ostenfeldii* blooms. Low concentrations of cellular PSP toxins were detected throughout the sampling period while higher toxin concentration occurred when night-time intensity of bioluminescence was >1 $nWcm^{-2}$ and could easily be detected by eye. However, more studies are needed on the relationship between bioluminescence intensity and cellular toxin concentration, both in cultures and in natural communities, to be able to improve the estimates of toxicity predictions from bioluminescence measurements. The fact that bioluminescence emission around 1 $nWcm^{-2}$ is detectable by eye further suggests a possibility to use less sensitive and lower cost optical instruments for bioluminescence detection.

The finding that bioluminescence was observed to be highly localised prior to the bloom peak, together with earlier findings showing that the distribution of *A. ostenfeldii* is highly localized in the Baltic Sea, is problematic with respect to monitoring as it suggests that monitoring should be conducted at a fine spatial resolution. The diurnal rhythm of bioluminescence should be taken into account when planning environmental monitoring of this phenomenon as it is not feasible to detect bioluminescence during the day. One solution could be to use complementary monitoring based on bioluminescence measurements and detection of *lcf* or another molecular marker to increase the chances of detecting *A. ostenfeldii*. The optical detection of bioluminescence provides an immediate answer if *A. ostenfeldii* is

present at a certain location at sufficient cell numbers. When detecting the *lcf* there is always a time lag between collecting the sample in the field and analysing it in the laboratory, but samples can be collected from wider areas also during daytime and at low *A. ostenfeldii* abundances. Therefore, detecting the *lcf* or another molecular marker would be useful in revealing new locations where *A. ostenfeldii* is present. Citizen observations of bioluminescence detected by eye could be an effective and cost-efficient way to monitor the long and complex coastline with adequate spatial coverage. However, informing citizens about the phenomenon and the risk of associated toxicity, and how and where it should be reported should be a continued effort, aided by press releases or through local information events.

The SDM of *A. ostenfeldii* offers new interesting possibilities in relation to monitoring as it can be used to target the monitoring efforts in the areas indicated as potentially highly suitable for *A. ostenfeldii*. Information about *A. ostenfeldii* presence in these discovered areas should be collected and used to validate or improve the model. The SDM could be further developed by including new observation data of *A. ostenfeldii* and by increasing the spatial coverage of environmental layers to be able to extend the model to cover, for example the coasts of Sweden and Estonia and eventually the whole Baltic Sea. This would help to further refine and validate the model results, as well as our understanding of *A. ostenfeldii* distribution in the Baltic Sea.

6 Acknowledgements

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