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From genes to communities: stress tolerance in eelgrass (*Zostera marina*)

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FROM GENES TO COMMUNITIES: STRESS TOLERANCE IN EELGRASS (*ZOSTERA MARINA*)

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ABSTRACT

Shallow coastal areas are dynamic habitats that are affected by a variety of abiotic and biotic factors. In addition to the natural environmental stress, estuarine and coastal seagrass ecosystems are exposed to effects of climate change and other anthropogenic impacts. In this thesis the effect of different abiotic (shading stress, salinity and temperature) and biotic stressors (presence of co-occurring species) and different levels and combinations of stressors on the performance and survival of eelgrass (*Zostera marina*) was assessed. To investigate the importance of scale for stress responses, varying levels of biological organization (genotype, life stage, population and plant community) were studied in field and aquarium experiments.

Light limitation, decreased salinity and increased temperature affected eelgrass performance negatively in papers I, II and III, respectively. While co-occurring plant species had no notable effect on eelgrass in paper IV, the presence of eelgrass increased the biomass of *Potamogeton perfoliatus*. The findings in papers II and III confirmed that more extreme levels of salinity and temperature had stronger impacts on plant performance compared to intermediate levels, but intermediate levels also had more severe effects on plants when they were exposed to several stressors, as illustrated in paper II. Thus, multiple stressors had negative synergetic effects. The results in papers I, II and III indicate that future changes in light climate, salinity and temperature can have serious impacts on eelgrass performance and survival.

Stress responses were found to vary among genotypes, life stages and populations in papers I, II and III, respectively, emphasizing the importance of study scale. The results demonstrate that while stress in general affects seagrass productivity negatively, the severity of effects can vary substantially depending on the studied scale or level of biological organization. Eelgrass genotypes can differ in their stress and recovery processes, as observed in paper I. In paper II, eelgrass seedlings were less prone to abiotic stress compared to adult plants, but stress also decreased their survival considerably. This indicates that recruitment and re-colonization through seeds might be threatened in the future. Variation among population responses observed in paper III indicates that long-term local adaptation under differing selection pressures has caused divergence in salinity tolerance between Baltic eelgrass populations. This variability in stress tolerance observed in papers I and III suggests that some eelgrass genotypes and populations have a better capacity to adapt to changes and survive in a changing environment.

Multiple stressors and biological level-specific responses demonstrate the uncertainty in predicting eelgrass responses in a changing environment. As eelgrass populations may differ in their stress tolerance both within and across regions, conservation strategies at both local and regional scales are urgently needed in order to ensure the survival of these important ecosystems.

Keywords: Abiotic stress, Biotic stress, Genotype, Irradiance stress, Life stage, Multiple stressors, Plant community, Population, Salinity, Seagrass, Species diversity, Temperature, Baltic Sea

SAMMANFATTNING (Swedish abstract)

Grunda kustområden är dynamiska habitat som påverkas av en mångfald av biotiska och abiotiska faktorer. Utöver den naturliga belastningen, utsätts de kustnära sjögräsängarna av en ökad mängd av antropogena effekter som är kopplade till klimatförändringen och andra mänskliga aktiviteter. Syftet med denna avhandling var att studera effekterna av abiotiska (ljusmängd, salinitet och temperatur) och biotiska (närvaron av andra fröväxtarter) stressfaktorer samt olika nivåer och kombinationer av dessa på ålgräsets (*Zostera marina*) produktion och överlevnad. För att utreda betydelsen av skala för ålgräsets stressrespons, inkluderades olika nivåer av ekologiska hierarkier; genotyp, livsstadie, population och växtsamhälle. Dessa nivåer studerades i fält- och akvarieexperiment.

Minskad ljusmängd, minskad salinitet och ökad temperatur påverkade ålgräset negativt i respektive arbeten I, II och III. Medan närvaron av andra fröväxtarter inte hade några märkbara effekter på ålgräset i arbete IV, ökade ålgräset biomassproduktion hos *Potamogeton perfoliatus*. Resultaten i arbete II och III påvisade att extrema nivåer av salinitet och temperatur hade starkare effekter på ålgräset jämfört med moderata nivåer. Då ålgräset var utsatt för flera olika stressfaktorer samtidigt, ledde moderata stressnivåer till signifikant negativa responser, som bevisades i arbete II. Resultaten från arbeten I, II och III indikerar att framtida förändringar kan ha allvarliga effekter på ålgräsets prestationsförmåga och överlevnad.

Ålgräsets stressrespons varierade bland genotyperna, livsstadierna och populationerna i respektive arbeten I, II och III. Resultaten demonstrerar att medan stress generellt påverkar sjögräsproduktivitet negativt, kan effekternas negativa inverkan variera beroende på valda angreppsätt och studieskala. Arbete I visade att olika ålgräsgenotyper kan skilja sig i sina stress- och återhämtningsprocesser. I arbete II uppvisade groddplantorna något större tolerans mot abiotisk stress jämfört med vuxna skott, men överlevnaden hos groddplantorna reducerades också märkbart. Detta indikerar att ålgräsets återkolonisering av områden där ålgräset minskat kan vara hotad i framtiden. Populationsspecifika responser på salinitetsstress, som observerades i arbete III, indikerar att lokaladaptation under olika selektionstryck har lett till skillnader i salinitetstolerans mellan norra och södra Östersjöns ålgräspopulationer. Variabilitet i stresstålighet som observerades i arbetena I respektive III antyder att vissa ålgräsgenotyper och –populationer har en större chans att adaptera sig till framtida miljöförändringar.

Att olika stressfaktorer har synergistiska effekter och att stressresponsen kan variera beroende på den studerade skalan och ekologiska nivån, poängterar hur svårt och osäkert det är att försöka förutsäga hur stor inverkan stress har i allt mer variabla kustmiljöer. Ålgräsets stresstolerans varierar lokalt och regionalt, vilket understryker betydelsen av att bevarandestrategier som öka sannolikheten för att dessa viktiga och unika ekosystem överlever.

Nyckelord: abiotisk stress, artssammansättning, biotisk stress, genotyp, livsstadium, ljusmängd, multipla stressfaktorer, population, salinitet, sjögräs, temperatur, växtsamhälle, Östersjön

TIIVISTELMÄ (Finnish abstract)

Rannikkoalueet ovat dynaamisia elinympäristöjä, joihin vaikuttaa laaja kirjo erilaisia abioottisia ja bioottisia tekijöitä. Meriruohojen sijoittuminen matalille rannikkoalueille altistaa ne lisäksi yhä lisääntyvälle määrälle ilmastonmuutoksen ja muiden ihmisen aiheuttamien muutosten vaikutuksille. Tässä väitöskirjassa tarkastellaan erilaisten abioottisten (valon määrä, veden suolapitoisuus sekä lämpötila) ja bioottisten (muiden kasvilajien läsnäolo) stressitekijöiden, stressitasojen ja stressiyhdistelmien vaikutuksia meriajokkaan (*Zostera marina*) tuotanto- ja selviytymiskykyyn. Tutkimusskaalan vaikutusten selvittämiseksi eriasteisia ekologisia hierarkioita (genotyyppejä, elinvaiheita, populaatioita ja kasviyhdyskuntia) tarkasteltiin neljässä erillisessä kenttä- ja akvaariokokeessa.

Alhainen valon määrä, alennettu suolapitoisuus ja korotettu lämpötila vaikuttivat negatiivisesti meriajokkaan suoriutumiskykyyn osatöissä I, II ja III. Muiden kasvilajien läsnäololla ei ollut näkyviä vaikutuksia meriajokkaaseen, mutta meriajokkaan läsnäolo lisäsi erään toisen kasvilajin biomassaa osatyössä IV. II ja III osatyö vahvistivat että korkeilla stressitasoilla oli suuremmat vaikutukset kasvien suoriutumiskykyyn kuin alhaisemmilla stressitasoilla, mutta myös keskitasoisella stressillä oli vakavia vaikutuksia mikäli kasvit altistettiin useammalle stressitekijälle, kuten osatyö II osoitti. Altistaminen usealle stressitekijälle vahvisti siis stressin haitallisia vaikutuksia. Osatöiden I, II ja III tulokset osoittavat, että tulevilla ympäristömuutoksilla voi olla vakavia vaikutuksia meriajokkaan tuotanto- ja selviytymiskykyyn.

Stressivaikutukset vaihtelivat genotyypistä, elinvaiheesta ja populaatiosta riippuen osatöissä I, II ja III, mikä korostaa tutkimusskaalan merkitystä. Tulokset osoittavat, että vaikka stressi yleisesti ottaen vähentää meriajokkaan tuotantokykyä, vaikutusten taso voi vaihdella huomattavasti tutkimusskaalasta riippuen. Osatyö I havainnoi että meriajokasgenotyypit voivat erota stressinsieto- ja palautumiskyvyssään. Osatyössä II meriajokkaan taimet olivat täysikasvuisiin kasveihin verrattuna jokseenkin vähemmän alttiita stressille, vaikka stressi laski myös taimien eloonjäämistodennäköisyyttä huomattavasti. Tämä ennakoi meriajokasniittyjen uudelleenasuttamistodennäköisyyden laskua tulevaisuudessa. Populaatioiden stressireaktioiden vaihtelevuus osatyössä III viittaa siihen, että paikallisadaptaatio eriävien valintapaineiden alla on johtanut eroavaisuuksiin suolapitoisuudensietokyvyssä. Nämä eroavaisuudet osoittavat, että joillakin genotyypeillä ja populaatioilla on paremmat mahdollisuudet sopeutua tuleviin ympäristöolosuhteisiin.

Stressitekijöiden monilukuisuus ja tutkimusskaalasta riippuvat eriävät stressireaktiot korostavat sitä, että meriajokkaan reaktioiden ennustaminen dynaamisessa elinympäristössä on epävarmaa. Meriajokasniittyjen keskinäiset erot stressinsietokyvyssä eri alueiden sisällä ja välillä korostavat sekä alueellisten, kansallisten että kansainvälisten suojelutoimien tärkeyttä näiden tärkeiden ja ainutlaatuisten niittyjen eloonjäämisen mahdollistamiseksi.

Avainsanat: abioottinen stressi, biottinen stressi, elinvaihe, genotyyppi, kasviyhdyskunta, lajimonimuotoisuus, lämpötila, meriruoho, monilukuinen stressi, populaatio, suolapitoisuus, valon määrä, Itämeri

ABSTRAKT (Danish abstract)

Lavvandede, kystnære områder repræsenterer dynamiske habitater, hvis organismer er påvirket af en lang række abiotiske og biotiske faktorer. Ålegræs (*Zostera marina*) udgør en vigtig primærproducent i sådanne områder på den nordlige halvkugle. Ud over de naturlige påvirkninger ålegræs udsættes for, eksponeres de også i stigende grad til antropogene påvirkninger, hvoraf nogle skyldes igangværende klima-forandringer. Målet med denne afhandling er at studere effekterne af forskellige abiotiske (lysmængde, salinitet og temperatur) og biotiske (tilstedeværelse af andre rodfæstede planter) stressfaktorer, samt forskellige niveauer og kombinationer af disse, på ålegræssets fitness. Studiet omfatter undersøgelser på en række forskellige økologiske hierarkier (dvs. af forskellige genotyper, livsstadier, populationer og samfund), for at undersøge hvorledes ålegræssets stressrespons påvirkes af skala. Studierne blev gennemført i forbindelse med fire forskellige felt- og akvarieeksperimenter.

Reduceret lysmængde, lav salinitet og forhøjet temperatur påvirkede ålegræs negativt (artiklerne I, II og III), mens tilstedeværelsen af andre plantearter ikke havde nogen påviselig effekt på ålegræs (artikel IV). Som forventet havde ekstreme stress-niveauer en større (negativ) effekt end mere moderate niveauer. Også moderate stress-niveauer førte imidlertid til målbare effekter, når planterne blev udsat for flere stressfaktorer på samme tid som vist i artikel II. Dette viser at effekterne forstærkes, når planten udsættes for flere stressfaktorer samtidigt. Resultaterne fra artikel I-III tyder på at igangværende (klima-)ændringer kan få alvorlige konsekvenser for ålegræssets fitness og overlevelse.

Stressresponset hos genotyper, livsstadier og populationer varierede (artikel I-III), hvilket understreger betydningen af at gennemføre studier på flere skalaer. Resultaterne viser at stress generelt påvirker ålegræs negativt, men at effekternes styrke kan variere afhængigt af den valgte studieenhed. Forskellige genotyper responderer forskelligt på stress i artikel I. Mens frøplanter var mindre sensitive overfor abiotisk stress end voksne skud i artikel II, reducerede øget stress imidlertid overlevelsen hos frøplanterne mærkbart. Dette medfører at gen-etablering af ålegræspopulationer i områder hvor fra planten er forsvundet, kan være truet i fremtiden. Variation i stressrespons mellem populationer i artikel III indikerer at adaptation under forskellige selektionstryk har fundet sted, og har medført forskelle i salinitets-tolerance mellem ålegræspopulationer fra forskellige lokaliteter i Østersøen. Denne variation i følsomhed antyder, at nogle genotyper og populationer har en større chance for at tilpasse sig ændringer og overleve i det fremtidige miljø.

Forskellige stressfaktorer kan således have synergetiske effekter, og stressresponset kan variere som funktion af den valgte studie-enhed. Dette viser hvor svært det er at forudsige effekterne af klimaændringer. Da ålegræssets respons til sådanne ændringer varierer mellem både genotyper og økotyper, bør fremtidige beskyttelses strategier foregå på regional, såvel som på interregional skala, i et forsøg på at sikre disse vigtige habitater for kommende generationer.

Nøgleord: abiotisk stress, artssammensætning, biotisk stress, genotype, livsstadium, lysmængde, multiple stressfaktorer, population, salinitet, samfund, temperatur, ålegræs, Østersøen

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I **Salo T**, Reusch TBH, Boström C. Genotype-specific responses to light stress in eelgrass (*Zostera marina*), a marine foundation plant. *Manuscript*.

II **Salo T**, Pedersen MF (2014) Synergistic effects of altered salinity and temperature on estuarine eelgrass (*Zostera marina*) seedlings and clonal shoots. J Exp Mar Biol Ecol 457: 143-150.

III **Salo T**, Pedersen MF, Boström C. Population specific salinity tolerance in eelgrass (*Zostera marina*). *Manuscript.*

IV **Salo T**, Gustafsson C, Boström C (2009) Effects of plant diversity on primary production and species interactions in brackish water angiosperm communities. Mar Ecol Prog Ser 296: 261-272.

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I Experiment design: **TS**, TBHR, CB. Performed the experiment: **TS**. Lab and data analyses: **TS**. Wrote the manuscript: **TS**, TBHR, CB.

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III Experiment design: **TS**, MFP. Performed the experiment: **TS**. Lab and data analyses: **TS**. Wrote the manuscript: **TS**, MFP, CB.

IV Experiment design: **TS**, CG, CB. Performed the experiment: **TS**, CG, CB. Lab and data analyses: **TS**. Wrote the manuscript: **TS**, CG, CB.

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1. INTRODUCTION

Coastal areas have for centuries been important regions for human populations in terms of food, transportation and other resource use and they have been popular areas for human settlement. Anthropogenic pressure on coastal areas has increased significantly over the past centuries and negative impacts of overexploitation of resources, destruction of habitats and pollution have led to increasing deterioration of coastal environment affecting coastal ecosystems negatively (Lotze et al. 2006).

Seagrasses are marine angiosperms that commonly form extensive meadows in estuarine and coastal waters across the globe. The primary production in seagrass ecosystems is high (Duarte & Chiscano 1999) and seagrass meadows provide habitat and food sources to a variety of marine organisms, and support high biodiversity (Orth et al. 1984). Seagrass meadows also have important part in coastal protection as they reduce current velocities, stabilize sediments and control for coastal erosion (Hemminga & Duarte 2000). They can also reduce nutrient load in coastal waters (Hemming & Duarte 2000) and function as carbon sink (Nelleman et al. 2009, Fourqurean et al. 2012).

Organisms inhabiting estuarine and coastal areas are affected by variety of environmental factors. Factors such as water movement, water salinity and temperature, available substrate for growth and light availability can limit seagrass growth and distribution, depending on the habitat requirements of the species or ecotype in question. In addition, the estuarine and coastal distribution of seagrasses makes them susceptible to increasing anthropogenic pressures and it has been estimated that globally as much as 65 % of seagrass meadows might have been lost due to human impacts (Lotze et al. 2006). Loss of seagrass ecosystems may lead to decrease in ecosystem services, threatening the functioning of coastal ecosystems (Orth et al. 2006, Waycott et al. 2009). While many anthropogenic impacts are known to impact seagrasses negatively, it is still unknown how much environmental changes seagrasses can tolerate.

This thesis investigates stress tolerance of eelgrass (*Zostera marina*). The summary introduces different aspects off the stress concept in biology with special emphasis on seagrasses. In the following summary effects of four stressors that are likely to become more important in the future due to the climate change are discussed at different levels of biological organization and dimension varying from genotypes to plant community.

1.1 Stress

1.1.1 Definition of stress

The original stress concept for organisms was formulated by Selye (1936) who defined stress as a "general alarm reaction to non-specific agents". Stress is difficult to define, and different definitions have been suggested during the past decades. Grime (1977), for example, defined plant stress as "external restrictions limiting biomass production of vegetation" while Rosen (1981) defined stress as "the gradient between ideal conditions and the ultimate limits of survival". Following these definitions, in this thesis, stress is considered as a factor, that can lead to decreased plant performance (e.g. slower growth) or even to increased mortality.

1.1.2 Stress responses

Organisms that are exposed to stress have four options: they can move, adapt (evolve), alter their phenotype, or die out. Plant mobility usually requires sexual reproduction and spatial dispersion of seeds affecting first subsequent generations. Also evolutionary adaptations take generations to gain. Thus, the initial acclimatization to stress is usually based on phenotypic plasticity. Phenotypic plasticity can be divided into two separate definitions: classical phenotypic plasticity, i.e. adjustment of phenotype to optimize plant performance in the prevailing environmental settings, and phenotypic buffering, i.e. maintaining plant performance at the original level despite changes in the environment (Reusch 2014).

Plant performance in response to a stressor will vary depending on the level of stress applied (Fig. 1). The more severe the stress, the more it will affect the plant and lead to lowered performance. When exposed to stress, plants will try to cope with it. The success is dependent on the phenotypic plasticity and the severity of the stress. Lichtenthaler (1996) described four different phases of extrinsic stress: Response phase, restitution phase, end phase and regeneration phase. In this classification the initial response is an alarm reaction with a decrease in performance. This phase is followed by an acclimatization process, where the plant will attempt to buffer its performance. Acclimatization can yield higher performance compared to pre-stress conditions, but might also yield negative net production. If the stress continues for too long or if the stress intensity is too high, the plant will eventually die. However, if the stress ends or declines enough, the plant might recover partly or completely and reach a new ambient state (Lichtenthaler 1996).

Seagrasses, as many other plants, are very plastic and can often alter their morphology and physiology substantially to optimize their performance in different environmental settings. For example, seagrasses have several ways to acclimatize to altered light conditions (see 1.2.1). They can increase or decrease the number and composition of pigments or alter leaf morphology (length, width), shoot density and leaf productivity (Lee et al. 2007), among others.

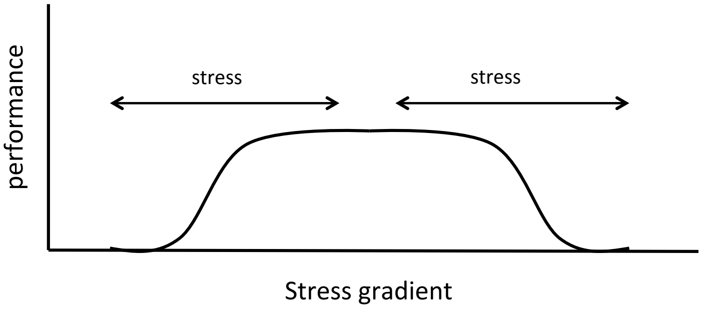

Fig. 1. Hypothetical performance curve in a gradient of stress. The arrows indicate stress, i.e. the gradient between optimal conditions (i.e. where performance is highest) and lethal conditions.

1.2 Common stressors in seagrass ecosystems

Shallow coastal areas are dynamic habitats that are affected by a variety of abiotic, biotic and anthropogenic factors. Some of these factors are described in the following text. These factors and their amplitude might vary both temporally and spatially and the multitude of different environmental and anthropogenic factors will determine whether a species can colonize a habitat and manage to grow and survive in that habitat. The amplitude and frequency by which certain environmental factors vary, may also limit the number of species that are able to survive under the altered conditions.

1.2.1 Abiotic factors

Despite the variety of abiotic factors that can affect seagrasses, most of them can affect photosynthesis and/or resource use and unfavorable levels can thus lead to decreased productivity in seagrass ecosystems. Compared to many other organisms the minimum light requirements of seagrasses are relatively high (Dennison et al. 1993) and light limitation is a common reason limiting growth in seagrass ecosystems. Reductions in light intensity can lead to decreased ratio between photosynthesis and respiration (P:R-ratio) and may yield negative net production (Lee et al. 2007). Decreases in irradiance levels may thus lead to decreased growth rate, increased mortality (Fig. 2, Dennison 1987, Lee et al. 2007) and changes in depth distribution (Duarte 1991), among others.

Increased temperature increases both plant respiration and gross photosynthesis. Respiration tends to increase more than photosynthesis with increasing temperature, resulting in a decreasing P:R-ratio (Marsh et al. 1986, Lee et al. 2007). Many temperate seagrass species have relatively low temperature optimum (Lee et al. 2007), and high temperature events can thus lead to decreased productivity (Nejrup & Pedersen 2008) and increased mortality (e.g. Reusch et al. 2005, Moore & Jarvis 2008, Nejrup & Pedersen 2008). Increased water temperature is often followed by low water oxygen concentration. While low water column oxygen levels *per se* can be harmful to seagrasses and decrease the productivity (Pulido & Borum 2010), the negative impacts of low oxygen levels are strengthened when combined with increased temperature (Raun & Borum 2013). High sediment sulfide levels have been reported to have serious impacts on seagrasses (Holmer et al. 2005). Low oxygen concentrations enhance the sulfate reduction and prevent oxidation of sediment sulfides back to sulfites (Libes 1992) and may lead to increased sulfide intrusion in rooted plants (Holmer et al. 2005).

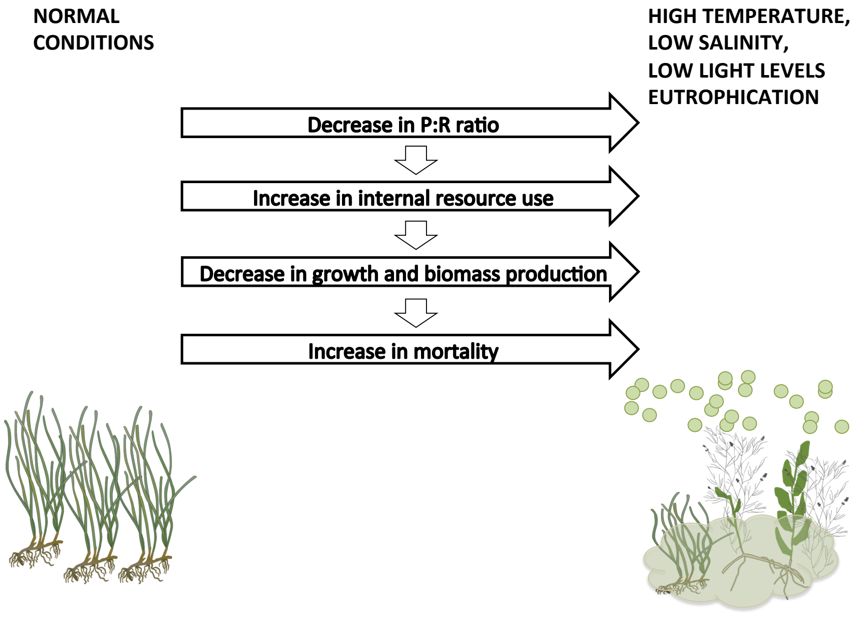

Fig. 2. Conceptual model of impacts of increased temperature, decreased salinity, decreased light levels and increased eutrophication on seagrass populations. The more simultaneously stressors are affecting the system, the larger the expected impact. Altered environmental conditions may lead to decreased eelgrass abundance and performance and increase competition with phytoplankton, opportunistic macroalgae and/or vascular plants. (Plant drawings taken from www.ian.umces.edu/imagelibrary).

Non-optimal salinities (hypo- or hyper-salinity) can also decrease seagrass productivity (Nejrup & Pedersen 2008). Changes in salinity can induce changes in pigmentation (Thorhaug et al. 2006) and lower the functioning of photosystems I and II (Touchette 2007). Altered salinity can also increase energy requirements of plants as energy is needed e.g. to maintain turgor pressure (Hellebust 1976, Kirst 1989).

Nutrient concentrations affect seagrasses both directly and indirectly through biotic interactions (for example due to shading) (see 1.2.2). Too low nutrient concentrations limit plant growth, while too high levels of certain nutrients (e.g. ammonium) may have a direct negative effect on seagrass performance due to toxic effects and/or an increased demand for C-skeletons needed for N-assimilation (Touchette & Burkholder 2007, Villazán et al. 2013).

Water movement is one of the key factors determining seagrass distribution. Low current velocity may eventually lead to carbon limitation and to decreased productivity due to increased leaf boundary layer thickness, while high current velocity and/or wave action increase erosion, alter sediment particle size and affect settling of seeds, anchoring of seedlings and patch size distribution and dynamics (Fonseca & Kenworthy 1987, Koch 2001).

1.2.2 Biotic factors

As light is one of the main factors controlling productivity and survival of aquatic plants, competition for light is very common in coastal habitats. Multi-specific seagrass meadows are relatively common (Duarte 2000), but in addition to co-occurring seagrass species or other rooted macrophytes (Fourqurean et al. 1995), also macroalgae, epiphytes and pelagic phytoplankton can compete with seagrasses for light. Drifting macroalgae mats have been reported to decrease seagrass growth and increase mortality due to both physical forces (Valdemarsen et al. 2010) and shading impacts (Rasmussen et al. 2012). Grazing by waterfowl (Hughes & Stachowicz 2004) or mesograzers (Valentine & Heck 1999) can remove large amounts of seagrass biomass while sediment reworking by benthic fauna may threaten earlier seagrass life stages (Valdemarsen et al. 2010). Outbreaks of plant pathogens, such as *Labyrinthula* sp., have caused massive seagrass diebacks in large geographical areas (den Hartog 1987, Vergeer & den Hartog 1994).

Not all biotic interactions are negative. Positive interactions between species can yield higher community productivity for example when species in a community differ in their resource use (Loreau 2000) and some species may facilitate each other (Cardinale et al. 2002) for example by stabilizing sediment and decreasing current velocities.

1.2.3 Anthropogenic factors

Anthropogenic increase in atmospheric CO_2-concentration has enormous impacts on marine and aquatic environments. Raised CO_2-concentration in seawater decreases pH of the water and increases the availability of inorganic carbon. Increased CO_2-concentration leads to increases in water and air temperature, which may in turn lead to altered oceanic circulations and changes in precipitation and wind patterns (Doney et al. 2010 and references therein). In coastal ecosystems these changes are believed to lead to higher average water temperature, increased frequency of heat events in summer, decreased sea ice-cover, changes in water salinity, increased wave activity, increase in storm events and increased variability in environmental factors, among others (Meier 2006, HELCOM 2007, Graham et al. 2008).

Other anthropogenic factors, such as nutrient pollution, are also deteriorating the growth environment for seagrasses. Eutrophication affects seagrass abundance and depth distribution negatively (Krause-Jensen et al. 2008). These negative impacts are mainly due to changes in light conditions as nutrient enrichment stimulates faster-growing algae, such as drift macroalgae, epiphytes and phytoplankton, all of which decreases water transparency (Sand-Jensen & Borum 1991). Negative impacts of eutrophication are reported in many areas and for example all sub-areas in the Baltic Sea are affected by eutrophication and decreased water transpacency (Andersen et al. 2011).

The alterations in environmental settings in coastal habitats due to ongoing and projected anthropogenic changes are likely to increase stress on organisms in coastal habitats. Thus, these changes are very likely to have large impacts on seagrass populations. While modest changes might be manageable for seagrass communities, larger changes or many simultaneous changes could exceed the tolerance limits of seagrasses. Modest changes in temperature could, for example, even result in increased productivity while larger increases are likely to lead to lethal temperatures for seagrasses.

1.3. Seagrass stress studies and knowledge gaps

The number of stress studies in seagrasses has increased notably during the last decades. When searching the Web of Science (accessed April 29, 2014) with the search words “seagrass AND stress” in total 139 experimental or comparative studies that fulfilled the search criteria were found (when excluding modeling studies). The average number of publications has increased from one per year in the late 1980’s to 16 papers per year in the early 2010’s (Fig. 3). The majority of the studies assessed either light (28%), temperature (21%) or salinity stress (20%). Notably, 72 % of the studies included only one stressor. In studies including multiple stressors, the combined effect of light and salinity, light and nutrients, and light and temperature were most common.

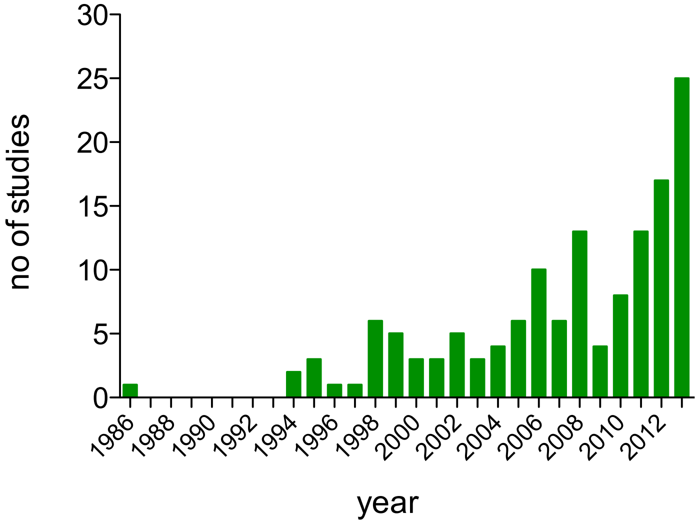

Fig. 3. Number of seagrass stress studies during years 1986-2013 in the Web of Science (accessed April 29, 2014) responding to the search words "seagrass AND stress" with the search. Modeling studies are excluded.

Non-optimal levels of most environmental factors reduce P:R-ratio and/or increase resource use (Fig. 2). When a plant is exposed to more than one stressor, negative stress effects are likely to increase. Such situation can yield to additive or multiplicative, i.e. synergistic or antagonistic, stress response. If the effect is additive, then the combined effect of two stressors corresponds to the sum of the two individual effects, while multiplicative effects are harder to predict. Synergistic effects are higher or more severe and antagonistic effects lower or less severe than additive effects (Gruner et al. 2008). For example, temperature has been shown to have negative interactive impacts with other environmental factors. Plants exposed to higher temperature have higher light requirement than those exposed to lower temperature (Lee et al. 2007, Moore et al. 2012) and increasing temperature leaves plants more sensitive to anoxia (Raun & Borum 2013). While the relative number of studies including multiple stressors has doubled from 16% to 32% during 1994-2003 to 2004-2013, most of the studies conducted are still estimating the impacts of only one stressor and the interactive impacts remain understudied. Global climate changes are expected to alter environment in many ways. To be able to estimate and manage biological consequences of anthropogenic impacts on coastal ecosystems, testing and evaluating seagrass tolerance for relevant stressors and the combination of these is necessary.

More than 55% of the 139 studies were conducted on *Posidonia oceanica*, *Z. marina* or *Thalassia testudinum* and less than 7% compared stress either between species, populations or stress type (e.g. continuous or pulsed stress). The majority of stress studies are still conducted using one species from one location, often ignoring the impact of life stage, population, species etc. on stress tolerance. For example, due to high natural mortality of early life stages in nature, life stages have been suggested to differ in their stress sensitivity. Yet, so far only few studies have compared the performance of seedlings and adult shoots (e.g. Biber et al. 2009, Raun & Borum 2013), and more comparative studies are needed to assess possible differences in stress sensitivity between different life stages and/or levels of biological organization, such as genotypes or spatially isolated populations.

Organisms that are distributed within their optimal range of an environmental factor are hypothesized to have higher acclimatization capacity to variable conditions than plants growing outside the optimal range (Touchette 2007). In addition, also local adaptation is important for stress tolerance. Edge populations or populations experiencing stress levels close to their tolerance limits might be better in adapting to further alterations in their environment compared to more central populations (Reusch 2014). Edge populations have also been suggested to be more likely to harbor genes specialized to the challenging environment (Johannesson & André 2006). So far it is still unknown how much edge populations and more central populations of seagrasses differ in their preference for environmental conditions and in their stress tolerance.

The suggested relationship between biodiversity and ecosystem functioning implies positive impacts of species diversity (Stachowicz et al. 2007). Also genetic diversity in seagrass ecosystems has been observed to enhance productivity (Hughes et al. 2009), resistance (Duffy 2006) and resilience, i.e. the capacity of a system to recover from a disturbance (Reusch et al. 2005, Ehlers et al. 2008). That the presence of several genotypes or individuals can alter the productivity and functioning of a seagrass meadow suggests that different genotypes differ in their stress tolerance. Comparative laboratory studies suggest that morphological and physiological responses may indeed be genotype dependent (Hughes et al. 2009, Tomas et al. 2011). Considering the importance of inter-genotypic differences for adaptive evolution and for species persistence, the subject is remarkably understudied. For example, comparisons in stress and recovery processes between genotypes are still more or less non-existing.

1.4 Aims of the thesis

The general aims of this thesis were to study how different abiotic (salinity, temperature and irradiance) and biotic (presence of co-occuring species) stressors and different levels of these stresses and combination of stressors affect performance and survival of eelgrass. To contribute to some of the other knowledge gaps mentioned above (1.3), the influence of studied scale in terms of biological organization and dimension in response to stress (i.e. genotype, life stage, population and plant community) was also assessed.

More specifically, the following research questions were assessed:

- Do different genotypes differ in their stress responses and in their recovery processes? (paper I)
- Do different stressors have interactive impacts on plant performance and do different life stages differ in their responses (paper II)
- Does previous adaptation or acclimatization impact plant performance and tolerance limits of plants and how important is the level of stress? (paper III)
- Is eelgrass productivity and resource use affected by co-occuring plant species and does community composition affect ecosystem functioning in mixed eelgrass ecosystems? (paper IV)

More specific aims and hypothesis are presented in respective papers I-IV.

2. MATERIAL AND METHODS

2.1 Model organism, eelgrass

Eelgrass (*Zostera marina* L.) was used as the model organism in the studies conducted in the framework for this thesis. Eelgrass is a temperate seagrass species with a wide distribution throughout the northern hemisphere (Den Hartog 1970). The southern distribution limit of the species is set by high summer water temperature, while low water temperatures in the northernmost part of the distribution area likely lead to reduced productivity (Clausen et al. in press). As a euryhaline species eelgrass has a wide salinity tolerance and it is found from fully oceanic to brackish waters (salinity 5.5) (e.g. Den Hartog 1970, Boström et al. 2003).

2.1 Study systems and populations

All the studies in this thesis were conducted in the Baltic Sea. The Baltic Sea has a strong salinity gradient with salinity changing along the Baltic from close to oceanic salinities in the Kattegat-Skagerrak area to very low salinity in the Gulf of Finland and Gulf of Bothnia. Eelgrass distribution in the Baltic Sea is limited to salinities higher than 5.5 (Boström et al. 2003). Eelgrass commonly forms mixed meadows together with freshwater angiosperms in the low saline areas of the Baltic Sea (for example see the study site description in paper IV). In areas with higher salinity eelgrass meadows are often mono-specific or eventually mixed with *Ruppia maritima*.

The connectivity between Baltic eelgrass populations is low and different sub-regions are genetically isolated from each other (Olsen et al. 2004, Johanneson & André 2006). The long-term isolation between regions and strong environmental gradients have likely enhanced local adaptation in the Baltic (Johanneson et al. 2011), and it is possible that eelgrass in different regions has developed to different ecotypes that differ in their tolerance limits for different stressors. At the same time, sexual reproduction is rare in the N Baltic where eelgrass persistence relies mainly on vegetative reproduction. This has resulted in reduced genotypic richness compared to the SW Baltic and the Atlantic area (Olsen et al. 2004, Reusch & Boström 2011).

To cover some of the spatial variation in environmental conditions and populations in the Baltic, studies were conducted both in Finland (N Baltic) and Denmark (SW Baltic). Field experiments in paper I and IV were conducted in Fårö, N Baltic, and the plants originated from Sandön and Fåro, respectively (Fig. 4). The study in paper II was conducted with plants originating from Isefjorden, while the plants used in paper III originated from Isefjorden, SW Baltic and Ängsö, N Baltic (Fig. 4). For coordinates and more detailed descriptions of the donor populations and sites, see the original papers (I-IV).

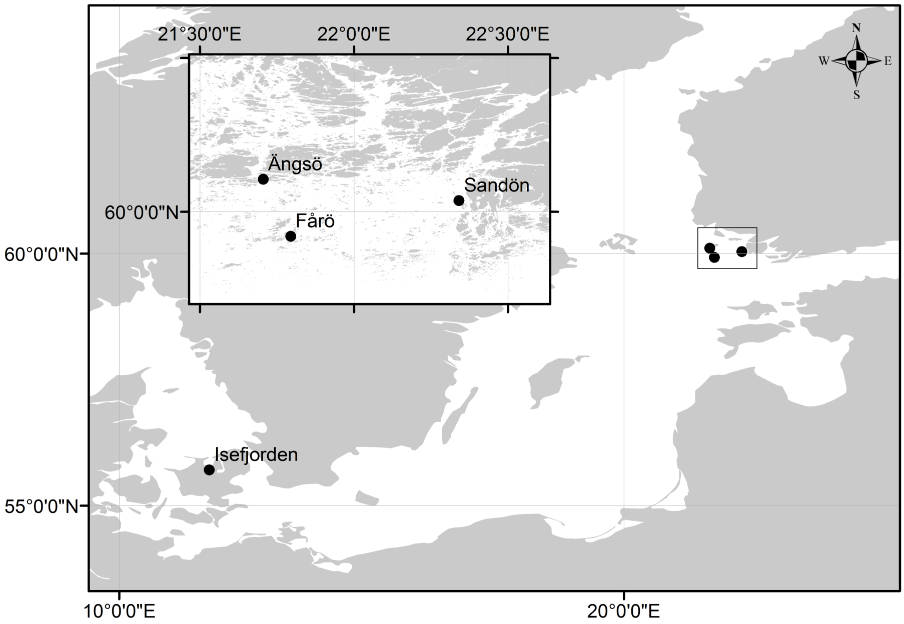

Fig. 4. Map over the donor locations (papers I-IV) and field study sites (papers I, IV) in N and SW Baltic Sea. The embedded map indicates the locations of N Baltic sites in more detail. (Map by MW Holm).

2.2 Experimental work

Studies in papers I and IV are field experiments and studies in papers II and III aquarium experiments. Each paper (I-IV) assesses the effects of stress on eelgrass performance: effects of light limitation and the recovery from such stress are assessed in paper I, combined effects of low salinity and increased temperature are studied in paper II, while altered salinity is assessed in more detail in paper III. The importance of co-occurring species is finally examined in paper IV. These experiments represent different scales of study: different genotypes (I), life stages (II), populations (III) and plant communities (IV) (Fig. 5). The experimental settings, response variables and data-analysis are summarized in Table 1.

Paper I

To study the effect of light limitation on different genotypes (I) donor genotypes were identified at Sandön (Fig. 4) by using 9 polymorphic microsatellite loci. Replicated monocultures (n=10) of four different genotypes were established in a common garden experiment at Fårö (Fig. 4). The experimental plots were allowed to recover from transplantation for four weeks, after which half of the replicate plots (n=5) for each genotype were shaded (78% less light compared to control plots) for four weeks and, then, subsequently allowed to recover under ambient light conditions for four weeks.

Paper II and III

As changes in temperature and salinity are impossible to assess in the field without simultaneously changing other parameters, the effects of these factors were studied in aquarium experiments. The singular and combined effect of salinity and temperature on different eelgrass life stages (II) were studied by transplanting seedlings (solitary shoots with sparse root development and an attached seed coat) and adult shoots (apical ramets separated from a genet) to aquaria (split plot design). Both life stages were exposed to different levels of salinity (5,12.5 and 20), temperature (15, 20 and 25°C) and all the possible combinations (9) of these for 5 weeks. The impact of altered salinity on different populations (III) was assessed by transplanting adult plants originating from two different populations, i.e. Ängsö and Isefjorden (Fig. 4), with different ambient salinities, 6 and 20, respectively. Plants were exposed to 8 different levels of salinity (i.e. 2, 4, 6, 9, 12.5, 15, 20 and 25) for five weeks. Target temperatures and salinities in these experiments were obtained by placing aquaria into larger containers equipped with both heating and cooling systems and by mixing seawater from the North Sea with tap water with naturally high DIC concentrations, respectively. Salinity and temperature were initially altered stepwise to allow plants to acclimatize for osmotic and thermal changes. To ensure sufficient DIC levels and water mixing, each aquarium was aerated constantly and 2/3 of the water was changes weekly to ensure adequate nutrient levels for plant growth. Light levels in aquaria experiments were ca. 120 µmol photons m^{-2} s^{1} PAR in 16:8 h light-dark cycle.

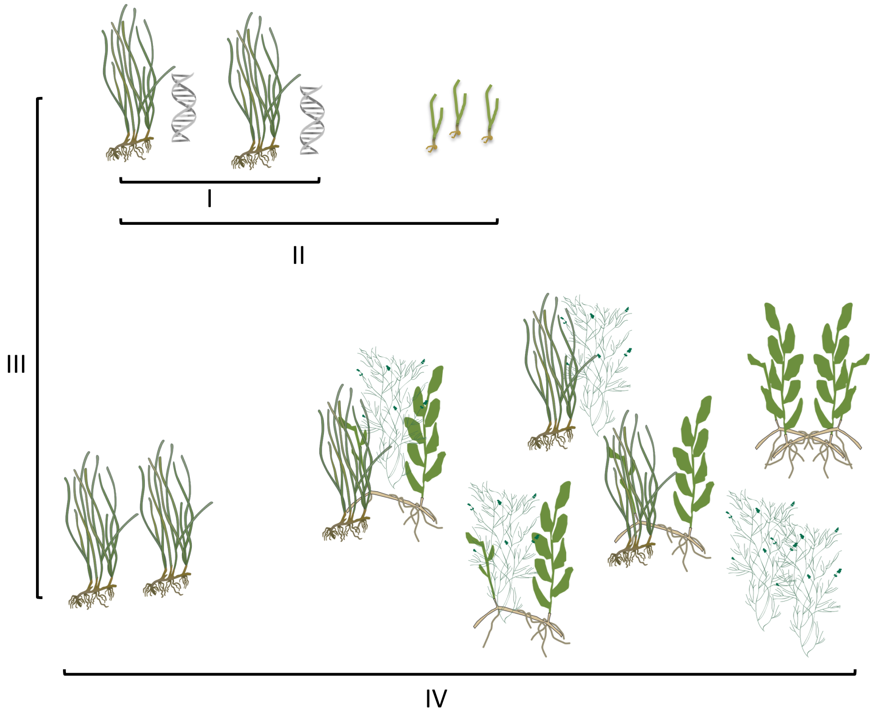

Fig. 5. A schematic picture illustrating the different scales of biological organization included in the different studies in papers I-IV: genotypes (I), life stages (II), populations (III) and plant communities (IV). All drawings from www.ian.umces.edu/imagelibrary, except for the seedling drawing.

Table 1. Summary of the experimental settings, response variables and data-analysis in the different papers I-IV

	I	**II**	**III**	**IV**
Stressor	Light limitation	Salinity Temperature Sal x temp	Salinity	Co-occurring plant species
Test-unit	Genotype	Life stage (seedling or adult shoot)	Population (low saline or high saline)	Plant species Community
Factors	Genotype (4 levels) Light (2 levels) Time (2 levels)	Salinity (3 levels) Temperature (3 levels) Life stage (2 levels)	Salinity (8 levels) Population (2 levels)	Species richness (3 levels) Species composition (7 levels)
Timescale	12 weeks	5 weeks	5 weeks	14 weeks
Experiment type	Field exp.	Aquaria exp.	Aquaria exp.	Field exp.
Experiment design	Full factorial repeated measures	Full factorial split plot	Full factorial split plot	Full factorial replacement design
Response variables	Gene expression (22 genes) Leaf elongation rate Shoot biomass Sucrose concentration in plant tissue	Mortality rate Leaf elongation rate Production of new leaves No of leaves per shoot Leaf necrosis Sucrose and starch concentration in plant tissue	Mortality rate Leaf elongation Production of new leaves No of leaves per shoot Leaf necrosis Sucrose concentration in plant tissue Chlorophyll fluorescence	Aboveground, belowground and total biomass Shoot density Spatial spread No of turions and tubers Porewater nutrient concentration
Data-analysis	Univariate permutational repeated measures ANOVA Linear regression ANOSIM SIMPER	Multivariate and univariate permutational ANOVA	Multivariate and univariate permutational ANOVA	1-sample t-test One way ANOVA Linear and quadratic regression, Additive partitioning D_i

Paper IV

The effect of co-occurring species and the importance of species identity and diversity on productivity (IV) were studied by establishing replicated (n=4) mono-, bi- and tricultures of eelgrass, *Potamogeton filiformis* and *P. perfoliatus* in all the possible (7) combinations (Fig. 5). The plots were established according to replacement design so that each plot had a total aboveground biomass of 30 g (VW). The plots were allowed to grow undisturbed for 14 weeks before assessing species ratios.

Each experimental plot or aquaria was always sampled at the end of the experiment (II-IV), except in paper I, where subsampling took place after both shading and recovery periods. At these time points samples were collected to analyze response variables varying from genetic to phenotypic. In more detail, relative gene expression (I), mortality rates or shoot density (II-IV), leaf elongation rate (I-III), aboveground (I, IV), belowground (IV) and total (IV) biomass, sucrose (I-III) and starch (II) concentrations in plant tissue, production of new leaves, no of leaves per shoot and leaf necrosis (II-III), chlorophyll fluorescence (III), number of turions and tubers, spatial spread and porewater nutrient concentration (IV) were assessed in respective experiments (Table 1).

2.4 Data-analysis

Both multivariate (II, III) and univariate (I-III) permutational analyses were conducted to study the impacts of stress and study unit. Multivariate analyses were used to evaluate the overall impact of the experimental factors on multiple response variables and these analyses were followed by univariate analyses to evaluate the specific impact of treatments factors on each response variable as suggested by Quinn and Keough (2002). The permutational analyses were conducted as repeated measures analyses to incorporate sampling time as a factor (I) or as partly nested analyses (II, III). In partly nested analysis, the effects were tested either against sub-plot or whole plot error, depending on the factor (Anderson et al. 2008). Aquarium was always considered a random factor, while all the rest of the factors were considered fixed. All the resemblance matrixes were based on Euclidean distance and all permutations were run 9999 times at significance level of $\alpha=0.05$. In case of missing data points (II), df's were adjusted for unbalanced design. Prior to conducting permutational analyses the dispersion of data were analyzed either by using MDS plots or conducting PermDisp- analyses (Anderson et al. 2008).

Univariate analysis of variance and regression analysis were used to inspect the effect of co-occurring plant species and plant community composition on different response variables (IV). Prior to the analyses, the distribution of the data and homogeneity of variances were inspected. If necessary, data were log-transformed to fulfill the assumptions of ANOVA. If assumptions could not be met, non-parametric Kruskal-Wallis test followed by Mann-Whitney U-test were used (IV). To estimate plant performance in mixed cultures and to assess components in net biodiversity effects, D_i (Loreau 1998) and additive partitioning (Loreau & Hector 2001), respectively, were calculated (IV). These indices were tested against 0.0 with 1-sample t-test. Dunn-Sidák method was used to correct for multiple t-tests comparisons (Sokal & Rolf 1994).

2.4.1 Data synthesis and effect size

In addition to the data-analysis performed in the individual papers, effect size-calculations were performed to compare and combine the results from the different studies (I-IV). Effect size reflects the magnitude of the studied treatment and it can be used to assess the consistency of results in different groups or studies, which is why it is commonly used in meta-analyses (Borenstein et al. 2009). As growth was one of the most common response variables in the different studies (I-IV) and as Grime (1977) emphasized the importance of stress for limiting biomass production (see 1.1.1), effect size calculations were based on leaf growth (I-III) and aboveground biomass (IV). Effect size was calculated as Hedges' g according to Nakagawa and Cuthill (2007) and Borenstein et al. (2009). Hedges' g for each factor and/or test unit were calculated by first calculating Cohen's d

$$\text{Cohen's d} = \frac{\overline{X_1 - X_2}}{S_{\text{within}}} \qquad \text{Equation 1}$$

where $\overline{X}_1$ is the sample mean of the stressed group, $\overline{X}_2$ is the sample mean of the control group and S_{within} is the pooled variance for these groups. S_{within} was calculated as

$$S_{\text{within}} = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}} \qquad \text{Equation 2}$$

where n_1 and S_1 and n_2 and S_2 are the sample sizes and standard deviations in the stressed group and in the control group, respectively. The variance of d was estimated as

$$V_d = \frac{n_1+n_2}{n_1 n_2} + \frac{d^2}{2(n_1+n_2)} \qquad \text{Equation 3}$$

As Cohen's d might overestimate the effect size in small samples (Nakagawa & Cuthill 2007), these were transformed to Hedge's g by using correction factor J

$$J = 1 - \frac{3}{4df - 1} \qquad \text{Equation 4}$$

where the degrees of freedom were calculated as n_1+n_2-2. Hedge's g and the variance for it were calculated according to Equations 5 and 6, respectively.

$$g = J \text{ x } d \qquad \text{Equation 5}$$

$$V_g = J^2 \text{ x } V_d \qquad \text{Equation 6}$$

The approximate 95% confidence intervals for the calculated effect sizes were estimated as

$$95\% \text{ CI} = g - t_{df} \text{ to } g + t_{df} \qquad \text{Equation 7}$$

where t_{df} is the t-value corresponding to the number of df's at $\alpha = 0.05$ (Nakagawa & Cuthill 2007).

In study I, separate effect sizes were calculated for different genotypes and for stress and recovery periods. In study II, effect sizes were calculated separately for different life stages for decreased salinity (pooled performance in medium and low salinity), increased temperature (pooled performance in medium and high temperature) and the combined effect of decreased salinity and increased temperature (pooled performance in medium and extreme treatments). In study III, salinities were grouped to low (2-6), medium (9-15) and high (20-25) and effect sizes were calculated separately for different populations in different salinity groups. In study IV, effect size was calculated for eelgrass growing together with either *P. filiformis* or *P. perfoliatus* and when grown together with both. To study the magnitude of biological effect of different stressors on eelgrass performance additional calculations with pooled genotypes (I), life stages (II), populations (III) and co-occuring species (IV) were conducted. Moreover, to inspect the relative importance of scale (*sensu* level of biological organization and dimension) on plant performance, calculations with pooled time points (I), temperatures and salinities (II, III) and plant communities (IV) were conducted. To be able to compare different factors, salinity and temperature, in study II, data for decreased salinity (II) were not pooled with altered salinities in study III for effect size calculations. To study the effect of plant community, also data on communities without eelgrass (i.e. monocultures of *P. filiformis* and *P. perfoliatus* and bicultures of *P. filiformis* and *P. perfoliatus*) were included. All the calculations were calculated against control treatments, except in calculations with mixed plant communities (IV), where calculations were compared against monoculture values.

3. RESULTS AND DISCUSSION

In this thesis I studied the impacts of four different potential stressors on eelgrass performance. Each study also compared different scale of biological organization. Both light limitation (I), decreased salinity (II, III) and increased temperature (II) affected eelgrass performance negatively, while co-occurring species had no detectable effect on eelgrass (IV), although the presence of eelgrass increased the biomass of one of the other plant species. As expected, more extreme stress levels had stronger impacts on plant performance compared to intermediate or low levels (III), but also, intermediate stress levels became more severe when plants were exposed to several stressors simultaneously (II). Thus, the negative effect of stress was strengthened synergistically when exposed to multiple stressors (II). Differences in responses were observed in all of the inspected scales, i.e. genotypes (I), life stages (II), populations (III) and communities (IV). The results demonstrate that while stress in general affects seagrass productivity negatively, the severity of effects can vary substantially depending on the study scale. This variability in stress tolerance increases the uncertainty in predicting the impact and future fate of eelgrass populations, because some genotypes or populations may be favored by future changes while others may disappear.

3.1 Stressor

3.1.1 Light limitation

Drifting macroalgae mats can heavily reduce irradiation levels and extensive algal biomass can lead up to 90 % reduction in light levels (Rasmussen et al. 2012). Such heavy shading reduces plant performance significantly (I, negative effect size for shading in Fig. 6a and 7a). In paper I both growth rates, aboveground biomass and plant tissue sucrose levels decreased significantly in response to shading (I), indicating that heavy shading reduced net photosynthesis to a level where plants had to mobilize internal carbon stores to sustain cellular metabolism. The observed depletion of sucrose storages in some plants (I) suggests that four weeks of heavy light limitation (78%) is close to lethal levels for northern Baltic eelgrass populations. While drifting algae mats may cover seagrass meadows for long time periods, small-scale spatial changes in drifting algae mat dynamics might limit the duration of light limitation for individual plants (Rasmussen et al. 2013). Water movement is a key component in drift algae movement (Rasmussen et al. 2013) and it can also affect the horizontal distribution of phytoplankton at the surface waters, especially if the plankton community is dominated by cyanobacteria (George & Edwards 1976). Increased wind speed and water movement could thus increase tolerance to shading by drifting algae and phytoplankton.

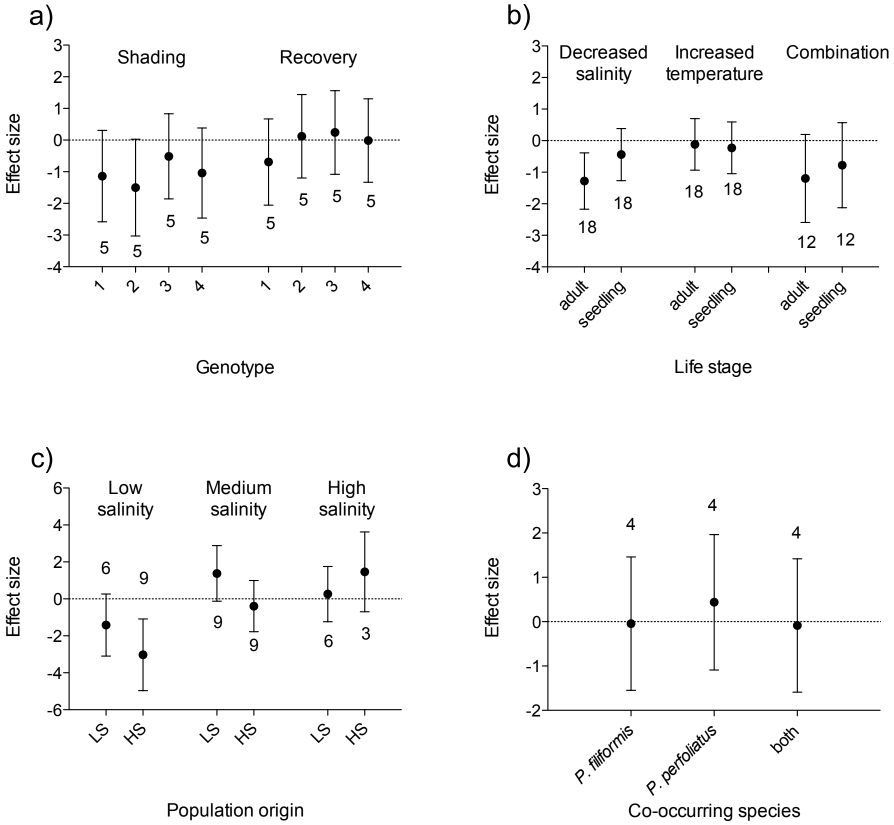

Fig. 6. Effect size ± 95% CI for results from the different papers (I-IV) indicating the biological importance of a) shading stress and recovery in four different genotypes (I), b) decreased salinity, increased temperature and the combination of these on adult plants and seedlings (II), c) salinity level on populations with different ambient salinites (III, LS=low ambient salinity, HS=high ambient salinity) and d) co-occurring species on eelgrass performance (IV). The data are leaf elongation data with an exception of d, where the data are aboveground biomass. The numbers below or above each data point indicate the sample size (n).

The recovery of growth rates (effect size close to zero in Fig. 6a), shoot biomass and tissue sucrose levels of shaded eelgrass plots when these were returned to control light levels (I) suggest that small eelgrass plots have the capacity to recover from close to lethal state in four weeks. Even faster recovery from heavy shading has been reported (Gustafsson & Boström 2013). The higher growth rate, biomass or higher sucrose concentrations (I) and significantly higher biomass (Gustafsson & Boström 2013) observed after recovery imply that the attempt to acclimatize to extreme low-light conditions, results in boosted production rates when plants are subsequently exposed again to higher light levels. Seagrasses can survive and recover from even longer light limitation if their rhizome connections to the surrounding meadow are kept intact (Collier et al. 2009). Seagrasses can translocate carbon between connected ramets (Marba et al. 2002), and a large clone/genet size could increase survival of the clone edges during light limitation stress, especially if light limitation is horizontally dynamic. Resource translocation among ramets could also help overcoming other spatially heterogenic stresses (Marba et al. 2002), such as co-occurring species depleting same resources. Resource translocation is, however, likely to be more important in species with long-lived rhizomes, such as *Posidonia oceanica,* compared to eelgrass.

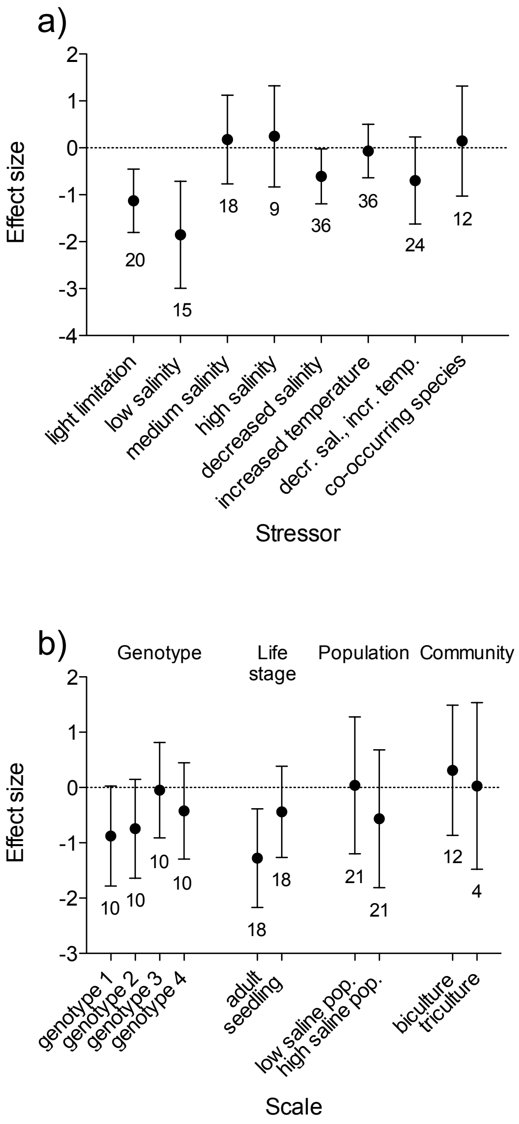

Fig. 7. Effect size ± 95% CI for results from the different papers (I-IV) indicating (a) the biological importance of different stressors and the level of salinity stress and (b) the variability in results depending on the choice of test unit or scale (*sensu* biological organization and dimension). All values are based on eelgrass leaf growth, except for co-occurring species in (a) that is based on aboveground biomass and community in (b), which is based on aboveground biomass of the whole plant community. The number below each data point indicates sample size (n).

3.1.2 Salinity, temperature and the combined effect

In addition to light limitation, salinity and temperature are among the most common factors affecting seagrass growth and distribution. Climate change is expected to induce changes in all these factors thus increasing stress in coastal communities (Doney et a. 2012). Both decreased salinity and increased temperature alone can have negative impacts on eelgrass performance (II, III, Nejrup & Pedersen 2008). The level of stress has a large impact on plant performance and when inspecting eelgrass performance over a broad range of salinities (III), medium and high salinity had no notable impact on leaf elongation, while low salinity led to highly reduced plant growth (Fig. 7a; both low salinity (III) and decreased salinity (II)). Combining both salinity and temperature stress can yield negative synergistic effects (II), even when stress levels are within the natural range for eelgrass. Comparison between the severity of salinity and temperature stress on leaf elongation (Fig. 6b, 7a) reveals that reduced salinity has more negative impacts than increased temperature, as the effect size (Fig. 7a) is more negative for the former compared to the latter. Pooling all the possible combinations of altered salinity and temperature indicates even more severe decrease in average performance and the biological effects vary from very negative (synergistic) to slightly positive (antagonistic) impacts (Fig. 7a). The increasing variance for effect size when combining stressors can partly result from lower number of observations used in the calculations (Nakawaga & Cuthill 2007), but the combination of different scenarios with differing plant responses is also likely to lead to increased variability in and decreased predictability of responses.

According to different climate scenarios, the ongoing climate change is likely to lead to increased sea surface temperatures and decreased salinity in the Baltic Sea area (Meier 2006, HELCOM 2007, Graham et al. 2008). While the salinity is expected to decrease, but remain relatively stable, the lowest salinities are expected to occur during summer months (Meier 2006), colliding with the peak growth season of eelgrass. Simultaneously, the sea surface temperature is expected to increase most during summer months (Meier 2006). As altered salinity and temperature can have synergistic impacts on eelgrass (II), a combination of these conditions might lead to extinctions of eelgrass in large areas. Decreases in seagrass productivity are likely also in areas with moderate changes in salinity or temperature (II). The severity of responses will depend on the “initial” ambient salinity and temperature conditions (III) and the adaption capacity of populations in question.

Some climate projections predict a decrease in precipitation in certain areas, e.g. Southern Baltic Sea (Graham et al. 2008), which could lead to increase in salinity (Meier 2006). As effects of increased temperature are less severe at higher salinities (II), an increase in salinity could perhaps alleviate future temperature stress, at least to some degree. While an increase in summer temperatures might lower production, increases in spring and autumn temperatures could lead to a prolonged growth season (Klausen et al. in press) and increased annual productivity. In some areas with very high summer temperatures seagrasses show bimodal growth patterns with two maximum growth peaks when conditions are closest to optimal and lowered production during the period with the highest water temperatures (Lee et al. 2007). However, plants from different temperature regions have been shown to differ in their capability to recover from temperature stress (Franssen et al. 2011, Winters et al. 2011), suggesting that it is not straight forward to expect that e.g. Baltic eelgrass populations can adapt to large increases in summer temperatures.

3.1.3 Co-occurring species

Seagrass meadows consisting of more than one species are relatively common (Duarte 2000). Thus, in addition to possible negative impacts of phytoplankton, epiphytes (Dennison et al. 1993) or macroalgae (Drouin et al. 2012), also co-occurring seagrass species or other submerged aquatic angiosperms can increase competition for resources in seagrass meadows. For example competition for nutrients and/or light can lead to exclusion of seagrass species with higher nutrient or light demand (Fourqurean et al. 1995).

The co-occurring angiosperm species do not necessarily lead to negative interactions between species. Species rich seagrass communities have in general higher production rates than species poor communities (Gamfeldt et al. 2014), indicating that different seagrass species may mutualistically affect each other. For example community productivity (Gustafsson & Boström 2010) and stability (Gustafsson & Boström 2012) were higher in mixed meadows compared to monocultures of eelgrass. Positive net impacts in species rich systems can originate from complementarity in resource use. For example, differences in rooting depths among species may lead to an increase in the total nutrient pool available for a meadow (Duarte et al. 2000). Facilitation may also occur through increased sediment stabilization and sheltering against physical stress (IV). However, as the neutral effect sizes in Fig. 6d and 7a suggest, co-occurring species do not necessarily have any notable impact on seagrasses (IV). Species richness effects depend on the selection and complementarity effects and whether these are positive, negative or neutral. Selection and complementarity effects can thus strengthen each others positive or negative effects or they can counteract each other and yield neutral or intermediate net effects (Hector et al. 2002).

Whether co-occurring species increase or decrease the productivity of a seagrass meadow depends both on the plant species composition (Gustafsson

& Boström 2000) and species identity (IV, Gustafsson & Boström 2010). In addition, also the density or biomass of different species in a community can affect the net effect of biodiversity (Gustafsson 2013). Thus, any change in the environmental conditions that would affect species unevenly could potentially alter the total interaction strength within that community and, thus, also the community functioning.

3.2 Scale

3.2.1 Genotypes

Earlier studies of stress tolerance and/or productivity in seagrass communities with different levels of genotypic diversity suggest that different genotypes harbor some ecologically relevant differences (e.g. Reusch et al. 2005, Duffy 2006, Ehlers et al. 2008, Hughes & Stachowicz 2009). While differences among eelgrass genotypes has been recorded previously (Hughes et al. 2009, Tomas et al. 2011), the results from study I confirm for the first time that seagrass genotypes indeed differ in their phenotypic performance during stress and in their recovery processes. In more detail, the studied genotypes differed in their sucrose concentrations both during and after shading. In particular, some genotypes were able to withstand shading stress and maintain sucrose concentrations similar to those in plants kept under control conditions while other genotypes depleted their sucrose levels almost completely (I). That the same genotype (genotype 3) sustaining high sucrose levels was also able to maintain almost normal growth rates, suggest that some genotypes are better in phenotypic buffering (see 1.1.2) and thus more probable to resist stress caused by light limitation. This is also illustrated in the effect size calculations (Fig. 6a, 7b): while the variability in effect sizes is very equal among genotypes, the average effects are slightly different indicating small differences between genotypes. While these differences may not be statistically significant for all variables (I), they may have some biological relevance for plant productivity and survival, among others. Differences between genotypes were also tentatively linked to patterns in relative gene expression (I) suggesting that the observed differences between phenotypic responses have genetic basis.

The observed variation in stress response and capacity for recovery among genotypes may partly explain the positive effects of high genotypic diversity during and after stress (Reusch et al. 2005, Duffy 2006, Ehlers et al. 2008). As the choice of genotype or genotypes in seagrass studies can influence results significantly (I, Hughes et al. 2009, Tomas et al. 2011), the genetic material included into ecological studies should be considered in detail. Including several genotypes to a study will likely increase the variance of the results, but simultaneously also give more realistic results to predict community response.

3.2.2 Life stages

Due to the low germination rates of seeds and low survival rates of seedlings in nature (Olesen 1999, Jarvis & Moore 2010), earlier life stages of seagrasses have been considered to be more sensitive to stress than adult and clonal shoots. In study II high temperature increased mortality of seedlings more than in adult plants. While the variability in responses to altered temperature and salinity is approximately similar for both adult plants and seedlings (Fig. 6b, 7b), both decreased salinity and the combination of decreased salinity and increased temperature have more negative impacts on adult plants than seedlings. Accordingly, in study II, decreased salinity had a more negative impact on adult plants than on seedlings. The small size and thin leaves of seedlings, resulting in a higher surface area to volume-ratio, is believed to be a key feature in seedlings tolerating stress better than large adult shoots (Raun & Borum 2013). The larger surface area to volume ratio in seedlings allows them to maintain higher internal oxygen pressures than adult shoots in the absence of light. This indicates that seedlings might be less vulnerable to low oxygen and high temperature stress (Raun & Borum 2013). The overall lower biomass of seedlings compared to adult plants could also ameliorate their stress tolerance for other abiotic stressors. The lower amount of belowground biomass lowers the need for rhizosphere oxygenation to prevent sulphide intrusion from the sediment while lower amounts of aboveground tissues reduce the need for extensive carbohydrate storage (Biber et al. 2009). Due to their small size, seedlings are easily shaded by clonal shoots when growing within a meadow, and settling in previously unvegetated areas expose them more to physical disturbances. While seed burial and long-term survival might be enhanced by activity of benthic organisms, some faunal species tend to bury seeds too deep for successful seed germination to take place (Delefosse & Kristensen 2012). Benthic fauna can also affect seedlings, as seedlings can be uprooted from the sediment or buried in the sediment as a result of extensive sediment reworking (Valdemarsen et al. 2010).

Flowering of adult shoots in seagrasses seems to be controlled mainly by water temperature (Phillips et al. 1983), and high temperatures may lead to abnormal flowering (De Cock 1981). In study II only few flowering shoots were observed. In nature extraordinary flowering in response to thermal stress has been observed in Mediterranean *P. oceanica* meadows (Diaz-Almela et al. 2007). Increasing water temperature might thus change the phenology of a species and alter the initiation of the flowering season to earlier. Also seed germination is increased in higher temperatures (Hootsmans et al. 1987). Some studies have found higher germination success in low salinity (Hootsmans et al. 1987), while Salo (unpublished data) observed 15-20 % higher germination rates in seeds incubated in salinity 20 compared to seeds kept in lower salinities (Fig. 8). Whether such differences in germination rates are population or area specific or depend on length of stratification period (mimicked winter conditions) or some other factors remain unclear and warrants further studies.

While seedlings might be more stress tolerant for some abiotic stressors compared to adult plants, the low overall success rate of seed germination and low tolerance of seedlings to physical disturbances decrease the efficiency of sexual reproduction. In many areas meadow maintenance is based on vegetative reproduction (Olesen 1999), while the production of seeds and seedlings are suggested to be more essential for long distance dispersal and re-establishment of meadows after major disturbances (Olesen & Sand-Jensen 1994, Olesen 1999, Greve et al. 2005, Jarvis & Moore 2010). Increasing frequency of stress events due to climate change and other anthropogenic impacts are therefore likely to increase the importance of sexual reproduction. While increasing temperature might enhance flowering (Phillips et al. 1983), the negative impacts of altered conditions on seedlings might decrease the likelihood for future re-colonization based on seeds and seedlings.

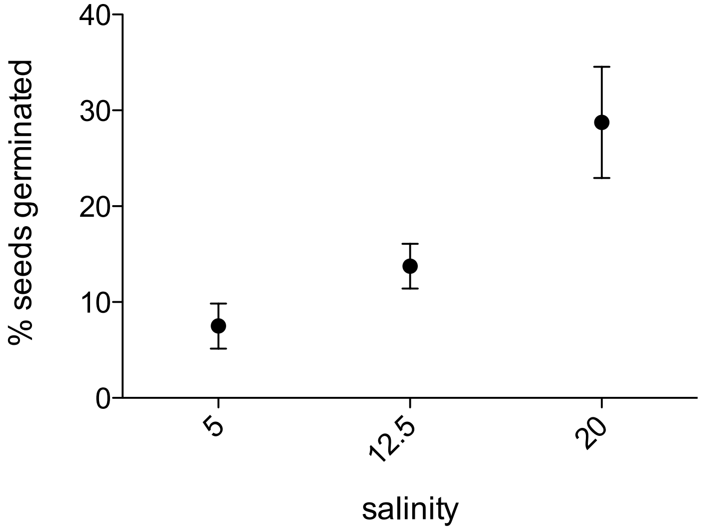

Fig. 8. Percentage (± SE) of seeds germinated in different salinities in 15°C after 5 months stratification in 3°C in darkness. (T. Salo, unpublished data).

3.2.3 Populations

When an environment is altered (e.g. increased water temperature), the selection pressure is changed to support or promote more plastic individuals and/or individuals with a larger buffering capacity in a certain trait (e.g. heat stress tolerance). If both the trait and the plasticity in that trait are inheritable, adaptive evolution can take place (Reusch 2014). In consequence, the following generations may be better adapted to the new conditions (e.g. higher temperature). Thus, genetic heterogeneity in combination with environmental stress should lead to divergence in genotypes and phenotypic plasticity and/or phenotypic buffering in areas with different selection pressures. Eventually this should lead to the development of different ecotypes and, at evolutionary time-scales, eventually new species.

The long term-isolation of Baltic eelgrass populations combined with a strong and stable salinity gradient, causing different selection pressures in different geographic areas, has likely lead to differences in salinity tolerances between populations. Populations adapted to low or high salinity differ indeed in their salinity tolerance (III). Less negative effect size for individuals from low salinity areas indicate that these plants tolerate low salinities better compared to individuals from high salinity areas (Fig. 6c). While individuals originating from low salinity areas have their production maximum at intermediate salinities, individuals from high salinity areas have higher production rates at higher salinities (Fig. 6c). The decreased production capacity of shoots originating from high salinity in low salinities compared to shoots from low salinity areas (Fig. 7b), indicates a horizontal shift (III, Kingsolver et al. 2004) in salinity tolerance among Baltic Sea eelgrass populations. Whether these differences are due to genetic divergence remain yet unsolved, but the results suggest that Baltic eelgrass populations might have developed into different ecotypes.

Seagrass populations from different locations can differ in their stress and recovery responses (Bergmann et al. 2010) and local adaptation has been shown to increase e.g. seagrass biomass (Hämmerli & Reusch 2002). Adaptation to the local environmental conditions might enhance the probability of a community to adapt to further changes (Reusch 2014). For example, the optimum temperature of different seagrass populations is partly dependent on the natural temperature regime experienced by those populations (Lee et al. 2007). Studies by Franssen et al. (2011) and Winters et al. (2011) suggest that previous adaptation to high temperatures can increase the chance of recovery after thermal stress. Similarily, the higher tolerance to hyposaline conditions in low saline eelgrass populations (III) suggest that these populations might have the capacity to adapt to future climate change induced declines in salinity. As the selection pressure and, thus, likely the adaptation of populations in the Baltic Sea has been towards tolerance of hyposalinity and not e.g. high temperatures, it is difficult to predict whether these populations are plastic enough to cope with all the future changes in their environment.

3.2.4 Plant communities

Plant community composition can affect community performance (e.g. IV, Kautsky 1991, Gustafsson & Boström 2010, 2012) and while presence of eelgrass may contribute significantly to the total biomass of a plant community, presence of other species, such as *P. filiformis*, can lead to increased shoot density (IV). In addition to impacts on plant community *per se* (IV, Gustafsson & Boström 2010), community composition can also affect other trophic levels, such as density and biomass of epifauna (Gustafsson & Boström 2009). Community performance depends on the abundance of different species (Kautsky 1991) and loss or decline of perennial habitat forming species in coastal communities can have tremendous impacts (Lilley & Schiel 2006). Any changes in seagrass communities are thus likely to affect the whole ecosystem. Accordingly, losses in eelgrass habitats have resulted in e.g. large changes in fish communities (Pihl et al. 2006).

Mixed meadows formed by eelgrass growing together with angiosperms with freshwater origin are common in the Northern Baltic Sea (IV, Boström et al. 2014). The expected future alterations in salinity (Meier 2006) are expected to induce changes in the species composition and distribution (Dippner et al. 2008). Decreased salinity is likely to increase the distribution and competitive ability of freshwater angiosperms, while growth of marine eelgrass might be suppressed both due to decreased salinity (III) and potentially by enhanced competition with freshwater species. On the other hand, presence of freshwater angiosperms in seagrass habitats has been recorded to enhance resistance of eelgrass to stress (Gustafsson & Boström 2013). Thus there is a chance that altered salinity conditions could boost such positive impacts induced by freshwater species growing together with eelgrass.

3.3 Climate change and eelgrass

Predicting future climate is very difficult and a variety of different climate scenarios have been suggested to Baltic Sea area alone (Meier 2006, HELCOM 2007, Graham et al. 2008, Meier et al. 2012). Climate scenarios and the magnitude of changes in them may vary a lot depending on the applied models. In general, climate change is expected to increase CO_2 levels and decrease pH of the water and alter the availability of inorganic carbon species. Other expected future changes are e.g. increased water temperature, altered salinity, decreased sea ice-cover, increased wave activity and an increased frequency in storm events (Meier 2006, HELCOM 2007, Graham et al. 2008, Doney et al. 2012). The increase in precipitation and run-off is likely to raise nutrient inputs into the Baltic Sea (HELCOM 2007). This consequently increases the frequency and intensity of phytoplankton blooms and, thus, alter the light climate. Thus, climate change is threatening to affect many environmental factors that also determine seagrass productivity and survival.

The impacts of single stressors are relatively easy to predict (I-III). For example, decreases in irradiance and salinity levels and and increases in water temperature are likely to reduce seagrass productivity (I-III). As combination of stressors may have e.g. negative synergistic impacts on organisms and result in larger stress effect than expected based on the effect of singular stressors (II), the combined effect of several alterations in coastal environment are much more difficult to predict. As climate change is also expected to increase the variability and decrease predictability of temporal changes in environment (Meier 2006, HELCOM 2007, Graham et al. 2008, Doney 2012), predicting future effects in response to changes in multiple environmental parameters is close to impossible.

All effects of climate change are, however, not necessarily degrading eelgrass performance. Higher seawater temperature may have a positive impact on eelgrass. Increased productivity and prolonged growth season at high latitudes can enable eelgrass to expand its distribution northwards where it is likely to provide new habitats in previously unvegetated areas (Clausen et al. in press). Changes in species range might also induce changes to the other end of the range. While eelgrass might be able to escape increasing temperatures northwards, the southern limit of the range distribution might suffer from losses if these populations cannot adjust to the altered conditions. Southern eelgrass populations have been shown to be better adapted to heat waves compared to more central populations (Franssen et al. 2011, Winters et al. 2011). Thus, the southern populations might have the capacity to adapt to the future conditions and sustain their current distribution. Future changes are, nevertheless, likely to decrease the productivity in these populations, especially during the peak heat season (Lee et al. 2007).

Some of the climate change impacts might help eelgrass to overcome other changes. In some areas seagrass productivity is limited by low inorganic carbon concentration (Lee et al 2007). Increase in seawater CO_2-concentration and change in pH is hypothesized to increase seagrass productivity in the future (Hall-Spencer et al. 2008, Kroeker et al. 2010) as both of these changes increase the availability of inorganic carbon species preferred by seagrasses. Small changes in the combined effects of salinity, temperature and light levels might be enough to reduce seagrass productivity (I-III) and yield a negative annual carbon balance. Increased CO_2 levels could thus allow seagrasses to boost their annual production and increase their carbon storages to increase survival and stress tolerance. The positive impacts of increased CO_2 levels might disappear during high water temperatures

(Eklöf et al. 2012), but could still help maintain positive annual net production.

Whether a population can adapt to the altered conditions depends – as mentioned previously - on the plasticity and the adaptive capacity of the genotypes in that population. While differences in genotypes, life stages and populations lower the predictability of community stress effects and responses, the same differences increase the possibility that some of the individuals or populations might survive future stress events. Fitness-related traits that increase the capacity of organisms to tolerate extreme environmental stress are expected to become more important in the future due to the climate chance (Jump & Peñuelas 2005). While genotypic differences are needed for adaptive evolution to take place, high genetic variability in a population does not necessarily guarantee that the variability is large enough for the population to keep up with the environmental change (Jump & Peñuelas 2005). There are also alternative ways of transferal of phenotypic traits besides DNA-encoded adaptive evolution. An example of such nongenetic carry-over effects is adaptation through trans-generational plasticity where the mother population acclimatizes itself and simultaneously “pre-adapts” the offspring to altered environmental conditions, e.g. increased temperature (Reusch 2014). These new tolerance ranges can then be carried over to the new generation. Such trans-generational plasticity is likely to be many times faster than “traditional” adaptive evolution (Reusch 2014). Unfortunately no studies assessing commonness of such carry over effects in seagrasses exist. Regardless, if the climate change exceeds the limits of plasticity of an organism, mortality selection will take place. Thus, it is likely that some local extinctions of seagrass populations and loss of genes will take place in the near future.

3.4 Future of eelgrass populations in the Baltic Sea

Eelgrass populations in the Baltic are already experiencing high levels of stress due to e.g. low salinity, low inorganic carbon concentrations (Hellbom & Björk 1999) and eutrophication (Baden et al. 2003, Krause-Jensen et al. 2008). Climate change is likely to increase stress both for eelgrass and other benthic organisms. While the co-occurring plant species may (Gustafsson & Boström 2010, 2013) or may not (IV) enhance productivity and resilience in eelgrass meadows, increased clonal growth (Reusch & Boström 2011) and low genetic diversity (Olsen et al. 2004, Johanneson & André 2006, Reusch & Boström 2011) minimize the possibility of evolutionary rescue of Baltic eelgrass populations. Increased clonality may increase the vulnerability of seagrass populations in marginal ecosystems (Procaccini et al. 2007). The low genetic diversity could thus indicate increased possibility of local extinctions of eelgrass populations in the near future.

Decline in eelgrass abundance can impact seagrass associated fauna and vegetation negatively (Heck et al. 2008 and references therein). Marine fauna in the Baltic are often generalists, and equal abundances of fauna are found in other macrophyte stands (e.g. *Fucus vesiculosus, P. perfoliatus* and *P. pectinatus*) compared to eelgrass beds (Gustafsson & Boström 2009, Gustafsson & Salo 2012). Thus, it is possible that other macrophyte species providing and sustaining same ecosystem services could take over the empty niche in case of local eelgrass extinctions take place. Due to low species richness of macrophytes in the Baltic that are capable to colonize sandy sediments in high salinity, such shift in macrophyte species would not be likely in all areas.

There are theories hypothesizing that populations, which experience stress levels close to their tolerance limit might be better in adapting to further alterations in environment compared to more central populations (Reusch 2014 and references therein). The Baltic populations have been suggested to harbor genes specialized to the challenging environment (Johanneson & André 2006). This together with previous rapid evolution in the Baltic Sea (Johanneson et al. 2011) suggest that it is not completely impossible that some Baltic eelgrass populations could be able to adapt or acclimatize to future conditions in the Baltic Sea.

4. MANAGEMENT IMPLICATIONS

Costal habitats are one of the key ecosystems controlling the impacts of climate change. Seagrass meadows function as natural CO_2-sinks (Fourqurean et al. 2012). Thus, protecting seagrass habitats and restoring lost seagrass meadows could reduce global CO_2 levels and alleviate climate change effects. Variability in storminess, wave activity and other physical forces is expected to increase in the future due to the climate change (Doney et al. 2012). Seagrass meadows also reduce current velocities and wave attenuation (reviewed by Koch 2001), stabilize sediments and control for coastal erosion (Hemminga & Duarte 2000). They are thus one of the key organism groups alleviating possible increase in storm impacts on coastal areas (Duarte et al. 2013). Thus, compared to seagrass restoration costs (see below), it would be logical and a relatively inexpensive option to protect these valuable plant communities.

Even though the species and genetic diversity for many organisms is lower in the Baltic compared to more central populations, some of the populations are likely to possess unique genes and genotypes specifically adapted to the Baltic Sea (Johanneson & André 2006). If Baltic eelgrass populations would go extinct, the likelihood for an equivalent habitat forming species taking over eelgrass meadows in marginal ecosystem with very low species diversity is extremely small (Johanneson et al. 2011). Emphasis should therefore be put on the conservation and protection of these isolated and unique seagrass populations. Conservation efforts should aim to secure sufficient variability in the gene pool in eelgrass populations both to enhance viability of eelgrass populations and to increase possibility for future adaptations in the changing environment. Due to the high isolation by distance in eelgrass (Olsen et al. 2004, Johanneson & André 2006) conservation efforts should be implemented simultaneously regionally and between regions taking into account population connectivity (Procaccini et al. 2007).

Seagrass restoration is expensive and restoration efforts do not often succeed as well as planned (Paling et al. 2009), why preserving existing seagrass populations is recommended. If seagrass meadows are restored, special emphasis should be put on choosing the donor population. While the donor population should be adapted to similar conditions to increase the success ratio of the restoration efforts (van Katwijk et al. 2009), care should be taken not to introduce “alien” genes to a population. Introduced genotypes might be a risk for the viability of the locally adapted populations as new gene flow might reduce the number of ”pre-adapted” genes in the population (Johanneson & André 2006, Procaccini et al. 2007). Such artificial change in gene pool can lead to loss of adaptations and genetic variation and cause changes in population composition and structure (Laikre et al. 2010), which could be hazardous in a marginal system like the Baltic Sea.

5. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The results from this thesis suggest that stress effect is not only highly dependent on the stressor and the level of stress, but the response to stress might vary substantially among genotypes, life stages and populations. Combining several stressors and different levels of stressors decreases the predictability of plant responses. Climate change is expected to alter shallow coastal environments in many ways simultaneously increasing the stochasticity in environmental conditions (e.g. Doney et al. 2012). Thus, predicting future changes and responses in seagrass ecosystems is very challenging. For example, while species diversity has been observed to increase resistance to stress (Gustafsson & Boström 2013), it is unknown how and whether decrease in salinity will impact these biotic interactions, and thorough holistic studies are needed in order to elucidate these aspects.

Due to the multitude of expected changes, future research should include studies with multiple stressors and instead of only concentrating on stable stress level, the effects of increase in variability should also be assessed. Further, estimating whether plasticity and adaptive capacity in seagrass populations are large enough to cope with future changes brings new challenges to the question of future status of seagrass meadows. Differing stress tolerance among genotypes (I) and populations (II) indicate that Baltic eelgrass populations harbor some genetic variability. However, whether the level of variability is large enough is still unknown. The work assessing evolutionary adaptation and evolutionary rescue in seagrasses is still very much non-existent (Reusch 2014). Thus, experiments spanning over several generations are needed to assess the rate of adaptation and likelihood for evolutionary rescue in seagrasses in changing environments.

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REFERENCES

Andersen JH, Axe P, Backer H, Carstensen J, Claussen U, Fleming-Lehtinen V, Järvinen M, Kaartokallio H, Knuuttila S, Korpinen S, Kubiliute A, Laamanen M, Lysiak-Pastuszak E, Martin G, Murray C, Møhlenberg F, Nausch G, Norkko A, Villnäs A (2011) Getting the measure of eutrophication in the Baltic Sea: towards improved assessment principles and methods. Biogeochemistry 106: 137-156.

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E. Plymouth, UK.

Baden S, Gullström M, Lundén B, Pihl L, Rosenberg R (2003) Vanishing seagrass (*Zostera marina*, L.) in Swedish coastal waters. Ambio 32: 374-377.

Bergmann N, Winters G, Rauch G, Eizaguirre C, Gu J, Nelle P, Fricke B, Reusch TBH (2010) Population-specificity of heat stress gene induction in northern and southern eelgrass *Zostera marina* populations under simulated global warming. Molecular Ecol 19: 2870-2883.

Biber PD, Kenworthy WJ, Paerl HW (2009) Experimental analysis of the response and recovery of *Zostera marina* (L.) and *Halodule wrightii* (Ascher.) to repeated light-limitation stress. J Exp Mar Biol Ecol 396: 110-117.

Borenstein M, Hedges LV, Higgins JPT (2009) Introduction to meta-analysis. Wiley, NJ, USA.

Boström C, Baden S, Krause-Jensen D (2003) The seagrasses of Scandinavia and the Baltic Sea. In: Green EP, Short FT, Spalding MD (eds.) World atlas of seagrasses: present status and future conservation. California Press.

Boström C, Baden S, Bockelmann A-C, Dromph K, Fredriksen S, Gustafsson C, Krause-Jensen D, Möller T, Nielsen SL, Olesen B, Olsen J, Pihl L, Rinde E (2014) Distribution, structure and function of Nordig eelgrass (Zostera marina) ecosystems: implications for coastal management and conservation. Aquatic Conserv Mar Freshw Ecosyst DOI: 10.1002/acq.2424

Cardinale BJ, Palmer MA, Collins SL (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415: 426-429.

Clausen KK, Krause-Jensen D, Olesen B, Marba N. Seasonality of eelgrass across gradients in temperature and latitude. Mar Ecol Prog Ser. In press. DOI: 10.3354/meps1080

Collier CJ, Lavery PS, Ralph PJ, Masini RJ (2009) Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. J Exp Mar Biol Ecol 370: 89-103.

De Cock AWAM (1981) Influence of temperature and variations in temperature on flowering in *Zostera marina* L. under laboratory conditions. Aquat Bot 10: 125-131.

Delefosse M, Kristensen E (2012) Burial of *Zostera marina* seeds in sediment inhabited by three polychaetes: laboratory and field studies. J Sea Res 71: 41-49.

Den Hartog C (1970) The sea-grasses of the world. North Holland Publ, Amsterdam.

Den Hartog C (1987) "Wasting disease" and other dynamic phenomena in *Zostera* beds. Aquat Bot 27: 3-14.

Dennison WC (1987) Effects of light on seagrass photosynthesis, growth and depth distribution. Aquat Bot 27: 15-26.

Dennison WC, Orth RJ, Moore KA, Stevenson JC, Carter V, Kollar S, Bergström PW, Batiuk RA (1993) Assessing water quality with submersed aquatic vegetation - Habitat requirements as barometers of Chesapeake Bay healt. Bioscience 43: 86-94.

Diaz-Almela E, Marbà N, Duarte CM (2007) Consequences of Mediterranean warming events in seagrass (*Posidonia oceanica*) flowering records. Global Change Biol 13: 224-235.

Dippner JW, Vuorinen I, Daunys D, Flinkman J, Halkka A, Köster FW, Lehikoinen E, MacKenzie BR, Möllmann C, Møhlenberg F, Olenin S, Schiedek D, Skov H, Wasmund N (2008) Climate-related marine ecosystem change. In: Bolle H-J, Menenti M, Rasool I (eds.). Assessment of Climate Change for the Baltic Sea Basin. Springer. Berlin.

Doney SC, Ruckelshaus M, Duffy JE, Barry JB, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais NN, Sydeman WJ, Talley LD (2012) Climate change impacts on marine ecosystems. Annu Rev Mar Sci 4: 11-37.

Drouin A, McKindsey CW, Johnson LE (2012) Detecting the impacts of notorious invaders: experiments versus observations in the invasion of eelgrass meadows by the green seaweed *Codium fragile*. Oecologia 168: 491-502.

Duarte CM (1991) Seagrass depth limits. Aquat Bot 40: 363-377.

Duarte CM (2000) Marine biodiversity and ecosystem services: an elusive link. J Exp Mar Biol Ecol 250: 117-131.

Duarte CM, Chiscano CL (1999) Seagrass biomass and production: a reassessment. Aquat Bot 65: 159-174.

Duarte CM, Terrados J, Agawin NSR, Fortes MD (2000) An experimental test of the occurrence of competitive interactions among SE Asian seagrasses. Mar Ecol Prog Ser 197: 231-240.

Duarte CM, Losada IJ, Hendriks IE, Mazarrasa I, Marbà N (2013) The role of coastal plant communities for climate change mitigation and adaptation. Nature Climate Change 3: 961-968.

Duffy JE (2006) Biodiversity and the functioning of seagrass ecosystems. Mar Ecol Prog Ser 311: 233-250.

Ehlers A, Worm B, Reusch TBH (2008) Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. Mar Ecol Prog Ser 355: 1-7.

Eklöf JS, Alsterberg C, Havenhand JN, Sundbäck K, Wood HL, Gamfeldt L (2012) Experimental climate change weakens the insurance effect of biodiversity. Ecol Lett 15: 864-872.

Fonseca MS, Kenworthy WJ (1987) Effects of current on photosynthesis and distribution of seagrasses. Aquat Bot 27: 59-78.

Fourqurean JW, Powell GVN, Kenworthy WJ, Zieman JC (1995) The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. Oikos 72: 349-358.

Fourqurean JW, Duarte CM, Kennedy H, Marbà N, Holmer M, Mateo MA, Apostolaki ET, Kendrick GA, Krause-Jensen D, McGlathery K, Serrano O (2012) Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience 5: 505-509..

Franssen SU, Gu J, Bergmann N, Winters G, Klostermeier UC, Rosenstiel P, Bornberg-Bauer E, Reusch TBH (2011) Transcriptomic resilience to global warming in the seagrass *Zostera* marina, a marine foundation species. PNAS 108: 19276-19281.

Gamfeldt L, Lefcheck JS, Byrnes JEK, Cardinale BJ, Duffy JE, Griffin JN (2014) Marine biodiversity and ecosystem functioning: what's known and what's next? PeerJ, doi: 10.7287/peerj.preprints.249v1

George DG, Edwards RW (1976) The effect of wind on the distribution of chlorophyll a and crustacean plankton in a shallow eutrophic reservoir. J Appl Ecol 13: 667-690.

Graham IP, Chen D et al. (2008) Projections of future anthropogenic climate change. In: Bolle H-J, Menenti M, Rasool I (eds.). Assessment of Climate Change for the Baltic Sea Basin. Springer. Berlin.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111: 1169-1194.

Greve TM, Krause-Jensen D, Rasmussen MB, Christensen PB (2005) Means of rapid eelgrass (*Zostera marina* L.) recolonisation in former dieback areas, Aquat Bot 82: 143-156.

Gruner DS, Smith JE, Seabloom EW, Sandin SA, Ngai JT, Hillebrand H, Harpole WS, Elser JJ, Cleland EE, Bracken MES, Borer ET, Bolker BM (2008) A cross-system synthesis of consumer and nutrient resource control on producer biomass. Ecol Lett 11: 740-755.

Gustafsson C (2013) Biodiversity and ecosystem functioning in angiosperm communities in the Baltic Sea. PhD-thesis, Åbo Akademi University, Åbo, Finland.

Gustafsson C, Boström C (2009) Effects of plant species richness and composition on epifaunal colonization in brackish water angiosperm communities. J Exp Mar Biol Ecol 382: 8-17.

Gustafsson C, Boström C (2010) Biodiversity influences ecosystem functioning in aquatic angiosperm communities. Oikos 120: 1037-1046.

Gustafsson C, Salo T (2012) The effect of patch isolation on epifaunal colonization in two different seagrass ecosystems. Mar Biol 159: 1497-1507.

Gustafsson C, Boström C (2013) Influence of neighboring plants on shading stress resistance and recovery of eelgrass, *Zostera marina* L. PLOSone 8: 1-11.

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia M-C (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454: 96-99.

Heck KL Jr., Carruthers TJB, Duarte CM, Hughes AR, Kendrick G, Orth RJ, Williams SW (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11: 1198-1210.

Hector A, Loreau M, Schmid B, and the BIODEPTH project (2002) Biodiversity manipulation experiments: studies replicated at multiple sites. In: Loreau M, Naeem S, Inchausti P (Eds.) Biodiversity and ecosystem functioning – synthesis and perspectives. Oxford University Press, New York.

HELCOM (2007) Climate change in the Baltic Sea Area – HELCOM Thematic Assessment in 2007. Balt. Sea Environ. Proc. No. 111.

Hellbom F, Björk M (1999) Photosynthetic response in *Zostera marina* to decreaseing salinity, inorganic carbon content and osmolality. Aquat Bot 65: 97-104.

Hellebust JA (1976) Osmoregulation. Ann Rev Plant Phys 27: 485-505.

Hemminga MA, Duarte CM (2000) Seagrass ecology. Cambridge University Press, Cambridge, UK.

Holmer M, Fredriksen MS, Møllegaard H (2005) Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. Aquat Bot 81: 367-379.

Hootsmans MJM, Vermaat JE, Van Vierssen W (1987) Seed-bank development, germination and early seedling survival of two seagrass species from the Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem.. Aquat Bot 28: 275-285.

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. PNAS 101: 8999-9002.

Hughes AR, Stachowicz JJ (2009) Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. Ecology 90: 1412-1419.

Hughes AR, Stachowicz JJ, Williams SL (2009) Morphological and physiological variation among seagrass (Zostera marina) genotypes. Oecologia 159:725-733.

Hämmerli A, Reusch TBH (2002) Local adaptation and transplant dominance in genets of the marine clonal plant *Zostera marina*. Mar Ecol Prog ser 242: 111-118.

Jarvis JC, Moore KA (2010) The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, *Zostera marina* populations following a large-scale decline. Hydrobiologia 649: 55-68.

Johannesson K, André C (2006) Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Mol Ecol 15: 2013-2029.

Johanneson K, Smolarz K, Grahn M, André C (2011) The future of Baltic Sea populations: local extinction or evolutionary rescue? AMBIO 40: 179-190.

Jump AS, Peñuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecol Lett 8: 1010-1020.

van Katwijk MM, Bos AR, de Jonge VN, Hanssen LSAM, Hermus DCR, de Jong DJ (2009) Guidelines for seagrass restoration: importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. Mar Poll Bull 58: 179-188.

Kautsky L (1991) In situ experiments on interrelationships between six brackish macrophyte species. Aquat Bot 39: 159-172.

Kingsolver JG, Izem R, Ragland GJ (2004) Plasticity of size and growth in fluctuating thermal environments: comparing reaction norms and performance curves. Integr Comp Biol 44: 450-460.

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. Annu Rev Plant Phys Plant Mol Biol 40: 21-53.

Koch EW (2001) Beyond light: physical, geological and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24: 1-17.

Krause-Jensen D, Sagert S, Schubert H, Boström C (2008) Empirical relationships linking distribution and abundance of marine vegetation to eutrophication. Ecol Indic 8: 515-529.

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13: 1419-1434.

Laikre L, Schmartz MK, Waples RS, Ryman N, the GeM working group (2010) Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. Trends Ecol Evol 25: 520-529.

Lee K-S, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. J Exp Mar Biol Ecol 350: 144-175.

Libes SM (1992) An introduction to marine biogeochemistry. John Wiley & Sons, Inc., New York.

Lichtenthaler HK (1996) Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol 148: 4-14.

Lilley SA, Schiel DR (2006) Community effects following the deletion of a habitat-forming alga from rocky marine shores. Oecologia 148: 672-681.

Loreau M (1998) Separating sampling and other effects in biodiversity experiments. Oikos 82: 600-602.

Loreau M (2000) Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91: 3-17.

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412: 72-76.

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation and recovery potential of estuaries and coastal seas. Science 312: 1806-1809.

Marba N, Hemminga MA, Mateo MA, Duarte CM, Mass YEM, Terrados J, Gacia E (2002) Carbon and nitrogen translocation between seagrass ramets. Mar Ecol Prog Ser 226: 287-300.

Marsh JA Jr, Dennison WC, Alberte RS (1986) Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). J Exp Mar Biol Ecol 101: 257-267.

Meier HEM (2006) Baltic Sea climate in the late twenty-first century: a dynamical downscaling approach using two global models and two emission scenarios. Climate Dynamics 27: 39-68.

Meier MHE, Müller-Karulis B, Andersson HC, Dieterich C, Eilola K, Gustafsson BG, Höglund A, Hordoir R, Kuznetsov I, Neumann T, Ranjbar Z, Savchuk OP, Schimanke S (2012) Impact of climate change on ecological quality indicators and biochemical fluxes in the Baltic Sea: a multi-model ensemble study. Ambio 41: 558-573.

Moore KA, Jarvis JC (2008) Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: implications for long-term persistence. J Coat Res 55: 135-147.

Moore KA, Shields EC, Parrish DB, Orth RJ (2012) Eelgrass survival in two contrasting systems: role of turbidity and summer water temperatures. Mar Ecol Prog Ser 448: 247-258.

Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev 82: 591-605.

Nejrup LB, Pedersen MF (2008) Effects of salinity and water temperature on the ecological performance of *Zostera marina*. Aquat Bot 88: 239-246.

Nelleman C, Corcoran E, Duarte CM, Valdés L, De young C, Fonseca L, Grimsditch G (Eds) (2009) Blue Carbon. A rapid response assessment. United Nations Environment Programme.

Olesen B (1999) Reproduction in Danish eelgrass (*Zostera marina* L.) stands: size-dependence and biomass partitioning. Aquat Bot 65: 209-219.

Olesen B, Sand-Jensen K (1994) Patch dynamics of eelgrass *Zostera marina*. Mar Ecol Prog Ser 106: 147-156.

Olsen JJ, Stam WT, Coyer JA, Reusch TBH, Billingham M, Boström C, Calvert E, Christie H, Granger S, La Lumière R, Milchakova N, Oudot-Le Secq M-P, Procaccini G, Sanjabi B, Serrão E, Veldsink J, Widdicombe S, Wyllie-Echeverria S (2004) North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. Mol Ecol 13: 1923-1941.

Orth RJ, Heck KL, Montfrans J (1984) Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator.prey relationships. Estuaries 7: 339-350.

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck KL Jr., Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. BioScience 56: 987-996.

Paling EI, Fonseca M, van KAtwijk MM, van Keulen (2009) Seagrass restoration. Coastal wetlands: an integrated ecosystem approach: 687-713.

Phillips RC, McMillan C, Bridges KW (1983) Phenology of eelgrass, *Zostera marina* L., along latitudinal gradients in North America. Aquat Bot 15: 145-156.

Pihl L, Baden S, Kautsky N, Rönnbäck P, Söderqvist T, Troell M, Wennhage H (2006) Shift in fish assemblage structure due to loss of seagrass *Zostera marina* habitats in Sweden. Esc Coast Shelf Sci 67: 123-132.

Proccaccini G, Olsen J, Reusch T (2007) Contribution of genetics and genomics to seagrass biology and conservation. J Exp Mar Biol Ecol 350: 234-259.

Pulido C, Borum J (2010) Eelgrass (*Zostera marina*) tolerance to anoxia. J Exp Mar Biol Ecol 358:8-13.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, UK.

Rasmussen JR, Olesen B, Krause-Jensen D (2012) Effects of filamentous macroalgae mats on growth and survival of eelgrass, *Zostera marina*, seedlings. Aquat Bot 99: 41-48.

Rasmussen JR, Pedersen MFP, Olesen B, Nielsen SL, Pedersen TM (2013) Temporal and spatial dynamics of ephemeral drift-algae in eelgrass, *Zostera marina*, beds. Est Coasts Shelf Sci 119: 167-175.

Raun AL, Borum J (2013) Combined impact of water column oxygen and temperature on internal oxygen status and growth of *Zostera marina* seedlings and adult shoots. J Exp Mar Biol Ecol 441: 16-22.

Reusch TBH (2014) Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. Evol Applications 7: 104-122.

Reusch TBH, Boström C (2011) Widespread genetic mosaicism in the marine angiosperm *Zostera marina* is correlated with clonal reproduction. Evol Ecol 25: 899-913.

Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. PNAS 102: 2826-2831.

Rosen BR (1981) The tropical high diversity enigma – the corals'-eye view. In: Forey PL (Ed.), The evolving biosphere. British museum (Natural History), Cambridge University Press.

Sand-Jensen K, Borum J (1991) Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat Bot 41: 137-175.

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138: 32.

Sokal RR, Rolf FJ (1994) Biometry: the principles and practice of statistics in biological research. 3rd ed. WH Freeman, New York.

Stachowicz JJ, Bruno JF, Duffy JE (2007) Understanding the effects of marine biodiversity on communities and ecosystems. Annu Rev Ecol Evol Syst 38: 739-766.

Thorhaug A, Richardson AD, Berlyn GP (2006) Spectral reflectance of *Thalassia testudinum* (Hydrocharitaceae) seagrass: low salinity effects. Am J Bot 93: 110-117.

Tomas F, Abbott JM, Steinberg C, Balk M, Williams SL, Stachowicz JJ (2011) Plant genotype and nitrogen loading influence seagrass productivity, biogeochemistry, and plant-herbivore interactions. Ecology 92: 1807-1817.

Touchette BW (2007) Seagrass-salinity interactions: physiological mechanisms used by submersed marine angiosperms for a life at sea. J Exp Mar Biol Ecol 350: 194-215.

Touchette BW, Burkholder JM (2007) Carbon and nitrogen metabolism in the seagrass, *Zostera marina* L.: environmental control of enzymes involved in carbonallocation and nitrogen assimilation. J Exp Mar Biol Ecol 350: 216-233.

Valdemarsen T, Canal-Vergés P, Kristensen E, Holmer M, Kristiansen MD, Flindt MR (2010) Vulnerability of *Zostera marina* seedlings to physical stress. Mar Ecol Prog Ser 418: 119-130.

Valentine JF, Heck KL Jr. (1999) Seagrass herbivory: evidence for the continued grazing of marine grasses. Mar Ecol Prog Ser 176: 291-302.

Vergeer LHT, den Hartog C (1994) Omnipresence of Labyrinthulaceae in seagrasses. Aquat Bot 48: 1-20.

Villazán B, Pedersen MF, Brun FG, Vergara JJ (2013) Elevated ammonium concentrations and low light form a dangerous synergy for eelgrass *Zostera marina*. Mar Ecol Prog Ser 493: 141-154.

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Cardinale A, Fourqurean JW, Heck KL Jr., Hugher AR, Kendrivk GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. PNAS 106: 12377-12381.

Winters G, Nelle P, Fricke B, Rauch G, Reusch TBH (2011) Effects of simulated heat wave on photophysiology and gene expression of high- and low-latitude populations of *Zostera marina*. Mar Ecol Prog Ser 435: 83-95.

From genes to community: stress tolerance in eelgrass (*Zostera marina*)

This phd-thesis compares the effects of stress on different eelgrass genotypes, life-stages and populations and estimates the importance of community composition and species diversity in submerged plant communities for production. The thesis further compares the importance of different singular and multiple stressors on eelgrass survival and productivity. The results suggest that stress responses vary both on genotype, life stage and population level, while species diversity may not have notable impact on community production. While the stress responses depend on the stressor and the level of stress, multiple stressors can cause more severe (negative synergistic) impacts compared to singular stressors.

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