

Seagrass Wasting Disease

Impact of abiotic factors and chemical defense

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"I know more than many, but still only just a little."

- Patrick Rothfuss

ABSTRACT

Eelgrass, *Zostera marina*, is the dominant seagrass species in the northern hemisphere, and forms large meadows that provide several important ecosystem services. In the 1930s about 90% of the *Z. marina* populations in the Atlantic Ocean were killed in an epidemic caused by the wasting disease pathogen *Labyrinthula zosterae*. Despite the devastating impact of this disease the host-pathogen interaction is still poorly understood, and there is limited knowledge about factors that influence the disease. In this thesis I study how abiotic factors can impact the relationship between *Z. marina* and *L. zosterae*, and investigate if the seagrass can defend itself against the pathogen through production of inhibitory compounds.

In two field studies I investigated if laboratory results, showing that low salinity mitigate *L. zosterae* infection, can be translated to the field. Surveys along the salinity gradient of the Swedish coast show that *L. zosterae* infection is high in high salinity areas (20 - 25 PSU), and that infection generally decreases or disappears at low salinity (<10 PSU). These findings suggest that low salinity areas can act as a refuge against seagrass wasting disease. However, in contrast to previous studies, salinities as low as 6 PSU do not always exclude infection in *Z. marina* meadows. The disease was detected, in low concentrations through DNA-based methods, in several meadows in the Baltic proper. Thus, it is possible that a *L. zosterae* strain has adapted to the low salinity environment of the Baltic Sea. This could lead to an increased distribution of the pathogen in areas previously considered to be protected from the pathogen, and potentially result in wasting disease outbreaks also in low salinity *Z. marina* meadows.

One way for *Z. marina* to withstand infection by *L. zosterae* is to be chemically defended through the production of secondary metabolites. All *Z. marina* shoots investigated in this thesis produced chemical compounds that inhibited *L. zosterae* growth. Laboratory experiments implied that the production is not induced by a high pathogen pressure, as previously suggested, but rather that *Z. marina* contains inhibitory compounds that function as a constitutive defense. Chemical analyses further detected three partially purified inhibitory fractions against the pathogen, and characterization of the compounds showed that these are new, yet unknown, compounds from *Z. marina*.

A laboratory infection experiment was performed to investigate how individual and interactive effects of extrinsic factors affect *L. zosterae* infection in *Z. marina*. The results showed a complex host-pathogen interaction, with several interactions between light, temperature, tissue damage, and infection that affected growth, production of inhibitory compounds, and gene expression in *Z. marina*. Overall, light intensity had the strongest effect on wasting disease, where reduced light intensity resulted in a significant increase in lesion coverage.

KEYWORDS: Seagrass wasting disease, *Zostera marina*, *Labyrinthula zosterae*, eelgrass, chemical defense, host-pathogen interaction, abiotic factors, infection

POPULÄRVETENSKAPLIG SAMMANFATTNING

Ålgräs (*Zostera marina*) är en marin fröväxt som bildar stora undervattensängar på grunda mjukbottnar och är den dominerande sjögräsarten på norra halvklotet och så även i Sverige. Dessa ängar bildar habitat för många organismer och är en speciellt viktig uppväxtmiljö för fiskar och ryggradslösa djur. Ålgräsängar bidrar med flera så kallade ekosystemtjänster, det vill säga tjänster som bidrar till människans välbefinnande. Bland annat så reducerar ålgräsängarna vattenrörelser och skyddar därmed kusten mot havets vågkraft. Ålgräsets rotsystem stabiliserar bottensedimentet och minskar resuspension av partiklar i vattenmassan, vilket ger klarare vatten. Ålgräsängarna binder även stora mängder kol och hjälper därmed till att minska växthuseffekten.

De senaste decennierna har stora delar av de globala sjögräsbestånden minskat, till stor del på grund av mänsklig påverkan och globala förändringar. En ytterligare men mindre undersökt orsak till sjögräsets försvinnande är angrepp av parasitsjukdomar. På 1930-talet försvann cirka 90 % av allt ålgräs längs både den amerikanska och europeiska atlantkusten i en epidemi som tros ha orsakats av parasiten *Labyrinthula zosterae*. Infektion av denna mikroskopiska organism resulterar i svarta irreguljära fläckar på ålgräsets gröna blad och sjukdomen benämns allmänt som *seagrass wasting disease*. Trots parasitangreppets förödande inverkan så är kunskapen om vad som påverkar relationen mellan ålgräset och parasiten liten. I denna avhandling har jag genomfört flertalet fältundersökningar och laboratorieexperiment för att öka förståelsen om hur abiotiska faktorer (salthalt, temperatur, ljus och vävnadsskador) påverkar interaktionen mellan ålgräset och *Labyrinthula*. Jag har även studerat om ålgräset kan motverka dessa parasitangrepp genom att producera kemiska försvarsämnen.

Tidigare laborativa studier har visat att låg salthalt kan bromsa infektionsgraden av *Labyrinthula* och i denna avhandling har jag undersökt om liknande resultat även kan observeras för olika salthaltsmiljöer ute i naturen. Undersökningarna skedde utmed den svenska kusten vars naturliga salthaltsgradient sträcker sig från nästan full oceaniskt salthalt på västkusten till brackvatten på östkusten. Resultaten visade att infektionen var hög i hög salthalt (20 – 25 ‰), medan den generellt var låg eller helt försvann i låg salthalt (<10 ‰). Detta innebär att områden med låg salthalt möjligtvis kan fungera som en fristad från dessa parasitangrepp för ålgräs. I motsats till tidigare studier visar denna avhandling dock att låg salthalt inte alltid förhindrar infektion av *Labyrinthula*. Angripna ålgrässkott hittades i flertalet ålgräsängar i norra Östersjön i områden med en salthalt på cirka 6 ‰. Det är möjligt att en stam av *Labyrinthula* har anpassat sig

till den låga salthalten i Östersjön, något som potentiellt kan leda till nya utbrott av *wasting disease* i områden som tidigare antagits vara skyddade från parasiten.

Ett vanligt sätt för växter att motstå angrepp av parasiter är att producera kemiska försvarsämnen som motverkar parasiternas tillväxt och spridning. Alla ålgräsplantor undersökta i denna avhandling producerade ämnen som minskade tillväxten av *Labyrinthula* och experimentella studier visade att ålgräset producerar dessa ämnen vare sig om *Labyrinthula* är närvarande eller ej, och till lika stor mängd oberoende av infektionsgrad. Vidare kemiska analyser indikerade att ålgräs producerar åtminstone tre olika ämnen som alla hindrar tillväxten av *Labyrinthula*, och karaktärerna av dessa försvarsämnen tyder på att det är tidigare okända kemiska ämnen från ålgräs.

Infektionsexperiment utfördes för att undersöka hur individuella och interaktiva effekter av temperatur, ljus och vävnadsskador, som liknar de som orsakas av båtpropellrar, påverkar infektionen av *Labyrinthula*. Resultaten visar att relationen mellan ålgräset och parasiten är komplex och att flertalet interaktioner mellan de olika faktorerna påverkar tillväxten av ålgräs, dess produktion av försvarsämnen, och även uttrycket av gener associerade med immunförsvaret, primära metabolismen och fenolsyntesen. Ljusintensiteten hade den största inverkan på *wasting disease*, och plantor utsatta för lågt ljus hade i genomsnitt 35 % högre sjukdomsspridning jämfört med plantor i normalt ljus.

LIST OF PAPERS

This thesis is based on the following papers, which is referred to in the text by their roman numerals:

PAPER I: **Jakobsson-Thor S.**, Toth G.B., Brakel J., Bockelmann A-C., Pavia H. in press. Seagrass wasting disease varies with salinity and depth in natural *Zostera marina* populations. *Marine Ecology Progress Series*.

PAPER II: **Jakobsson-Thor S.**, Toth G.B., Pavia H. Seagrass wasting disease along a naturally occurring salinity gradient. *Manuscript*.

PAPER III: **Jakobsson-Thor S.**, Toth G.B., Brakel J., Pavia H. Effects of temperature, light and tissue damage on seagrass wasting disease in *Zostera marina*. *Manuscript*.

PAPER IV: **Jakobsson-Thor S.**, Poulin R.X., Toth G.B., Kubanek J., Pavia H. Chemical defense against the wasting disease pathogen *Labyrinthula zosterae* in eelgrass, *Zostera marina*. *Manuscript*.

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BACKGROUND

Seagrasses

Seagrasses are a polyphyletic group of marine vascular plants belonging to the order Alismatales (den Hartog & Kuo 2007). Seagrasses consists of about 60 different species worldwide divided in four families: Zosteraceae, Cymodoceaceae, Posidoniaceae, and Hydrocharitaceae (Les et al. 1997, den Hartog & Kuo 2007). However, there is still a debate whether the families Ruppiaceae and Zannichelliaceae, which can inhabit both marine and fresh water environments, also should be included (Waycott et al. 2007). Seagrasses have evolved from land plants to tolerate marine conditions and are the only group of vascular plants that successfully can live fully submerged in the saline environment of the ocean. Although seagrasses have evolved from different lineages, they have all been exposed to similar evolutionary pressure resulting in convergent evolution of multiple traits, e.g. salt tolerance, extensive root and rhizome systems, hydrophilous pollination, and gas exchange from the seawater through the leaf surface (e.g. Cox 1988, Kuo & den Hartog 2007, Olsen et al. 2016).

Seagrasses are important foundation species, forming extensive meadows on intertidal and subtidal soft bottom areas that host a large diversity of invertebrates and fish (e.g. Boström & Bonsdorff 1997, Jackson et al. 2001). Seagrass meadows are considered to be one of the most valuable ecosystems in the world (Costanza et al. 2014), and provide a multitude of ecosystem services (Nordlund et al. 2016), including coastal protection, sediment accumulation and stabilization (Barbier et al. 2011), acting as a nursery grounds for commercially important species of fish and shellfish (Orth et al. 1984), and constituting one of the most significant carbon sinks in coastal areas (Duarte et al. 2005).

The temperate seagrass *Zostera marina* L. (Fig. 1A), commonly known as eelgrass, is the dominant seagrass species in the northern hemisphere. *Z. marina* has a wide distributional range and can be found in coastal waters of the Pacific and Atlantic Ocean (Fig. 2, Green & Short 2003, Short et al. 2010). *Z. marina* meadows (Fig. 1B) grows from the intertidal down to 10 - 15 m depth (Borum & Greve 2004, Short et al. 2007, Boström et al. 2014), and the morphology of the plants vary within *Z. marina*'s distribution: plant size generally increases in the northern altitudes and in deeper meadows (Krause - Jensen et al. 2000, Short et al. 2007). In Sweden, where the studies in this thesis were performed, meadows are found in sites with salinities from 5 to 25 PSU (Boström et al. 2014), from high salinity waters on the west coast, to brackish environments in the Baltic Sea (Johannesson et al. 2011). The Swedish coast has a low tide (maximum 0.3 m)

and, as a result, *Z. marina* meadows grow subtidally. However, air pressure and wave action has an added affect on the sea level along the Swedish coast (Johannesson 1989) and extreme weather can result in an emergence of shallow growing shoots.

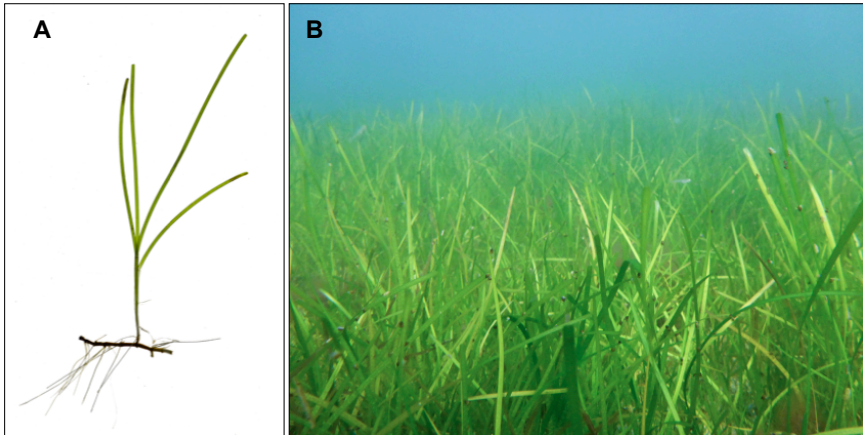


Figure 1. *Zostera marina* A) shoot, and B) meadow.



Figure 2. Worldwide distribution of *Zostera marina* marked in yellow (Short et al. 2010).

Over the last decades there has been a large decline of the *Z. marina* populations and other seagrass species (Waycott et al. 2009 and references within) due to multiple stressors such as sediment and nutrient runoff, physical disturbance,

invasive species, commercial fishing practices, aquaculture, overgrazing, algal blooms, global warming, and disease (Orth et al. 2006).

***Labyrinthula zosterae* and seagrass wasting disease**

Seagrasses are a general host to protists of the genus *Labyrinthula* (Vergeer & den Hartog 1994), where the pathogenic *Labyrinthula zosterae* D. Porter & Muehlstein, 1991 (Fig. 3A) causes black necrotic lesions on *Z. marina* leaves (Fig. 3B), commonly referred to as seagrass wasting disease (Muehlstein et al. 1991, 1992). *L. zosterae* is the believed agent of the 1930s wasting disease epidemic, resulting in an approximately 90% decline of the *Z. marina* populations along both the European and American coasts of the Atlantic Ocean (Petersen 1934, Renn 1935, Muehlstein 1989). In the 1980s new disease outbreaks occurred on the American east coast (Short et al. 1986, Short et al. 1988), and since then, studies have confirmed that *L. zosterae* is omnipresent in *Z. marina* meadows (Bockelmann et al. 2013, Groner et al. 2014, Groner et al. 2016).

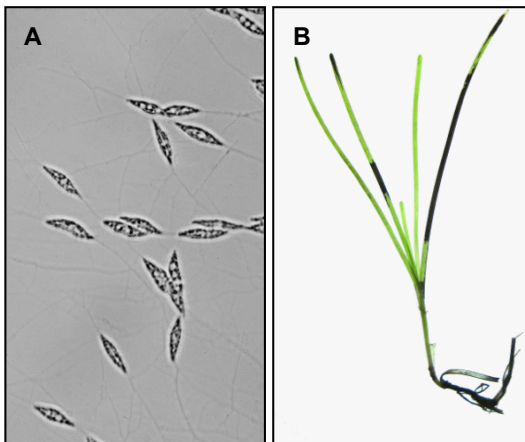


Figure 3.
Labyrinthula zosterae A) cells, and B) lesions on a *Zostera marina* shoot.

Labyrinthula. zosterae belongs to the class Labyrinthulomycetes, which consists of colony forming stramenopiles (Beakes et al. 2014). The classification of these organisms was for long questioned and their endophytic capacities along with biflagellate zoospores in some groups initially placed them among the Fungi. The genus *Labyrinthula* consists of several endophytic species. They are spindle shaped and excrete plasma membrane extensions called an ectoplasmic net via an organelle named the bothrosome (Porter 1972). The ectoplasmic net facilitate motility of *Labyrinthula* cells as well as digestion of organic material (Raghukumar 2002). The genus *Labyrinthula* has been found in terrestrial and aquatic environments, associated with both flora and fauna (Vergeer & den Hartog 1994, Raghukumar 2002, Bigelow et al. 2005, Wahid et al. 2007) and

several species of *Labyrinthula* have been detected in seagrasses around the world, but only a few are pathogenic in nature (Bockelmann et al. 2012, Martin et al. 2016). Both non-pathogenic and pathogenic species of *Labyrinthula* have been detected in *Z. marina*, and multiple species of *Labyrinthula* can exist in a *Z. marina* shoot simultaneously (Bockelmann et al. 2012).

Factors affecting *Labyrinthula zosterae* infection

The presence of *L. zosterae* cells does not always result in signs of the disease (e.g. Bockelmann et al. 2013, Burge et al. 2013). Opportunistic pathogens, like *L. zosterae*, are organisms that generally are omnipresent in the environment, and where infection only leads to severe disease under specific conditions, e.g. when the resistance by the host is low (Scheffer 1991, Burge et al. 2013). Due to *L. zosterae*'s potentially devastating effects on seagrass meadows, researchers have studied the underlying reasons for its pathogenicity, with the hope of discovering what made it become pandemic in the past, and the estimated likelihood of it happening again. The recurrent virulent outbreaks of *L. zosterae* may be due to weakening of the *Z. marina* plants from adverse environmental changes (e.g. Tutin 1938). Stressors caused by a changing environment, e.g. heat stress, reduced light and salinity, and lowered pH, are all factors that potentially can increase pathogen susceptibility for an organism (Chakraborty et al. 2000, Anderson et al. 2004).

Salinity

It is still not known what caused *L. zosterae* to become pandemic in the 1930s (Short et al. 1987), but many of the surviving or less effected *Z. marina* populations were found in areas with low salinity (e.g. Cottam 1935, Young 1943, Stevens et al. 1950, Rasmussen 1977, den Hartog 1987), leading to the hypothesis that low salinity mitigates the infection (Young III 1938, Young 1943). Several studies have therefore investigated whether variation in salinity affect the virulence of the pathogen (Young 1943, Short et al. 1986, Short et al. 1988, Giesen et al. 1990, Burdick et al. 1993), and it has been reported that *Labyrinthula spp.* growth is greatly reduced at salinities below 10 PSU (Young 1943, Muehlstein et al. 1988, Martin et al. 2009). Additionally, laboratory experiments have found a correlation between salinity and lesion coverage (Burdick et al. 1993), and a positive linear relationship between salinity and *L. zosterae* lesion area (McKone & Tanner 2009), further supporting the hypothesis that low salinity is a limiting factor for *L. zosterae* infection. A yearlong field survey showed that wasting disease increased during increasing salinity events in a *Z. marina* meadow in Great Bay, New Hampshire, USA, and that prolonged disease was sustained during salinities above 20 PSU (Burdick et al. 1993). The impact of *Labyrinthula sp.* disease on the subtropical seagrass *Thalassia*

testudinum K. D. Koenig, 1805 has also been found to be low in areas below 15 PSU (Blakesley et al. 2002), but to my knowledge quantitative measurements on how *L. zosterae* disease and pathogen abundance varies in different salinity environments among natural *Z. marina* populations is still missing, and was therefore investigated in **paper I** and **II** of this thesis.

Light

A correlation between *L. zosterae* infection rate and water depth has been detected in intertidal *Z. marina* meadows in the San Juan archipelago, USA (Groner et al. 2014). Shoots growing higher up in the intertidal region, and consequently with higher light availability, had a higher degree of *L. zosterae* infection compared to deeper populations. It is unclear if *L. zosterae* infection at different depths can be linked to light intensity due to the low number of experimental studies. So far infection by *Labyrinthula* sp. in *T. testudinum* has been negatively correlated with canopy light (Trevathan-Tackett et al. 2013), and a laboratory study by Vergeer et al. (1995) showed that an increase in irradiance decreased lesion coverage on *Z. marina*. However, the irradiance levels in Vergeer et al. (1995) were lower than the levels found in the *T. testudinum* meadows (Trevathan-Tackett et al. 2013). Whether *L. zosterae* infection is higher on *Z. marina* shoots at shallower depths in non-tidal systems, like the ones found in Sweden, is not known. This was investigated in **paper I** in the present thesis, along with the effect of light on *L. zosterae* infection (**paper III**).

Temperature

The influence of temperature on wasting disease dynamics has been poorly investigated, and most data on temperature effects are correlational (Rasmussen 1977, Giesen et al. 1990, Bull et al. 2012). For example, mass mortality of eelgrass caused by wasting disease in Denmark during the 1930s was connected to unusually high water temperatures (Rasmussen 1977). Furthermore, temperature correlates with *L. zosterae* abundance in natural *Z. marina* populations, and the pathogen is more active during warmer months of the year (Bockelmann et al. 2013). However, laboratory studies show that *Labyrinthula* sp. infection on the Mediterranean seagrass species *Cymodocea nodosa* Ascherson, 1870 and *Posidonia oceanica* Delile, 1813 decreases at higher temperatures (Olsen & Duarte 2015, Olsen et al. 2015), whereas a moderate temperature increase does not affect lesion size in *T. testudinum* (Bishop 2013). Additional laboratorial studies are needed to determine how increased temperature affects *L. zosterae* infection in *Z. marina*, and were investigated in **paper III** of this thesis.

Tissue damage

Tissue wounding caused by physical/mechanical damage is related to increased risk of pathogen infection in terrestrial plants (Maleck & Dietrich 1999), but this issue has not been addressed for seagrasses with one exception. In a study on *T. testudinum* in Florida Bay, Bowles and Bell (2004) showed that plants subjected to simulated herbivory were more likely to be infected by *Labyrinthula* sp. The Swedish northwestern coast is a popular recreational boating area, and large parts of the coastline are exploited by human construction, with 74% of the docks and 91% of the marinas located in *Z. marina* meadows (Eriander 2016). The effect of boat traffic and damage by boat propellers on *Z. marina* plants is therefore potentially high, and shoots with damaged leaves are frequent in some highly trafficked areas (personal observation). No study has, to my knowledge, investigated if the degree of *L. zosterae* infection in *Z. marina* increase in damaged shoots similar to the increase found in *T. testudinum* (Bowles & Bell 2004). This was investigated in **paper III**.

Plant defense

One way for *Z. marina* to withstand infection by *L. zosterae* is to be chemically defended through the production of secondary metabolites. Extracts and isolated compounds from several seagrass species show inhibitory effects against associated microorganisms and pathogens (Zidorn 2016). For example, extracts from two species (*Halodule beaudettei* den Hartog, 1964 and *Syringodium filiforme* Kützing, 1860) both deter a stramenopile pathogen (*Schizochytrium aggregatum* Goldstein & Belsky, 1964) related to *Labyrinthula* (Engel et al. 2006). Chemical defenses against *L. zosterae* are, however, still poorly understood and no specific defense compound against the pathogen has been identified. Phenolic acids have been the main focus in previous research on seagrass defenses against pathogenic *Labyrinthula* species (Buchsbaum et al. 1990, Vergeer et al. 1995, Vergeer & Develi 1997, Steele et al. 2005, McKone & Tanner 2009, Trevathan-Tackett et al. 2015, Groner et al. 2016), and phenolic acid concentration have been found to be higher in diseased plants in natural *Z. marina* populations (Vergeer & Develi 1997). The phenolic acid concentrations also increases in response to *L. zosterae* inoculation (McKone & Tanner 2009). It has therefore been suggested that phenolic acids limit the spread of the pathogen (Buchsbaum et al. 1990, Vergeer et al. 1995, Vergeer & Develi 1997, McKone & Tanner 2009). The phenolic acids caffeic acid isolated from *Z. marina* (Vergeer & Develi 1997), and p-coumaric acid, p-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and vanillin isolated from *T. testudinum* (Trevathan-Tackett et al. 2015) have all showed inhibitory effects against *Labyrinthula* spp. However, increased phenolic acid production in *T. testudinum* is only found in

infected leaves above the lesions, not below (Steele et al. 2005). The authors therefore suggest that phenolic acids are not produced in a response to pathogenic *Labyrinthula sp.* infection, but rather is a pseudo-induction caused by a disruption in carbohydrate allocation within a leaf leading to higher abundance of carbon above the lesions and thereby an accumulation of phenolic acids. Furthermore, additional unidentified compounds with inhibitory effects towards *Labyrinthula sp.* has been isolated from *T. testudinum* (Trevathan-Tackett et al. 2015). No study has, to my knowledge, investigated if other compounds produced by *Z. marina* except for phenolic acids inhibit *L. zosterae* infection. In this thesis, the presence of inhibitory compounds against *L. zosterae* growth was investigated in natural *Z. marina* populations (**paper I** and **II**), and the production of these compounds in response to abiotic factors was further studied in laboratory experiments (**paper III**). The inhibitory compounds were isolation and characterized in **paper IV**.

AIMS OF THIS THESIS

The overall objective of the thesis was to gain a better understanding of how abiotic factors influence *Labyrinthula zosterae* infection in *Zostera marina*, and whether plant resistance can reduce infection. This was achieved by studying infection both in natural populations and in laboratory experiments.

More specifically, the aim of each paper in the thesis was:

PAPER I: To investigate if *Labyrinthula zosterae* infection varies in natural *Zostera marina* populations between high and low salinity areas, between different water depths, and whether resistance to *L. zosterae* infection depends on pathogen pressure.

PAPER II: To investigate if *Labyrinthula zosterae* infection correlates with salinity in natural *Z. marina* populations over a larger geographical scale, and whether resistance to *L. zosterae* infection depends on pathogen pressure.

PAPER III: To experimentally investigate individual and interactive effects of temperature, light, and tissue damage on *Labyrinthula zosterae* infection, and how these stressors affect *Z. marina*'s production of inhibitory compounds and response to infection in terms of gene expression.

PAPER IV: To isolate and characterize chemical defense compounds against *Labyrinthula zosterae* produced by *Zostera marina*.

METHODS

In this section I summarize the methods most used in the thesis. For more details see the specific papers.

Quantifying *Labyrinthula zosterae* and wasting disease

Labyrinthula zosterae is an opportunistic pathogen and infection does not always lead to disease (Bockelmann et al. 2013, Burge et al. 2013). Additionally, necrotic lesions on seagrass leaves may have other causes than *L. zosterae* disease and have been linked to e.g. salinity stress, (Biebl & McRoy 1971), and temperature (Collier & Waycott 2014). It is therefore important to measure both pathogen load and the resulting disease symptoms when investigating infection by *L. zosterae* or other pathogenic *Labyrinthula* spp. (Bergmann et al. 2011).

Lesion coverage measures the extent of necrotic lesions on a seagrass shoot, and is the commonly used measurement of *Labyrinthula* spp. disease. It can be performed in two different ways, either by measuring the percent lesion coverage on the most infected leaf of the shoot (wasting index), or by measuring the percent lesion coverage on the whole shoot (whole shoot wasting index, Burdick et al. 1993). *L. zosterae* infection spreads through leaf-to-leaf contact and disease is usually more prominent on mature, terminal leaves and wasting index therefore gives higher disease values, compared to whole shoot wasting index that incorporate younger uninfected leaves in the measurement (Burdick et al. 1993). Due to the high turnover rate of *Z. marina* leaves (Olesen & Sand-Jensen 1994), a shoot can replace old infected leaves with new uninfected ones, thus reducing lesion coverage and ensuring its survival. In this thesis the whole shoot wasting index, hereafter referred to as lesion coverage, was used to measure the effect of disease on a shoot as a whole (**paper I**, **paper II**, **paper III**, and **paper IV**). Lesion coverage measurements were achieved by photographing each *Z. marina* shoot on a light table, and lesion area and whole shoot area was calculated using ImageJ software.

Several techniques have been applied in previous studies to confirm the presence of the pathogen in seagrass tissue. One of the widely used methods is to culture *Labyrinthula* spp. from a piece of the investigated seagrass shoot, as described in the section about *L. zosterae* isolation and culturing below, and to verify the presence or absence of the pathogen using microscopy (Muehlstein et al. 1988, Short et al. 1993, Durako & Kuss 1994, Vergeer & den Hartog 1994, Bockelmann et al. 2013, Trevathan-Tackett et al. 2013). Two methods, histology and quantitative PCR (qPCR), have been used to actually quantify pathogen abundance in seagrass tissue (Bergmann et al. 2011, Brakel et al. 2014, Groner et al. 2014, Groner et al. 2016, Brakel et al. 2017). Histology makes it possible to

identify individual *L. zosterae* cells within seagrass tissue using microscopy (Groner et al. 2014), whereas the qPCR assay identifies and quantifies the pathogen from *L. zosterae* DNA extracted from seagrass tissue using genetic markers (Bergmann et al. 2011).

In **paper I**, **paper II**, **paper III**, and **paper IV** of this thesis the qPCR assay was used to identify the pathogen, and *L. zosterae* concentration was quantified according to the protocol described in Bockelmann et al. (2013). The third leaf of each *Z. marina* shoot was freeze-dried and homogenized. DNA was extracted from 2 - 3 mg sample using Invisorb Spin DNA Extraction Kit, or Invisorb Spin Plant Mini Kit (Strattec Molecular, Germany) according to the manufacturer's protocol, with the exception that 1 μ L salmon sperm was added to saturate the silica columns with unspecified DNA. DNA was eluted in 100 μ L elution buffer. In the cases when the Invisorb Spin DNA Extraction Kit was used the target DNA was further purified using a one-step PCR inhibitor removal kit (Zymo Research, USA). RT-qPCR was performed on a StepOne Plus q-PCR machine (Applied Biosystems, USA) with the forward primer Laby_ITS_Taq_f: TTGAACGTAACATTCGACTTTCGT and the reverse primer Laby_ITS_Taq_r: ACGCATGAAGCGGTCTTCTT, along with the probe Laby_ITS_Taq_pr: TGGACGAGTGTGTTTTG that carried the fluorescence label 6-Fam at the 59 end and the dark quencher BHQ-1 at the 39 end. Reactions were carried out in a 20 μ L reaction volume: 10 μ L TaqMan universal Master Mix (Applied Biosystems, now Life Technologies), 2 μ L template DNA, 2.4 μ L (5 μ M) each of the two primers, 2.4 μ L Milli-Q H₂O and 0.8 μ L probe (5 μ M). The thermo-cycling program was 2 min at 50°C and 10 min at 95°C, followed by 48 cycles at 95°C for 15 s and 60°C for 1 min. Each sample ran in technical triplicates and a standard curve with known *L. zosterae* cell concentrations and a negative control containing Milli-Q H₂O instead of template DNA ran alongside the samples on each qPCR plate.

***Labyrinthula zosterae* isolation and culturing**

Labyrinthula zosterae used in **paper I**, **paper II**, **paper III**, and **paper IV** was isolated from *Z. marina* shoots haphazardly collected in the archipelago outside of the Tjärnö marine laboratory, Sweden. *Z. marina* leaves with apparent *L. zosterae* lesions were cut into 3 cm pieces and surface sterilized in 0.5% sodium hypochlorite for 20 s, rinsed in distilled water for 10 s, and submerged in filtered seawater (0.2 μ m) for 1 min (Bockelmann et al. 2013). The pieces were placed on agar plates containing serum-seawater agar (SSA) consisting of filtered seawater (0.2 μ m), 12 g l⁻¹ agar-agar, 1 g l⁻¹ glucose, 0.1 g l⁻¹ peptone, and 0.1 g l⁻¹ yeast extract, 3 mg l⁻¹ germanium dioxide, 25 mL l⁻¹ streptomycin/penicillin (10 000 units penicillin and 10 mg streptomycin mL⁻¹), and 10 ml l⁻¹ horse

serum. The agar solution was autoclaved and cooled down to 50°C before antibiotics and horse serum were added. *L. zosterae* was maintained by transferring 7 mm diameter plugs from the edge of the growing cultures and placing them face down in the center of new agar plates every third week. The agar plates were sealed with parafilm and the cultures were incubated in the dark at 25°C.

Chemical extractions and chemical defense assays

The investigation of inhibitory compounds against *L. zosterae* produced by *Z. marina* was conducted with an open approach, rather than using a priori assumptions about defensive roles of specific groups of compounds. Initial extraction of potential inhibitory compounds was performed to achieve a recovery of *Z. marina* metabolites of a broad polarity range. Thus, freeze-dried and homogenized plant leaves were extracted by soaking the *Z. marina* tissue in dichloromethane, dichloromethane/methanol 1:1, and methanol for 1 hour each on a shaker table. However, further investigation showed that dichloromethane/methanol 1:1 extracted the inhibitory compounds well, and was therefore used as the solvent mixtures for crude extractions in **paper I**, **paper II**, **paper III**, and **paper IV** of this thesis.

Bioassay-guided fractionation

A bioassay-guided fractionation approach was applied to isolate and characterize the inhibitory compounds against *L. zosterae* produced by *Z. marina* (**paper IV**). Approximately 5 kg *Z. marina* leaves was coarsely homogenized and extracted trice in methanol/dichloromethane 1:1 for 1 h. Separation of *Z. marina* compounds was achieved by applying several fractionation techniques: liquid-liquid separation, size exclusion, normal phase chromatography, and reverse phase high performance liquid chromatography (HPLC) equipped with a diode array detector. The inhibitory effect of the fractions was investigated using a *L. zosterae* growth assay (see bioassays below). Additional characterization of the inhibitory compounds was made with nuclear magnetic resonance spectroscopy and HPLC mass spectrometry.

Bioassays

The inhibitory effect of the extracted *Z. marina* metabolites (**paper I**, **paper II**, **paper III**, and **paper IV**) on *L. zosterae* growth was tested using a modified version of the bioassay described by (Martin et al. 2009). The bioassay was adapted to a smaller scale to minimize the use of the extracted plant material for each replicate. Thus, 1 mL liquid media, SSA medium minus agar (see above), containing 1% dimethyl sulfoxide and 1 mL volumetric aliquot of the extract of interest, were transferred into 6 well plates ($\text{\O} = 35 \text{ mm}$). *L. zosterae* was

transferred to the wells by taking 7 mm diameter plugs from cultures growing on agar plates (Fig. 4A), prepared as above, and placed face down in the center of each well. Media containing 1% dimethyl sulfoxide served as a control. The well plates were incubated in the dark for 16 - 24 h at 25°C after which the media was removed. To determine *L. zosterae* growth the outer edge of the *L. zosterae* colony growing attached to the bottom of the well plate was outlined with a marker and photographed (Fig. 4B), and the total area of the *L. zosterae* colony was thereafter measured using ImageJ software. *L. zosterae* growth on *Z. marina* extracts was compared to growth in control wells to determine the inhibitory effect of the extracts.

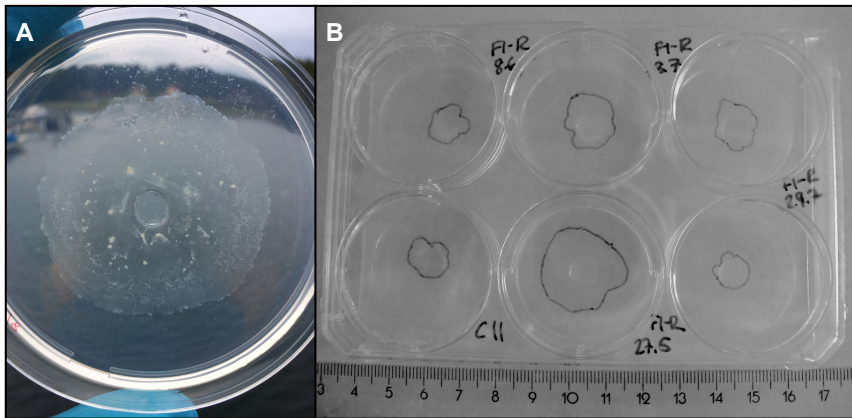


Figure 4. A) *Labyrinthula zosterae* culture on an agar plate, and B) *L. zosterae* growth assay.

***Labyrinthula zosterae* infection experiments**

Laboratory infection experiments were carried out in order to investigate the susceptibility to *L. zosterae* infection among *Z. marina* populations from different salinity environments (**paper I**, and **paper II**), and to test how the interaction between *Z. marina* and *L. zosterae* is affected by different extrinsic factors (temperature, light, and tissue damage) (**paper III**). After epibiont removal, *Z. marina* shoots were planted in individual 6 L containers by submerging the shoot rhizomes in 600 mL sterilized sediment. The containers were placed in a flow through system with filtered surface water. Shoots were infected by attaching *L. zosterae* vectors in the form of *L. zosterae* covered agar plugs ($\text{Ø} = 5 \text{ mm}$), taken from the edge of *L. zosterae* colonies growing on agar plates (see above), to the middle part of the third youngest leaf (Fig. 5). Control shoots received agar plugs without *L. zosterae*. Shoots were photographed (Fig. 5) and ImageJ software was used to calculate the percent coverage of the wasting disease lesions as described above.

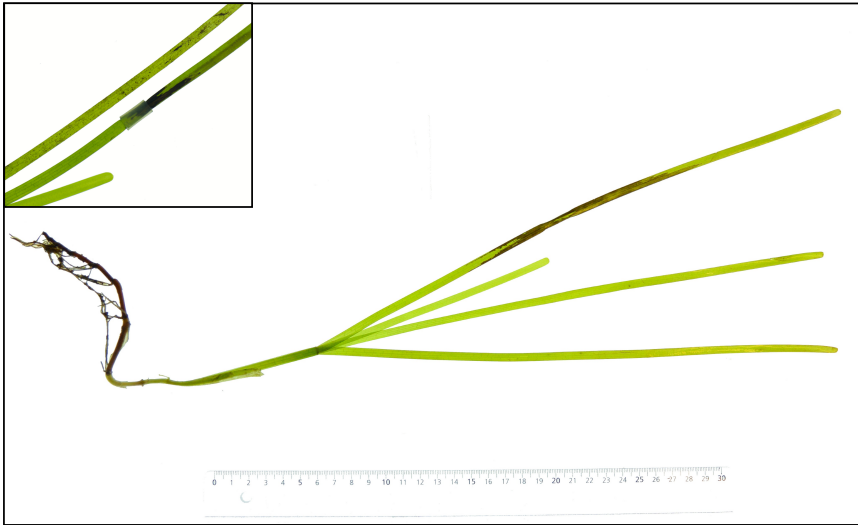


Figure 5. Photograph of a *Z. marina* shoot following experimental *L. zosterae* infection. Upper left corner shows attachment of a *L. zosterae* vector with a piece of silicon tubing enclosed around the leaf.

Previous infection experiments have applied similar methodology as in this thesis, but have used other types of *Labyrinthula* spp. vectors. Short et al. (1987) used autoclaved pieces of seagrass leaves, which they reinfected with *L. zosterae* by placing them on a *L. zosterae* covered agar plate. A similar approach using gauze pieces instead of leaves have also been used (Brakel et al. 2014). Artificial vectors were used instead of biological vectors in this thesis to minimize variation between replicates due to biological interactions between *L. zosterae* and the vector material. In addition, we were not able to make gauze vectors function reliably in our experiments (17% success rate) and instead chose to use agar plugs (100% success rate) (Table 1).

MAIN RESULTS AND DISCUSSION

Field distribution

When this thesis was initiated no study had investigated the distribution of *L. zosterae* infection in *Z. marina* in Swedish waters. The results in **paper I** and **II** showed that the pathogen is omnipresent in *Z. marina* shoots in high salinity environments along the Swedish coast. Prevalence of necrotic lesions symptomatic for wasting disease was also high in these areas, although lesion coverage on the *Z. marina* shoots was generally low ($\bar{x} = 3.8\%$). Overall, our results show that *Z. marina* meadows in high salinity areas on the Swedish west coast have a high prevalence of *L. zosterae* infection compared to what has been reported from other regions (Bockelmann et al. 2013, Groner et al. 2016).

Labyrinthula zosterae infection generally decreases with decreasing salinity along the Swedish coastline and prevalence of the pathogen in *Z. marina* meadows in salinities between 7 PSU and 10 PSU is low (Fig. 6, **paper II**). In Åhus and Kalmarsund (7 PSU) no *L. zosterae* cells were detected. However, we found that *L. zosterae* was present in *Z. marina* plants from two areas in the low salinity environment (6 PSU). This is, to my knowledge, the first report of *L. zosterae* in the Baltic Proper, an area with constantly low salinity, although other *Labyrinthula* spp. have been isolated from *Z. marina* in the Baltic Sea in Åland and Finland (Bockelmann et al. 2013, Lindholm et al. 2016).

Previously, a non-pathogenic *Labyrinthula* sp. has been identified in both high (20 – 25 PSU) and low (5 PSU) salinities (Bockelmann et al. 2012), demonstrating a wide salinity tolerance in this genus. It is therefore possible that a strain (or a few strains) of *L. zosterae* has adapted to the low salinity in the Baltic Sea. *L. zosterae* cells were isolated at a relatively low concentration in the low salinity populations compared to the average cell concentration found in the high salinity populations. Lesion coverage was also low in the low salinity meadows, although no statistically significant difference was detected in comparison with lesion coverage on high salinity populations. This implies that the *L. zosterae* strain(s) existing in low salinity meadows could have stronger negative effects on the host than its high salinity counterpart, resulting in pronounced disease symptoms despite its low cell concentration. Further studies investigating pathogen performance in different salinities as well as their effects on the seagrass host could provide a better understanding of the adaptation and ecological significance of low salinity *L. zosterae* strains from the Baltic Sea.

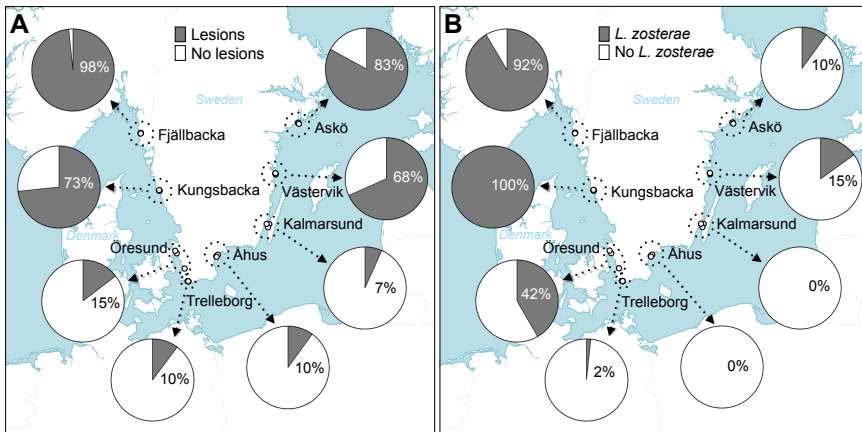


Figure 6. Prevalence of *L. zosterae* infection on *Z. marina* shoots along the Swedish coast, measured as a) lesion prevalence, and b) *L. zosterae* cell prevalence (**paper II**). Pie carts show prevalence on 60 shoots collected from three meadows per area. Prevalence numbers are shown for infected shoots.

Field and laboratory experiments further showed that *Z. marina* shoots from low salinity can become extensively infected by *L. zosterae* when transferred to high salinity environments (**paper I** and **II**). This suggests that the low infection observed in the low salinity environments is not because these shoots are inherently more resistant against the pathogen. These findings instead corroborate previous studies where low salinity has been found to reduce *Labyrinthula spp.* growth in laboratory experiments (Muehlstein et al. 1988, Martin et al. 2009). The results from **paper I** and **II** together suggest that low salinity areas generally can act as a refuge against seagrass wasting disease due to the poor performance of the pathogen in low salinity, as previously suggested (Young 1943, McKone & Tanner 2009), also in Swedish *Z. marina* meadows. However, an adaptation of *L. zosterae* to the low salinity environment of the Baltic Sea, as indicated by results in **paper II**, could lead to a wider distributional range of *L. zosterae* also into areas previously considered to be protected from the pathogen.

Impact of light, temperature and tissue damage

The study in **paper III** is one of the first to investigate interactive effects of multiple factors on *Labyrinthula sp.* infection in seagrass systems (see review by Sullivan et al. in press), and shows that changes in extrinsic factors can impact the host-pathogen interaction between *Z. marina* and *L. zosterae* on a relatively short time scale (7 days). However, contrary to our hypothesis, reduced light, increased temperature, and tissue damage did not have additive or synergistic effects on *L. zosterae* infection in *Z. marina*. This corroborate the results from

the only previous published study investigating the effect of multiple factors on *Labyrinthula* sp. infection in seagrass (*T. testudinum*), where the effect of single factors did not differ from the effect of cumulative stressors (Bishop et al. 2017). Instead, light intensity had the largest effect on *L. zosterae* infection in *Z. marina*, and light also impacted *Z. marina* growth and expression of genes associated with immune defense, phenol synthesis and primary metabolism. However, there were also a large number of interactions between the tested factors (light, temperature, tissue damage, and infection), highlighting the complexity of this host-pathogen system (Fig. 7, **paper III**).

Low light significantly increased lesion coverage with $\approx 35\%$. The low light treatment also significantly increased the *L. zosterae* cell concentration in *Z. marina*, but only under ambient temperatures. *Z. marina* shoots experiencing ambient temperature had slightly ($\approx 7\%$ higher mean value), but still significantly, more *L. zosterae* cells under reduced light conditions compared to shoots kept at ambient light. At higher temperatures lesion coverage did not correlate with *L. zosterae* cell concentration, and instead the same level of *L. zosterae* cells lead to higher lesion coverage for shoots subjected to reduced light conditions compared to shoots at ambient light. Furthermore, at ambient temperatures, the effect of reduced light was approximately four times greater on lesion coverage than on pathogen load, showing that a relatively small increase in cell concentration can have large impact on the degree of disease symptoms in this system, or that *Z. marina* is more sensitive to *L. zosterae* cells in a light limiting environment.

The effects of light intensity on *L. zosterae* infection in the experiments of **paper III** support previous laboratory findings, showing that reduced light increases lesion coverage (Vergeer et al. 1995). A consistent pattern has been found in natural *T. testudinum* populations, with increased lesion coverage on shoots at lower light intensities (Trevathan-Tackett et al. 2013). However, in **paper I** it was found that in natural populations, shallow-growing *Z. marina* shoots, exposed to high light levels, show larger lesion coverage compared to deeper growing shoots receiving less light. Together, this suggests that other factors, apart from light, influences the different degree of *L. zosterae* infection between water depths in natural *Z. marina* populations on the Swedish west coast.

As previously detected (Brakel et al. 2014, Brakel et al. 2017), expression of genes associated with immune defense generally was reduced in *Z. marina* following *L. zosterae* infection (**paper III**). However, the result furthermore showed that interactive affects between *L. zosterae* inoculation and extrinsic factors can alter this response. This was also observed for genes associated with

primary metabolism, where *L. zosterae* inoculation changes the expression, but only at reduced light conditions. Similar responses have been detected in *Arabidopsis thaliana*, where heat stress and draught can alter gene expression in virus-treated plants (Prasch & Sonnewald 2013). The results show that the gene response towards *L. zosterae* infection is depending on prevailing environmental conditions.

Although extrinsic factors clearly can affect the host-pathogen interaction between *Z. marina* and *L. zosterae* (Fig. 7, **paper III**), no single factor investigated in this thesis, or previously studied, have been shown to alter the virulence of *L. zosterae* to a level similar to the one observed in the wasting disease outbreak in the 1930s. It is still possible that adverse environmental changes can facilitate future wasting disease outbreaks, and other explanatory

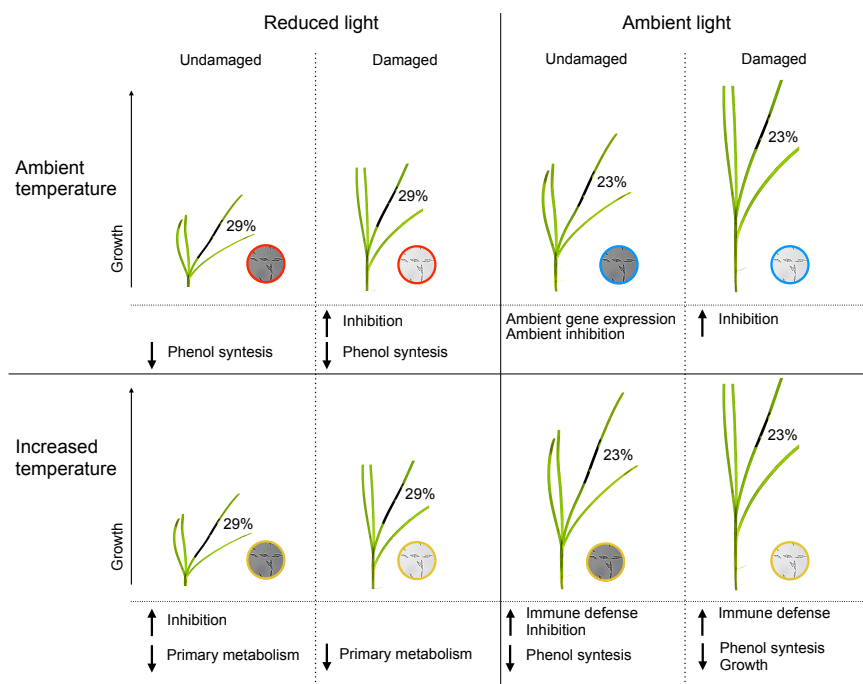


Figure 7. Summary of the significant effects of light, temperature and tissue damage on the host-pathogen interaction between *Z. marina* and *L. zosterae* following nine days of *L. zosterae* inoculation (**paper III**). Mean lesion coverage are given in percentage, *L. zosterae* cell concentration: ambient = blue circle border, increased = red circle border, intermediate = yellow circle border. Dark grey circles have significantly higher cell concentration than light grey circles for each combination of light and temperature. *L. zosterae* growth inhibition by *Z. marina* extracts, and up or downregulation of gene groups in relation to control shoots (i.e. undamaged *Z. marina* shoots at ambient light and temperature) are shown below each treatment.

factors than e.g. temperature, light, and salinity should be explored. In general, the influence of biotic interactions between *Z. marina* and other organisms on wasting disease is also largely unstudied. A recent study on the east coast of detected a correlation between epiphyte coverage and disease probability in natural *Z. marina* meadows (Groner et al. 2016), confirming the need of a more comprehensive understanding of biotic interactions in this system. Thus, future multi-factorial studies might benefit from including both abiotic and biotic factors. Furthermore, while studies have compared the impact of *Labyrinthula* spp. infection on different seagrass individuals and populations (e.g. Bockelmann et al. 2013, Trevathan-Tackett et al. 2013, Groner et al. 2014) the impact of different *L. zosterae* strains has been poorly studied. There is a large diversity of pathogenic and non-pathogenic *Labyrinthula* species worldwide (Bockelmann et al. 2012, Martin et al. 2016), and seagrass susceptibility to *L. zosterae* can be strain specific (Garcias-Bonet et al. 2011, Groner et al. 2014). It is possible that the presence of more virulent *L. zosterae* strains was one of the contributing factors to wasting disease outbreaks in the past. Future in-depth studies focusing on the role of *L. zosterae*'s genetic and functional variation for the outcome of this host-pathogen interaction are therefore needed.

Inhibitory compounds

All *Z. marina* shoots investigated in this thesis produced inhibitory compounds against *L. zosterae* (**paper I, II, III, and IV**). Furthermore, the inhibitory effects of extracted *Z. marina* metabolites did not differ among shoots with different pathogen pressure *in situ* (**paper I and II**) or in laboratory experiments (**paper III**), suggesting that the inhibitory compounds act as a constitutive defense in *Z. marina*. Further isolation of the compounds identified three partially purified fractions with inhibitory effect against the pathogen (**paper IV**), and ¹H NMR spectra analyses indicated that the compounds are present in low natural concentrations. Thus, despite an initial extraction of approximately 5 kg *Z. marina* wet weight, the molecular structures of the inhibitory compounds against *L. zosterae* could not be fully determined. Nevertheless, UV spectral analyses, mass spectrometry and NMR spectroscopy, showed that the characteristics of the inhibitory compounds differ from that of known phenolic acids found in seagrasses (**paper IV**), a group of compounds which previously have been suggested to function as a chemical defense against the pathogen (Buchsbaum et al. 1990, Vergeer et al. 1995, Vergeer & Develi 1997, McKone & Tanner 2009). Instead, the results suggest that yet unknown metabolites can act as a defense against *L. zosterae* in *Z. marina*. Further elucidation of the partially purified fractions isolated in this thesis is needed to identify the chemical species with inhibitory effects against *L. zosterae* in *Z. marina*, and this would probably require extraction of an even larger quantity of *Z. marina* tissue. Once the

compounds have been fully identified, the potential role of these compounds in *Z. marina* populations in different parts of world and in other seagrass-*Labyrinthula* systems could be further investigated. Whether these compounds function as a chemical defense *in situ*, and not only in a laboratory setting also warrants further investigation.

CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis shows that *L. zosterae* is omnipresent in *Z. marina* meadows in high salinity environments in Swedish waters, while most of the low salinity areas have no or low infection, and could possibly act as a refuge for *Z. marina* plants from *L. zosterae* infection (**paper I** and **II**). Low salinity meadows could therefore become important source populations for re-establishments of *Z. marina* after possible wasting disease outbreaks in the future, and care should be taken to protect such areas. It is for example important when considering future restoration efforts of seagrass meadows in low salinity areas, where introduction of infected *Z. marina* shoots should be avoided. Furthermore, future establishments of marinas and docks in low salinity meadows along the west coast should be carefully considered and avoided if possible.

The surveys in the Baltic Sea showed, however, that even a salinity as low as 6 PSU does not always exclude *L. zosterae* infection in *Z. marina* meadows (**paper II**). Although the prevalence of infection was lower on *Z. marina* shoots in Askö and Västervik compared to in the high salinity environments on the west coast, these findings suggest that *L. zosterae* strains have the potential to adapt to the low salinity in the Baltic Sea. This could in turn imply a wider distribution of *L. zosterae* to areas previously considered to be protected from infection, and impact the ability of low salinity areas to shelter *Z. marina* from the pathogen in the future.

Different light intensities can probably not in itself explain the different degree of *L. zosterae* lesions on *Z. marina* shoots growing at different depths. While low light intensity increased lesion coverage in laboratory experiments (**paper III**), disease symptoms are larger on shallower growing shoots, with higher light intensity, in natural *Z. marina* meadows than on deeper growing shoots receiving less light (**paper I**). Any indirect effects of light on disease would require further studies, and the reason behind the increased disease on shallow shoots currently remains unknown.

Zostera marina produces inhibitory compounds against *L. zosterae*, and our results suggest that they function as a constitutive defense (**paper IV**). Although not fully identified, the characteristics of these compounds suggest that they are

novel compounds from *Z. marina*, rather than the phenolic acids that previously have been suggested to function as defense compounds. These results also show that it is important to apply an open approach, without preconceived notions about the identity of the compounds with inhibitory effect, when studying chemical defenses in seagrass-*Labyrinthula* systems. Further elucidation of the three partially purified inhibitory fractions isolated in this study is crucial to allow for more advanced tests of the function of these compounds within the plant, and to further investigate possible local adaptations in terms of chemical defenses towards *L. zosterae* in eelgrass populations.

Multifactorial investigations of the effect of light, temperature, and tissue damage on *L. zosterae* infection showed that low light significantly increased lesion coverage (**paper III**). However, a large number of interactive effects between the tested factors also affect *L. zosterae* cell concentration, production of inhibitory compounds, *Z. marina* growth, and expression of genes associated with immune defense, phenol synthesis, and primary metabolism. These findings highlight the complexity of this system and shows that multi-factorial investigations are needed to understand the impact of abiotic factors on seagrass-*Labyrinthula* interactions.

Although *L. zosterae* is omnipresent in *Z. marina* meadows in Sweden in the studied areas of this thesis (**paper I and II**), more detailed surveys of wasting disease, both geographically and over longer time periods, are lacking and currently no monitoring program of this disease exists in Sweden. To better understand the disease dynamics and how *L. zosterae* infection affect *Z. marina* meadows in Sweden, I propose that investigations of seagrass wasting disease should be considered and included in future monitoring of the seagrass health in Swedish waters.

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REFERENCES

- Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution* 19:535-544
- Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecol Monogr* 81:169-193
- Beakes GW, Honda D, Thines M (2014) 3 Systematics of the Straminipila: Labyrinthulomycota, Hyphochytriomycota, and Oomycota. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and Evolution*, Vol 7A. Springer Berlin Heidelberg, p 39-97
- Bergmann N, Fricke B, Schmidt MC, Tams V and others (2011) A quantitative real - time polymerase chain reaction assay for the seagrass pathogen *Labyrinthula zosterae*. *Molecular ecology resources* 11:1076-1081
- Biebl R, McRoy CP (1971) Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Marine Biology* 8:48-56
- Bigelow DM, Olsen MW, Gilbertson RL (2005) *Labyrinthula terrestris* sp. nov., a new pathogen of turf grass. *Mycologia* 97:185-190
- Bishop N, Martin DL, Ross C (2017) Effects of multi-stress exposure on the infection dynamics of a *Labyrinthula* sp.-turtle grass pathosystem. *Marine Ecology Progress Series* 581:119-133
- Bishop ND (2013) The effects of multiple abiotic stressors on the susceptibility of the seagrass *Thalassia testudinum* to *Labyrinthula* sp., the causative agent of wasting disease. Master thesis, University of North Florida
- Blakesley BA, Berns DM, Merello MF, Hall MO, Hyniova J (2002) The dynamics and distribution of the slime mold *Labyrinthula* sp. and its potential impacts on *Thalassia testudinum* populations in Florida. In: Greening HS (ed) *Seagrass Management: It's Not Just Nutrients!* Tampa Bay Estuary Program, 2000 Aug 22-24; St. Petersburg, FL., p 199-207
- Bockelmann A-C, Tams V, Ploog J, Schubert PR, Reusch TBH (2013) Quantitative PCR reveals strong spatial and temporal variation of the wasting disease pathogen, *Labyrinthula zosterae* in northern European eelgrass (*Zostera marina*) beds. *PLOS ONE* 8:e62169
- Bockelmann AC, Beining K, Reusch TBH (2012) Widespread occurrence of endophytic *Labyrinthula* spp. in northern European eelgrass *Zostera marina* beds. *MEPS* 445:109-116
- Borum J, Greve TM (2004) The four European seagrass species. In: Borum J, Duarte CM, D. K-J, Greve TM (eds) *European seagrasses: an introduction to monitoring and management. The M&MS project*, p 1
- Boström C, Bonsdorff E (1997) Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L.) beds in the northern Baltic Sea. *Journal of Sea Research* 37:153-166
- Boström C, Baden S, Bockelmann AC, Dromph K and others (2014) Distribution, structure and function of Nordic eelgrass (*Zostera marina*)

- ecosystems: implications for coastal management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 24:410-434
- Bowles JW, Bell SS (2004) Simulated herbivory and the dynamics of disease in *Thalassia testudinum*. *Marine Ecology-Progress Series* 283:127-132
- Brakel J, Werner FJ, Tams V, Reusch TBH, Bockelmann A-C (2014) Current European *Labyrinthula zosterae* Are Not Virulent and Modulate Seagrass (*Zostera marina*) Defense Gene Expression. *PLOS ONE* 9:e92448
- Brakel J, Reusch TBH, Bockelmann A-C (2017) Moderate virulence caused by the protist *Labyrinthula zosterae* in ecosystem foundation species *Zostera marina* under nutrient limitation. *Marine Ecology Progress Series* 571:97-108
- Buchsbaum RN, Short FT, Cheney DP (1990) Phenolic-nitrogen interactions in eelgrass, *Zostera marina* L.: possible implications for disease resistance. *Aquatic Botany* 37:291-297
- Bull JC, Kenyon EJ, Cook KJ (2012) Wasting disease regulates long-term population dynamics in a threatened seagrass. *Oecologia* 169:135-142
- Burdick DM, Short FT, Wolf J (1993) An index to assess and monitor the progression of wasting disease in eelgrass *Zostera marina*. *Marine Ecology Progress Series* 94:83-90
- Burge CA, Kim CJS, Lyles JM, Harvell CD (2013) Special issue oceans and humans health: the ecology of marine opportunists. *Microbial ecology* 65:869-879
- Chakraborty S, Tiedemann AV, Teng PS (2000) Climate change: potential impact on plant diseases. *Environmental pollution* 108:317-326
- Collier CJ, Waycott M (2014) Temperature extremes reduce seagrass growth and induce mortality. *Marine pollution bulletin* 83:483-490
- Costanza R, de Groot R, Sutton P, van der Ploeg S and others (2014) Changes in the global value of ecosystem services. *Global Environmental Change* 26:152-158
- Cottam C (1935) Wasting disease of *Zostera marina*. *Nature* 135:306-306
- Cox PA (1988) Hydrophilous pollination. *Annual Review of Ecology and Systematics* 19:261-279
- den Hartog C (1987) "Wasting disease" and other dynamic phenomena in *Zostera* beds. *Aquatic Botany* 27:3-14
- den Hartog C, Kuo J (2007) Taxonomy and biogeography of seagrasses. In: Larkum AWD, Orth RJ, Duarte C (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, p 1-23
- Duarte CM, Middelburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences* 2:1-8
- Durako MJ, Kuss KM (1994) Effects of *Labyrinthula* infection on the photosynthetic capacity of *Thalassia testudinum*. *Bulletin of Marine Science* 54:727-732
- Engel S, Puglisi MP, Jensen PR, Fenical W (2006) Antimicrobial activities of extracts from tropical Atlantic marine plants against marine pathogens and saprophytes. *Marine Biology* 149:991-1002
- Eriander L (2016) Restoration and management of eelgrass (*Zostera marina*) on the west coast of Sweden. Doctorial thesis, University of Gothenburg

- Giesen WBJT, Van Katwijk MM, Den Hartog C (1990) Temperature, salinity, insolation and wasting disease of eelgrass (*Zostera marina* L.) in the Dutch Wadden Sea in the 1930's. Netherlands journal of sea research 25:395-404
- Green EP, Short FT (2003) World Atlas of Seagrasses, Vol. University of California Press, Berkeley, USA
- Groner ML, Burge CA, Couch CS, Kim CJS and others (2014) Host demography influences the prevalence and severity of eelgrass wasting disease. Diseases of Aquatic Organisms 108:165-175
- Groner ML, Burge CA, Kim CJS, Rees E and others (2016) Plant characteristics associated with widespread variation in eelgrass wasting disease. Diseases of Aquatic Organisms 118:159-168
- Jackson EL, Rowden AA, Attrill MJ, Bossey SJ, Jones MB (2001) The importance of seagrass beds as a habitat for fishery species. Oceanography and marine biology 39:269-304
- Johannesson K (1989) The bare zone of Swedish rocky shores: why is it there? Nordic Society Oikos 54:77-86
- Johannesson K, Smolarz K, Grahm M, André C (2011) The future of Baltic Sea populations: local extinction or evolutionary rescue? Ambio 40:179-190
- Krause - Jensen D, Middelboe AL, Sand - Jensen K, Christensen PB (2000) Eelgrass, *Zostera marina*, growth along depth gradients: upper boundaries of the variation as a powerful predictive tool. Oikos 91:233-244
- Kuo J, den Hartog C (2007) Seagrass morphology, anatomy, and ultrastructure. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands, p 51-87
- Les DH, Cleland MA, Waycott M (1997) Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (seagrasses) and hydrophily. Systematic Botany 22:443-463
- Lindholm T, Lindqvist C, Sjöqvist C (2016) Occurrence and Activity of Slime Nets, *Labyrinthula* sp. Among Aquatic Plants in Cold and Oligohaline Baltic Sea Waters. Annales Botanici Fennici 53:139-143
- Maleck K, Dietrich RA (1999) Defense on multiple fronts: how do plants cope with diverse enemies? Trends in plant science 4:215-219
- Martin DL, Boone E, Caldwell MM, Major KM, Boettcher AA (2009) Liquid culture and growth quantification of the seagrass pathogen, *Labyrinthula* spp. Mycologia 101:632-635
- Martin DL, Chiari Y, Boone E, Sherman TD and others (2016) Functional, Phylogenetic and Host-Geographic Signatures of *Labyrinthula* spp. Provide for Putative Species Delimitation and a Global-Scale View of Seagrass Wasting Disease. Estuaries and Coasts 39:1403-1421
- McKone KL, Tanner CE (2009) Role of salinity in the susceptibility of eelgrass *Zostera marina* to the wasting disease pathogen *Labyrinthula zosterae*. Marine Ecology Progress Series 377:123-130
- Muehlstein LK, Porter D, Short FT (1988) *Labyrinthula* sp., a marine slime mold producing the symptoms of wasting disease in eelgrass, *Zostera marina*. Marine Biology 99:465-472
- Muehlstein LK (1989) Perspectives on the wasting disease of eelgrass *Zostera marina*. Diseases of aquatic organisms 7:211-221

- Muehlstein LK, Porter D, Short FT (1991) *Labyrinthula zosterae* sp. nov., the causative agent of wasting disease of eelgrass, *Zostera marina*. *Mycologia* 83:180-191
- Muehlstein LK (1992) The host-pathogen interaction in the wasting disease of eelgrass, *Zostera marina*. *Canadian Journal of Botany* 70:2081-2088
- Nordlund LM, Koch EW, Barbier EB, Creed JC (2016) Seagrass Ecosystem Services and Their Variability across Genera and Geographical Regions. *PLOS ONE* 11:e0163091
- Olesen B, Sand-Jensen KAJ (1994) Demography of shallow eelgrass (*Zostera marina*) populations--shoot dynamics and biomass development. *Journal of Ecology* 82:379-390
- Olsen JL, Rouzé P, Verhelst B, Lin Y-C and others (2016) The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature* 530:331-335
- Olsen YS, Duarte CM (2015) Combined effect of warming and infection by *Labyrinthula* sp. on the Mediterranean seagrass *Cymodocea nodosa*. *Marine Ecology Progress Series* 532:101-109
- Olsen YS, Potouroglou M, Garcias-Bonet N, Duarte CM (2015) Warming reduces pathogen pressure on a climate-vulnerable seagrass species. *Estuaries and coasts* 38:659-667
- Orth RJ, Heck KL, Vanmontfrans J (1984) Faunal Communities in Seagrass Beds - A Review of the Influence of Plant Structure and Prey Characteristics on Predator - Prey Relationship. *Estuaries* 7:339-350
- Orth RJ, Carruthers TJB, Dennison WC, Duarte CM and others (2006) A global crisis for seagrass ecosystems. *BioScience* 56:987-996
- Petersen HE (1934) Wasting disease of eelgrass (*Zostera marina*). *Nature* 134:143-144
- Porter D (1972) Cell division in the marine slime mold, *Labyrinthula* sp., and the role of the bothrosome in extracellular membrane production. *Protoplasma* 74:427-448
- Prasch CM, Sonnewald U (2013) Simultaneous application of heat, drought, and virus to *Arabidopsis* plants reveals significant shifts in signaling networks. *Plant Physiology* 162:1849-1866
- Raghukumar S (2002) Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). *European journal of protistology* 38:127-145
- Rasmussen E (1977) The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. In: McRoy CP, Helffferich C (eds) *Seagrass ecosystems: a scientific perspective*, Vol 4. M. Dekker, New York, USA, p 1-51
- Renn CE (1935) A mycetozoan parasite of *Zostera marina*. *Nature* 135:544-545
- Scheffer R (1991) Role of toxins in evolution and ecology of plant pathogenic fungi. *Cellular and Molecular Life Sciences* 47:804-811
- Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. *Journal of Experimental Marine Biology and Ecology* 350:3-20

- Short FT, Mathieson AC, Nelson JI (1986) Recurrence of the eelgrass wasting disease at the border of New Hampshire and Maine, USA. *Marine Ecology Progress Series* 29:89-92
- Short FT, Muehlstein LK, Porter D (1987) Eelgrass wasting disease: cause and recurrence of a marine epidemic. *The Biological Bulletin* 173:557-562
- Short FT, Ibelings BW, Den Hartog C (1988) Comparison of a current eelgrass disease to the wasting disease in the 1930s. *Aquatic Botany* 30:295-304
- Short FT, Porter D, Iizumi H, Aioi K (1993) Occurrence of the eelgrass pathogen *Labyrinthula zosterae* in Japan. *Dis Aquat Org* 16:73-77
- Short FT, Carruthers TJR, Waycott M, Kendrick GA and others (2010) *Zostera marina*. The IUCN Red List of Threatened Species 2010:e.T153538A4516675
- Steele LT, Caldwell M, Boettcher A, Arnold T (2005) Seagrass-pathogen interactions: 'pseudo-induction' of turtlegrass phenolics near wasting disease lesions. *Marine Ecology Progress Series* 303:123-131
- Stevens NE, Ellis HR, Stevens RB (1950) Wasting and recovery of *Zostera marina* on the Atlantic coast of the United States. *Plant Disease Reporter* 34:357-362
- Sullivan BK, Trevathan-Tackett SM, Neuhauser S, Govers LL (in press) Host-pathogen dynamics of seagrass diseases under future global change. *Marine Pollution Bulletin*
- Trevathan-Tackett SM, Lauer N, Loucks K, Rossi AM, Ross C (2013) Assessing the relationship between seagrass health and habitat quality with wasting disease prevalence in the Florida Keys. *Journal of Experimental Marine Biology and Ecology* 449:221-229
- Trevathan-Tackett SM, Lane AL, Bishop N, Ross C (2015) Metabolites derived from the tropical seagrass *Thalassia testudinum* are bioactive against pathogenic *Labyrinthula* sp. *Aquatic Botany* 122:1-8
- Tutin TG (1938) The autecology of *Zostera marina* in relation to its wasting disease. *New Phytologist* 37:50-71
- Vergeer LHT, den Hartog C (1994) Omnipresence of Labyrinthulaceae in seagrasses. *Aquatic Botany* 48:1-20
- Vergeer LHT, Aarts TL, de Groot JD (1995) The 'wasting disease' and the effect of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquatic Botany* 52:35-44
- Vergeer LHT, Develi A (1997) Phenolic acids in healthy and infected leaves of *Zostera marina* and their growth-limiting properties towards *Labyrinthula zosterae*. *Aquatic Botany* 58:65-72
- Wahid MI, Yoshikawa T, Sakata T (2007) Bacteriolytic activity and growth of marine isolates of labyrinthulids on dead bacterial cells. *Fisheries Science* 73:1286-1294
- Waycott M, Procaccini G, Les DH, Reusch TBH (2007) Seagrass evolution, ecology and conservation: a genetic perspective. In: Larkum AWD, Orth RJ, Duarte CM (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, The Netherlands, p 25-50

- Waycott M, Duarte CM, Carruthers TJB, Orth RJ and others (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* 106:12377-12381
- Young EL (1943) Studies on *Labyrinthula*. The etiologic agent of the wasting disease of eel-grass. *American Journal of Botany* 30:586-593
- Young III EL (1938) *Labyrinthula* on Pacific coast eel-grass. *Canadian Journal of Research* 16:115-117
- Zidorn C (2016) Secondary metabolites of seagrasses (Alismatales and Potamogetonales; Alismatidae): Chemical diversity, bioactivity, and ecological function. *Phytochemistry* 124:5-28