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MACROALGAL DEFENSES AGAINST HERBIVORY: CAUSES AND CONSEQUENCES OF INTRASPECIFIC VARIATION

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ABSTRACT

In marine benthic communities, herbivores consume a considerable proportion of primary producer biomass and, thus, generate selection for the evolution of resistance traits. According to the theory of plant defenses, resistance traits are costly to produce and, consequently, inducible resistance traits are adaptive in conditions of variable herbivory, while in conditions of constant/strong herbivory constitutive resistance traits are selected for. The evolution of resistance plasticity may be constrained by the costs of resistance or lack of genetic variation in resistance. Furthermore, resource allocation to induced resistance may be affected by higher trophic levels preying on herbivores.

I studied the resistance to herbivory of a foundation species, the brown alga *Fucus vesiculosus*. By using factorial field experiments, I explored the effects of herbivores and fish predators on growth and resistance of the alga in two seasons. I explored genetic variation in and allocation costs of resistance traits as well as their chemical basis and their effects on herbivore performance. Using a field experiment I tested if induced resistance spreads via water-borne cues from one individual to another in relevant ecological conditions.

I found that in the northern Baltic Sea *F. vesiculosus* communities, strength of three trophic interactions strongly vary among seasons. The highly synchronized summer reproduction of herbivores promoted their escape from the top-down control of fish predators in autumn. This resulted into large grazing losses in algal stands. In spring, herbivore densities were low and regulated by fish, which, thus,enhanced algal growth. The resistance of algae to herbivory increased with an increase in constitutive phlorotannin content. Furthermore, individuals adopted induced resistance when grazed and when exposed to water-borne cues originating from grazing of conspecific algae both in the laboratory and in field conditions. Induced resistance was adopted to a lesser extent in the presence of fish predators.

The results in this thesis indicate that inducible resistance in *F. vesiculosus* is an adaptation to varying herbivory in the northern Baltic Sea. The costs of resistance and strong seasonality of herbivory have likely contributed to the evolution of this defense strategy. My findings also show that fish predators have positive cascading effects on *F. vesiculosus* which arise via reduced herbivory but possibly also through reduced resource allocation to resistance. I further found evidence that the spread of resistance via water-borne cues also occurs in ecologically realistic conditions in natural marine sublittoral. Thus, water-borne induction may enable macroalgae to cope with the strong grazing pressure characteristic of marine benthic communities. The results presented here show that seasonality can have pronounced effects on the biotic interactions in marine benthic communities and thereafter influence the evolution of resistance traits in primary producers.

TIIVISTELMÄ

Merenpohjan eliöyhteisöissä kasvinsyöjät kuluttavat suuren osan perustuottajien biomassasta ja voimakkaan kasvinsyönnin tuottama luonnonvalinta onkin johtanut perustuottajien resistenssipiirteiden evoluutioon. Silloin kun kasvinsyönnin voimakkuus vaihtelee ajallisesti ja paikallisesti ja kun resistenssistä on kustannuksia, kasvin oletetaan puolustautuvan indusoituvan resistenssin avulla. Jatkuvan tai voimakkaan kasvinsyönnin puolestaan oletetaan johtavan konstitutiivisen resistenssin evoluutioon. Resistenssin evoluutiota voivat rajoittaa resistenssin kustannukset tai geneettisen muuntelun puute. Resistenssipiirteiden ilmenemiseen voivat vaikuttaa myös kasvinsyöjiä saalistavat pedot.

Väitöskirjassani tutkin resistenssipiirteiden evoluutiota monivuotisella ruskoleviin kuuluvalla *Fucus vesiculosus* rakkolevällä. Luonnossa tehdyillä kokeilla tutkin kasvinsyöjien ja kasvinsyöjiä saalistavien kalojen vaikutusta rakkolevän kasvuun ja resistenssiin eri vuodenaikoina. Tutkin myös resistenssipiirteiden muuntelun määrää ja allokaatiokustannuksia sekä resistenssin kemiallista perustaa ja sen vaikutusta kasvinsyöjien kasvuun ja lisääntymiseen. Testasin luonnonolosuhteissa, leviääkö rakkolevän resistenssi vesivälitteisten yhdisteiden avulla yksilöstä toiseen.

Havaitsin, että vuodenaikaisuus pohjoisella Itämerellä vaikuttaa lajien välisten vuorovaikutusten voimakkuuteen. Kasvinsyöjien yhtäaikainen lisääntyminen kesällä näkyi syksyllä niiden suurina tiheyksinä ja rakkolevän suurena kulutuksena. Keväällä kasvinsyöjätiheydet olivat pieniä. Kalat eivät vaikuttaneet kasvinsyöjätiheyksiin syksyllä, mutta niiden saalistus vähensi kasvinsyöjiä keväällä, mikä paransi rakkolevän kasvua. Rakkolevä puolustautui kasvinsyöjiä vastaan. Korkea konstitutiivinen fenolisten yhdisteiden, florotanniinien, määrä lisäsi levien resistenssiä. Sekä kasvinsyöjien syönti että syödystä naapurilevästä peräisin olevat vesivälitteiset yhdisteet indusoivat rakkolevän resistenssin niin laboratorio- kuin luonnonolosuhteissa. Resistenssin indusoituminen oli heikompaa kalojen läsnä ollessa.

Tulokseni viittaavat siihen, että rakkolevän indusoituva resistenssi on sopeuma pohjoisen Itämeren vaihtelevaan kasvinsyöntiin. Resistenssin kustannukset ja kasvinsyönnin vuodenaikaisuus ovat todennäköisesti suosineet resistenssin plastisuuden evoluutiota. Kalat vaikuttavat positiivisesti rakkolevään paitsi vähentämällä kasvinsyöntiä mahdollisesti myös vähentämällä resurssien allokointia resistenssiin. Vesivälitteinen makrolevien resistenssin leviäminen todennäköisesti edesauttaa niitä selviämään merenpohjan leväyhteisöille tunnusomaisesta voimakkaasta kasvinsyönnistä. Väitöskirjani tulokset osoittavat, että lajien välisillä vuorovaikutussuhteilla ja niiden vuodenaikaisella vaihtelulla on keskeinen merkitys merenpohjan eliöyhteisöjä muokkaavana tekijänä.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following publications which are referred to in the text by their Roman numerals.

I. Haavisto, F., Välikangas, T. & Jormalainen, V. (2010). Induced resistance in a brown alga: phlorotannins, genotypic variation and fitness costs for the crustacean herbivore. *Oecologia*, 162, 685-695.

II. Haavisto, F. & Jormalainen, V. (2014). Seasonality elicits herbivores' escape from trophic control and favors induced resistance in a temperate macroalgae. *Ecology*, 95, 3035-3045.

III. Haavisto, F., Koivikko, R. & Jormalainen, V. Defensive role of macroalgal phlorotannins: costs and benefits under natural herbivory. *Submitted manuscript.*

IV. Haavisto, F. & Jormalainen, V. Water-borne defense induction of a rockweed in the wild. *Manuscript*.

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Contributions to the original publications

	I	**II**	**III**	**IV**
Original idea	VJ	FH VJ	FH VJ	VJ FH
Experiments	FH TV VJ	FH VJ	FH VJ	FH VJ
Chemical analyses			RK	
Statistical analyses	FH VJ	FH	FH VJ	FH
Writing	FH VJ TV	FH VJ	FH VJ RK	FH VJ

FH = Fiia Haavisto, VJ= Veijo Jormalainen, RK= Riitta Koivikko,
TV= Tommi Välikangas

1. INTRODUCTION

Natural populations are composed of individuals which differ in their morphology, behavior, or physiology. A part of these differences originates from the genetic variation among individuals, which serves as the raw material for natural selection and enables a species to adapt to its environment. However, intraspecific variation can also arise as a result of interactions with the environment, particularly via plastic responses, in which an individual genotype expresses different phenotypes depending on its abiotic or biotic environment (Bradshaw1965; Callahan et al. 2008). Phenotypic plasticity has been reported from a wide range of organisms in diverse ecosystems. Because this plasticity often has a genetic basis, it can also be adaptive (Agrawal 2001, Pigliucci 2005).

The key issue in the study of phenotypic plasticity is recognition of the conditions that favor its evolution and of the factors that constrain it. In principle, phenotypic plasticity is a mechanism for adaptation to a spatially or temporally variable environment (Bradshaw 1965, Dudley & Schmitt 1996; Padilla & Savedo 2013; Murren et al. 2015). Under these circumstances, it is considered a cost-saving strategy, for which benefits arise because resources can be invested only on demand, and not allocated continuously as for costly fixed traits (Tollrian & Harvell 1999; Auld et al. 2010; Steiner & Auld 2012). A critical component of adaptive phenotypic plasticity is that the individual is able to correctly assess changes in the environment and adjust its phenotype accordingly (Padilla & Savedo 2013; Murren et al. 2015). However, this is not always the case, as several factors may constrain the production of the optimal phenotype. There may be, for instance, costs associated with plasticity, or limitations due to the lag time between detection of the environmental cue and production of the new phenotype, which could lead to a mismatch between the environment and the phenotype (DeWitt 1998; Callahan et al. 2008; Auld et al. 2010). In addition, adaptive plasticity can be constrained by a lack of genetic variation, genetic covariation of plasticity with other traits, or random processes such as genetic drift or hitchhiking (Steiner & Auld 2012). Understanding the evolution of plasticity thus requires information on the adaptive nature of the plastic traits, quantification of the genetic variation in plasticity, and knowledge of the frequency of spatial and temporal variation in the environment (Murren et al. 2015).

In this thesis, I explored intraspecific variation in the resistance of a marine macroalga to herbivory in the highly seasonal northern Baltic Sea. My main aims were to determine i) whether there is plasticity in macroalgal resistance to herbivores, i.e. inducible resistance, ii) the environmental cues that trigger this resistance (e.g., the action of herbivory itself or water-borne cues from nearby grazing), and iii) the circumstances in which it is adopted in natural populations. I further explored how this variation affects the second trophic level, herbivores, and if it is altered by the presence of a third trophic level, fish predators. I quantified genetic variation in two types of resistance traits: those that are fixed and constitutively expressed, and those that are induced. In general, genetic variation within a population can be crucial for its persistence, as heritable variation is a prerequisite for adaptation to new environmental challenges. For this reason, the presence and magnitude of intraspecific genetic variation has been the focus of much recent research, particularly in the face of widespread anthropogenic ecosystem change. Additionally, it is increasingly acknowledged that intraspecific variation in the defenses of primary producers have significant ecological repercussions for higher trophic levels, with the potential to alter community composition and function (Agrawal et al. 2005; Poelman et al. 2008). To address my study questions, I employed some of the theoretical framework initially developed to explain intraspecific variation in resistance in terrestrial plants.

1.1 Theory of plant defenses

In both terrestrial and marine environments, herbivores have major impacts on the growth, reproduction, and survival of primary producers (Cyr & Pace 1993; Gruner et al. 2008; Poore et al. 2012). They are an important link between primary producers and higher trophic levels, and thus, can affect the species assemblages and the productivity of ecosystems (Poore et al. 2012). The selective pressure to escape from herbivores has led to the evolution of a wide variety of structural and chemical resistance traits in primary producers (Karban & Baldwin 1997; Amsler & Fairhead 2006; Amsler 2008; Schaller 2008; Barton 2016), which reduce herbivory by decreasing a herbivore's performance or its preference for a plant. These traits can be constitutive, i.e. present at all times, or inducible, i.e. altered on demand (Karban & Baldwin 1997, Karban & Myers 1989, Karban 2011). Note, however, that resistance, particularly induced resistance, may not necessarily result in defense. Instead, the term induced defense is reserved for situations in which induced resistance to herbivory delivers clear fitness benefits to the plant (Karban & Myers 1989).

Several defense allocation theories aim to explain inter- and intraspecific variation in plant resistance. Most of these rely on the assumption that defense is costly for a plant (reviewed in Herms & Mattson 1992; Stamp 2003; Pavia & Toth 2008; Pavia et al. 2012). For example, resource-based allocation costs can arise due to limitations in the availability of resources for different functions, such as growth, reproduction, and the production or maintenance of defensive metabolites. Opportunity costs come into play when resource investments in resistance at early life-stages accumulate later in life (Strauss et al. 2002). Furthermore, resistance can have indirect ecological costs which become apparent only in interactions with other species (Strauss et al. 2002). Of these, allocation costs are the most studied, and they have been detected as trade-offs between constitutive and inducible resistance to herbivores (Koricheva et al. 2004; Kempel et al. 2011; Campbell & Kessler 2013; Moreira et al. 2014), and between plant resistance and other fitness components (Strauss et al. 2002; Mooney et al. 2010). Costs are often, however, difficult to recognize due to variations in overall resource availability. Therefore, their detection often depends on the environmental and ecological context in which they are measured (Koricheva 2002; Strauss et al. 2002).

The optimal defense model presumes that herbivores are the main selective force shaping plant resource allocation to secondary defense metabolites. Given that resources are limited and that resistance has costs, resources are allocated via natural selection between resistance and other needs in order to maximize the fitness of an individual plant (McKey 1974, Feeny 1976, Rhoades 1979). This model also makes predictions about within-plant variation in resistance, stating that allocation to resistance should increase with the fitness value of the plant part, and with the risk of herbivory in the absence of defensive metabolites. The induced defense model (Karban & Baldwin 1997) shares many of these assumptions and could be considered a sub-model (Stamp 2002). However, it further predicts that, because resistance traits are costly for the plant, constitutive defenses are favored under conditions of strong and/or constant grazing pressure, while induced defenses are favored when the grazing pressure varies in time and/or space. In addition, induced resistance should be triggered by a reliable cue of herbivory which accurately predicts future increases in grazing damage. Furthermore, in order to decrease future grazing damage, the defense mechanism should negatively affect herbivore preference and/or performance.

1.2 Interactions in marine benthic communities

1.2.1 Herbivory

In marine benthic communities, herbivory on primary producers is generally high (Hay 1991, Cyr and Pace 1993, Gruner et al. 2008). A recent quantitative synthesis of over 600 herbivore-exclusion experiments in marine benthos showed that, globally, herbivores remove on average 68% of primary producer biomass (Poore et al. 2012). Because of their large size, macrograzers such as herbivorous fish, urchins, and mollusks are able to consume considerable amounts (Hay 1996). However, marine benthic communities also harbor mesograzers (<2.5 cm in size), such as crustacean isopods and amphipods, and gastropods, which often occur in high densities (Brawley 1992) and, despite their small size, can have considerable impacts on primary producers (Poore et al. 2012). For example, high herbivore densities can also lead to occasional overgrazing events (Steneck et al. 2002; Leidenberger et al. 2012). However, even low-to-moderate herbivore densities and less widespread grazing damage can result in serious fitness costs for macrophytes when herbivores cluster on certain individuals to feed, or when grazing results in thallus breakage (Krumhansl & Scheibling 2011) or the loss of valuable (e.g., photosynthetically active) tissues (Poore et al. 2014). In natural macrophyte populations, grazing is rarely constant, but varies over temporal (e.g., Viejo and Åberg 2003, Whalen et al. 2013) and spatial scales (Korpinen et al. 2007a, Jormalainen and Ramsay 2009), among individuals (Wright et al. 2004; Tomas et al. 2011), or among different parts within individuals (Pavia et al. 2002; Lima et al. 2008). Because of this, selection for resistance traits in marine macrophytes is also likely to vary over multiple spatial and temporal scales.

1.2.2 Macroalgal resistance to herbivory

Global grazing impacts on marine macrophytes are most strongly affected by the traits of producers (morphologically based functional groups, taxonomy), rather than environmental factors (water temperature, latitude, nutrient availability) or grazer taxa (Poore et al. 2012). Thus, a producer's nutritional quality, resistance traits, and grazing tolerance are of major importance in determining the outcomes of interactions with consumers. In macroalgae, over 3000 natural metabolites (e.g., terpenes, polyketides, nonribosomal peptides, alkaloids, shikimates) have been described, with most research focusing on their applications for pharmaceutical use or as antifouling agents (Maschek & Baker 2008). Some of these metabolites act as constitutive defense mechanisms against consumers while others are induced in response to grazing (Amsler & Fairhead 2006; Toth & Pavia 2007; Amsler 2008; Jormalainen & Honkanen 2008; Pavia et al. 2012). The first evidence of induced resistance in marine macroalgae was reported by Van Alstyne (1988); two more-recent meta-analyses demonstrated that induced resistance is common in this group, particularly in brown algae and, to a lesser extent in green algae and red algae (Toth & Pavia 2007; Jormalainen & Honkanen 2008). In addition, some macroalgae use activated defenses, in which a constitutively present precursor is transformed into a more repellent metabolite on demand (Paul & Van Alstyne 1992; Jung et al. 2002; Van Alstyne & Houser 2003).

Induced resistance is not only employed in response to direct grazing damage, but may be triggered by cues in the environment that indicate an increased risk of herbivory. Research on terrestrial plants has shown that several species commonly detect volatile organic cues released in the grazing of neighboring plants, and consequently increase their own resistance before they are actually attacked (Karban 2014). Some plants even increase their resistance in response to olfactory cues originating from the herbivore itself (Helms et al. 2013). Toth and Pavia (2000) were the first to demonstrate that macroalgae are able to increase their resistance in response to water-borne cues originating from the grazing of conspecifics. The ability to elevate the level of defense before an actual attack allows the cue-receiving individual to not only avoid the costs of constitutive defenses, but also to avoid the fitness costs of biomass loss during the first encounter with grazers, as occurs when defense is induced by direct grazing (Karban & Baldwin 2007). Likewise, the cue-emitter may benefit if info-chemicals are used to spread systemic resistance within a plant to parts of limited or slow vascular connections (Karban et al. 2006, Frost et al. 2007, Heil & Silva Bueno 2007, Rodŕiguez-Saona et al. 2009). This could be especially beneficial for marine macroalgae (Toth and Pavia 2000), which often have a poorly developed internal transportation system and thus, a limited ability to induce systemic resistance (Hurd et al. 2014; Hemmi et al. 2004; but see Thomas et al. 2014 for kelps). Empirical evidence of macroalgal "communication" shows that while some species respond to these water-borne cues (Rohde et al. 2004; Diaz et al. 2006; Macaya et al. 2005; Yun et al. 2007), others are insensitive to them (Sotka et al. 2002, Weidner et al. 2004; Rohde & Wahl 2008), with the outcome depending on both the algal species (Toth 2007; Yun et al. 2012) and the herbivore involved (Rohde et al. 2004).

1.2.3 Intraspecific variation in macroalgal resistance

Individuals of macroalgal species may vary in their nutritional quality or in physical and chemical resistance to herbivory. Genetic variation in secondary metabolites (Pavia et al. 2003; Wright et al. 2004, Dworjanyn et al. 2006, Jormalainen et al. 2003; Jormalainen and Honkanen 2004; Johannesson 2012) and in plant quality for the herbivores (Jormalainen et al. 2008, Jormalainen and Ramsay 2009, Tomas et al. 2011) contributes to this variation. While there are several studies in terrestrial plants showing natural genetic variation in induced resistance (e.g. Karban & Baldwin 1997, Underwood 2000; Agrawal et al. 2002; Holeski et al. 2010), there are almost no studies addressing genetic variation in induced resistance in macroalgae. Moreover, it is possible that induced resistance through water-borne cues varies among different genotypes. For instance, terrestrial plants have been shown to be able to fine-tune their resistance induction, and some plants are able to differentiate cue source based on their relatedness to the emitter and to respond more strongly to the cues emitted by their close relatives (Karban & Shiojiri 2009; Karban et al. 2013).

Besides the genetic component, there may also be an environmental component to intraspecific variation in inducible resistance. Indeed, abiotic factors such as light limitation (Weinberger et al. 2011; McDowell et al. 2015) and the presence of pollutants (Warneke & Long 2014) have been reported to decrease the defensive response in some species. In addition, some algae demonstrate cue-specificity, and do not induce resistance when mechanically damaged (Pavia & Toth 2000, Rohde et al. 2004), or induce resistance differently depending on the herbivore species present (Pavia & Toth 2000; Rohde et al. 2004; Long et al. 2007). Resistance induction has also been observed to vary spatially among populations subjected to different herbivore pressures (Long & Trussell 2007), and temporally over the duration of an experiment (Toth & Pavia 2007; Flöthe et al. 2014b). The inconsistent and sometimes contradictory results obtained from some species suggest that the ability to respond to water-borne cues varies among populations, with some responding (Rohde et al. 2004; Yun et al. 2007, Yun et al. 2012; Haavisto et al. 2010) and others not (Long & Trussell 2007; Flöthe et al. 2014a). These among-population differences, in turn, may be due to variation in such factors as disturbance frequency, density of the macroalgal stand, and changes in grazing pressure. All of these have the ability to affect the predictability and reliability of the cue and thus generate among-population variation in the strength of selection for water-borne resistance induction.

Induced resistance in primary producers may also be affected by the third trophic level, that of predators. Trophic cascades, in which the predator indirectly induces positive effects on the primary producer level by either limiting herbivore densities or by altering herbivore behavior, are well documented in both terrestrial and marine ecosystems (Pace et al. 1999, Shurin et al. 2002, Borer et al. 2005; Estes et al. 2011). Price et al. (1980) suggested that by affecting herbivory, the third trophic level might modify plant resource allocation to defense. This could be especially true in the case of induced resistance, as the induction and relaxation of resistance is assumed to follow changes in the risk of herbivory (Karban and Baldwin 1997). Examples of this have been found in terrestrial plant-insect systems, in which the activities of predators and parasitoids affected grazing damage on plants, and consequently reduced the plants' induced resistance (Griffin and Thaler 2006, Poelman et al. 2011). Likewise, Reynolds and Sotka (2011) showed that water-borne cues from predatory fish decreased grazing by amphipods, which, in turn, led to a weakening of the induced resistance in the brown alga *Sargassum filipendula*. Despite the prevalence of trophic cascades in benthic marine systems (Shurin et al. 2002), the generality of the effects of the third trophic level on macrophyte quality is not well understood (Jormalainen & Honkanen 2008; Pavia et al. 2012).

The majority of studies on induced resistance in macroalgae have been conducted in the laboratory, with the goal of describing this defense strategy in different species (Pavia et al. 2012). Indeed, a recent review revealed that only a handful of studies on phenotypic plasticity in marine macroalgae were based on field experiments (Padilla & Savedo 2013). Water-borne induction has been explored almost exclusively in controlled laboratory settings (Toth & Pavia 2000; Sotka et al. 2002; Rohde et al. 2004; Diaz et al. 2006; Macaya et al. 2005; Toth 2007; Yun et al. 2007; Rohde & Wahl 2008; Yun et al. 2012, Flöthe et al.2014a). Thus, the link between naturally varying herbivory and induced resistance, which has been well documented in some terrestrial ecosystems (e.g., Karban et al. 1999; Long et al. 2011; Wieski et al. 2014) remains poorly explored in marine macroalga-herbivore interactions. Consequently, we lack information on the main selective forces that favor induced resistance, as well as the relevance of the induced defense strategy and water-borne signaling in natural populations.

1.3 Aims of the thesis

I have explored ecology and evolution of macroalgal resistance against herbivory in the framework of the optimal defense model (McKey 1974; Feeny 1976; Rhoades 1979) and the induced defense model (Karban & Baldwin 1997). My study species is the long-living marine brown alga *Fucus vesiculosus* (L), which is considered as an important foundation species in Baltic Sea rocky littoral habitats (Kautsky 1992; Jormalainen & Honkanen 2008, Wahl et al. 2011). *F. vesiculosus* populations in the northern Baltic Sea offer an interesting framework to study evolution of herbivore resistance as there is great seasonal variation in growth and reproduction of both the alga (Bäck et al.1991), and its herbivore, the crustacean isopod *Idotea balthica* (Pallas) (Salemaa 1979, Salemaa 1986, Korpinen et al. 2010) generating temporal variation in the strength of species interactions. *I. balthica* is a generalist but prefers *F. vesiculosus* as a host (Jormalainen et al. 2005), and is the main herbivore of the species. Consequently, my focus has been on the interaction of these two species.

My first aim was to test, whether induced resistance is triggered by cues that reliably signal the risk of herbivory, and whether it negatively affects herbivore preference and performance (**I**). Second, in a field experiment, I explored whether induced resistance in *F. vesiculosus* is adopted according to spatial and temporal variation of herbivory in natural population, or altered by the third trophic level. By comparing the variation in induced resistance to the frequency of herbivory, my aim was to recognize the main selective forces behind the plasticity of resistance (**II**). To estimate the evolutionary potential of resistance traits, I explored the magnitude of genetic variation in fixed and plastic resistance traits (**I-IV**). The key assumption in the theory of plant defenses is the costs of resistance (McKey 1974; Feeny 1976; Rhoades 1979; Karban & Baldwin 1997), and to address this, I explored the benefits and costs of phlorotannin production and herbivore resistance in a natural environment with varying abiotic conditions and herbivory (**III**, **IV**). Finally, earlier laboratory studies have shown that *F. vesiculosus* is able to sense and use water-borne cues originating from the grazing of conspecific algae to increase resistance before the arrival of the herbivore (Rohde et al. 2004; Yun et al. 2007; Yun et al. 2012). My last aim was to test, whether the resistance induction spreads via water-borne cues also in natural conditions of marine sublittoral, and over what distances (**IV**).

I carried out a combination of field and laboratory experimentation to specifically address the following questions (the chapter numbers given in brackets).

i) Is the induced resistance cue-specific, and does it possess costs for the herbivore in terms of reduced performance? (I)

ii) Do herbivores and the third trophic level impact the performance and induction of resistance in natural macroalgal populations? (II)

iii) Is there genetic variation in resistance to herbivory (I, II, IV)?

iv) What is the role of phlorotannins in herbivore resistance (I, III, IV)?

v) Are phlorotannin production and herbivore resistance costly (III, IV)?

vi) Does induced resistance spread via water-borne cues in natural conditions? (IV)

2. MATERIALS AND METHODS

2.1 Study area

My study area is in the Archipelago Sea in southwestern Finland, a part of the northern Baltic Sea (Fig.1). The area is characterized by low water salinity (5.5-6.5 psu), the absence of tides, and very strong seasonality. Favorable summer months, with seawater temperatures occasionally reaching 20°C, are followed by harsh winters during which wide areas of the sea are often covered with ice. Species diversity is lower compared to the southern Baltic Sea, where salinity is higher, or to oceanic systems (Bonsdorff 2006). Food webs tend to be simpler but the commonly occurring species are often highly abundant.

I collected *F. vesiculosus* individuals for laboratory and field experiments from two sites in the Archipelago Sea. For the experiments in chapters I and IV, I collected the genotypes from the island of Korsholm (WGS 60° 8.32'N, 22° 17.3'E) comprising a large and abundant *F. vesiculosus* population less than10 kilometers from the mainland. The collection site of the algae used in the experiments in chapters II and III was situated south of Korppoo around the island of Trutkobben (WGS84 60° 4.7′ N, 21° 32.4′ E). The field experiment in chapters II and III was located in a shallow bay on Bränskär Island (WGS84 60°5.21′ N, 21°57.86′E) at a site with a mixed bottom substrate and a diverse assembly of both the macroalgae and angiosperms. The field experiment in chapter IV was carried out in Seili Island in a sandy bay (WGS84: 60°14.4' N, 21°58.0' E) lacking natural *Fucus* population. All laboratory bio-assays (testing herbivore preference and performance) were done in the Archipelago Research Institute in Seili Island (Fig 1.)

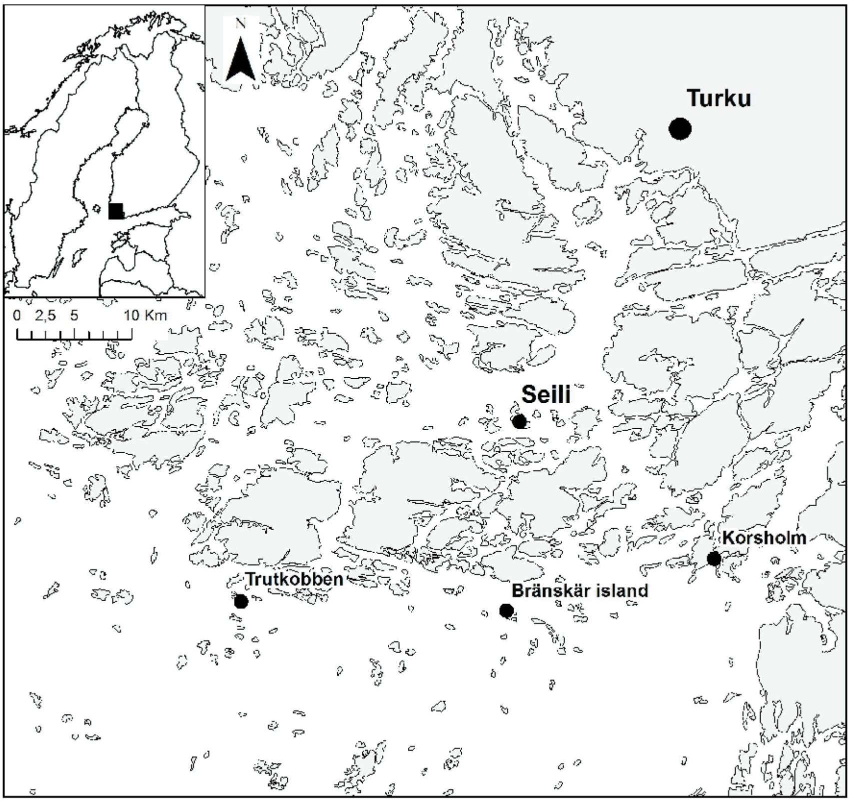

Figure 1. Map of the sampling and field experiment sites in the Archipelago Sea of the northern Baltic Sea.

2.2 Study species

Fucus vesiculosus L. (Fucales, Phaeophyceae), or bladderwrack, is a perennial brown alga growing on rocky shores in the Baltic Sea, where it offers a year-round source of habitat and food for invertebrates and fish, and a substrate on which other algae can grow (Kautsky et al. 1992; Jormalainen & Honkanen 2008, Wahl et al. 2011; Fig. 2). The species is dioecious, and in the study area it reproduces sexually (Bäck et al. 1991). Reproduction and growth are concentrated in the favorable spring and summer months, while little growth occurs in the winter. When it is not actively growing, the alga uses storage compounds like mannitol for the maintenance of essential functions; these compounds also support the growth peak in early spring (Lehvo et al. 2001; Kraufvelin et al. 2012).

In the last three decades, *F. vesiculosus* has declined in abundance, which has been mainly attributed to eutrophication coinciding with high herbivore abundances (Kangas et al.1982; Haahtela 1984; Korpinen et al. 2007c). In the outer Archipelago Sea, where the decline was most pronounced (Kangas et al.1982), the populations still have not recovered, and in the study area the species forms beds down to a depth of only about three meters (Snickars et al. 2014). While abiotic factors like salinity, wave exposure, and light influence the distribution of *F. vesiculosus* (Kautsky et al. 1992, Rinne et al. 2011), herbivores are also able to affect the abundance of the alga, both in the colonization stage (Korpinen et al. 2007b) and in mature stands of adult individuals (Jormalainen & Ramsay 2009). Despite occasional overgrazing events which result in localized destruction of *F. vesiculosus* stands, the species is rather resistant to grazing which indicated into adoption of some anti-herbivore adaptations (Salemaa 1986; Kangas 1982). Indeed, previous studies have demonstrated that in herbivore grazing *F. vesiculosus* adopts induced resistance to reduce further herbivory (e.g., Hemmi et al. 2004, Rohde et al. 2004, Yun et al. 2007, Long et al. 2007, Rohde and Wahl 2008, Flöthe et al. 2014 a; Flöthe et al. 2014 b). Furthermore, in the study area, *F. vesiculosus* contains high quantities of phlorotannins (8-14% of the dry weight), phenolic compounds unique to brown algae. Phlorotannin content varies among different parts of the alga (Tuomi et al. 1989), and the total content and composition of different polymers varies also among genotypes (Koivikko et al. 2008). Genotypes with high phlorotannin content suffer less herbivory and biofouling (Jormalainen et al. 2008; Jormalainen & Ramsay 2009). Furthermore, a phlorotannin-rich diet has been shown to reduce the food assimilation efficiency of herbivores (Jormalainen et al. 2005) and impair their growth (Jormalainen et al. 2011).

Herbivore populations in the northern Baltic Sea consist of small-sized mesograzers such as gastropods, amphipods, and isopods (Boström & Bonsdorff 1997; Korpinen et al. 2010). However, the main species responsible for grazing on adult *F. vesiculosus* is the crustacean isopod *Idotea balthica* Pallas, a widespread marine isopod which is abundant in true marine environments (35‰ salinity) but also persists in marginal populations in the brackish waters of the Gulf of Finland (3.5‰ salinity) (Salemaa 1986; Leidenberger et al. 2012; Fig. 2). *I. balthica* is described as a key species in the ecological interactions of the Baltic Sea, with the potential to cause occasional localized destruction of *F. vesiculosus* stands (reviewed in Leidenberger et al. 2012). In the study area, the species has a lifespan of 13-15 months with one major, highly synchronized reproductive period: juveniles are released in July, and the majority of adults die before autumn (Salemaa 1979). The newly-born juveniles feed first on filamentous algae, mainly *Cladophora glomerata* (L.), and then move, often at high densities, to feed on *F. vesiculosus* in late August-September (Jansson and Matthiesen 1971, Salemaa 1979). The population density of the new autumn isopod generation drops during winter, with about one-tenth surviving to the next reproductive period (Salemaa 1978; Korpinen et al. 2010). The environmental factors behind this decline are not known, but biotic factors such as predation and intraspecific competition have been suggested (Salemaa 1979).

Nearly twenty fish species are known to consume *I. balthica* (Salemaa 1986; Leidenberger et al. 2012). The most prominent fish species in the shallow waters of the study area are three-spined and nine-spined sticklebacks (*Gasterosteus aculeatus* L. and *Pungitius pungitius* L., respectively) and sand goby (*Pomatoschistus minutus* Pallas). Other common species are black goby (*Gobius niger* L.), bleak (*Alburnus alburnus* L.), eelpout (*Zoarces viviparus* L.), perch (*Perca fluviatilis* L.), and roach (*Rutilus rutilus L.*) (Rajasilta et al. 1999; Gagnon et al. 2015; Mustamäki et al. 2015; personal observations). Fish predation on herbivores has been shown to trigger trophic cascades that result in the enhanced colonization success of *F. vesiculosus* germlings (Korpinen et al. 2007b) and in alteration in the species composition of algae (Gagnon 2016).

Figure 2. The study species *Fucus vesiculosus L.* is a perennial brown alga which dominates shallow rocky shores in the Baltic Sea, where it is considered an important habitat-forming foundation species. The crustacean isopod *Idotea balthica* Pallas is a widespread marine omnivore that prefers *F. vesiculosus* as a host. In the northern Baltic Sea, grazing damage on adult *F. vesiculosus* is mainly caused by *I. balthica*, which occasionally occurs in very high densities and causes localized destruction of *Fucus* belts. Photograph of *I. balthica* by Veijo Jormalainen.

2.3 Manipulation of algae

In chapter I, my objective was to test the cue-specificity of induced resistance in *F. vesiculosus*. For this, I tested if induced resistance was differently expressed in the algae that were exposed to herbivore grazing, artificial damage, pure herbivore presence and to water-borne cues released by the grazing of conspecifics. I further tested whether induced resistance caused performance costs for *I. balthica*. I conducted the manipulations in chapter I, and the consecutive herbivore preference bio-assays exploring induced resistance (I, II, IV) in laboratory conditions in 1-liter glass containers under greenhouse light at 15 °C.

I performed manipulative field experiments in three chapters (II, III, and IV). For the manipulations, I used steel-framed cages which were either open or covered with different-sized mesh-nets that prevented the access of herbivores, fish, or both to the algae (Fig. 3a). In chapters II and III, I manipulated the access of naturally occurring herbivores and fish predators to the algae in two different seasons to explore seasonal variation in herbivory on *F. vesiculosus*, as well as variation in the trophic cascades caused by herbivore-consuming fish. In chapter IV, I explored the spread of resistance via water-borne cues in natural conditions. As a cue-source, I used experimental cages in which *I. balthica* was enclosed, at natural densities, to feed on *F. vesiculosus.* The cue-receiving algae were protected from natural herbivores within cages covered with small-sized mesh-net (Fig. 3a), and were placed in the vicinity of a cage that contained algae and actively feeding herbivores (Fig. 3b).

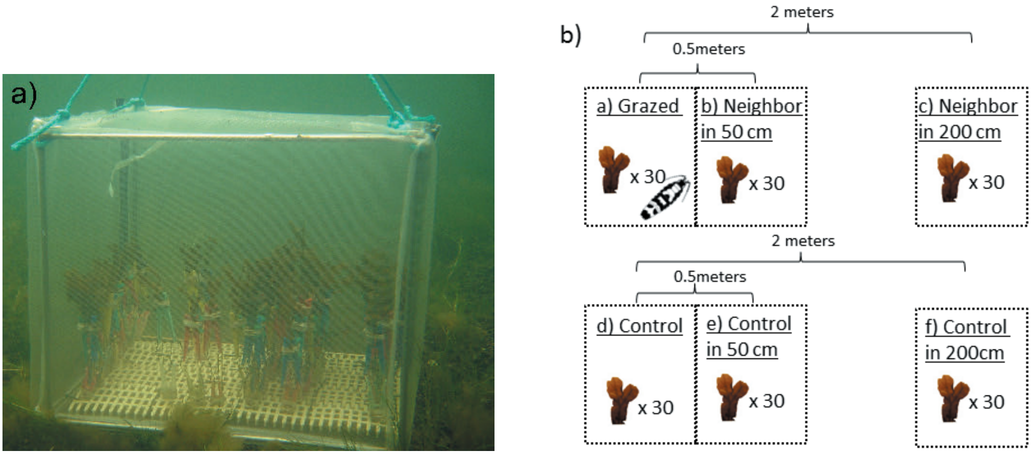

Figure 3. a) An example of field manipulation: a herbivore exclusion cage containing pieces of different *F. vesiculosus* genotypes (II, IV). **b)** The experimental setup used to explore the spread of induced resistance via water-borne cues (IV). In **b),** pieces from 30 algal genotypes were replicated in each cage and exposed to water-borne cues originating from grazed algae at a distance of either 50 or 200 cm. Photograph by Veijo Jormalainen.

To estimate genetic variation in algal resistance traits and growth, I used clonal material with pieces of individual algae were replicated within and among treatments (I, II, III, and IV; Fig. 3a). In the study area, *F. vesiculosus* reproduces sexually, and the phenotypes in the natural population comprise genetically unique individuals (Serrão et al.1997). In my work, I defined a genetic individual as an entity growing from a single thallus branch in a holdfast, according to earlier studies (Honkanen & Jormalainen 2005; Jormalainen & Ramsay 2009). I used the term *genotype* to encompass all the algal parts that originated from a single genetic individual. Due to short-range gamete dispersal (<10 m; Serrão et al.1997), individuals growing closely together might be close relatives.

To avoid the confounding effects of estimating genetic variation from closely related individuals, I always collected different genotypes 10-100 meters from each other. The estimation of genetic effects from clonal material presumes that carry-over effects from the environment of the clonal parent are minimal (Schwaegerle et al. 2000). To minimize such effects, I reared the pieces of all genotypes in a common environment before each experiment, either in flow-through mesocosms situated outdoors for two to eight weeks (I, II, III), or under natural conditions (IV). This length of time was sufficient for any induced resistance that may have been present at the time of collection (due to, for example, grazing) to relax (Rohde & Wahl 2008; Flöthe et al. 2014b). To obtain estimates of genetic variation, I replicated pieces of each genotype within and among treatments. This approach allowed me to separate the variance components due to genotype (G), environment (E), or the interaction of the two (G x E) (Thompson 1991). This approach yields "heritability in the broad sense", which includes effects due to dominance and epistasis, and thus is equivalent to or larger than the additive genetic variation (Falconer & Mackay 1996).

2.4 Measured traits

I explored grazing damage and growth of *F. vesiculosus* by measuring several traits: the number of apical tips, biomass, thallus area, and the length of undamaged vegetative branches (Table 1.). In *Fucus*, the meristematic cells are located in apices, and part of these initiates the transformation into receptacles, the reproductive structures (Bäck et al. 1991). Thus, the number of apices is a strong indicator of both future growth potential and reproductive output, and can be used as a proxy for algal fitness (Jormalainen and Honkanen 2004). To estimate grazing damage, I measured the biomass and thallus area of herbivore-exposed algae; in addition, I estimated algal growth in the presence of herbivores by measuring the length of the undamaged vegetative branches. These values were all compared with those obtained from algae growing in the absence of herbivores. In chapter II, I collected herbivores from the experimental cages and counted fish at the site in order to determine the species composition and densities of these two groups.

As measures of resistance to herbivory, I used estimates of the change in biomass, the number of apical tips, or thallus area. In chapters II and III, due to pronounced grazing damage on the algae, I used the values for change in biomass and numbers of apices directly as measures of resistance. In chapter IV, I corrected biomass and apical changes measured in herbivory with growth that was measured in the absence of herbivores. I also used growth estimates taken in the absence of herbivores to quantify the costs of phlorotannin production and resistance (III, IV).

To explore induced resistance, throughout the thesis I conducted pairwise herbivore preference bio-assays using a similar protocol. An individual *I. balthica* male was offered two marked apical pieces, one from the herbivory treatment and the other from the control treatment. The paired pieces were similar in size and always came from a single genotype, in order to control for genotypic variation in quality. The change in biomass of each piece was recorded over five days. I further explored the effect of induced resistance on herbivore performance by measuring *I. balthica*'s somatic growth and egg production over one intermolt period (I). I used this approach because measures of herbivore feeding preference or performance provide information on the food quality from the herbivore's perspective, and therefore, this method is considered to be more informative than measures of a single resistance-related trait (e.g., concentration of a chemical compound) (Hay et al. 1998).

To study the function of phlorotannins in resistance, I measured phorotannins in the herbivory treatments (including both the constitutive and induced phlorotannins), and in the absence of herbivores (including the constitutive phlorotannins only). Earlier studies that quantified phlorotannins and induced resistance (Yates & Peckol 1993; Hemmi et al. 2004; Long et al. 2007) yielded variable results motivating me to further explore their role in induced resistance. The total phlorotannin content was determined with the Folin-Ciocalteu method (as in Koivikko 2008). Furthermore, to study whether herbivory triggers qualitative differences in the assemblage of individual and similar sized phlorotannins, HPLC-analyses (High-Performance Liquid Chromatographic) were run for a small laboratory-manipulated set of algae with method described in Koivikko et al. (2007).

Table 1. Summary of traits measured on *F. vesiculosus* and *I. balthica* in different chapters to quantify herbivore resistance, growth and induced resistance in the algae. Phlorotannins were quantified to explore their role in *F. vesiculosus* resistance.

	Traits in algae				Induce resistance		Phlorotannins
Chapter	Biomass	Apical tips	Apical length	Thallus area	Herbivore preference	Herbivore performance	
I					X	X	X
II	X	X			X		
III			X	X			X
IV	X	X	X		X		X

2.5 Statistical analyses

I used general linear or generalized linear mixed models, in which the parameter estimation was based on the restricted maximum-likelihood method. When measuring changes in the size of the algae or the herbivore, I used ANCOVA models in which initial size was a covariate (I, II, IV). In the models, the experimental treatment was a fixed factor and the experimental unit (e.g., cage nested under treatment) was a random blocking factor. In addition, I treated genotype as a random factor. Models initially included all interactions with the fixed factors. In the analyses of herbivore preference bio-assay data, I further included the container as a within-subject effect in order to account for the interdependency of measures of the two algal pieces in the same container. I simplified the full models by eliminating the random factors and interactions that did not significantly contribute to the model fit on the basis of the Akaike Information Criterion (AIC) (Littell et al. 2006). I derived estimates of significance for random factors using likelihood-ratio tests (Bolker et al. 2009) and for fixed factors using pairwise comparisons with Tukey-Kramer tests (T-K). When the treatment interacted significantly with the covariate, I compared the treatment effects at different covariate values (10%, 25%, median, 75% and 90% quantiles of the covariate distribution) (I, II, IV). For the reaction norms and genetic correlations, I derived model estimates for the genotypes (e.g., biomass, phlorotannins) as the "best linear unbiased predictors" (BLUP, Littell et al. 2006) (II, III, IV). When the genotype or the genotype-by-treatment interacted with the initial size, I accounted for this by subtracting BLUPs over adjusted covariate value (e.g. median) (II, IV). For some models, the prerequisite of variance homoscedasticity among the grouping factors was not met and I then applied a model with heterogeneous variances (Littell et al. 2006) (II, IV). I conducted all the analyses with the SAS 9.2 or SAS 9.4 package.

3. RESULTS AND DISCUSSION

3.1 Variation in herbivory

Herbivory on *F. vesiculosus* varied over several different scales: with the size of the algae, among different parts, among genotypes, spatially within the shore, with the presence of herbivore-eating fish predators, and between the two seasons of the year (II, IV). In the natural population, the most pronounced variation in herbivory was due to seasonal variation in herbivore population densities. In autumn, the density of summer-born *I. balthica* was almost 400 individuals per square meter, while by the next spring the population had declined to 5% of this. The high *I. balthica* densities in autumn caused strong grazing pressure on *F. vesiculosus*, and algae lost, on average, 86% of the apical tips required for further growth and reproduction. Instead, during the next spring there was very little grazing damage. The strong seasonality in herbivore densities also determined the influence of the third trophic level. Fish predators had no regulatory effect over herbivores at high densities, but triggered a trophic cascade at the low herbivore densities in spring (II).

These results highlight the importance of the seasonal dynamics of mesograzer populations on the outcome of interactions among three trophic levels in the marine temperate littoral zone. Due to its synchronized reproduction (Salemaa 1979) and large production of offspring (Hemmi & Jormalainen 2004), *I. balthica* was able to escape control by predation, which is typically strong in marine benthic communities (Shurin et al. 2002; Borer et al. 2005). This resulted in strong grazing pressure and, thus, a period of strong selection for macroalgal resistance. The escape from predator control seen here could be further promoted by the ongoing eutrophication of the Baltic Sea, which increases abundance of filamentous green algae, the preferred food source for juvenile isopods, and may thus increase herbivore survival (Kangas et al. 1982). Furthermore, high nutrient availability for both filamentous algae and *F. vesiculosus* increases their quality as food, and enhances both the growth and reproduction of *I. balthica* (Hemmi & Jormalainen 2002; Hemmi & Jormalainen 2004). These factors may promote the occasionally high *I. balthica* densities observed in the Baltic Sea (Leidenberger et al. 2012). While fish were unable to control the herbivores at their high autumn densities, predation has been implicated in the wintertime decline of *I. balthica* (Salemaa 1978, Jormalainen et al. 1995). It is therefore possible that predators were able to suppress the herbivore population later in the winter, outside the temporal scope of this experiment (II).

3.2 Induced resistance as a response to grazing

Over the course of my thesis, I consistently found that grazing by herbivores induced resistance in *F. vesiculosus* (Fig. 4). Resistance induction was cue-specific, and was triggered by either direct grazing or water-borne cues from grazing, but not by the simple presence of herbivores or by simulated grazing (I, IV). Furthermore, the induction of resistance via grazing and water-borne cues resulted in fitness costs for the herbivore in terms of reduced egg production (I).

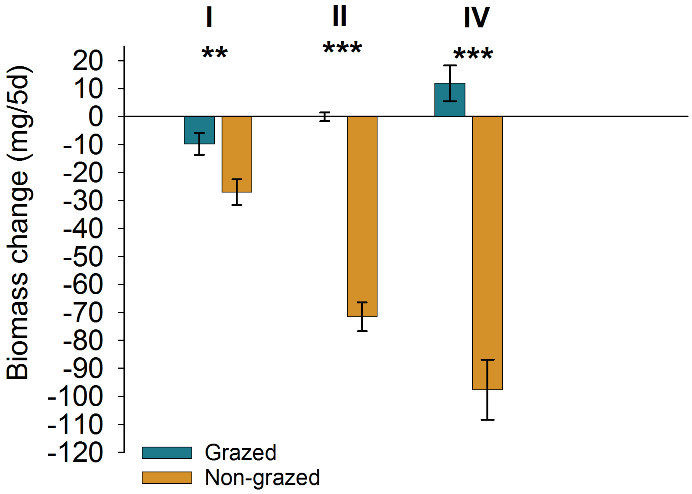

Figure 4. Summary of results from three different preference bio-assays (in Chapters I, II, IV) exploring the induction of resistance in *F. vesiculosus* as a response to direct grazing. Induction of resistance was measured in preference bio-assays where *I.balthica* male was offered an apical tip from an alga exposed to herbivores (Grazed) or excluded from grazing (Non-grazed). The biomass changes (mean ± SE) of the pieces were recorded over five days. Prior to the bio-assays the grazed algae underwent different grazing treatments: in I, the algae was grazed by *I. balthica* for five days in laboratory settings (n= 105), in II, by natural herbivory in autumn owing *I. balthica* densities of 391 individuals/m^2 (n= 102) and in IV, for 25 days in the experimental cages in the field with *I. balthica* density of 550/m^2 (n= 92). The non-grazed algae were manipulated in the same conditions but without herbivores. Significant differences are shown by asterisks ***P<0.0001, **P<0.01.

In the natural populations, I found that the induction of resistance followed the patterns of seasonal variation in grazing pressure: strong induction during high herbivory in autumn, and no induction during low herbivory in spring (II). Indeed, at high herbivore densities, the strong induced resistance was independent of the smaller-scale variation in grazing damage observed within the shore and among genotypes (II, IV). However, induced resistance during autumn was affected by the third trophic level, the fish predators, as the number of algae showing induced resistance was smaller when these predators were present than when they were absent (II).

My results support the induced defense model (Karban & Baldwin 1997) by showing that induced resistance in *F. vesiculosus* is triggered by cues from herbivory, and that algal resistance is increased through a decrease in herbivore preference and performance (I). I further found that induced resistance varied with seasonal variation in herbivore density and grazing (II). However, at high herbivore densities, induction was independent of the grazing damage (II, IV). In some plant species, resistance induction occurs when a certain threshold is met in the amount of biomass lost (Underwood 2000), or the amount of triggering cue received. Here, the lack of correlation between damage and resistance induction suggests that this threshold in *F. vesiculosus* might be very low, either in terms of the amount of grazing damage or the water-borne cues that trigger induction. The strong reduction in further herbivory on the induced algae indicates that induced resistance is a very efficient resistance strategy, and is likely important in minimizing mortality during the periods of high grazing pressure (II, IV). Furthermore, by decreasing herbivore performance, induced resistance may suppress the population growth rate of *I. balthica* and reduce future grazing pressure (I).

The model of Karban & Baldwin (1997) predicts that induced resistance is favored in environments in which herbivory varies temporally or spatially, and where the cues from herbivory reliably predict the probability of further grazing. Here, the main variation in herbivory was seasonal, and arose from the population dynamics of *I. balthica*. Induced resistance in *F. vesiculosus* followed this seasonal variation, which suggests that synchronous breeding of *I. balthica* in the littoral of the northern Baltic may be an important force selecting for plasticity in algal resistance. Due to the synchronous herbivore breeding, grazing pressure is seasonally predictable; the damage from the first juveniles to move from filamentous algae to feed on *F. vesiculosus* in the autumn offers a reliable cue for the oncoming increase in grazing. The magnitude of an attack, however, remains somewhat unpredictable due to spatial (Salemaa 1978; Vesakoski 2009) and among-year variation in *I. balthica* densities (Gagnon et al. 2016). Selection that includes both predictable and unpredictable components, such as that observed here, has been suggested to favor the evolution of induced resistance (Tollrian and Harvell 1999). The benefit of plasticity likely arises in the spring and summer months, when herbivore densities are low and resources can be allocated to reproduction and vegetative growth instead of to constitutive defenses.

The presence of fish predators reduced the magnitude of the induced resistance in *F. vesiculosus*. Fish predation did not, however, decrease the total grazing damage on the algae. A likely explanation for the decrease in induction is that fish triggered spatial variation in grazing by efficiently removing herbivores over a very small spatial scale within the experimental cages. Consequently, some of the algae suffered so little grazing damage that resistance was not induced. Two observations in my data support this explanation. First, I found a marginally significant tendency for spatial variation in grazing to increase in the presence of fish. Second, there were more non-induced algae in the presence of fish. These observations are consistent with the idea that fish may cause small-scale spatial variation in grazing damage and thereby increase the number of non-induced algae. Thus, the third trophic level may affect induced resistance in plant populations not just via decreased grazing *per se* but also via changes in the pattern of damage accumulation among the different individuals.

3.3 Induced resistance via water-borne cues

In addition to direct grazing, resistance in *F. vesiculosus* was also induced by water-borne cues originating from grazed conspecifics, both in the laboratory assays and in the natural environment of the marine sublittoral (I, IV). In the natural setting, the spread of resistance via water-borne cues occurred over a distance of ≤2 m, but varied spatially. Induction was weaker in the algae that were induced through water-borne cues than in the directly grazed algae, but it was consistently expressed among genotypes (IV). Moreover, the performance of *I. balthica* was reduced when feeding on algae in which resistance was induced by water-borne cues (I).

My findings are in agreement with earlier laboratory studies (Rohde et al. 2004; Yun et al. 2007; Yun et al. 2012) in showing that *F. vesiculosus* is able to sense and use the information carried by water-borne cues to increase resistance before an herbivore attack. My results further demonstrate that, in natural settings, these cues spread over ecologically relevant scales and result in the induction of non-attacked individuals in a dense macroalgal stand. This suggests that when large macroalgal stands are subjected to herbivore attacks, especially of the synchronized type observed here, an entire stand could become induced via the gradual spread of the cue. This strategy may enable the algae to withstand occasional intense grazing pressure (Hay 1991; Poore et al. 2012). Most marine herbivores are generalists (Hay 1996), and thus the spread of induced resistance over a macroalgal stand could motivate them to feed on other food sources or to move to other, as-yet un-induced stands. Furthermore, the negative impacts of induced resistance on herbivore performance (Toth et al. 2005) are likely amplified if the spread of resistance deteriorates food quality over large stands. This could result in stronger effects on herbivore population size and increased prevention of future grazing damage than can be anticipated from short-term laboratory experiments.

In addition, I found that *F. vesiculosus* is able to fine-tune its resistance depending on the cue received. Resistance induction was stronger in the directly grazed algae than in those induced via water-borne cues (IV). The difference might imply that the allocation of resources to costly defenses increases as the reliability of the induction cue increases (Karban & Baldwin 2007). Here, loss of biomass together with possible chemical cues in herbivore saliva or other excretions likely comprise a more reliable cue of the immediate threat, and thus trigger stronger resource allocation to defense, than water-borne cues do. Weaker water-borne induction could be a way to conserve some resources for defense in anticipation of an actual attack, and demonstrate one way in which macroalgae could optimize the allocation of costly resources for defense.

3.4 Genetic variation in growth and resistance

I found a genetic component to the variation in *F. vesiculosus*' overall resistance to herbivores (including both constitutive and induced resistance), in resistance induced by grazing, and in phlorotannin content, but not in resistance induced by water-borne cues (Table 2). The amount of genetic variation depended on the measure of resistance (apical tips or biomass) and on the resistance trait. When I measured resistance over the whole individual, in terms of the remaining biomass or thallus area in herbivory, genetic variation accounted for 22-38% of the variation in resistance (Table 2, Fig.5a). This corresponds well with previous measurements conducted both in mesocosms and in the field (Jormalainen et al. 2008; Jormalainen and Ramsay 2009). However, when resistance was measured as the number of remaining apical tips in herbivory, the contribution of genetic variation was somewhat smaller. This is likely because herbivores demonstrate a strong preference for the apices, and due to this, most of the variation was eliminated in this measure (II). However, as the number of apical tips is an important fitness component (Jormalainen and Honkanen 2004), even a small degree of genetic variation influencing their loss could result in fitness differences and selection among genotypes. Furthermore, I found intraspecific variation in phlorotannin content, to which genetic variation contributed considerably (Table 2). This is in accordance with earlier studies showing that phlorotannins vary widely among genotypes (Jormalainen et al. 2003; Jormalainen & Honkanen 2008; Koivikko et al. 2008; Jormalainen et al. 2011). As genetic variation is a prerequisite for the evolution of traits, the results here show that most of the measured resistance traits in *F. vesiculosus* have strong potential for continued evolution.

Table 2. Degree of genetic determination of different traits of *F. vesiculosus*. Percent (%) is the proportion of genotypic variance (V_G) in relation to the total phenotypic variance (V_P). Resistance was measured as the complement of the loss to herbivory of apical tips, biomass, or thallus area. Growth and constitutive phlorotannin content were measured in the absence of herbivores.

Trait	Measure	V_G/V_P (%)	Chapter
Total resistance (Constitutive and induced)			
	Apical tips	10 - 15	II, IV
	Biomass	22 - 38	II, IV
	Thallus area	25	III
Induced resistance in direct grazing	Herbivore preference	10	I, II
Induced resistance, water-borne cues	Herbivore preference	0	IV
Growth (in the absence of herbivores)	Apical tips	5.8 - 15	II, IV
	Length of branches	13	IV
	Biomass	8.3 -31	II, IV
Phlorotannins	Induced and constitutive	44	III
	Constitutive	22 - 32	III, IV

After encountering grazers, the majority of the *F. vesiculosus* genotypes demonstrated induced resistance and after induction I observed very little variation among genotypes in their palatability (Fig. 5b, I, IV), and little genotypic variation in the induced resistance (Table 2). The lack, or minimal amount, of genetic variation could be a result of directional selection on this trait which has eliminated variation and driven it to fixation (Agrawal et al. 2002). It is possible that the prominent spatial and temporal variation in herbivory within the *Fucus* populations of the northern Baltic (Salemaa 1978; Salemaa 1979; Korpinen et al. 2010, Vesakoski 2009; Gagnon et al. 2016) has caused strong directional selection for plasticity in resistance, and consequently led to reduced variation in the induced resistance. To address this, comparative studies are needed of genetic variation in induced resistance among populations with varying consumer pressure, in order to clarify whether the small degree of variation is the result of selection or random processes.

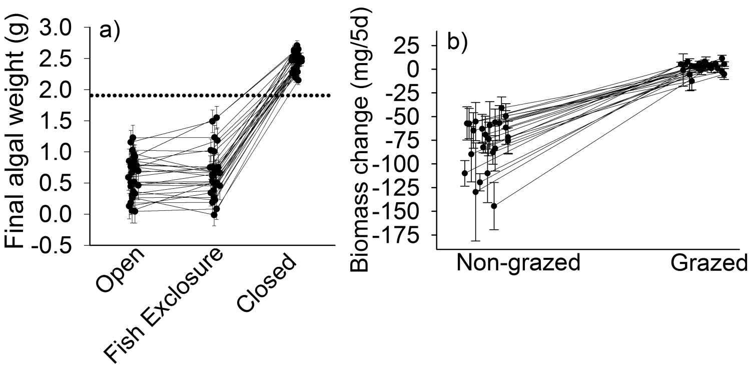

Figure 5. Genotypic variation in **a)** resistance to herbivores (including constitutive and induced resistance) during a natural peak in grazing pressure (II) and in **b)** induced resistance of *F. vesiculosus*. Total resistance **(a)** was measured as the difference in final biomass of the genotypes after 25 days with herbivores and fish present (open), with only herbivores present (fish exclosure), or with both fish and herbivores absent (closed). The dotted line shows the median initial biomass, for which the final biomass of each genotype was adjusted. Induced resistance **(b)** was measured via herbivore consumption (change in algal biomass) of pieces originating from non-grazed (from closed cages) and grazed algae (data pooled from fish exclosure and open cages). Each dot is the biomass change of one genotype (mean ± SE), and the lines connect values from the same genotype from the two treatments.

3.5 Phlorotannins as a resistance trait

Herbivory on algal tissues decreased with increased constitutive phlorotannin content in laboratory-run feeding trials and in the field during the autumn conditions of high herbivory. This suggests that constitutive phlorotannins contribute to herbivore resistance in *F. vesiculosus*. Under conditions of low spring herbivory, though, there was no relationship between grazing damage and constitutive phlorotannin content (III). Furthermore, I consistently found throughout my thesis work that grazing resulted in an increase in induced phlorotannin content (Fig. 6, I, III, IV). However, I also found that the relationship between the induction of phlorotannins and the induction of resistance was not straightforward. In chapter I, direct grazing resulted in the induction of both increased phlorotannin content and resistance. In addition, the mere presence of herbivores, without grazing, was sufficient to induce an increase in phlorotannin content. However, herbivore presence had no effect on the induction of resistance. Likewise, in chapter III, the magnitude of the increase in induced phlorotannin content did not covary with the strength of resistance induction. Finally, in chapter IV, algae in which resistance was induced by water-borne cues did not show an increase in phlorotannin content. However, the HPLC analysis that separated individual or similar-sized phlorotannin polymers showed that both grazing and the reception of water-borne cues from grazed individuals did cause changes in the composition of the phlorotannin profile (III).

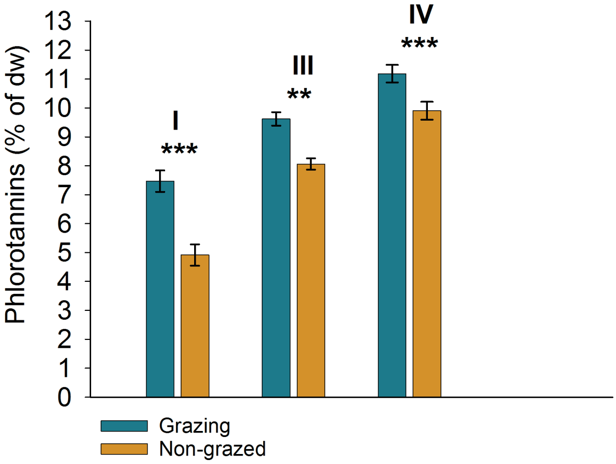

Figure 6. Summary of the results from three experiments (in chapters **I, III, IV**) which explored the induction of phlorotannins in *F. vesiculosus* that was subjected to direct grazing. In **I,** the grazed algae were exposed to *I. balthica* for five days in a laboratory setting (n = 105); in **III,** to natural herbivory with *I. balthica* densities of approximately 391 individuals/m^2 (n = 102); and in **IV**, to 25 days in experimental cages in the field, with *I. balthica* present at a density of 550/m^2 (n = 92). Significant differences are shown with asterisks: ***P<0.0001, **P<0.01.

In showing that high phlorotannin content increases *F. vesiculosus*' resistance to herbivory, my results are consistent with those of earlier studies (Jormalainen et al. 2008, Jormalainen & Ramsay 2009, Nylund et al. 2012). However, the benefit of high constitutive phlorotannin content, in terms of herbivore resistance, was not evident in the wild during the low herbivory season. Thus, the selection for high constitutive phlorotannin levels in natural populations likely varies seasonally with herbivory (III). Indeed, high levels of constitutive phlorotannins would offer a larger selective advantage during periods of intense herbivory because the algae would benefit from immediate and ever-present resistance, in contrast to the time lag inherent in induced resistance (Rohde & Wahl 2008, Flöthe et al. 2014b).

My findings also imply that the quantitative induction of phlorotannins does not exclusively explain induced resistance in *F. vesiculosus*. However, the results from my analyses of phlorotannin profiles suggest that at least part of the induction of resistance may be due to qualitative differences in the phlorotannin assemblage, rather than increases in total quantity. Instead, quantitative increases in phlorotannins were linked to grazing in both the laboratory (I) and field assays (III, IV). This may indicate a role for phlorotannins in other herbivory-related processes, such as wound healing (Halm et al. 2011; Lüder & Clayton 2004). Phlorotannins are also exuded into the water surrounding algae (Jennings & Steinberg 1994; Koivikko et al. 2005), where they have been shown to repel herbivores (Pereira et al. 2015). Thus, an increase in phlorotannin content due to grazing, and the consequent increase in exudation (Koivikko et al. 2005), may affect biotic interactions within the water column, particularly through the spread of waterborne resistance to neighboring individuals.

3.6 Costs of resistance

In chapters III and IV, I explored whether phlorotannin production and herbivore resistance incurred costs for the algae. I found that when algae were exposed to intense autumn herbivory, there was a genetic tradeoff between the levels of induced and constitutive phlorotannins. There was, however, no correlation between growth and either constitutive or induced phlorotannins. In the low-herbivory conditions of the following spring, I found a tendency for a negative correlation between growth and phlorotannin content (III). In addition, in the field experiment conducted in spring, I found a strong negative correlation between total herbivore resistance and growth (IV).

The detection of trade-offs often depends on the specific fitness component that is measured (Strauss et al. 2002) or on the environmental conditions in which measurements take place (Steinberg 1995, Pavia et al. 1999). My results support earlier conclusions that there are costs involved in phlorotannin production (Yates & Peckol 1993, Pavia et al. 1999; Johannesson et al. 2012) and in herbivore resistance (Jormalainen & Ramsay 2009), as assumed by the optimal defense model. My findings also show, however, that the realization of these costs varies with seasonal variation in herbivory and resource allocation in the algae. In the highly seasonal northern Baltic Sea, growth of *F. vesiculosus* is most intense during the spring and summer months (Bäck et al. 1999; Kraufvelin et al. 2012), while in autumn, growth is limited by light availability and the differentiation of the reproductive tissue (receptacles) begins (Bäck et al. 1999: Lehvo et al. 2001). The absence of an apparent trade-off between growth and phlorotannin production in the autumn is likely the result of a shift in resource allocation from growth to other needs, meaning that growth in autumn would be a very poor currency with which to measure costs. These results highlight the importance of considering seasonal variation in patterns of grazing pressure and life-history traits when measuring the benefits and trade-offs of defensive metabolites in natural populations.

4. CONCLUSIONS AND FUTURE DIRECTIONS

Here, I have tested assumptions of both the optimal defense model and the induced defense model (Karban & Baldwin 1997) in the interactions between a marine herbivore and its macroalgal host in the seasonal northern Baltic Sea. I have quantified variation in the extent to which macroalgal resistance to herbivores is triggered by the herbivores themselves, and investigated the influence of the third trophic level on this interaction. My results show that seasonality in the northern Baltic Sea can have a strong impact on three-trophic-level interactions in macrophyte communities. I found that the highly synchronized summer reproduction of the mesograzer *Idotea balthica* enabled it to escape from the trophic control of fish predators, thus resulting in large grazing losses in *F. vesiculosus*. However, in the following spring, herbivore densities were low, and fish were able to regulate the herbivore population and thus enhance algal growth. My findings imply that temporally synchronized mesograzer reproduction could, in certain situations, promote herbivore escape from the top-down control of predatory fish. This, in turn, could contribute to the often-observed high grazing losses in littoral macrophyte assemblages in temperate seas (Poore et al. 2012). The results here also demonstrate that in temperate marine systems, the strength of three-trophic-level interactions can vary considerably among seasons, and thus indicate that caution is required in interpreting field experiments conducted at a single point in time, as they may not give a correct or complete representation of the strength of species interactions.

I suggest that phenotypic plasticity in *F. vesiculosus*' herbivore resistance is an important defense strategy in the northern Baltic Sea environment with temporally varying herbivory. My findings further show that many of the preconditions of adaptive phenotypic plasticity are fulfilled here. First, resistance to herbivory and phlorotannin production as a resistance trait are costly for algae (III, IV). Herbivory in a natural population varied both temporally and spatially, with herbivores having the ability to occasionally cause substantial damage to algae as well as to generate selection for the evolution of resistance (II). *F. vesiculosus* was able to adjust its phenotype according to cues that reflected changes in the risk of grazing (I, IV). Furthermore, the variation in induced resistance followed seasonal variation in grazing pressure in natural populations (II). Overall, the resistance induced by grazing seemed to be very efficient: it resulted in the strong deterrence of herbivores and in reduced herbivore performance (I, II, IV). While the fitness benefits of plasticity in resistance remain to be verified experimentally, my results imply that induced resistance in *F. vesiculosus* is an adaptation to the variable herbivory of the northern Baltic Sea.

F. vesiculosus is able to use water-borne info-chemicals released in the grazing of conspecifics to increase resistance prior to the arrival of an actual herbivore attack. The results presented here comprise the first evidence that this water-borne induction is also relevant under ecologically realistic conditions in natural macroalgal populations (IV). This suggests that the spread of water-borne induction may enable macroalgal stands to cope with the strong grazing pressure characteristic of marine benthic communities (Hay 1991, Poore et al. 2012). While the use of info-chemicals to increase resistance is well established in terrestrial plants (Karban et al. 2014), the generality of this phenomenon in natural populations of marine macroalgae needs to be verified. Future studies are clearly required to identify the chemical structure of the info-chemicals involved in water-borne induction. Furthermore, the key question regarding the evolutionary plausibility of warning neighboring individuals about upcoming herbivory has remained largely unanswered in terrestrial systems (Mescher & De Moraes 2015), and is basically unexplored in the marine macroalgae. Controversy exists about whether the emitted cues are instead targeted to the individual itself, for instance, to aid the spread of systemic resistance, or whether the emitter benefits through kin selection by increasing the resistance of related individuals growing nearby (Heil et al. 2014). These ideas need further testing in natural populations, and offer interesting targets for future studies, particularly in the marine realm.

The results in this thesis highlight the overall importance of variation in temperate marine sublittoral communities: variation in abiotic environments, in biotic interactions, and in the heredity of resistance traits in primary producers. In the highly seasonal northern Baltic Sea, variation in the abiotic environment has shaped life-history strategies of key species in littoral communities. I show here that this seasonality can have a strong impact on the realization of life history trade-offs, the strength of three-trophic-level interactions, and on selective pressures for herbivore resistance traits in primary producers. The keys to understanding the evolutionary potential of a species and the forces driving evolutionary processes in natural populations can be found in recognizing the degree of genetic variation and environmental heterogeneity in these systems. The results of this work emphasize the necessity of incorporating these components into field studies in order to gain a better understanding of the interactions in temperate marine sublittoral communities.

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REFERENCES

Agrawal, A.A. (2001). Phenotypic plasticity in the species interactions and evolution of species. *Science*, 294, 321-326.

Agrawal, A.A., Conner, J.K., Johnson, M.T.J. & Wallgrove, R. (2002). Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution*, 56, 2206-2213.

Amsler, C.D. (eds.) (2008). *Algal chemical ecology*. London, Springer.

Amsler, C.D. & Fairhead, V.A. (2006). Defensive and sensory chemical ecology of brown algae. *Advances in Botanical Research*, 43, 1-91.

Auld, J.R., Agrawal, A.A. & Relyea, R.A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B-Biological Sciences*, 277, 503-511.

Barton, K.E. (2016). Tougher and thornier: general patterns in the induction of physical defence traits. *Functional Ecology*, 30, 181-187.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J-S.S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24, 127–135.

Bonsdorff, E. (2006). Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem. *Journal of Experimental Marine Biology and Ecology*, 330, 383-391.

Borer, E.T., Seabloom, E.W., Shurin, J.B., Anderson, K.E., Blanchette, C.A., Broitman, B., Cooper, S.D. & Halpern. B. S. (2005). What determines the strenght of a trophic cascade? *Ecology*, 86, 528-537.

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.

Brawley, S. H. (1992). Mesoherbivores. In: John, D.M., Hawkins, S.J. & Price, J.H. (eds). Plant-animal interactions in the marine benthos. Clarendon Press, Oxford, pp. 235-263.

Bradshaw, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13, 115-155.

Bäck, S., Collins, J.C.& Russell, G. (1991). Aspects of the reproductive biology of *Fucus vesiculosus* from the coast of SW Finland. *Ophelia*, 34, 129–141.

Callahan, H.S., Maughan, H. & Steiner, U.K. (2008). Phenotypic plasticity, costs of phenotypes, and costs of plasticity. *Annals of the New York Academy of Sciences*, 1133, 44-66.

Campbell, S.A. & Kessler, A. (2013). Plant mating system transitions drive the macroevolution of defense strategies. *Proceedings of the National Academy of Sciences of the United States of America*. 110, 3973-3978.

Cyr, H. & Pace, M.L. (1993). Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature*, 361, 148–150.

DeWitt, T.J., Sih, A. & Wilson, D.S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13, 77-81.

Díaz, E., Güldenzoph, C., Molis, M., McQuaid, C. & Wahl, M. (2006). Variability in grazer-mediated defensive responses of green and red macroalgae on the south coast of South Africa. *Marine Biology*, 149, 1301-1311.

Dudley, S.A. & Schmitt, J. (1996). Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist*, 147, 445-465.

Dworjanyn, S. A., Wright, J. T., Nicholas, A. P., de Nys, R. & Steinberg, P.D. (2006). Cost of chemical defense in the red alga *Delisea pulchra*. *Oikos*, 113, 13–22.

Estes. J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R. & Wardle, D.A. (2011). *Trophic downgrading of planet earth*. *Science*, 333, 301–306

Falconer, D.S. & Mackay, T.F.C. (1996). *Introduction to quantitative genetics.*4th edn. Longman Group Ltd, Harlow, Essex, UK.

Feeny, P. (1975). Biochemical coevolution between plants and their insect herbivores. In Gilbert, L.E. & Raven, P.H. (eds.) Coevolution of animals and plants. University of Texas Press, Austin, pp. 3-19.

Flöthe, C.R., John, U. & Molis, M. (2014a). Comparing the relative importance of water-borne cues and direct grazing for the induction of defenses in the brown seaweed *Fucus vesiculosus*. *PLoS ONE*, 9, e109247.

Flöthe, C.R., Molis, M. & John, U. (2014b). Induced resistance to periwinkle grazing in the brown seaweed *Fucus vesiculosus* (Phaeophyceae):

Molecular insights and seaweed-mediated effects on herbivore interactions. *Journal of Phycology*, 50, 564-576.

Frost, C.J., Appel, H.M., Carlson, J.E., De Moraes C.M., Mescher, M.C. & Shultz, J.C. (2007). Within-plant signalling via volatiles overcomes vascular constraints on systemic signaling and primes responses against herbivores. *Ecology Letters*, 10, 490-498.

Gagnon, K. (2016). Top-down and bottom-up impacts of the great cormorant (*Phalacrocorax carbo sinensis*) on coastal benthic communities in the Baltic Sea. *Annales Universitatis Turkuensis*, Turku.

Gagnon, K., Yli-Rosti, J. & Jormalainen, V. (2015). Cormorant-induced shifts in littoral communities. *Marine Ecology Progress Series*, 541, 15-30.

Gagnon, K., Sjöroos, J., Yli-Rosti, J., Stark, M., Rothäusler, E. & Jormalainen, V. (2016). Nutrient enrichment overwhelms top-down control in algal communities around cormorant colonies. *Journal of Experimental Marine Biology and Ecology*, 476, 31-40.

Griffin, C.A.M. & Thaler, J.S. (2006). Insect predators affect plant resistance via density- and trait-mediated indirect interactions. *Ecology Letters*, 9, 338–346.

Gruner, D.S., Smith, J.E., Seabloom, E.W., Sandin, S.A., Ngai, J.T., Hillebrand, H., Harpole, W.S., Elser, J.J.,Cleland, E.E., Bracken, M.E.S., Borer, E.T. & Bolker, B.M. (2008). A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters*, 11, 740–755.

Haahtela, I. (1984) A hypothesis of the decline of bladder wrack (*Fucus vesiculosus* L.) in SW Finland in 1975-1981. *Limnologica* , 15, 345-350.

Hay, M.E. (1991). Marine-terrestrial contrast in the ecology of plant chemical defenses against herbivores. *Trends in Ecology & Evolution*, 6, 362-365.

Hay, M.E. (1996). Marine chemical ecology: what's known and what's next? *Journal of Experimental Marine Biology and Ecology*, 200, 103-134.

Hay, M.E., Stachowicz, J.J., Cruz-Rivera, E., Bullard, S., Deal, M.S. & Lindquist, N. (1998) Bioassays with marine and freshwater macroorganisms. In: Haynes KF & Millar JG (eds.) Methods in chemical ecology. Kluwer Academic Publishers, Norwell, pp. 39-141.

Halm, H., Lüder, U.H. & Wiencke,C. (2011). Induction of phlorotannins through mechanical wounding and radiation conditions in the brown macroalga *Laminaria hyperborea*. *European Journal of Phycology*, 46,16-26.

Helms, A.M., De Moraes, C.M., Tooker, J.F. & Mescher, M.C. (2013). Exposure of *Solidago altissima* plants to volatile emissions of an insect antagonist (*Eurosta solidaginis*) deter subsequent herbivory. *Proceedings of the National Academy of Sciences of the United States of America*.110, 199-204.

Heil, M. & Silva Bueno, J.C. (2007). Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 5467-5472.

Hemmi, A. & Jormalainen, V. (2002). Nutrient enhancement increases performance of a marine herbivore via quality of its food alga. *Ecology*, 83, 1052–1064.

Hemmi, A. Honkanen, T., & Jormalainen, V. (2004). Inducible resistance to herbivory in *Fucus vesiculosus*- duration, spreading and variation with nutrient availability. *Marine Ecology Progress Series*, 273, 109-120.

Herms, D.A. & Mattson, W.J. (1992). The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, 67, 283-335.

Holeski, L.M., Chase-Alone, R., & Kelly, J.K. (2010). The genetics of phenotypic plasticity in plant defense: trichome production in *Mimulus guttatus*. *American Naturalist*, 175, 391-400.

Honkanen, T. & Jormalainen, V. (2005). Genotypic variation in tolerance and resistance to fouling in the brown alga *Fucus vesiculosus*. *Oecologia*, 144, 196-205.

Hurd, C.L., Harrison, P.J., Bischof, K. & Lobban, C.S. (2014). *Seaweed ecology and physiology*. 2nd edn. Cambridge University Press, United Kingdom.

Jansson, A-M. & Matthiesen, A.S. (1971). On the ecology of young *Idotea* in the Baltic. In: Crisp, D.J.(eds.). Fourth European Marine Biology Symposium. Cambridge University Press, London, pp. 71-88.

Jennings, J.G. & Steinberg, P.D. (1994). The in situ exudation of phlorotannins from the sublittoral kelp *Ecklonia radiata*. *Marine Biology*, 121, 349-354.

Johannesson, K., Forslund, H., Capetillo, N.Å., Kautsky, L., Johansson, D., Pereyra, R.T. & Råberg S. (2012). Phenotypic variation in sexually and asexually recruited individuals of the Baltic Sea endemic macroalga *Fucus radicans*: in the wild and after growth in a common-garden. *BMC Ecology*. 12, 2.

Jormalainen, V. & Ramsay, T. (2009). Resistance of the brown alga *Fucus vesiculosus* to herbivory. *Oikos*, 118, 713-722.

Jormalainen, V. & Honkanen, T. (2008). Macroalgal chemical defenses and their roles in structuring temperate marine communities. In Amsler, C.D. (ed.) Algal Chemical Ecology. Springer, Germany, pp. 57-89.

Jormalainen, V. & Honkanen, T. (2004). Variation in natural selection for growth and phlorotannins in the brown alga *Fucus vesiculosus. Journal of Evolutionary Biology*,17, 807-820.

Jormalainen, V., Wikström, S.A. & Honkanen, T. (2008). Fouling mediates grazing: intertwining of resistances to multiple enemies in the brown alga *Fucus vesiculosus, Oecologia.* 155, 559-569.

Jormalainen, V., Koivikko, R., Ossipov, V. & Lindqvist, M. (2011). Quantifying variation and chemical correlated of bladderwrack quality- herbivore population makes a difference. *Functional Ecology*, 24, 900-909.

Jormalainen,V., Honkanen,T., Vesakoski, O. & Koivikko, R. (2005). Polar extracts of the brown alga *Fucus vesiculosus* (L.) reduce assimilation efficiency but do not deter the herbivorous isopod *Idotea balthica* (Pallas). *Journal of Experimental Marine Biology and Ecology*, 317,143-157.

Jormalainen, V., Honkanen, T., Koivikko, R., Eränen, J. (2003). Induction of phlorotannin production in a brown alga: defense or resource dynamics? *Oikos*, 103,640-650.

Jung, V., Thubaut, T., Meinesz, A. & Pohnert, G. (2002). Comparison of the wound-activated transformation of caulerpenyne by invasive and noninvasive *Caulerpa* species of the Mediterranean. *Journal of Chemical Ecology*, 28, 2091-2105.

Kangas, P., Autio, H., Hällfors, G., Luther, H., Niemi, Å. & Salemaa, H. (1982). A general model of the decline of *Fucus vesiculosus* at Tvärminne, south coast of Finland in 1977-81. *Acta Botanicum Fennici*, 118, 1-27.

Karban, R. (2014). Volatile communication between plants that affects herbivory: a meta-analysis. *Ecology Letters,* 17, 44–52.

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, 25, 339–347.

Karban, R. & Shiojiri, K. (2009). Self-recognition affects plant communication and defense. *Ecology Letters*, 12, 502-506.

Karban, R. & Baldwin, I.T. (1997). *Induced responses to herbivory*. The University Chicago Press, Chicago, Illinois, USA.

Karban, R. & Myers, J.H. (1989). Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, 20, 331-348.

Karban, R., Agrawal, A.A, Thaler, J.S. & Adler, L.S. (1999). Induced plant responses and information content about risk of herbivory. *Trends in Ecology & Evolution*, 14, 443–447.

Karban, R., Shiojiri, K., Huntzinger, M. & McCall A.C. (2006). Damage-induced resistance in sagebrush: volatiles are key to intra- and interplant communication. *Ecology*, 87, 922-930.

Karban, R., Shiojiri, K., Ishizaki, S., Wetzel, W.C. & Evans, R.Y. (2013). Kin recognition affects plant communication and defence. *Proceedings of the Royal Society B-Biological Sciences*, 280, 20123062.

Kaustky, H., Kautsly, L., Kautsly, N. & Lindblad, C. (1992). Studies on the *Fucus vesiculosus* community in the Baltic Sea. *Acta Phytogeographica Suecica*, 78, 33-48.

Kempel A, Schädler M, Chrobock T, Fischer M & Kleunen, M. (2011). Tradeoffs associated with constitutive and induced plant resistance against herbivory. *Proceedings of the National Academy of Sciences of the United States of America,*108, 5685-5689.

Koivikko, R. (2008). Brown algal phlorotannins. Improving and applying chemical methods. Turun yliopiston julkaisuja, Turku.

Koivikko, R., Eränen, J., Loponen, J. & Jormalainen,V. (2008). Variation of phlorotannins among three populations of *Fucus vesiculosus* as revealed by HPLC and colorimetric quantification. *Journal of Chemical Ecology*. 34, 57-64.

Koivikko, R., Loponen, J., Pihlaja, K. & Jormalainen, V. (2007). High-performance liquid chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculosus*. *Phytochemical Analyses*, 18, 326-332.

Koivikko, R., Loponen, J., Honkanen, T. & Jormalainen, V. (2005) Contents of soluble cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implication of their ecological functions. *Journal of Chemical Ecology*, 31, 195-212.

Koricheva, J. (2002). Meta-analysis of sources of variation in fitness costs of plant herbivore defenses. *Ecology*, 83, 176-190.

Koricheva, J, Nykänen, H. & Gianoli, E. (2004). Meta-analysis of trade-offs among plant antiherbivore defenses: Are plants Jacks-of-All-Trades, Masters of all? *American Naturalist*, 163, E64-E75.

Korpinen, S., Jormalainen, V. & Honkanen, T. (2007a). Effects of nutrients, herbivory, and depth on the macroalgal community in the rocky sublittoral. *Ecology*, 88, 839-852.

Korpinen, S., Jormalainen, V. & Honkanen, T. (2007b). Bottom–up and cascading top–down control of macroalgae along a depth gradient. *Journal of Experimental Marine Biology and Ecology*, 343, 52–63.

Korpinen, S., Honkanen, T., Vesakoski, O., Hemmi, A., Koivikko, R., Loponen, J. & Jormalainen, V. (2007c). Macroalgal communities face the challenge of changing biotic interactions: review with the focus on the Baltic Sea. *Ambio*, 36, 203-211.

Korpinen, S., Jormalainen, V. & Pettay, E. (2010). Nutrient availability modifies species abundance and community structure of *Fucus*-associated littoral benthic fauna. *Marine Environmental Research*, 70, 283–292

Kraufvelin,P., Ruuskanen,A.T., Bäck,S. & Russell,G. (2012). Increased seawater temperature and light during early springs accelerate receptacle growth of *Fucus vesiculosus* in the northern Baltic proper. *Marine Biology*, 159, 1795-1807.

Krumhansl, K.A. & Scheibling, R.E. (2011). Spatial and temporal variation in grazing damage by the gastropod *Lacuna vincta* in Nova Scotian kelp beds. *Aquatic Biology*, 13, 163-173.

Lehvo, A., Bäck, S. & Kiirikki, M. (2011). Growth of *Fucus vesiculosus* L. (Phaeophyta) in the northern Baltic Proper: energy and nitrogen storage in seasonal environment. *Botanica Marina*. 44, 345-350.

Leidenberger, S., Harding, K. & Jonsson P.R. (2012). Ecology and distribution of the isopod genus *Idotea* in the Baltic Sea: Key species in a changing environment. *Journal of Crustacean Biology*, 32, 359-381.

Lima, L.M. de S., Alor, R., Uriostequi, R., Murray, S.N. & Pereira, R.C. (2008). Within-plant variation in palatability and chemical defenses in the green seaweed *Avrainvillea elliottii*. *Botanica Marina*, 51, 21-25.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. & Schabenberger, O. (2006). SAS for mixed models, Second Edition. SAS Institute Inc., Cary, NC

Long, J.D., Mitchell, J.L. & Sotka, E.E. (2011). Local consumers induce resistance differentially between *Spartina* populations in the field. *Ecology*, 92, 180-188.

Long, J.D. & Trussell, G.C. (2007). Geographic variation in seaweed induced responses to herbivory. *Marine Ecology Progress Series,* 333: 75-80.

Long, J.D., Hamilton, R.S. & Mitchell, J.L. (2007). Asymmetric competition via induced resistance: specialist herbivores indirectly suppress generalist preference and populations. *Ecology*, 88, 1232-1240.

Lüder, U.H. & Clayton, M.N. (2004). Induction of phlorotannins in the brown macroalga *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory- the first microscopic study. *Planta*, 218, 928-937.

Macaya, E.C. Rothäusler, E., Thiel, M., Molis, M. & Wahl, M. (2005). Induction of defenses and within-alga variation of palatability in two brown algae from the northern-central coast of Chile: Effects of mesograzers and UV radiation. *Journal of Experimental Marine Biology and Ecology*, 325, 214-227.

Maschek, J.A. & Baker, B.J. (2008). The chemistry of algal secondary metabolism. In Amsler, C.D. (eds.) Algal Chemical Ecology. Springer, Germany, pp. 1-24.

McDowell, R.E., Amsler, M.O., Li, Q., Lancaster Jr, J.R. & Amsler, C.D. (2015). The immediate wound-induced oxidative burst of *Saccharina latissima* depends on light via photosynthetic electron transport. *Journal of Phycology*, 51, 431-441.

McKey, D. (1974). Adaptive patterns in alkaloid physiology. *American Naturalist*, 108, 305-320.

Mooney, K.A., Halitschke, R., Kessler, A. & Agrawal, A.A. (2010). Evolutionary trade-off in plants mediate the strength of trophic cascades. *Science*, 327, 1642-1644.

Moreira, X., Mooney, K.A., Rasmann, S., Petry,W.K., Carrillo-Gavilán, A., Zas, R., Sampedro,L. (2014). Trade-offs between constitutive and induced defences drive geographic and climatic clines in pine chemical defences. *Ecology Letters*, 17, 537-546.

Murren, C.J., Auld, J.R., Callahan, H., Ghalambor, C.K., Handelsman, C.A., Heskel, M,A., Kingsolver, J.G., Maclean, H.J., Masel, J., Maughan, H., Pfennig, D.W., Relyea, R.A., Seiter, S., Snell-Rood, E., Steiner, U.K. & Schlichting, C.D. (2015). Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity*, 1-9.

Mustamäki, N., Jokinen, H., Scheinin, M. Bonsdorff, E. & Mattila, J. (2015). Seasonal small-scale variation in distribution among depth zones in a coastal Baltic Sea fish assemblage. *ICES Journal of Marine Science*, doi: 10.1093/icesjms/fsv068.

Nylund, G.M., Pereyra, R.T., Wood, H.L., Johannesson, K. & Pavia, H. (2012). Increased resistance towards generalist herbivory in the new range of a habitat-forming seaweed. *Ecosphere 3*: UNSP 125.

Paul, V.J. & Van Alstyne, K.L. (1992). Activation of chemical defenses in the tropical green algae *Halimeda* spp. *Journal of Experimental Marine Biology and Ecology*. 160, 191-203.

Pavia, H. & Toth, G.B. (2008). Macroalgal models in testing and extending defense theories. In Amsler, C.D. (ed.). Algal Chemical Ecology. Springer, Germany, pp. 147-172.

Pavia, H., Toth, G.B. & Åberg, P. (2002). Optimal defense theory: elasticity analyses as a tool to predict intraplant variation in defenses. *Ecology*, 83, 891-897.

Pavia, H., Toth, G.B., Lingren, A. & Åberg, P. (2003). Intraspecific variation in the phlorotannin content of the brown alga *Ascophyllum nodosum*. *Phycologia*, 42, 378-383.

Pavia, H. & Toth, G.B. (2000). Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology*, 81, 3212-3225.

Pavia, H., Toth, G.B., Lingren, A. & Åberg, P. (2003). Intraspecific variation in the phlorotannin content of the brown alga *Ascophyllum nodosum*. *Phycologia*, 42, 378-383.

Pavia, H. Toth, G.B. & Åberg, P. (1999). Trade-offs between phlorotannin production and annual growth in natural populations of the brown seaweed *Ascophyllum nodosum*. *Journal of Ecology*, 87, 761-771.

Pavia, H., Baumgartner, F., Cervin, G., Enge, S., Kubanek, J., Nylund, G.M. et al. (2012). Chemical defenses against herbivores. In Brönmark, C. & Hansson, L-A. (eds.) *Chemical ecology in aquatic systems*. Oxford University Press Inc., New York, USA, pp. 210-235.

Padilla, D.K. & Savedo, M.M. (2013). A systematic review of phenotypic plasticity in marine invertebrate and plant systems. *Advances in Marine Biology*, 65, 67-94.

Pace, M. L., Cole, J.J., Carpenter, S. R. & Kitchell, J.F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution*, 14, 483-488.

Pereira, M., Tala, F., Fernández, M. & Subida, M.D. (2015). Effects of kelp phenolic compounds on the feeding-associated mobility of the herbivore snail *Tegula tridentata*. *Marine Environmental Research*, 112, 40-47.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution*, 9, 481-486.

Poelman, E.H., van Loon, J.J.A. & Dicke, M. (2008). Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends in Plant Science*, 13, 534-541.

Poelman, E.H., Zheng, S.-J., Zhang, Z., Heemskerk, N.M., Cortesero, A.-M & Dicke, M. (2011). Parasitoid-spesific induction of plant responses to parasitized herbivores affects colonization by subsequent herbivores. *Proceedings of the National Academy of Sciences of the United States of America* 108, 19647-19652.

Pohnert, G. & Boland, W. (2002). The oxylipin chemistry of attraction and defense in brown algae and diatoms. *Natural Product Report*, 19,108-122.

Poore, A.G.B., Gutow, L., Pantoja, J.F., Tala, F., Madariaga, D.J. & Thiel, M. (2014). Major consequences of minor damage: impacts of small grazers on fast-growing kelps. *Oecologia*. 174, 789-801.

Poore, A.G.B., Campbell, A.H., Coleman, R.A., Edgar, G.J., Jormalainen, V., Reynolds, P.L., Sotka, E.E., Stachowicz, J.J., Taylor, R.B., Vanderklift, M.A.& Duffy, J.E. (2012). Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology letters*, 15, 912-922.

Price, P.W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N. & Weis. A. E. (1980). Interactions Among Three Trophic Levels: Influence of Plants on Interactions Between Insect Herbivores and Natural Enemies. *Annual Review of Ecology and Systematics*, 11, 41-65.

Rajasilta, M., Mankki, J., Ranta-Aho, K. & Vuorinen, I. (1999). Littoral fish communities in the Archipelago Sea, SW Finland: A preliminary study of changes over 20 years. *Hydrobiologia*, 393, 253-260.

Reynolds, P.L. & Sotka, E.E. (2011). Non–consumptive predator effects indirectly influence marine plant biomass and palatability. *Journal of Ecology*, 99, 1272–1281.

Rhoades, D.F. (1979). Evolution of plant chemical defense against herbivores. In Rosenthal, G.A., Janzen, D.H. (eds.). Herbivores: Their interaction with secondary plant metabolites. Academic Press, New York, pp. 3-54.

Rinne, H., Salovius-Lauŕen, S. & Mattila, J. (2014). The occurrence and depth penetration of macroalgae along environmental gradients in the northern Baltic Sea. Estuarine, Coastal and Shelf Science, 94, 182-191.

Rodriguez-Saona, C.R., Rodriguez-Saona, L.E. & Frost, C.J. (2009). Herbivore-induced volatiles in the perennial shrub *Vaccinium corymbosum* and their role in inter-branch signaling. *Journal of Chemical Ecology.* 35, 163-175.

Rohde, S. & Wahl, M. (2008). Temporal dynamics of induced resistance in a marine macroalga: Time lag of induction and reduction in *Fucus vesiculosus. Journal of Experimental Marine Biology and Ecology*. 367, 227-229.

Rohde, S., Molis, M. & Wahl, M. (2004). Regulation of anti-herbivore defence by *Fucus vesiculosus* in response to various cues. *Journal of Ecology*, 92, 1011-1018.

Salemaa, H. (1986). *Ecological and genetic adaptations of Idotea spp. (Isopoda, Crustacea)*. Yliopistopaino, Helsinki.

Salemaa, H. (1979). Seasonal variability in the colour polymorphism of *Idotea baltica* (Isopoda) in the northern Baltic. *Hereditas*, 90, 51-57.

Salemaa, H. (1978). Geographical variability in the colour polymorphism of *Idotea balthica* (Isopoda) in the northern Baltic. *Hereditas*, 88, 165–182.

Schaller, A. (2008). *Induced plant resistance to herbivory*. Springer.

Serrão, E.A., Kautsky, L., Lifvergren, T. & Brawley, S. (1997). Gamete dispersal and pre-recruitment mortality in Baltic *Fucus vesiculosus*. *Phycologia Suppl.*, 36, 101-102.

Schwaegerle, K.E., McIntyre, H. & Swingley, C. (2000). Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. *Evolution*, 54, 452-461.

Shurin, J.B., Borer, E.T., Seabloom, E.W., Anderson, K.,Blanchette, C.A., Broitman, B., Cooper, S.D. & Halpern, B.S. (2002). A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, 5, 785-791.

Snickars, M., Rinne, H., Salovius-Laurén, S., Arponen, H. & O'Brien, K. (2014). Disparity in the occurrence of *Fucus vesiculosus* in two adjacent areas of the Baltic Sea- Current status and outlook for the future. *Boreal Environment Research*. 19, 441-451.

Sotka, E.E., Taylor, R.B. & Hay, M.E. (2002). Tissue-specific induction of resistance to herbivores in a brown seaweed: the importance of direct grazing versus waterborne signals from grazed neighbors. *Journal of Experimental Marine Biology and Ecology*, 277, 1-12.

Stamp, N. (2002). Out of the quagmire of plant defence hypotheses. *The quarterly review of biology*. 78, 23-55.

Steinberg, P.D. (1995). Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata*. *Oecologia*. 102, 169-173.

Steiner, U.K. & Auld, J.R. (2012). Why is the jack of all trades a master of none? Studying the evolution of inducible defences in aquatic systems. In: Brönmark, C. & Hansson, L-A. (eds.) *Chemical ecology in aquatic systems*. Oxford University Press Inc., New York, USA, pp. 172-183.

Steneck, R., Graham, M.H., Bourque, B.J., Corbett, D., Erlandson, J.M., Estes, J.A., Tegner, M.J. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. *Marine Sciences Faculty Scholarship*, Paper 65.

Strauss, S.Y., Rudgers, J.A., Lau, J.A. & Irwin, R.E. (2002). Direct and ecological costs of resistance to herbivory. *Trends in Ecology & Evolution*. 17, 278-85.

Thomas, F., Cosse, A., Le Panse, S., Kloareg, B., Potin, P. & Leblanc, C. (2014). Kelps feature systemic defense responses: insights into the evolution of innate immunity in multicellular eukaryotes. *New Phytologist*, 204, 567-576.

Thompson, J.D. (1991). Phenotypic plasticity as a component of evolutionary change. *Trends in Ecology and Evolution*, 6, 246-249.

Tollrian, R, Harvell, C.D. (1999). *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton, New Jersey.

Tomas, F., Abbott, J.M., Steinberg, C., Balk, M., Williams, S.L. & Stachowicz, J.J. (2011). Plant genotype and nitrogen loading influence seagrass productivity, biochemicstry, and plant-herbivore interactions. *Ecology*, 92, 1807-1817.

Toth, G.B. (2007). Screening for induced herbivore resistance in Swedish intertidal seaweeds. *Marine Biology*. 151, 1597-1604.

Toth, G.B. & Pavia H. (2007). Induced herbivore resistance in seaweeds: a meta-analysis. *Journal of Ecology.*, 95, 425-434.

Toth, G.B. & Pavia H. (2000). Water-borne cues induce chemical defense in a marine alga (*Ascophyllum nodosum*). *Proceedings of the National Academy of Sciences of the United States of America*, 97, 14418-20.

Toth, G.B., Langhamer, O. & Pavia, H. (2005). Inducible and constitutive defenses of valuable seaweed tissues: consequences for herbivore fitness. *Ecology*, 86, 612-618.

Tuomi, J., Ilvessalo, H., Niemelä, P. & Jormalainen, V. (1989) Within-plant variation in phenolic content and toughness of the brown alga *Fucus vesiculosus* L. *Botanica Marina*, 32, 505-509.

Underwood, N. (2000). Density dependence in induced plant resistance to herbivore damage: threshold, strength and genetic variation. *Oikos*, 89, 295–300.

Van Alstyne, K.L. (1988). Herbivore grazing increases polyphenolic defenses in the intertidal brown alga *Fucus distichus*. *Ecology*, 69, 655-633.

Van Alstyne, K.L. & Houser, L.T. (2003) Dimethylsulfide release during macroinvertebrate grazing and its role as an activated chemical defense. *Marine Ecology Progress Series,* 250, 175-181.

Vesakoski, O. (2009). Local and sexual divergence in host-use traits and anti-predator adaptations in the marine herbivore *Idotea balthica*. Turun yliopisto, Turku.

Viejo, R. M. & Åberg, P. (2003) Temporal and spatial variation in the density of mobile epifauna and grazing damage on the seaweed *Ascophyllum nodosum*. *Marine Biology*.142, 1229-1241.

Wahl, M., Jormalainen, V., Eriksson, B.K., Coyer, J.A., Molis, M., Schubert, H., Dethier, M., Karez, R., Kruse, I., Lenz, M., Pearson, G., Rohde, S., Wikström, S.A., Olsen, J.L. (2011). Stress ecology in *Fucus*: abiotic, biotic and genetic interations. *Advances in Marine Biology*, 59, 37-105.

Warneke, A.M. & Long, J. (2014). Copper contamination impairs herbivore initiation of seaweed inducible defenses and decreases their effectiveness. *PloS ONE*, 10, e0135395.

Weidner, K., Lages, B.G., de Gama, B.A.B., Molis, M., Wahl, M. & Pereira, R.C. (2004). Effect of mesograzers and nutrient level on induction of defenses in several Brazilian macroalgae. *Marine Ecology Progress Series*. 283, 113-125.

Weinberger, F., Rohde, S., Oschmann, Y. Shahnaz, L., Dobretsov, S. & Wahl, M. (2011). Effects of limitation stress and of disruptive stress on induced antigrazing defense in the bladder wrack *Fucus vesiculosus*. *Marine Ecology Progress Series*, 427, 83-94.

Whalen, M.A., Duffy, J.E. & Grace, J.B. (2013). Temporal shifts in top-down vs. bottom-up control of epiphytic algae in a seagrass ecosystem. *Ecology*, 94, 510-520.

Wieski, K. & Pennings, S. (2014). Latitudinal variation in resistance and tolerance to herbivory of a salt marsh shrub. *Ecography*, 37, 763-769.

Wright, J.T., de Nys, R., Poore, A.G.B. & Steinberg, P.D. (2004). Chemical defense in a marine alga: Heritability and the potential for selection by herbivores. *Ecology*, 85, 2946-2959.

Yates, J.L. & Peckol, P. (1992). Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. *Ecology*. 74, 1757-1766

Yun, H.Y., Engelen, A.H. Santos, R.O. & Molis, M. (2012). Water-borne cues of non-indigenous seaweed mediate grazer-deterrent responses in native seaweeds, but not vice versa. *PLoS ONE*, 7, e38804.

Yun, H.Y., Cruz, J., Treitchke, M., Wahl, M. & Molis, M. (2007). Testing for the induction of anti-herbivory defences in four Portuguese macroalgae by direct and water-borne cues of grazing amphipods. *Helgoland Marine Research*, 61, 203-209.