

# Effects of the non-indigenous Harris mud crab on eelgrass growth

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Master's thesis

Environmental and marine biology

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### Abstract

Biological invasions are occurring at increasingly fast rates, and they are among the most serious threats to biodiversity. Introduced species interact with local communities, potentially altering the food web structure and functioning. The Harris mud crab (*Rhithropanopeus harrisii*) is an introduced species in the Baltic Sea, where its distribution range is expanding. Since there are no native crab species in the northern Baltic Sea, the mud crab is expected to affect the structure and functioning of native ecosystems. It utilises a range of habitats and was recently discovered in eelgrass (*Zostera marina*), an important foundation species, in its introduced range. Mud crab predation on invertebrate grazers and mussels could induce a trophic cascade through top-down processes, which would negatively affect eelgrass by promoting filamentous algae and altering sediment nutrient levels. Previous studies suggest the mud crab can induce trophic cascades in the Baltic Sea, where the simple food web structures are vulnerable to change. Effects of the mud crab in eelgrass have not previously been studied. Here, I conducted a field survey to measure mud crab densities in an eelgrass meadow. Then, I studied the potential occurrence of a mud crab induced trophic cascade and direct physical disturbance to eelgrass through an aquarium experiment. The density of mud crabs in an eelgrass meadow in the Archipelago Sea was 21 crabs/m<sup>2</sup>, which was five times higher than the last sampling in 2015. The mud crabs readily consumed invertebrates in the aquarium experiment, but no trophic cascade occurred; the mud crab did not affect porewater nutrients, algal growth or eelgrass growth. Light availability was instead the determining factor for eelgrass and algal growth. The burrowing behaviour of the mud crab did disturb eelgrass and caused uprooting of planted shoots. This is the first record of mud crabs uprooting eelgrass shoots. The density of mud crabs in the Baltic Sea is generally poorly known and the data available have been collected using varying methods, making comparisons difficult. Here, I present an estimate of mud crab densities based on core samples that sample both burrowed and active crabs to accurately reflect the population structure and density. Mud crab burrowing behaviour could disturb eelgrass, especially transplanted shoots that are not stabilised by complex root and rhizome structures. Further studies are needed to determine the occurrence of trophic cascades and uprooting of shoots in field conditions.

**Keywords:** • non-indigenous species • mud crab • *Rhithropanopeus harrisii* • eelgrass • trophic interactions • top-down • uprooting • density • physical disturbance

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## 1. Introduction

### 1.1 Non-indigenous species

Ecosystems are formed by a network of multiple interactions between species and their environment, and these interactions result in a flow of energy through food webs. Changing the interactions can have unpredictable far-reaching effects, which may resonate across trophic levels. Biological invasions caused by the introduction of non-indigenous species (NIS) are especially effective at inducing changes in recipient ecosystems (Carlton 1996, Paolucci et al. 2013). NIS are species or lower taxa that have been moved outside of their natural range, intentionally or unintentionally resulting from human activities (Olenin & Minchin 2011). Sometimes the true origin of a species is unclear, then it is referred to as a cryptogenic species (Carlton 1996). NIS can potentially alter local biodiversity and modify the structure, functions and services that the ecosystems provide (Strayer 2012).

Biological invasions are considered one of the most serious threats to biodiversity, second only to habitat destruction and loss (Niemivuo-Lahti 2012). The rate at which biological invasions are occurring is rapidly increasing (Leppäkoski & Olenin 2000a, Ojaveer & Kotta 2015) and constitutes an unprecedented form of global change (Ricciardi 2007). Currently, 193 NIS and cryptogenic species have been recorded in the Baltic Sea (AquaNIS 2020). The number has drastically increased since 2001, when 100 recorded species were listed (Leppäkoski et al. 2002). The main global vector of NIS transfer is ship traffic (Molnar et al. 2008, Bailey 2015), an activity that has increased fourfold between 1992 and 2012 (Tournadre 2014). The main ways in which NIS have been introduced to the Baltic Sea are through intentional introduction (e.g. for aquaculture), transportation of the habitat itself containing NIS (e.g. ship fouling or ballast water), and through opening canals and rivers that allow active or passive natural dispersal (Leppäkoski et al. 2002).

The spread of NIS simultaneously adds common species and removes unique or endemic species (Ruiz et al. 1997). Thereby, it is facilitating a subtle long-term process of homogenization of the world's biodiversity leading to increased homogeneity in the ecosystems (McKinney & Lockwood 1999). Generally, NIS have increased local species richness in the Baltic Sea, but they have also caused local declines of several native species (Leppäkoski et al. 2002). Many of the non-indigenous species in the Baltic Sea originate from warmer areas (Leppäkoski et al. 2002). Therefore, rising temperatures and changes in salinity conditions can potentially influence the invasion patterns of NIS in the Baltic (Leppäkoski et al. 2002, Holopainen et al. 2016). If global warming continues, warm-water species are more likely to establish in large areas of the Baltic Sea (Holopainen et al. 2016).

Through their effects on native ecosystems, NIS also further threaten the sustainability of natural resources (Ojaveer & Kotta 2015). Thus, several international policy/legislative frameworks and tools have been developed (Ojaveer & Kotta 2015). For example, the EU Marine Strategy Framework Directive (MSFD) (European Commission 2008) explicitly requires the impacts of NIS

to be evaluated and embedded into an ecosystem context. The European Commission Decision contains two criteria for assessing the impacts of NIS (Descriptor 2, European Commission 2010) in relation to the progress of achieving good environmental status. The criteria regard the abundance of NIS and their environmental impact at the level of species, habitats and ecosystems, which will be addressed in this thesis. Research into the effects of NIS in the Baltic Sea is essential to develop an understanding of the potentially far-reaching effects of NIS in the Baltic Sea and globally, and to enable appropriate management.

## 1.2 The Baltic Sea

The Baltic Sea is one of the world's largest brackish-water sea areas, covering almost 400 000 km<sup>2</sup>. The area is non-tidal and shallow. It is a unique area, influenced by strong environmental gradients (e.g. temperature, salinity, exposure) that determine the species distribution (Snoeijs-Leijonmalm & Andrén 2017). These gradients can be described as three-dimensional (longitude, latitude, depth) and they vary temporally over years, seasons and days. This environmental variability in the Baltic Sea offers hospitable conditions for NIS originating from across the world, displaying varying ecological tolerances (Leppäkoski & Olenin 2000a). Furthermore, the species richness in the Baltic Sea is naturally low, which allow NIS to colonize empty niches (Paavola et al. 2005). The biological diversity of the Baltic Sea originates to a large extent from NIS (Leppäkoski & Olenin 2000a). NIS often provide a new function (e.g. feeding strategy) or a new trophic level, thus they can potentially restructure the local community (Leppäkoski et al. 2002). The Baltic Sea serves as both a recipient and donor area, which makes it an important node in the global network of NIS transfers (Leppäkoski & Olenin 2000b).

The semi-enclosed sea is subjected to multiple anthropogenic pressures, and it is sensitive to stressors such as pollution due to its characteristics. These characteristics include a long water residence time (30-40 years), shallow depth and a large catchment area (Korpinen et al. 2012). The catchment area covers 1 700 000 km<sup>2</sup>, which is large compared to the volume of the Baltic Sea. The catchment area is also densely populated, with 85 million inhabitants (HELCOM 2018). The effects of anthropogenic pressures reflect on the degraded state of the sea; long-term trends are dominated by large-scale eutrophication, accumulation of contaminants and over-exploited fish populations (HELCOM 2018). The associated increase in phytoplankton and ephemeral macroalgae is considered one of the most serious threats to the environment in the Baltic Sea (Bonsdorff et al. 1997, Jansson & Dahlberg 1999, Snoeijs-Leijonmalm et al. 2017). Eutrophication has been a prevailing issue for decades. Although the annual nutrient input into the Baltic Sea has decreased, the status of the marine environment has not improved, and most areas are still suffering from eutrophication (HELCOM 2018)

### 1.3 The mud crab

The Harris mud crab (*Rhithropanopeus harrisii* Gould, 1841), hereafter referred to as the mud crab, is an introduced species in the Baltic Sea, native to the northwest Atlantic (from Canada to the Gulf of Mexico) (Williams 1984). The mud crab has been present in the southern Baltic Sea since the 1950s or earlier (Turoboyski 1973), but during recent years it has expanded northwards and was first observed in the Finnish Archipelago Sea in 2009 (Karhilahti 2010). Shortly afterwards, the crab was discovered in the Gulf of Riga as well (Kotta & Ojaveer 2012). The mud crab has rapidly increased in abundance and extended its distribution area to several habitats after the first observation in Finland (Fowler et al. 2013). It is a relatively small species, reaching a carapace width of 26 mm in the Baltic Sea (Turoboyski 1973).

The mud crab is the first and, so far, only crab species to become established in the northern Baltic Sea (Elmgren & Hill 1997, Boström 2003, Bonsdorff 2006, Gagnon & Boström 2016). Therefore, the introduction of the mud crab into the area can be assumed to alter functional links in the food web and affect the community structure and functioning (Forsström et al. 2015, Gagnon & Boström 2016). The lack of competition from native crab species in the northern Baltic Sea can potentially further facilitate its expansion (Gagnon & Boström et al. 2016). The mud crab has been identified as a potentially or locally harmful non-indigenous species in the Baltic Sea and is included in Finland's National Strategy on Invasive Species (Niemi-Laitinen 2012).

Mud crabs utilise available habitats in an opportunistic manner. In their native range they are typically found in habitats that offer shelter, e.g. oyster reefs or other natural structures that form cavities, but these structures are a limiting resource in the northern Baltic Sea (Fowler et al. 2013). Mud crabs occur in several types of habitats in the northern Baltic Sea, e.g. in reed belts (*Phragmites australis* (Cav.) Trin. ex Steud), bladderwrack (*Fucus vesiculosus* Linnaeus, 1753) beds, buried in muddy bottoms, hiding under small rocks and are even found at exposed hard bottom sites (Fowler et al. 2013). More recently, it was discovered that mud crabs also utilise eelgrass (*Zostera marina* Linnaeus, 1753) as a habitat in the Archipelago Sea (Gagnon & Boström 2016). Mud crabs occur in seagrass meadows in their native range as well, but this is the first time they have been recorded in seagrass in their introduced area (Gagnon & Boström 2016). This broad range of habitat use suggests that mud crabs colonize almost all available types of habitat in Finnish coastal waters (Fowler et al. 2013).



**Figure 1.** A large male mud crab which was used in this study.

The mud crab is one of the most widely distributed brachyuran crab species in the world (Roche & Torchin 2007). Generalist species, such as the mud crab, tend to be successful invaders (Weis 2010). The mud crab possesses many characteristics of a successful invader, such as high reproductive capacity, a planktonic larval stage, omnivorous feeding habits and tolerance to a wide range of temperature (1-35 °C) and salinity (1-40 ‰) (Turoboyski 1973). Mud crabs can even survive short periods of being frozen (Turoboyski 1973). Mud crabs are present at depths down to 20 meters (Hegele-Drywa & Normant 2014), and at low temperatures they migrate to deeper waters and bury themselves in the sediment (Turoboyski 1973).

The mud crab is omnivorous and feeds on sessile and mobile macroinvertebrates, as well as algae and dead organic matter (Turoboyski 1973, Hegele-Drywa & Normant 2009, Forsström et al. 2015). It is an efficient predator of littoral grazers and mussels (Forsström et al. 2015). The mud crab readily consumes gammarid amphipods (*Gammarus* spp.) isopods (*Idotea* spp.), blue mussels (*Mytilus trossulus* Gould, 1850/*Mytilus edulis* Linnaeus, 1758) and gastropods (*Theodoxus fluviatilis* Linnaeus, 1758) (Forsström et al. 2015, Jormalainen et al. 2016). Mud crabs prefer to prey upon small and medium sized prey, which suggests that they could potentially alter the prey population size structure (Forsström et al. 2015).

Several studies have identified the mud crab as an omnivore, however, Aarnio et al. (2015) suggest the trophic level of this novel species might be more complicated than this, as stable isotope analyses suggest that the mud crab undergoes an ontogenetic diet shift as it grows. The isotope signatures position small individuals (< 12 mm) among primary consumers, while large individuals (> 12 mm) are more similar to secondary consumers (Aarnio et al. 2015). In other words, small crabs primarily feed on detritus and plant matter, while larger individuals prey on other animals. The reason mud crabs undergo this dietary shift remains unclear, but it could for example be due to size-specific nutritional requirements related to moulting and growth (Aarnio et al. 2015).

Mud crabs contribute to secondary production as they are consumed by different predatory species in the Baltic Sea, including fish such as the fourhorned sculpin (*Myoxocephalus quadricornis* Linnaeus, 1758), perch (*Perca fluviatilis* Linnaeus, 1758) and roach (*Rutilus rutilus* Linnaeus, 1758) (Ovaskainen 2015, Puntilla-Dodd et al. 2019) as well as seabirds such as goldeneyes (*Bucephala clangula* Linnaeus, 1758) and great cormorants (*Phalacrocorax carbo sinensis* Staunton, 1796) (Forsström et al. 2015 and references therein).

## 1.4 Eelgrass

Seagrass meadows are critically important ecosystems across the globe. They provide a multitude of ecosystem services, such as nursery and feeding areas, high faunal production and diversity, carbon sequestration and storage, primary production, sediment stabilization and nutrient recycling (Duarte 2002, Green & Short 2003). The complex three-dimensional habitat they form supports diverse infaunal and epifaunal communities on otherwise species-poor sandy substrates (Boström & Bonsdorff 1997, Boström et al. 2006, Jephson et al. 2008). Eelgrass is the only seagrass species in the Baltic Sea, and forms some of the most diverse marine ecosystems in Scandinavia (Boström et al. 2014). Seagrasses are foundation species that provide critically important habitat; however, they are threatened by several factors including human-mediated stressors such as overfishing, eutrophication and habitat destruction (Duarte 2002, Boström et al. 2014). They are also affected by biological stressors such as disease and factors related to human mediated climate change (Orth et al. 2006). Biological invasions also pose a potential threat, as they have been shown to have multiple negative impacts on the native community structure and functioning in seagrass ecosystems around the world (Williams 2007).

Significant global declines of seagrass ecosystems, in areal extent and habitat value, have been experienced over the past decades (Orth & Moore 1983, Short & Wyllie-Echeverria 1996, Waycott et al. 2009). The rapid rates at which seagrass decline is progressing makes them among the most threatened ecosystems on the planet (Orth et al. 2006, Hughes et al. 2009, Waycott et al. 2009). The rate of decline in seagrass meadows has accelerated during the past century, but the largest losses have occurred after 1980 (Waycott et al. 2009). However, a recent trend reversal has been documented in Europe (de los Santos et al. 2019). It has been estimated that 35 % of seagrass area has been lost globally during the period 1879-2006 (Waycott et al. 2009). In Europe, the largest percentage of seagrass area decline have occurred in the Baltic Sea (de los Santos et al. 2019). Large-scale losses have been documented in Denmark since the early 1900s where an estimated 80 % of eelgrass area has been lost (Boström et al. 2003). In addition, local losses of 60-100 % of eelgrass area have been reported from Sweden, Poland and Lithuania (Baden et al. 2003, Boström et al. 2003).

The eelgrass meadows in the northern Baltic Sea generally have low genetic diversity and mainly reproduce vegetatively due to the low salinity (Reusch et al. 1999). At its northernmost distribution range (southern Finland; Boström et al. 2003), large meadows often consist of one or only a few



clones and they can be more than 1000 years old (Reusch et al. 1999). Due to the low genetic diversity, these low-salinity populations may be at considerable risk to human impacts and climate change (Reusch et al. 1999). In the case of large scale-declines in eelgrass distribution in this area, the probability of recolonization is very low, and recovery would be slow (Reusch et al. 1999). The recovery process is further complicated by a combination of environmental factors (Krause-Jensen et al. 2012), such as the relative abundance of top predators, intermediate predators and mesograzers which mediate macroalgal blooms (Hughes et al. 2013). The importance of an ecosystem approach to understand and predict seagrass losses and recovery has been highlighted through a growing body of literature providing evidence for bottom-up and/or top-down control (Crowder & Norse 2008, Foley et al. 2010).

According to the Red list of Finnish species, eelgrass is listed as “near threatened” (Hyvärinen et al. 2019). Eelgrass meadows are included in legislation that concern coastal areas through several EU directives, such as the Habitats Directive, the Water Framework Directive, the Nitrate Directive and the Marine Strategy Framework Directive. Furthermore, HELCOM also recognises the need to protect and monitor eelgrass ecosystems, however, without any legislative power.

### 1.5 Top-down control and trophic cascades

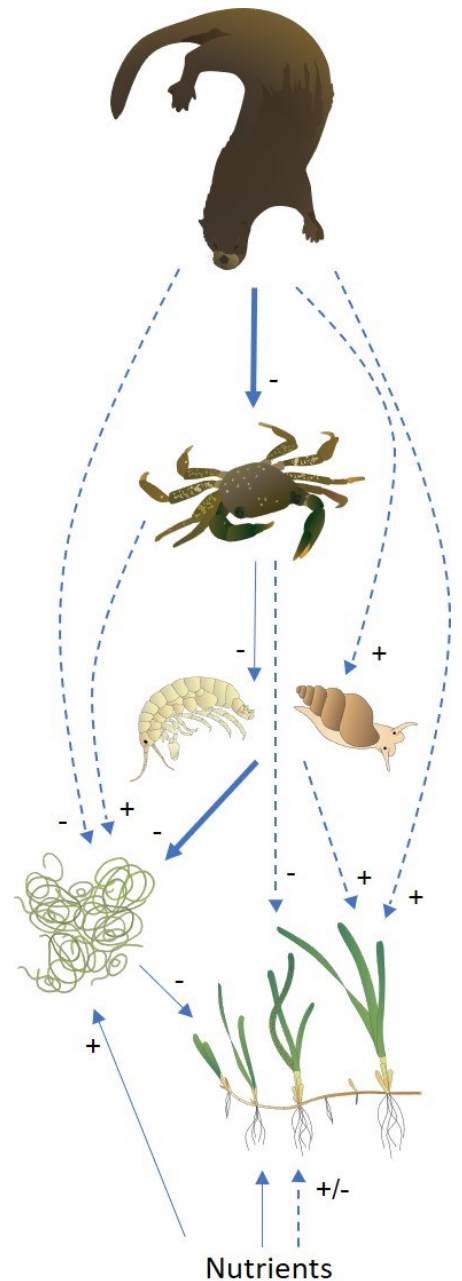
Trophic cascades have been identified in a wide range of aquatic and terrestrial systems (Pace et al. 1999). A trophic cascade occurs when changes on one trophic level in the food web resonate across more than one trophic link (Carpenter & Kitchell 1993). Trophic cascades are stronger and more likely to occur in marine than terrestrial ecosystems, due to trophic links generally being stronger in marine benthic systems (Strong 1992, Shurin et al. 2002, Shurin et al. 2006, Leroux & Loreau 2008). It has been suggested that trophic cascades are limited to simple ecosystems (Strong et al. 1992), however, recent findings suggest that they can occur in diverse communities with complex food webs as well, if the interaction strength is skewed toward only a few functionally dominant species (Pace 1999, Moksnes et al. 2008, Hughes et al. 2013).

The importance of higher trophic levels in regulating communities and ecosystem functioning has gained more recognition recently. For a long time, the mechanisms limiting the size of natural populations were assumed to be regulated mainly by primary producers or the input of limiting nutrients (bottom-up control), meaning that nutrient supply enhances primary production which resonates to higher trophic levels (Banse 2007). In contrast, higher trophic levels can also regulate lower trophic levels through predation (top-down control) ( Hairston et al. 1960). Declines in top-predators may cause mesopredator release, which is an increase in smaller predators (e.g. mud crab) (Frank et al. 2005, Sieben et al. 2011, Nilsson et al. 2019). High abundances of mesopredators can significantly reduce the densities of mesograzers, which release algae from grazer control and allows algae to thrive in response to elevated nutrient levels (Moksnes et al. 2008, Baden et al. 2010).

Drifting mats of filamentous algae have become increasingly common in the Baltic Sea (Vahteri et al. 2000, Lehvo & Bäck 2001). If algae are allowed to grow, they can form mats of unattached drifting algae which risk shading other macrophytes, and they can form hypoxic conditions when decaying (Valiela et al. 1997). Hypoxic conditions can reduce the ability of mesograzers to control algal growth, by limiting mesograzer populations (Valiela et al. 1997). In the absence of top-down control, mesograzer populations are able to respond to enhanced resource availability by increasing in abundance and hence control algal growth, even under increased nutrient loading (Hughes et al 2004, Valentine & Duffy 2006).

Trophic cascades through top-down processes have been identified in seagrass meadows, where extensive seagrass decline has occurred as a result. Along the Swedish west coast, the decline in piscivorous fish led to a mesopredator release, which was linked to an increase in macroalgal blooms (Eriksson et al. 2009) and extensive eelgrass loss (Moksnes et al. 2008, Baden et al. 2010, Baden et al. 2012). The area was affected by eutrophication from before, but the severe effects of algal blooms in eelgrass meadows only appeared after the decline in piscivorous fish, which subsequently caused mesograzer populations to almost disappear (Baden et al. 2012). Trophic cascades have also been recorded in the context of recovering seagrass ecosystems. The recovery of a top predatory sea otter (*Enhydra lutris* Linnaeus, 1758) population facilitated eelgrass recovery in a highly nutrient loaded estuary in California (Hughes et al. 2013). The sea otters had mitigating effects on nutrient loading indirectly through the predation on crabs (mesopredators), which consequently allowed mesograzer populations to recover and better control the development of algae (Figure 2, Hughes et al. 2013).

Furthermore, field studies on the mud crab in the northern Baltic Sea have identified trophic cascades, which have most likely been caused by the introduction of the mud crab (Jormalainen et al. 2016, Kotta et al. 2018). Jormalainen et al. (2016) recorded a shift in community structure in rocky *F. vesiculosus* habitats, where an ongoing invasion of mud crabs caused a pronounced decrease in amphipod and isopod populations, which allowed epiphytes to prosper and subsequently *F. vesiculosus* growth rate declined. Kotta et al. (2018) documented similar trends where the mud crab exerted strong top-down control, which resulted in a decline in richness and biomass of benthic



**Figure 2.** Top-down and bottom up processes involved in a trophic cascade which benefitted eelgrass, induced by the recovery of the top predatory sea otter (*E. lutris*). The figure is based on results and an illustration from Hughes et al. (2013). Thick solid arrows indicate increasing direct effects, thin solid arrows indicate decreasing direct effects and dashed arrows indicate indirect effects. The positive and negative effects on trophic guilds are marked with plus and minus symbols.

invertebrates as well as an increase in phytoplankton biomass and pelagic nutrients.

Both top-down and bottom-up processes regulate marine communities, and it can be hard to separate the processes since they interact. The recognition of top-down impacts are however becoming evident and there are studies stating that top-down processes are at least as important as bottom-up processes in seagrass ecosystems, and that the positive effects of epiphyte grazers are comparable in magnitude to the negative effects of nutrient enrichment in seagrass ecosystems (Hughes et al. 2004, Östman et al. 2016). The interactions between top-down and bottom-up processes are complex and context dependent (Baden et al. 2010). We are just beginning to understand the complexity of effects from large-scale changes on trophic levels for the status of marine communities, where local conditions (e.g. nutrient loads) may interact with the release of consumer control (Scheffer et al. 2005, Casini et al. 2008, Östman et al. 2016).

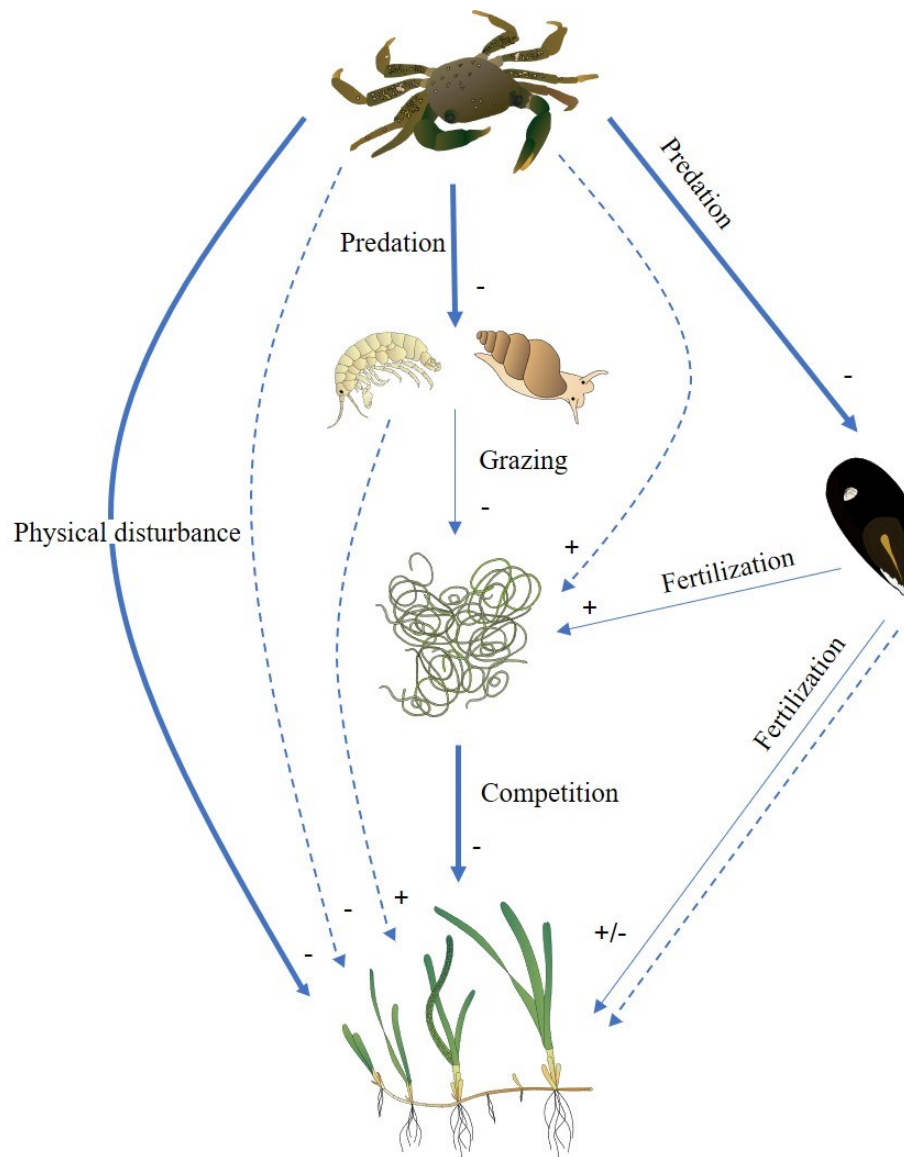
## 1.6 Aim of the thesis

Previous studies suggest that the mud crab can alter ecosystem functioning and structure, to the extent that trophic cascades can arise (Jormalainen et al. 2016, Kotta et al. 2018). Since the mud crab is a novel mesopredator in the northern Baltic Sea, it may add to the predation pressure on important eelgrass mesograzers and thereby indirectly affect eelgrass, as illustrated in Figure 3. In this thesis I studied the interactions between the mud crab, eelgrass, mussels and mesograzers. The mud crab consumes mesograzers, such as *Gammarus* spp. and *T. fluviatilis*, which effectively control algal growth (Hughes et al. 2004, Eriksson et al. 2011). Mud crab predation on mesograzers could alleviate grazing pressure on algae, allowing algae to thrive and potentially compete with eelgrass for light and nutrients, which would negatively affect eelgrass growth (Baden et al. 2010, 2012, Hughes et al. 2013, Jormalainen et al. 2016).

Mussel biodeposition of faeces and pseudofaeces fertilize the sediment and enhance eelgrass growth (Reusch et al. 1994, Peterson & Heck 2001, Wall et al. 2008). Mud crab consumption of mussels could therefore disrupt this bottom-up process and lead to decreased fertilization in sediments, which could negatively affect eelgrass growth. Furthermore, mud crabs could potentially disturb and uproot eelgrass due to their burrowing behaviour. The effect of mud crabs in eelgrass ecosystems has not yet been studied, therefore this thesis is a contribution to filling this knowledge gap. The aim of this thesis was to study the potential effects of the mud crab on eelgrass growth, including indirect effects through predation on grazers and/or mussels and direct effects through physical disturbance (Figure 3).

### Research questions

1. What is the natural density of mud crabs in an eelgrass meadow in the Archipelago Sea?
2. Does the mud crab indirectly affect eelgrass through a trophic cascade?
3. Does the mud crab directly affect eelgrass through physical disturbance?



**Figure 3.** Hypothesised top-down and bottom-up processes that are studied in this thesis. Thick solid arrows indicate increasing direct effects, thin solid arrows indicate decreasing direct effects and dashed arrows indicate indirect effects. The positive and negative effects on trophic guilds are marked with plus and minus symbols.

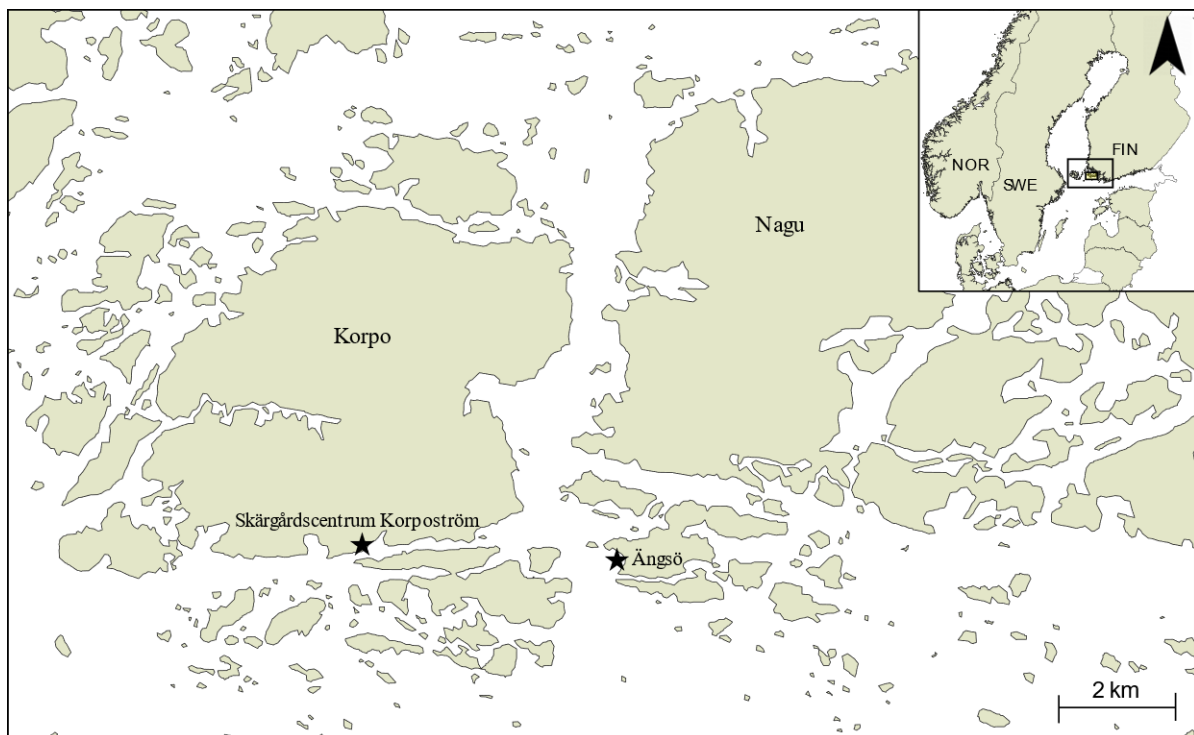
## 2. Material and methods

### 2.1 Study site and ecosystem

The study site is a semi-sheltered bay at Ängsö (60° 06'31"N; 21°42'45" E), in the Archipelago Sea (Figure 4). The eelgrass meadow studied here is interspersed with sandy patches (Gagnon & Boström 2016) and grows mixed with stands of other co-occurring plant species (e.g. *Stuckenia pectinata* L. Börner, *Zannichellia palustris* L.) (Gustafsson & Boström 2014) at 2.5–5 meters depth

(Gagnon & Boström 2016). It covers an area of about 7 hectares (Gagnon & Boström 2016), with a mean shoot density of 285 shoots  $m^{-2}$  (in June 2017) (Gagnon et al. unpublished data). The salinity in the area ranges between 5.5–7 psu and the annual surface water temperature range is 0–22 °C. During summer months the eelgrass meadow is often covered by drifting filamentous algae (Gustafsson & Boström 2014, Gagnon et al. 2017), which was confirmed by our observations during sampling in May when dense algal mats were present.

The site is well studied from previous sampling and experiments that have been conducted in the meadow and surrounding area since 2004 (Boström et al. 2006, Gustafsson & Boström 2014, Duffy et al. 2015, Gagnon & Boström 2016, Röhr et al. 2016). Common eelgrass epifauna in this area consist of the mesograzers *Idotea* spp., *Gammarus* spp., *T. fluviatilis*, *Hydrobia* spp. and the filter feeder *M. trossulus* (Gagnon et al. 2019). These above mentioned mesograzers play an important role in consuming periphyton and filamentous algae (Neckles et al. 1993).



**Figure 4.** The study site at Ängsö (right side) and the facilities of Skärgårdscentrum Korpoström (left side) are marked with stars. Map by K. Gagnon.

## 2.2 Mud crab density

The density of mud crabs in the eelgrass meadow at Ängsö was studied in the field using scuba diving on 6 June 2019. Randomly placed core samples were taken within the eelgrass meadow. In total 60 core samples were taken; three samples were always taken next to each other and placed in the same mesh bag. Altogether, there were 20 mesh bags, containing 3 core samples per bag. The



total area sampled thus equals to 0.52 m<sup>2</sup>. The core (∅ 10.5 cm) was manually pushed 10 cm deep into the sediment before the content was placed in the mesh bag (mesh size = 0.5 cm, which allowed sediment to wash out of the sample bag). Small juvenile crabs could have exited through the mesh, thus this sampling was aimed at adult crabs. The samples were treated directly after sampling, during which the crabs were counted and sexed based on their abdominal structure. The density of mud crabs at this site was previously studied in 2015 by Gagnon & Boström (2016), when mud crabs were noted in eelgrass for the first time outside of its native range.

### 2.3 Experiment design and setup

An aquarium experiment was set up to answer research questions 2–3. The experiment was conducted at the flow-through aquarium facility at Skärgårdscentrum Korpoström. The dimensions of the aquaria were 30 b x 20 l x 50 h cm. They were exposed to natural sunlight in an indoor facility and supplied with seawater from a nearby harbour, the salinity was 6.4 psu at the beginning of the experiment. The lower section of the windows in the facility were covered in an attempt to limit differences in light conditions among the aquariums. The experiment ran for 40 days from 22 May–1 July 2019. Seven treatments were carried out (see Table 1), each with five replicates. The 35 aquariums were placed in two rows, using a semi-randomized design.

**Table 1.** Table of treatments, abbreviations and the organisms included respectively. From here on, the treatments will be referred to according to the abbreviations below. *Zostera* refers to eelgrass (*Z. marina*), invertebrates include *T. fluviatilis* and *Gammarus* spp. Mussels refer to *M. trossulus* and the crabs are mud crabs. N = 5 for each treatment.

Treatment abbreviation	Organisms
Z	<i>Zostera</i>
ZI	<i>Zostera</i> + invertebrates
ZIC	<i>Zostera</i> + invertebrates + crab
ZM	<i>Zostera</i> + mussels
ZMC	<i>Zostera</i> + mussels + crab
ZMI	<i>Zostera</i> + mussels + invertebrates
ZMIC	<i>Zostera</i> + mussels + invertebrates + crab

Mud crabs were collected for the purpose of the experiment during the period 15 April–7 May 2019 in Turku, Kaarina and Parainen. Crab traps consisting of baskets filled with rocks and ceramic pot shards were used to trap mud crabs (Figure 5). The crabs were kept separated by sex in aquariums, which were filled with seawater and oxygenated by air pumps until the start of the experiment. As substrate they were provided with sand, pot shards and rocks, and they were fed with defrosted chironomid larvae and fresh *Gammarus* spp. or *M. trossulus* regularly, which they consumed readily. The crabs were starved for 24 hours prior to the experiment. Only male crabs with carapace width >15 mm were used in the experiment, due to the difference in diet between large and small

crabs (Aarnio et al. 2015). Since we studied the effects of predation, we wanted to make sure the crabs were carnivorous (i.e. > 12 mm carapace width) (Aarnio et al. 2015). Also, males tend to be more active than females during this time, since females may be gravid and thus spend more time in shelter caring for their eggs (Turoboyski 1973). Two males were used per aquarium, one larger (20–23 mm) and one smaller (15–19 mm).



**Figure 5.** One of the crab traps used to collect mud crabs for the aquarium experiment.

Sediment (sand) and eelgrass shoots were collected from a shallow area in Ängsö (Figure 4). The sediment was sieved through 1 mm to eliminate infauna and litter before being placed in the aquariums into a 10-cm thick layer. It was allowed to stabilize in the aquariums for two days before eelgrass was planted. Eelgrass was collected by scuba diving, then carefully cleaned from epifauna and epiphytes before being planted into the aquariums. Nine natural eelgrass shoots were planted in each aquarium, along with three artificial eelgrass shoots (to measure epiphyte colonisation). The artificial eelgrass shoots consisted of individual 30 cm long wrapping strings folded in half and anchored to a plastic mesh.

The eelgrass was allowed to acclimate for four days before grazers and mussels were added to the respective treatments. Blue mussels (*M. trossulus*) and grazers (gastropods *T. fluviatilis* and gammarids *Gammarus* spp.) were collected from *F. vesiculosus* stands and hard surfaces in the vicinity of Korpoström. Mussels larger than the crabs were excluded, because the crabs would likely not be able to open them. The grazers and mussels were allowed to settle for 30 hours before the start of the experiment when mud crabs were added. Densities of the organisms used in the experiment were determined based on existing data on their natural densities in the Ängsö eelgrass meadow (see Table 2). Four data loggers (HOBO Pendant<sup>®</sup> Temperature/Light Data Logger 64 K, Onset, USA) were deployed throughout the experiment in control treatments (two in each row, in the control aquariums to avoid interfering with epifauna) to measure light and temperature.

**Table 2.** Number of organisms and the densities included in the experimental treatments compared to natural densities in the field. Field data on grazers and mussels from Gagnon et al. (unpublished data), eelgrass and crab densities from Gagnon & Boström (2016) and crab densities from the present study marked with an asterisk.

Organism	Aquarium (no. ind.)	Aquarium (m <sup>-2</sup> )	Field (m <sup>-2</sup> )
Mud crab	2	33	4, 21*
<i>Gammarus</i> spp.	5	83	31
<i>T. fluviatilis</i>	7	117	142
<i>M. trossulus</i>	62 (22 g ww)	1033 (367 g ww)	272
Eelgrass	9 (+ 3 artificial)	150 (200)	20–400

## 2.4 Execution of the experiment and treatment of samples

At the end of the experiment the aquariums were emptied, and remaining organisms were counted. *M. trossulus*, *Gammarus* spp. and *T. fluviatilis* that were not found were assumed to have been consumed by mud crabs. *M. trossulus* with broken shells were also considered consumed. It should be noted that *Gammarus* spp. reproduced during the experiment, leading to very high population densities in both control and crab treatments (see appendix Figure A), which is why only adult *Gammarus* spp. were analysed.

Sediment organic content was measured by taking sediment samples in the beginning and the end of the experiment. The loss-on-ignition (LOI) method was used to determine the total organic content of the sediment (see Schumacher 2002). The samples were dried at 100 °C for 24 hours, and then weighed. Thereafter, the samples were burned at 520 °C for 3 hours and weighed again. The difference in weight before and after burning represents the amount of organic content in the sediment. Organic content was calculated using the formula below:

$$OC \% = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Nutrient concentrations in the sediment was studied by taking porewater samples. The samples were taken at the end of the experiment by inserting a rhizon porewater sampler into the sediment at the same depth as the eelgrass rhizomes. The rhizon was connected by a syringe to a glass jar with a rubber lid which had been decompressed, the air pressure difference allowed suction of porewater into the jar. The samples were frozen and later analysed for ammonium (NH<sup>+</sup><sub>4</sub>) and phosphate (PO<sub>4</sub><sup>3-</sup>) concentrations, by the accredited laboratory of Lounais-Suomen vesi- ja ympäristötutkimus Oy, using continuous flow analysis.

The amount of epiphytes was determined by scraping epiphytes off the artificial shoots. The epiphytes were then dried in 60 °C for 48 hours, then weighed. Loose filamentous algae in the aquariums were collected to study macroalgal blooms, by sieving (1 mm) the content of the water



column and the surface layer of sediment in the aquarium at the termination of the experiment. The collected filamentous algae were then dried in 60 °C for 48 hours then weighed.

Six days before the experiment was terminated, three shoots of eelgrass from each aquarium were punctured with a syringe near the base of the shoot. This created a scar on the leaves, which was used as a marker to determine the growth of the leaves (see Short & Duarte 2001). After the experiment was terminated, the parts of the leaves which had grown since the puncturing were dried at 60 °C for 48 hours, and then used for calculating growth rate ( $\text{mg dw}^{-1} \text{ day}^{-1}$ ). The shoot lengths of the punctured eelgrass shoots were also measured, to study overall growth. Eelgrass shoots that uprooted were counted over the course of the whole experiment.

## 2.5 Statistical methods

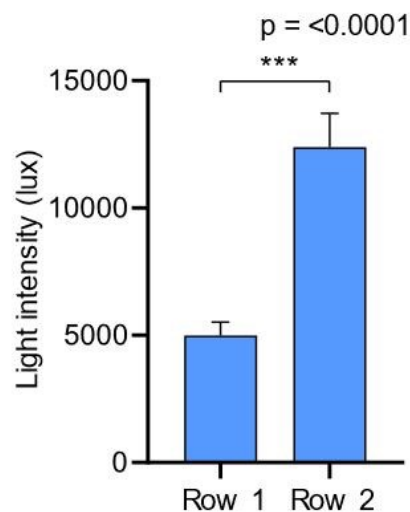
The data from the experiment were analysed in R (version 3.6.2, R Core Team 2020) using a linear mixed model (LMM), generalized linear models (GLM), generalized linear mixed models (GLMM) and Bayesian generalized linear models according to Table 3. Additionally, a Wilcoxon rank sum test with continuity correction was performed to analyse the difference in mean light intensity between rows (placement of aquarium) (Figure 6), using the `wilcox.test` function in the native “stats” package. The mean values from two data loggers on each row were compared between rows.

To determine if data fulfilled assumptions for parametric testing, Shapiro-Wilk and Levene’s tests were used prior to analysis. The fixed factors that were included in the LMM, GLM and GLMM models are treatment and row. Row was included as, despite our attempts at limiting differences in light conditions, row 1 had significantly lower light intensity than row 2 ( $W = 864.5$ ,  $Z = -4.01$ ,  $r = 0.71$ ,  $n = 33$ ,  $p < 0.0001$ , Figure 6, Appendix Figure B). Aquarium was included as a random factor to account for pseudoreplication in cases where several samples were taken from the same aquariums (i.e. for eelgrass growth and shoot length). The significance of the fixed factors and interactions were assessed using the `Anova` function in the package “car” (Fox & Weisberg 2011). Non-significant higher-level interactions were removed in the models, and AIC (Akaike's Information Criterion) values of full and simplified models were compared. The models with the lowest AIC values were selected.

For the mixed models (LMM and GLMM) I used the `lmer` and `glmer` functions in the R package “lme4” (Bates et al. 2015). For GLMs I used the function `glm` in the native “stats” package. A small value (+1) was added to measurements of filamentous algae, to fit the assumptions of a gamma distribution. Bayesian generalized linear models were used to analyse *T. fluviatilis* and *M. trossulus* using the function `bayesglm` in the package “arm” (Gelman & Yu-Sung 2020). The reason why a regular logistic regression could not be applied to these two analyses in the way as e.g. *Gammarus* spp. was analysed, was due to an issue with data separation, which occurs when a predictor variable perfectly predicts the outcome variable (UCLA: Statistical Consulting Group n.d.). In this case data

separation occurred in *T. fluviatilis* and *M. trossulus* because all values in one grazer treatment were zeros, and the differences between mussel treatments were distinctly different. To determine the fit of the Bayesian generalized linear models as well as the regular logistic regressions, validity tests for the fitted models were conducted based on randomized quantile residuals in the package “statmod” (Dunn & Smyth 1996).

Type I anova in the “stats” package, function `anova`, (R Core Team 2020) was used to report results (Table 4) for all but the mixed models. The mixed models were analysed using type II anova (Wald chisquare test) in the “car” package (Fox & Weisberg 2011) with the function `Anova`. The difference between the two is the order in which the factors are taken into account. To explore significant effects between treatments, Tukey all-pair comparisons were performed by using the function `glht` within the package “multcomp” (Hothorn et al. 2008). Lastly, the function `cld` within the same package was used to visualize the results from the all-pair comparisons as letters representing significant differences in the result figures.



**Figure 6.** Light intensity (mean  $\pm$  SE,  $n = 33$ , lux) at 14:00 on the two rows.

**Table 3.** The statistical methods, explanatory variables and error distributions used to analyse respective response variables. Plus symbols indicate fixed factors and asterisks indicate interactions, random factors are indicated within parentheses as random.

Response variable	Statistical method	Explanatory variable	Distribution
<i>T. fluviatilis</i>	Bayesian generalized linear model	Treatment	Binomial with a logit function
<i>Gammarus</i> spp.	Generalized linear model	Treatment	Binomial with a logit function
<i>M. trossulus</i>	Bayesian generalized linear model	Treatment	Binomial with a logit function
Sediment organic content	Generalized linear model	Row + Treatment	Gaussian with an identity function
Ammonium	Generalized linear model	Row * Treatment	Gaussian with an identity function
Phosphate	Generalized linear model	Row + Treatment	Gaussian with an identity function
Epiphytes	Generalized linear model	Row * Treatment	Gamma with a log link function
Filamentous algae (+1)	Generalized linear model	Row + Treatment	Gamma with a log link function
Eelgrass growth	Generalized linear mixed model	Row + Treatment + Aquarium (random)	Gamma with a log link function
Shoot length	Linear mixed model	Row + Treatment + Aquarium (random)	Normal
Uprooted shoots	Generalized linear model	Crab presence	Binomial with a logit function

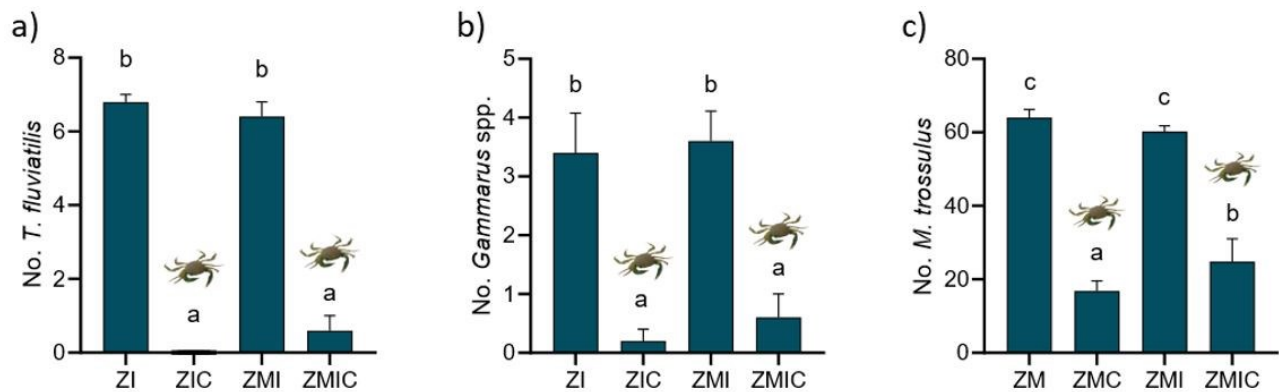
### 3. Results

#### 3.1 Mud crab density

The density of mud crabs in the eelgrass meadow at Ängsö was 21 crabs/m<sup>2</sup>. Within the 20 samples taken, a total of 11 crabs were found. The proportion of males and females was close to equal: 45 % were females, 55 % were males.

#### 3.2 Predation

Mud crabs readily consumed the animals present in respective experimental treatments (Figure 7a-c, Table 4). The number of *T. fluviatilis* was significantly lower in the treatments including crabs (ZIC and ZMIC) compared to control treatments (ZI and ZMI) ( $p < 0.001$ , Table 4, Figure 7a). Similarly, the number of *Gammarus* spp. decreased in the crab-invertebrate treatments ZIC ( $p = 0.002$ , Table 4, Figure 7b) and in ZMIC ( $p < 0.001$ , Table 4). *M. trossulus* was consumed in both crab treatments (ZMC and ZMIC,  $p < 0.0001$ , Table 4) compared to respective control treatments, but to a significantly higher degree in ZMC than in ZMIC ( $p = 0.003$ , Table 4, Figure 7c), where more food resources were available.



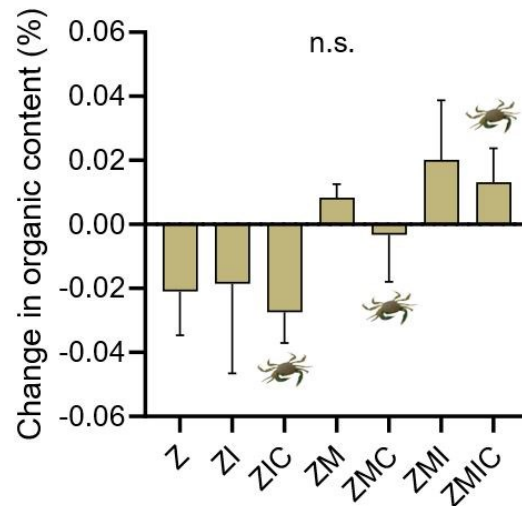
**Figure 7.** Number of a) *T. fluviatilis*, b) *Gammarus* spp. and c) adult *M. trossulus* (mean  $\pm$  SE) in each aquarium per treatment after the experiment. Different letters indicate significant differences ( $p < 0.05$ ) between treatments (for treatments, see Table 1).

**Table 4.** Results from separate linear models (see Table 3) on the effects of treatment and row on the response variables from the experiment. Significant effects are marked in bold. Degrees of freedom are given as numerator df, denominator df. F-test statistics are given for the fixed factors in GLM models with gamma or gaussian distribution.  $\chi^2$ - test statistics of likelihood ratio- tests are given for the factors in models using a binomial distribution and in mixed models.

Response variable	df	<i>F</i>	$\chi^2$	<i>P</i>
<i>T. fluviatilis</i>				
Treatment			143.22	< <b>0.0001</b>
<i>Gammarus</i> spp.				
Treatment			46.02	< <b>0.0001</b>
<i>M. trossulus</i>				
Treatment			797.06	< <b>0.0001</b>
Sediment organic content				
Row	1, 32	3.25		0.08
Treatment	6, 26	2.27		0.07
Ammonium				
Row	1, 32	12.21		<b>0.002</b>
Treatment	6, 26	3.15		<b>0.024</b>
Row : Treatment	6, 20	4.66		<b>0.004</b>
Phosphate				
Row	1, 31	1.38		0.25
Treatment	6, 25	0.84		0.55
Epiphytes				
Row	1, 33	21.40		<b>0.0001</b>
Treatment	6, 27	8.93		< <b>0.0001</b>
Row : Treatment	6, 21	2.10		0.096
Filamentous algae (+1)				
Row	1, 33	33.02		< <b>0.0001</b>
Treatment	6, 27	8.74		< <b>0.0001</b>
Eelgrass growth				
Row			34.44	< <b>0.0001</b>
Treatment			7.84	0.25
Shoot length				
Row			24.34	< <b>0.0001</b>
Treatment			2.18	0.90
Uprooted shoots				
Treatment			25.74	< <b>0.0001</b>

### 3.3 Sediment organic content

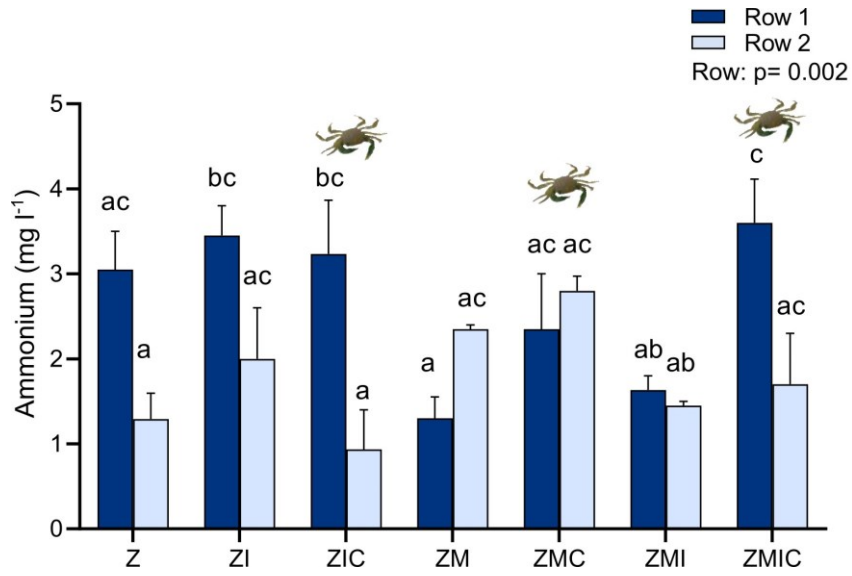
There were no significant differences in sediment organic content between treatments when comparing values between the start and the end of the experiment ( $p = 0.07$ , Table 4). There was, however, a weak trend indicating that the sediment organic content increased as *M. trossulus* were included and decreased when invertebrate grazers or mud crabs were included (Figure 8).



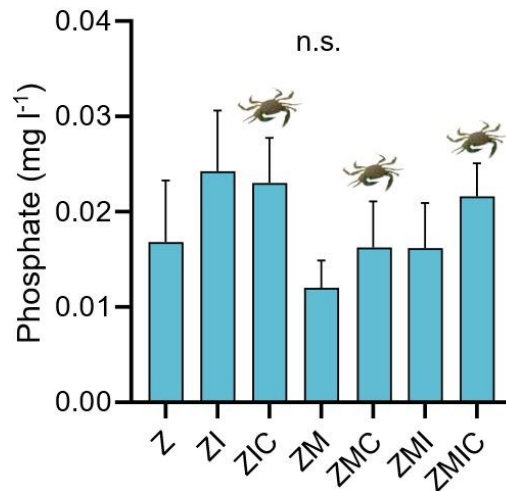
**Figure 8.** Change in sediment organic content (%) over the course of the experiment (mean  $\pm$  SE). N.s. = non-significant.

### 3.4 Porewater nutrients

There was a significant interaction effect between row and treatment ( $p = 0.004$ , Table 4, Figure 9) on ammonium concentrations. The mud crab had no clear effect on ammonium concentrations, except on row 1 where there was a significant increase between the treatments ZMI and ZMIC ( $p = 0.017$ , Table 4). There was no effect of treatment ( $p = 0.55$ , Table 4, Figure 10) nor row ( $p = 0.25$ , Table 4) on phosphate concentrations in the porewater, but the concentrations followed the same pattern as ammonium when visually assessed.



**Figure 9.** Ammonium concentrations ( $\text{mg l}^{-1}$ ) in the sediment porewater at the end of the experiment (mean  $\pm$  SE). Different letters indicate significant differences ( $p < 0.05$ ) between treatments.

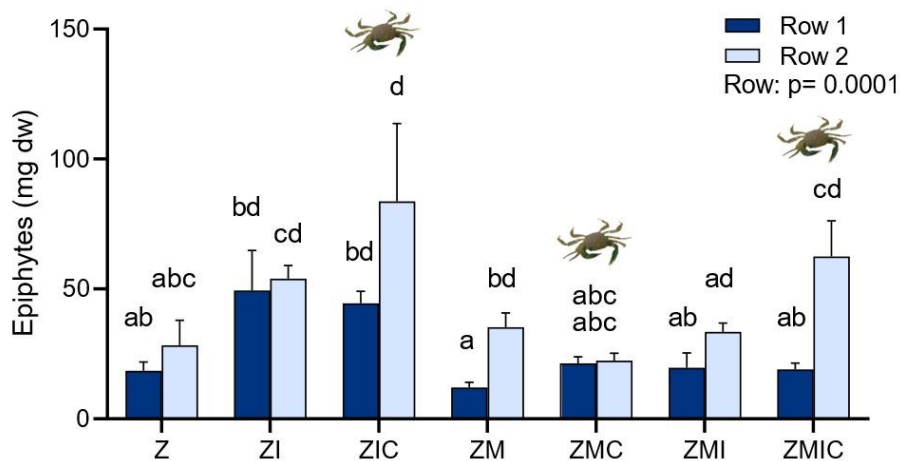


**Figure 10.** Phosphate concentrations ( $\text{mg l}^{-1}$ ) in the sediment porewater at the end of the experiment (mean  $\pm$  SE). N.s. = non-significant.

### 3.5 Epiphytes and filamentous algae

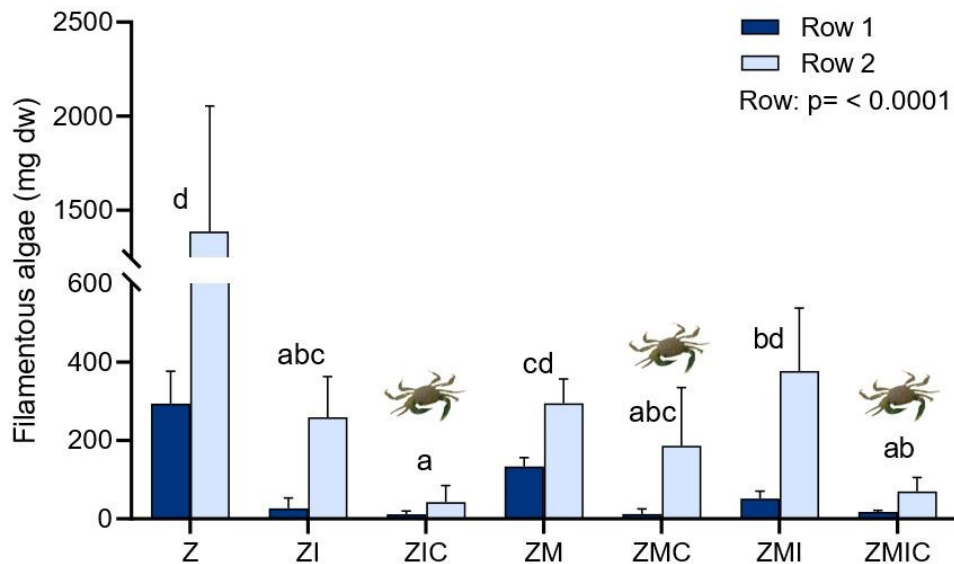
The amount of epiphytes on the artificial eelgrass shoots was significantly affected by both row ( $p = 0.0001$ , Table 4, Figure 11) and treatment ( $p < 0.0001$ , Table 4). The smallest amounts of epiphytes were found in the mussel treatment (ZM) on row 1 and the largest in the invertebrate grazer and crab treatment (ZIC) on row 2. On row 1, ZM differs significantly from both ZI ( $p < 0.001$ , Table 4) and ZIC ( $p < 0.001$ , Table 4). On row 2, ZIC differs significantly from Z ( $p = 0.03$ , Table 4) and ZMC ( $p < 0.019$ , Table 4). Also, the amount of epiphytes varied greatly within the treatment ZMIC between rows ( $p < 0.01$ , Table 4). The general trend was that invertebrate grazer treatments had more epiphytes than mussel treatments, and no effects from the mud crabs were detected.

The amount of loose filamentous algae in the water column of the aquariums varied between rows ( $p < 0.0001$ , Table 4) and treatments ( $p < 0.0001$ , Table 4, Figure 12). The largest amount of filamentous algae was found in the control treatment Z, with eelgrass alone, especially on row 2. Z differed from ZI ( $p = 0.006$ , Table 4), ZIC ( $p < 0.001$ , Table 4), ZMC ( $p < 0.001$ , Table 4) and ZMIC ( $p < 0.001$ , Table 4). Furthermore, ZIC, which had the overall smallest amounts of filamentous algae, differed from ZM ( $p < 0.001$ , Table 4) and ZMI ( $p = 0.03$ , Table 4). ZM had more algae than ZMIC ( $p = 0.013$ , Table 4). No differences caused by the mud crabs were detected.



**Figure 11.** Amount of epiphytes (mg dw) attached to artificial eelgrass shoots at the end of the experiment (mean  $\pm$  SE). Different letters indicate significant differences ( $p < 0.05$ ) between treatments.

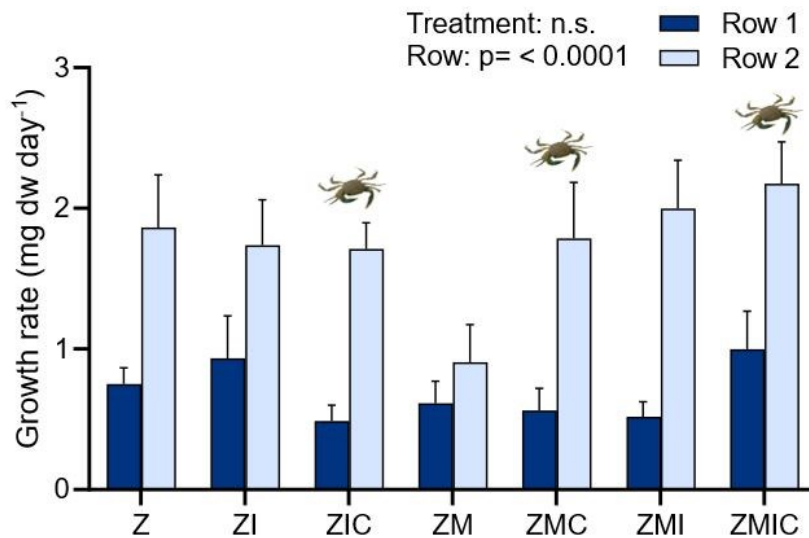




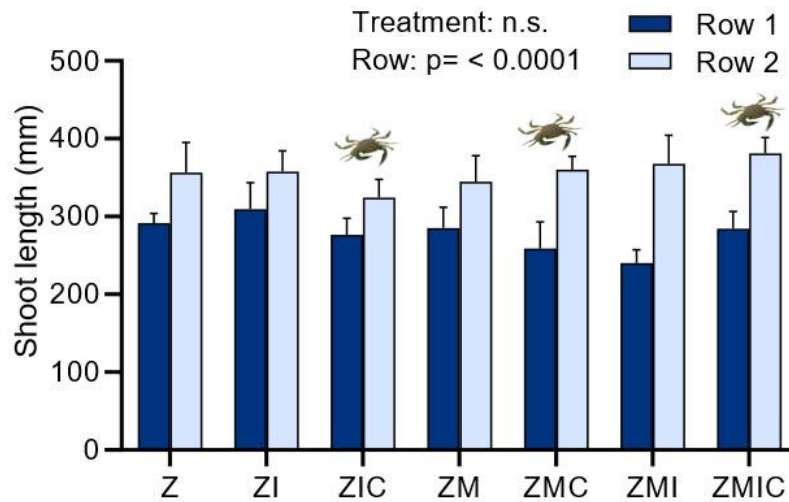
**Figure 12.** Amount of loose filamentous algae (mg dw) in the water column of the aquariums at the end of the experiment (mean  $\pm$  SE). Different letters indicate significant differences ( $p < 0.05$ ) between treatments.

### 3.6 Eelgrass growth and shoot length

There were no significant differences in eelgrass growth rate between treatments ( $p = 0.25$ , Table 4, Figure 13), the growth rate was instead controlled by the light conditions of the row ( $p < 0.0001$ , Table 4). Similarly, shoot length was not affected by treatment ( $p = 0.90$ , Figure 14, Table 4), but instead by row ( $p < 0.0001$ , Table 4).



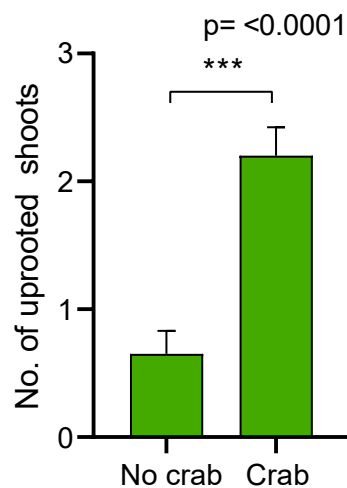
**Figure 13.** Eelgrass growth rate (mg dw) per day (mean  $\pm$  SE).



**Figure 14.** Eelgrass shoot length (mm, mean  $\pm$  SE) at the end of the experiment.

### 3.7 Uprooted shoots

The number of shoots that came loose during the experiment was significantly higher in treatments where mud crabs were included ( $p < 0.0001$ , Table 4, Figure 15).



**Figure 15.** Total number of shoots uprooted per aquarium during the experiment (mean  $\pm$  SE). No crab  $n = 20$ , Crab  $n = 15$ .

## 4. Discussion

The aim of this thesis was to study the potential effects of the mud crab on eelgrass growth, through trophic cascades or direct physical disturbance. To gain a better understanding of the occurrence of mud crabs in eelgrass, mud crab densities were sampled in an eelgrass meadow where an established mud crab population exists. An aquarium experiment was conducted to study the indirect and direct effects of mud crabs on eelgrass for the first time. The density of mud crabs found at Ängsö in an eelgrass meadow was 21 crabs/m<sup>2</sup>. The aquarium experiment showed no indirect effects on eelgrass by mud crabs through a trophic cascade, but the mud crabs physically harmed eelgrass through their burrowing behaviour, which caused eelgrass shoots to uproot.

### 4.1 Mud crab density

The density of mud crabs (21 crabs/m<sup>2</sup>) in the eelgrass meadow at Ängsö was five times higher than previous estimates (4 crabs/m<sup>2</sup>) by Gagnon & Boström (2016). The higher density found in this study likely reflects population growth in the study site as well as more accurate sampling methods. The sampling methods differ between the previous and present study, which should be considered when comparing the results. In Gagnon & Boström (2016), the mud crabs were collected through epifaunal sampling. Because mud crabs burrow in the sediment, we chose to study the densities using core samples and were thereby able to collect crabs hiding in the sediment. The density found in this study reflects the density of adult mud crabs, since the mesh size of the sample bags (5 mm) could have allowed juveniles to exit. Taking the potential loss of juveniles into consideration, the density could be a slight underestimation.

The density of mud crabs in the Baltic Sea is not well known, as they are not targeted in monitoring programs (Lokko et al 2018). Indications from a pilot experiment using artificial reefs in the north-eastern Baltic sea, show that the number of mud crabs per m<sup>2</sup> seafloor area is anywhere from a few to 1000 crabs (Lokko et al. 2018 and references therein). More conservative estimates have been made in Gulf of Gdańsk (Poland), where samples that were taken by bottom dredging showed maximum densities of only 0.19 crabs/m<sup>2</sup> in Puck Bay and 0.05 crabs/m<sup>2</sup> in Gdynia and Sopot (Hegele-Drywa & Normant 2014). In the Dead Vistula (Poland), a density of 0.5–6 crabs/m<sup>2</sup> was estimated based on bottom dredging samples (Turoboyski 1973). In the Taman Bay in the Sea of Azov the density of mud crabs was estimated to 1.4 crabs/m<sup>2</sup>, by visual assessments along transects (Zalota et al. 2016). In comparison to the recorded mud crab densities in the southern Baltic Sea and the Sea of Azov, the densities found at Ängsö are much higher than has been found. However, the methods used in the above-mentioned studies differ from the one used in this study, which should be taken into consideration. The lack of targeted NIS monitoring and surveillance is one of the main challenges in the Baltic Sea, thus future monitoring and standardization of methods are called for to combat data scarcity as well as variability in methods and data quality (Ojaveer et al. 2014, Lehtiniemi et al. 2015).

Most of previous studies and monitoring have used traps to estimate mud crab abundance in terms of catch per unit effort (CPUE) (e.g. Roche et al. 2009, Fowler et al. 2013, Forsström et al. 2018). CPUE is commonly used for monitoring populations of mobile species, e.g. fish (Olin et al. 2016, George et al. 2019). It is easier, quicker and cheaper than scuba diving for core samples. The downside is that CPUE cannot be used to compare densities, i.e. the average number of individuals per given area. The core samples used in the present study provide an accurate estimation of population density and structure, by sampling both active crabs moving around and passive crabs hiding in the sediment. Based on the results from the present study, core samples can successfully be used as a method for studying mud crab densities in soft bottom habitats.

The ratio of female and male mud crabs was close to balanced between the sexes in the Ängsö eelgrass meadow. Malmberg (2020) found almost exclusively male crabs outside of eelgrass meadows, while females were more common inside the eelgrass meadow, showing a sex dependent difference in habitat occupancy. Male mud crabs are known to be more active and move around, while females stay in sheltered areas (Turoboyski 1973), which could explain why females were more common inside than outside an eelgrass meadow. Male crabs have been disproportionately found in trapping studies (Czerniejewski & Rybczyk 2008 and references therein, Ovaskainen 2015, Malmberg 2020), which can be explained by spatial and temporal variations, population structure as well as potential method bias. Since male mud crabs are more motile, they are possibly more likely to be caught. These sex dependent differences in activity and habitat occupancy should be considered when sampling. Thus, direct sampling, such as core sampling, allows for a more precise selection of habitat (e.g. inside vs. outside of vegetation) and accurate sampling of crabs both on and in the sediment.

## 4.2 Predation on invertebrates did not induce a trophic cascade

The mud crabs consumed *M. trossulus*, *T. fluviatilis* and *Gammarus* spp. as expected based on previous studies that have identified said organisms as some of their main food sources (Hegele-Drywa & Normant 2009, Forsström et al. 2015). However, this did not cause a trophic cascade and thus contradicts the hypothesis (Figure 2). To answer research question number two, the mud crab did not indirectly affect eelgrass growth through a trophic cascade. One explanation as to why no trophic cascade occurred, relates to the fact that *M. trossulus*, *T. fluviatilis* and *Gammarus* spp. did not facilitate eelgrass growth as hypothesised based on previous studies. In the following section I will discuss potential reasons why the invertebrates did not facilitate eelgrass and thus why no trophic cascade occurred.

### 4.2.1 Grazers and mussels did not enhance eelgrass growth

Mussels alter the sediment biogeochemistry through biodeposition of faeces and pseudofaeces, which enrich sediments with nutrients (Kautsky & Evans 1987, Vinther et al. 2012). The nutrients

excreted by mussels mainly consist of nitrogen and phosphorus (Kautsky & Wallentinus 1980, Kautsky & Evans 1987), but they are typically measured as ammonium and phosphate, respectively, as they are the common forms of the elements in benthic coastal ecosystems and the main forms of nutrients used by plants (Touchette & Burkholder 2000, Iheagwara et al. 2013, Metzger et al. 2019). Biodeposits by *M. trossulus* create favourable substrates for sediment bacteria, which generates increased mineralisation and regeneration of nutrients (Stock & Albers 2000). Thus, biodeposition may increase nutrient availability and can stimulate eelgrass growth (Reusch et al. 1994, Peterson & Heck 2001a, Carroll et al. 2008, Wall et al. 2008), as hypothesised in this study. The mussels did not enhance eelgrass growth in this experiment, and porewater nutrient concentrations did not rise in mussel treatments. In contrast, the lowest porewater nutrient concentrations and eelgrass growth rates were found in the mussel treatment (ZM, Figure 8–9 and 12), although they were not statistically significant from the control treatment.

It is possible that mussels in this case induced sulphide stress, which would explain the low growth rate of eelgrass. The biodeposits from mussels may turn the sediments sulphidic ( $S^{2-}$ ) as a consequence of enhanced sulphate ( $SO_4^{2-}$ ) reduction rates (Vinther et al. 2008). Sulphate reduction occurs when anaerobic sulphate-reducing microorganisms respire, as they utilize sulphate as a terminal electron acceptor and produce hydrogen sulphide ( $H_2S$ ) (Muyzer & Stams 2008). Since sulphide is toxic to plants, enhanced sulphide pools can negatively affect eelgrass growth and photosynthetic activity (Holmer & Bondgaard 2001) and lead to increased seagrass mortality (Koch et al. 2007). Plant tissues are more susceptible to sulphide intrusion in conditions where oxygen levels are low (Pedersen et al. 2004). Since *M. trossulus* biodeposits on the surface of the sediment, it may lead to lower oxygen levels near the plant meristems due to increased sediment respiration, which makes the meristems more vulnerable (Vinther et al. 2012). In this study, sulphide concentrations were not measured, and it is therefore not possible to draw any final conclusions on whether sulphide stress occurred or not.

The porewater concentrations of ammonium and phosphate did not indicate elevated nutrient concentrations in mussel treatments, instead they were low (Figure 8–9). This does not necessarily fully explain the nutrient levels in the aquariums and does not rule out the possibility of harmful amounts of nutrients accumulating in the aquariums. It is possible that ammonium was converted to nitrite or nitrate through a nitrification process carried out by microorganisms, which would explain why the fertilization of mussels is not detected and why ammonium levels are lowest in mussel treatments. Nitrification is an oxidation process where ammonium ( $NH_4^+$ ) is converted to nitrite ( $NO_2^-$ ) and then to nitrate ( $NO_3^-$ ) (Ward 2008). However, ammonium can also convert directly to nitrogen ( $N_2$ ) in anoxic conditions, through a process called anammox (van de Graaf et al. 1995). The concentrations of nitrite, nitrate or nitrogen were not analysed in this study. Nitrogen exists in several chemical forms, of which ammonium is one (Gruber 2008). In the present study, only ammonium was analysed, since it is the main source of inorganic nitrogen and the preferred nitrogen source for eelgrass (Short & McRoy 1984, Touchette & Burkholder 2000).

Another possible reason that could explain the low ammonium and phosphate concentrations in the mussel treatment (ZM) could be that the nutrients were used by loose filamentous algae or epiphytes, which are fast growing and take up ammonium much more efficiently than seagrasses (Cornelisen & Thomas 2002). According to my observations, large amounts of filamentous algae developed early in the experiment in the mussel treatments. However, the amounts of filamentous or epiphytic algae were not particularly high in ZM at the time of sampling. It is noteworthy, that the sampled loose filamentous algae are not equal to the total amount of algae formed in the aquariums over time. Throughout the experiment, floating algae were removed from the top of the tank to prevent them from clogging the overflow filter. Also, some of the filamentous algae were removed at the point when eelgrass shoots were punctured, due to the movements of our hands in the aquariums even though caution was taken. However, all aquariums were subjected to this same disturbance and it should thus be standardized.

The mesograzers *T. fluviatilis* and *Gammarus* spp. feed on fast growing algae (Eriksson et al. 2011), and normally control algal growth. However, in this study the treatments including mesograzers (ZI and ZIC) contained the largest amounts of epiphytes. This can be explained by the high densities of *Gammarus* spp. which formed in the aquariums over time as they reproduced. The high densities of *Gammarus* spp. likely produced large amounts of faeces, which would add nutrients to the aquariums and facilitate epiphyte growth. Interestingly, the same trend does not appear regarding loose filamentous algae, where the amounts of filamentous algae in ZI and ZIC did not increase compared to other treatments. This suggests either that the mesograzers preferred to feed on loose filamentous algae rather than epiphytes, or that epiphytes benefitted to a larger extent from faeces excreted by *Gammarus* spp., possibly due to the epiphytes position in the water column. The fact that *Gammarus* spp. were able to produce such an amount of offspring, also shows how the mud crabs were not able to control a fertile population under the experimental time period, even though they did consume a large proportion of the grazers (see appendix Figure A). However, in natural conditions other predators would add to the predation pressure. Furthermore, it is noteworthy that the faeces and associated nutrients would not accumulate in such a way in natural conditions.

#### 4.2.2 Other implications of mud crab predation

*M. trossulus* was consumed to a significantly lower degree in the treatment with all prey species present (ZMIC) than in the treatment with only mussel and crab (ZMC). Thus, *M. trossulus* may have experienced lower predation risk due to higher alternative prey availability in the ZMIC treatment (Nordberg & Schwarzkopf 2019), or the difference can be explained by a potential preference for soft shelled prey. Forsström et al. (2015) found a similar trend, suggesting that the mud crab prefers soft-shelled crustaceans such as *Gammarus* spp. over hard-shelled *M. trossulus*. Similar preferences for soft-shelled prey have been observed in other crab species (Buck et al. 2003). It has been suggested that crabs might avoid hard shells because of the risk of claw damage from breaking them open (Juanes & Hartwick 1990).

Since the mud crab is a novel predator in the northern Baltic Sea, it is likely that the prey do not recognize the mud crab as a threat and thus lack anti-predation behaviour (Cox & Lima 2006, Sih et al. 2010, Anton et al. 2020). This phenomenon is especially pronounced in contexts where the introduced predator lacks native congeneric relatives in the recipient community (Anton et al. 2020). Since there are no native crab species in the northern Baltic Sea, the prey would likely not recognize the mud crab as a threat (Forsstöm et al. 2015). This would partly explain the mud crabs ability to catch *Gammarus* spp., which are highly mobile organisms. The high densities of *Gammarus* spp. also likely made them easier to catch in the confined area of the aquariums.

#### 4.3 The importance of interspecies interactions for seagrass

The largest amounts of loose filamentous algae formed in the control treatment (Z) with only eelgrass, which shows that eelgrass does not do well alone. Eelgrass does not naturally occur alone, but it interacts with a range of species from different functional groups of flora and fauna (Boström et al. 2006, Gustafsson & Boström 2011, 2013). Several studies have explored the importance of biodiversity and interspecies interactions in seagrass ecosystems (e.g. Hughes et al. 2004, Baden et al. 2010, Duffy et al. 2015). In general, high biodiversity facilitates healthier and more productive ecosystems that are more resilient to disturbance (e.g. Hughes et al. 2008, Hensel & Silliman 2013). The accumulation of filamentous algae in the treatment Z in the absence of grazers is in line with previous studies that have highlighted the importance of grazers in seagrass communities (Valentine & Duffy 2006, Duffy et al. 2015). Grazers control algal growth and thus decrease competition between seagrass and algae for light and nutrients (Neckles et al. 1993). Simultaneously, eelgrass offers shelter and food resources to grazers and other organisms (Boström & Mattila 1999, Boström et al. 2006, Voigt & Hovel 2019).

Mussel treatments contained less filamentous algae than the control treatment (Z), even though mussels could have fertilized algae in the aquariums (Vinther et al. 2008). However, suspension feeders like *M. trossulus* remove suspended particulate matter, such as phytoplankton, from the water column and thus increase water clarity (Jørgensen 1990, Chowdhury et al. 2016). This way *M. trossulus* could have removed algal propagules. Besides filtering the water and fertilizing the sediments, mussels contribute to several indirect effects in seagrass systems. They increase the structural complexity of the habitat, as they add surface for settlement and shelter (Zhang & Silliman 2019). The increased habitat complexity may indirectly reduce the predation pressure on mesograzers, as they are provided with more hiding places (Peterson & Heck 2001). Larger populations of mesograzers would be more efficient at controlling epiphytes and filamentous algae, which would allow more light to reach the seagrass (Östman et al. 2016).

Previous studies on the relationship between bivalves and seagrass have yielded varying results, ranging from positive and neutral to negative (e.g. Reusch et al. 1994, Carroll et al. 2008, Vinther et al. 2012, Castorani et al. 2015). Mussel fertilization may be harmful to seagrass especially in eutrophic conditions (Vinther et al. 2008, Vinther & Holmer 2008, Vinther et al. 2012). However, a



recent review by Gagnon et al. (2020) showed that 51 % of studies on seagrass-bivalve relationships have reported positive interactions, and only 24 % negative interactions. Thus, the interaction is often positive, but highly context dependent. The present study adds to the growing literature on context dependent outcomes regarding seagrass-bivalve interactions.

Even though loose filamentous algae prospered in the control treatment (Z), the amount of epiphytes remained similar to other treatments. It is possible that loose filamentous algae and epiphytes competed for light and nutrients, which could explain why loose filamentous algae thrived, but epiphytes did not. The macroalgal bloom formed by loose filamentous algae could have shaded epiphytes that grow below them on the eelgrass leaves. Macroalgal blooms require higher nutrient loading rates to form than epiphytic algae, due to higher individual biomass, lower nutrient uptake rates and longer turnover times but persist a long time (Havens et al. 2001).

Macroalgal blooms caused by eutrophication are becoming increasingly common and constitute one of the most serious problems in the Baltic Sea (Bonsdorff et al. 1997, Jansson & Dahlberg 1999, Lehvo & Bäck 2001). The macroalgae eventually accumulate and form drifting algal mats, which shadow and suffocate underlying flora and fauna (Valiela et al. 1997). Shading is stressful and detrimental to eelgrass, but eelgrass resistance to shading is greater when it grows in polycultures (Gustafsson & Boström 2013). Eelgrass commonly occurs in polycultures in the Baltic Sea, consisting of 5–10 angiosperms, which together have positive effects on eelgrass performance (Salo et al. 2009, Gustafsson & Boström 2011, Gustafsson & Boström 2013). This further highlights the importance of facilitative effects generated by biodiversity. Furthermore, seagrass interacts with microbes, which provide e.g. enhanced nutrient availability, phytohormone production and defence against pathogens (Tarquinio et al. 2019). Together these interactions can increase ecosystem resilience and form a more productive eelgrass meadow.

#### 4.4 The influence of light

Light availability is the primary determining factor for seagrass productivity (Dennison & Alberte 1985, Zimmerman et al. 1995), thereby it naturally affected eelgrass growth rates in this experiment. Furthermore, light intensity affected porewater nutrient concentrations. The ammonium concentrations were generally higher in low light intensity (row 1) in the absence of mussels. In contrast, ammonium concentrations were slightly higher in high light (row 2) than low light (row 1) in mussel treatments (ZM and ZMC). Castorani et al. (2015) also found an interaction effect between light intensity and ammonium porewater concentration from mussel biodeposition. Castorani et al. (2015) suggested that the interaction between light and mussel fertilization could be due to changes in eelgrass metabolism, leading to a reduced uptake of ammonium. Using metabolomic analysis, Hasler-Sheetal et al. (2016) revealed a cryptic interaction between light conditions and mussel fertilization on eelgrass, which did not show when applying traditional approaches. The analysis showed that mussel fertilization stimulated eelgrass nitrogen and energy metabolism under high light conditions. However, in low light conditions the mussels disrupted



eelgrass metabolism, enhancing responses against sulphide toxicity and causing tissue deterioration and inhibited oxidative energy metabolism (Hasler-Sheetal et al. 2016). Similar changes in eelgrass metabolism may have occurred in this experiment as well, which could explain the interaction between light and ammonium.

Eutrophication has caused low light conditions as well as hypoxia in large areas of the Baltic Sea (HELCOM 2018), which may shift the interaction between bivalves and seagrass from mutualistic to antagonistic (Vinther et al. 2008, Vinther & Holmer 2008, Vinther et al. 2012). The shift may be induced due to enhanced sulphide pools caused by mussel fertilization, and in hypoxic and low light conditions eelgrass is more vulnerable to sulphide intrusion. The lowered light conditions in the Baltic Sea has widescale effects on macrophytes, and one of them is decreased maximum depth distribution. For example, on the Swedish west coast where large eelgrass losses have occurred, the depth distribution of eelgrass has decreased with roughly two meters (Moksnes et al. 2018).

The environmental context is an important determinant for the strength, mechanisms and sign of interspecies interactions (Menge & Sutherland 1987, Connolly & Roughgarden 1999, Norkko et al. 2006, Gammal et al. 2019). In the present study, the environmental context depended on treatments and light intensity. Light (i.e. row) significantly affected porewater nutrients, algal growth and eelgrass growth and was thus one of the most prominent determining factors in this experiment. The environmental context such as light limitation (abiotic stress) can affect the direction of species interactions, according to the stress gradient hypothesis (Bertness & Callaway 1994, Bertness & Hacker 1994). The stress gradient hypothesis predicts that facilitative interactions become increasingly important in conditions where abiotic stress is elevated (Bertness & Callaway 1994, Bertness & Hacker 1994). Often habitat-modifying organisms, such as mussels or crabs, are able to both alleviate and exacerbate environmental stressors, potentially forming particularly complex species interactions (Kawai & Tokeshi 2007). In this study, eelgrass was most likely stressed by mussel fertilization, light limitation and physical disturbance by crabs. Thus, eelgrass was subjected to several co-occurring stress pathways (fertilization, disturbance), which together with light formed complex interactions.

#### 4.5 Physical disturbance caused uprooting of eelgrass shoots

Crabs are effective burrowers that rework sediment through bioturbation, which can affect sediment organic content (Botto and Iribarne, 2000), biogeochemistry, porewater characteristics and nutrient benthic fluxes (Gilbert et al. 1998, Fanjul et al. 2007, 2011) as well as erosion (Farron et al. 2020) and microtopography (Qiu et al. 2019). The burrowing behaviour is in some cases known to damage vegetation (Davis et al. 1998, Garbary et al. 2014, Howard et al. 2019, Derksen-Hooijberg et al. 2019).

The mud crab caused an increase in porewater ammonium concentrations, but only on row 1 between the treatments ZMI and ZMIC. Thus, the effect of mud crab bioturbation on sediment nutrients remained limited. Bioturbation by macrofaunal species are known to influence sediment nutrient and oxygen fluxes (Gilbert et al. 1998, Needham et al. 2011). Furthermore, other burrowing crab species have been shown to alter porewater characteristics, increased sediment oxygenation and influenced organic matter degradation rates (Fanjul et al. 2007, 2011, Needham et al. 2011, Giorgini et al. 2019). Mikkola (2016) did not find any effects of the mud crab on sediment nutrient levels. However, the results from the present study suggest that the mud crab could affect porewater nutrients to some degree, although it is likely context dependent as the effect only appeared between two treatments and only on one row.

The mud crabs caused uprooting of eelgrass shoots in the aquarium experiment (Figure 14), due to their burrowing behaviour. It was clear that the mud crabs preferred to seek shelter in the corners of the aquariums, where they dug pits in the sediment, changing the sediment landscape in the aquariums (Figure 15). This is the first record of mud crabs dislodging planted eelgrass shoots. At least one other invasive crab species is known to damage eelgrass beds through physical disturbance. The European green crab (*Carcinus maenas*), which has been introduced worldwide, damages eelgrass beds when it digs burrows in the sediment and shreds plants which has led to losses of eelgrass beds and interfered eelgrass restoration projects (Davis et al. 1998, Malyshev & Quijón 2011, Garbary et al. 2014, Howard et al. 2019). Furthermore, loss of eelgrass caused by the European green crab has likely resulted in a trophic cascade, as fish abundance and biomass have drastically decreased in areas where the European green crab has removed eelgrass (Matheson et al. 2016).



**Figure 16.** A picture showing the extent of which the mud crabs were able to modify the landscapes in the aquariums with their burrowing behaviour. Notice how the rhizomes of some of the eelgrass shoots are exposed and coming loose, and some shoots are partly buried in the sediment. In the bottom right corner one mud crab is visible.

Bioturbation by mud crabs had similar uprooting effects on eelgrass plants in this experiment as in the above-mentioned studies, even though the mud crab is a significantly smaller crab species than the European green crab, which can reach over 90 mm in carapace width (Grosholz & Ruiz 1996). However, further studies are needed to determine if the mud crab uproots eelgrass in field conditions. The confinement in small aquariums may affect the burrowing behaviour of mud crabs, since they were concentrated to the corners of the aquariums. Also, natural eelgrass meadows have complex root and rhizome structures which make them harder to dislodge than the transplanted eelgrass used in this experiment. In field conditions, the mud crab might not necessarily uproot eelgrass, but it may damage roots and rhizomes while burrowing, since they are within depths that coincide with mud crab burrowing. However, transplanting shoots is a common method used in restoration projects. In this context, transplanted shoots are sensitive to bioturbation by the mud crab, in a similar way as the European green crab can remove transplanted eelgrass (Davis et al. 1998).

Restoration projects are becoming increasingly important, as environmental degradation proceeds globally and valuable seagrass habitats disappear (Valdez et al. 2020). Restoration efforts are implemented worldwide to mitigate and/or compensate for losses of these important habitats; however, the success rates are low, and the efforts are expensive (van Katwijk et al. 2016). Only 37 % of seagrass restoration efforts have been successful, which is explained by scale of effort, site characteristics and planting methods (van Katwijk et al. 2016). Non-indigenous species (NIS) such as the mud crab can interfere with seagrass restoration in several ways. When seagrass is transplanted it is susceptible to NIS, especially if seagrass is planted in low densities (Ceccherelli et al. 2000). As previously mentioned, NIS can physically disturb restoration efforts, causing e.g. uprooting of shoots (Davis et al. 1998). Additionally, restoration efforts may facilitate spread of NIS when moving seagrass from donor to recipient area, especially if transplants come from distant donor sites and bring sediments (Wasson et al. 2001). NIS should be taken into consideration in restoration and preferably they should not be present as they increase the risk of damage on the restoration. Furthermore, facilitative interactions may be incorporated in the restoration effort to improve restoration success. However, Gagnon et al. (2020) suggest that studies on seagrass-bivalve interactions including only native species had significantly more positive outcomes than studies including NIS. Thus, the mud crab should be considered a potential disturbance in conservation and restoration efforts of valuable eelgrass habitat, as it could physically harm eelgrass or affect biodiversity and ecosystem functioning in unknown ways.

#### 4.6 Methodological limitations and considerations for future research

This was to our knowledge the first study to investigate the potential top-down effects of mud crabs in an eelgrass community and first to find mud crabs can uproot planted eelgrass shoots. However, the limitations of this study must be addressed. First of all, nutrients seemed to accumulate in the aquariums, causing epiphytes to flourish in grazer treatments, which contradicts the general concept of grazers removing epiphytes. It is possible that the fertilization effect of *M. trossulus* was affected

similarly by the aquariums. Since the reproductive *Gammarus* spp. formed high population densities, which in this case may have contributed to nutrient accumulation, it could in future studies be considered to keep the sexes separated and to not use gravid females.

The study was conducted on a small spatial and temporal scale. The relatively small size of the aquariums could have influenced how the nutrients accumulate, possibly through hydrodynamics inside the aquariums or due to low rates of water exchange. The fresh seawater entered the aquariums from above and exited from above. Therefore, it is possible that only the upper layer of the water column exchanged properly, potentially allowing nutrients to accumulate at the bottom. Interestingly, Gagnon et al. (unpublished) found that *M. trossulus* facilitates eelgrass growth by fertilizing the sediments, by conducting an aquarium experiment in the same facilities as the present study but using three times larger aquariums (60 x 30 cm). This further supports the theory that the size of the aquariums could have influenced the results. The spatial scale is an important factor to consider, as it may influence the results in any type of study (Thrush 1999). Additionally, the temporal scale may have been too short for this type of study, since negative effects on eelgrass from stressors such as NIS likely accumulate over time. Short-term aquarium experiments are useful for showing short-term mechanisms, but field experiments are necessary for showing long-term cumulative impacts of stressors such as the mud crab. Regarding the field sampling of mud crabs, the spatial scale could have been extended to include more than one eelgrass meadow, to better reflect mud crab densities in eelgrass beds in the Archipelago Sea overall.

Furthermore, the light conditions were difficult to standardize along the two rows of aquariums. However, this allowed us to also study the effect of light availability on the response variables in this study, and e.g. revealed an interesting interaction between light conditions and porewater nutrients. The effects of varying light conditions could have been minimized by choosing a different experimental design. A block design could have been applied to eliminate differences depending on the placement of the aquarium. However, a block design would have come with constraints to the number of treatments and replicates that could be performed simultaneously in the limited space and time. In that case the number of treatments would have had to be reduced. Many of the issues faced in this experiment, such as nutrients accumulating, could have been avoided by opting for a field cage experiment. Nevertheless, field experiments of that kind come with their own risks and challenges. However, this study presents new findings as well as points out knowledge gaps and can thus provide a base for future research.

As in general with experimental trials, caution should be taken when generalizing the results and applying them to natural systems. Future research is needed to disentangle the effects of mud crab predation on invertebrates in field conditions. It seems unlikely that a drastic decrease in mesograzers and *M. trossulus* would not resonate over trophic levels. Thus, further research is warranted. Field experiments would allow natural water exchange and prevent any excess accumulation of nutrients. Additionally, the physical disturbance from the mud crabs which caused eelgrass shoots to uproot should be tested in field conditions in both ambient eelgrass beds as well as restored plots. The former would be necessary to understand from a management perspective and

the latter from a restoration point of view. To fully understand the impacts of this novel species in the Baltic Sea, its effects on different trophic levels need to be studied in the different invaded communities, such as other soft and hard bottom habitats.

## 5. Conclusions

The density of mud crabs found within an eelgrass meadow at Ängsö was 21 crabs/m<sup>2</sup>, which is a higher density compared to previous findings within the Baltic Sea (Turoboyski 1973, Hegele-Drywa & Normant 2014, Gagnon & Boström 2016). However, differences in sampling methods make comparisons difficult, thus a standardized method which allows for comparison of densities is needed. Core samples as used in the present study can be used to accurately estimate adult mud crab density and population structure.

Mud crabs readily consumed invertebrates in the aquarium experiment, however, this did not cause a trophic cascade. *M. trossulus*, *Gammarus* spp. and *T. fluviatilis* did not facilitate eelgrass growth, thus predation on them did not affect eelgrass. *M. trossulus* likely caused sulphide stress instead of facilitating eelgrass, such reversed interaction has been identified previously in eutrophic conditions (Vinther et al. 2012). Nutrients seemed to accumulate in the aquariums, which explains e.g. how grazers caused epiphytes to flourish. This study contributes to the growing literature on the importance of context dependency in determining seagrass interactions with other species. Light was the determining factor for eelgrass and algal growth, and it interacted with porewater ammonium concentrations. The mud crab caused changes in porewater ammonium in one instance, although they were limited to one treatment and appeared to depend on light conditions. Due to confounding factors in the experiment, the possibility of trophic cascades occurring in the field due to mud crab induced top-down processes in eelgrass cannot be excluded but warrants further studies.

Furthermore, the mud crab physically disturbed eelgrass and caused uprooting, due to its burrowing behaviour. Other crab species are known to physically harm seagrass and have caused major damage to natural seagrass meadows as well as seagrass restorations (Davis et al. 1998, Howard et al. 2019). Especially transplanted seagrass may be vulnerable to disturbance; thus, the mud crab should be carefully considered in such a context. This is the first record of mud crabs dislodging eelgrass, although this needs further testing in field conditions.

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## Swedish summary- svensk sammanfattning

### Den introducerade slamkrabbans effekt på ålgräsets tillväxt

Slamkrabban (*Rhithropanopeus harrisi* Gould, 1841) är en introducerad art i Östersjön, som härstammar från den nordamerikanska östkusten (Williams 1984). Arten har förekommit i södra Östersjön sedan 1950-talet eller tidigare (Turoboyski 1973) och har nyligen etablerat sig i norra Östersjön, där den första gången påträffades i finska Skärgårdshavet år 2009 (Karhilahti 2010). I norra Östersjön finns naturligt inga andra arter av krabbor (Elmgren & Hill 1997, Bonsdorff 2006) och således utgör slamkrabban en ny funktionell roll som kan förändra funktionella länkar i födoväven och påverka samhällsstrukturen (Forsström et al. 2015, Gagnon & Boström 2016). Slamkrabban har därmed identifierats som en potentiellt eller lokalt skadlig introducerad art (Niemi-Lahti 2012). Slamkrabban är en opportunistisk art som utnyttjar en mångfald av olika habitat och födokällor. Nyligen påträffades slamkrabban första gången i en ålgräsäng i dess introducerade område (Gagnon et al. 2016). Som födokälla konsumerar slamkrabban detritus, alger och evertebrater (Turoboyski 1973, Hegele-Drywa & Normant 2009, Forsström et al. 2015). Några av dess vanligaste byten består av blåmusslor (*Mytilus trossulus*), gastropoder (*Theodoxus fluviatilis*) och gammarider (*Gammarus* spp.), som upprätthåller viktiga funktioner för bland annat ålgräset (Gagnon & Boström 2016).

Tidigare studier indikerar att slamkrabban kan orsaka trofiska kaskader (Jormalainen et al. 2016, Kotta et al. 2018). En trofisk kaskad sker då förändringar på en trofisk nivå leder till effekter över flera än en trofisk länk (Carpenter & Kitchell 1993). Eftersom slamkrabban är en ny predator i Östersjön, kan den orsaka ökat predationstryck på herbivorer. Effekterna av slamkrabban i ålgräsängar är okända. Syftet med denna avhandling var därför att studera slamkrabbans potentiella effekter på ålgräset, vilket inkluderar indirekta effekter genom en trofisk kaskad och direkta effekter genom fysisk störning.

Frågeställningarna i avhandlingen är:

1. Vad är den naturliga densiteten av slamkrabbor i en ålgräsäng i Skärgårdshavet?
2. Kan slamkrabban indirekt påverka ålgräsets tillväxt negativt genom en trofisk kaskad?
3. Kan slamkrabban fysiskt störa eller skada ålgräset genom att den gräver i sedimentet?

För att svara på fråga 1 studerades slamkrabbans densitet i en ålgräsäng vid Ängsö (Figur 3), där sampel av sediment togs med hjälp av proppar (ø 10,5 cm). Sammanlagt togs 60 slumpmässigt placerade sampel i ängen, totalt 0,52 m<sup>2</sup> undersöktes. Krabborna räknades och könsbestämdes. För att svara på frågorna 2–3 utfördes ett akvarieexperiment i Skärgårdscentrum Korpoströms genomflödesanläggning. Experimentet bestod av sju behandlingar med fem replikat. Akvarierna placerades på två rader, som herefter benämns rad. Behandlingarna innehöll olika kombinationer av slamkrabba, *M. trossulus*, *Gammarus* spp. och *T. fluviatilis* tillsammans med naturligt och artificiellt ålgräs (för att provta epifyter) (Tabell 2). Experimentet pågick i 40 dagar, varefter de

kvarvarande organismerna räknades och sampel togs för att undersöka sedimentets organiska halt, näring i porvatten (ammonium och fosfat), samt mängden lösa trådalger och epifyter. Ålgräsets tillväxt undersöktes genom att sex dagar före experimentets avslut punktera ålgrässkottets nedre del, vilket bildade ett ärr som användes som markör för att beräkna tillväxten per dag. Under experimentets gång räknades även antal ålgrässkott som lossnat ur sedimentet. Data från akvarieexperimentet analyserades i R med linjära modeller som bestod av generaliserade linjära modeller (GLM), bayesiansk GLM och blandade linjära och generaliserade modeller (GLMM och LMM) (se Tabell 3). Som förklarande variabler inkluderades behandling, rad och akvarium (då flera sampel tagits från samma akvarium). Rad inkluderades eftersom det fanns en skillnad i ljusintensiteten mellan raderna som akvarierna placerades på (Figur 6). Skillnaden i ljusintensiteten mellan raderna undersöktes med ett Wilcoxon rank sum test.

Densiteten av slamkrabbor i ålgräsängen vid Ängsö uppmättes till 21 krabbor/m<sup>2</sup>. I akvarieexperimentet konsumerade slamkrabban en väsentlig andel av bytesdjuren *M. trossulus*, *Gammarus* spp. och *T. fluviatilis* (Figur 7 a-c, Tabell 4), men detta orsakade ingen trofisk kaskad, enligt resultaten nedan. Behandlingarna hade ingen signifikant effekt på den organiska halten i sedimentet, men en trend visade att behandlingar med endast ålgräs eller ålgräs tillsammans med betare (*T. fluviatilis*, *Gammarus* spp.) tenderade att minska i organisk halt över tid, medan behandlingar som inkluderade *M. trossulus* tenderade att öka i organisk halt över tid (Figur 8, Tabell 4). Slamkrabban hade ingen tydlig inverkan på koncentrationen av ammonium i porvatten, förutom på rad 1 där en signifikant skillnad mellan behandlingarna ZMI och ZMIC fanns (Figur 9, Tabell 4). Inga signifikanta skillnader förekom i koncentrationen av fosfat mellan behandlingarna (Figur 10, Tabell 4). Mängden lösa trådalger och epifyter påverkades inte av slamkrabban, men det fanns signifikanta skillnader mellan rad och mellan övriga behandlingar (Figur 11–12, tabell 4). Ålgräsets tillväxt per dag och den totala längden av ålgrässkotten påverkades inte av behandlingarna, men raden hade en betydande inverkan på vardera (Figur 13–14, Tabell 4). Antalet ålgrässkott som lossnat under experimentets gång var signifikant högre i behandlingar som inkluderade slamkrabba, än i behandlingar utan slamkrabba (Figur 15, Tabell 4).

Slamkrabbors densitet i Östersjön är dåligt känd, till vilket denna studie bidrar med data. Densiteten vid Ängsö (21 krabbor/m<sup>2</sup>) är mycket hög jämfört med uppmätt densitet i studier från Polen (0,5–6 krabbor/m<sup>2</sup> och 0,05–0,19 krabbor/m<sup>2</sup> (Turoboyski 1973, Hegele-Drywa & Normant 2014, respektive)) eller i tidigare undersökningar vid Ängsö (4 krabbor/m<sup>2</sup> (Gagnon & Boström 2016)). I akvarieexperimentet uppstod ingen trofisk kaskad, vilket till stor del kan bero på att *T. fluviatilis*, *Gammarus* spp. och *M. Trossulus* inte hade de gynnande effekterna på ålgräset som förväntades. *M. Trossulus* förväntades öka mängden tillgänglig näring till akvarierna och gynna ålgräsets tillväxt, men kan istället ha orsakat stress för ålgräset, eftersom höga näringshalter kan leda till ackumulering av sulfider i sedimentet som skadar växten. Betarna (*T. fluviatilis*, *Gammarus* spp.) förväntades ha minskat på mängden alger (lösa trådalger och epifyter), men istället ökade de, högst sannolikt på grund av att *Gammarus* spp. förökade sig i akvarierna och avföringen (näring) från de stora populationerna möjliggjorde alg-tillväxt. Slamkrabban orsakade genom sitt grävande beteende att fler ålgrässkott lossnade ur sedimentet. Åtminstone en annan krabbart, *Carcinus maenas*, har



dokumenterats ha liknande effekter som har stört restaureringsprojekt och skadat naturliga ålgräsängar (Davis et al. 1998, Garbary et al. 2014, Howard et al. 2019). Detta är dock första gången som slamkrabban observerats orsaka sådan fysisk störning hos ålgräs, och fortsatta undersökningar i fält krävs.

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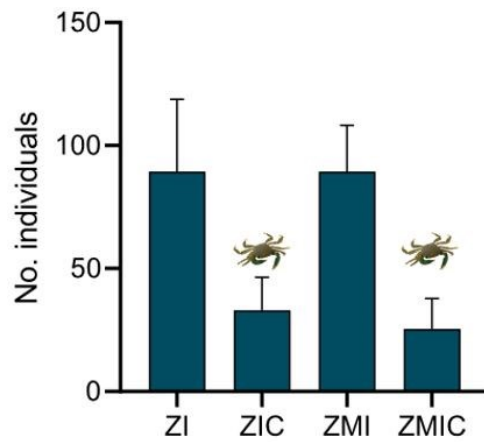
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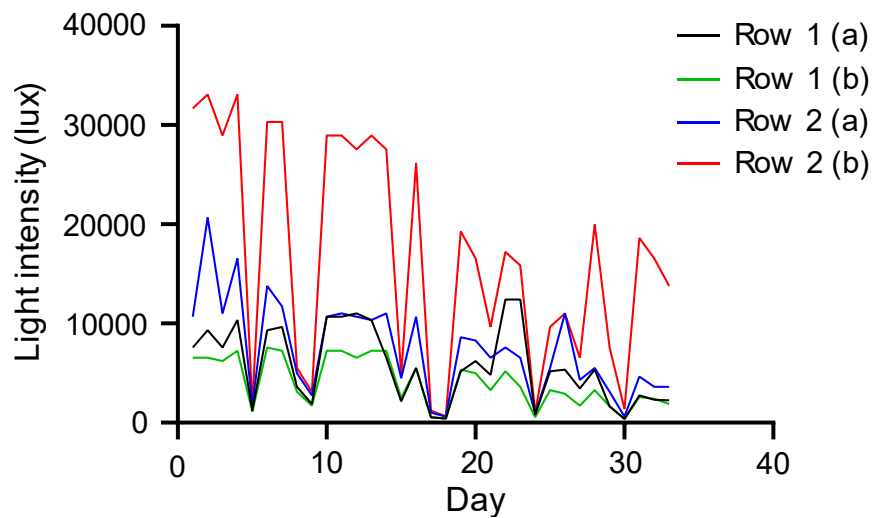
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## Appendix



**Figure A.** Total number of *Gammarus* spp. (mean  $\pm$  SE) at the end of the experiment, including both adults and juveniles. Only 5 (adult) *Gammarus* spp. were included in the beginning of the experiment.



**Figure B.** Light intensity at 14:00 (mean  $\pm$  SE, N= 33, lux) plotted for each data logger (two on each row).