

**Effects of separate and combined predation by the invasive mud crab (*Rhithropanopeus harrisi*) and the native fish (*Perca fluviatilis*) on the marine isopod (*Idotea balthica*) in the northern Baltic Sea**

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Introductions of novel predators have caused dramatic declines and extinctions of native prey species worldwide. This also concerns the littoral community of the northern Baltic Sea, where the functionally novel predatory mud crab *Rhithropanopeus harrisii* has caused a rapid reduction in the abundance and diversity of the native key herbivores. Predator invasion success may be attributed to prey naivete, or the absence of predator recognition and appropriate anti-predator behaviour of native prey due to the lack of co-evolution. There is a knowledge gap regarding the combined effect of invasive and native predators on prey. If the combined mortality effect is synergistic, it could lead to drastic declines in native prey populations. In this thesis, I have examined the effects of separate and combined predation by the invasive mud crab and the native perch *Perca fluviatilis* on the mortality of the marine herbivorous isopod *Idotea balthica*. I also observed the anti-predator behaviour of the isopod to evaluate its naivete. For this experiment, I collected all the animals from the Archipelago Sea. I conducted a predation experiment in the laboratory and compared mortality rates of the isopods preyed on by mud crabs only, perch only, and both the predator species together. Based on a survival analysis, both the separate and combined predation by the two predators caused a significant mortality on the isopods. However, the two predators together consumed less isopods than was expected based on how much they consumed separately. This suggests that there was an antagonistic interaction between the predators, which led to a reduction in their combined effect. As the two predators were never observed to interfere each other directly, the reduced prey mortality is possibly due to a trait-mediated indirect interaction, involving a behavioural modification between the predators and the prey. These results have important community- and ecosystem-level implications. Although the mud crab is an efficient predator that may benefit from the possible naivete of the isopods, when both predators co-occur, they cause lower mortality on the isopods. Thus, the perch and other similar native predators may have a key role in the ecosystem of the northern Baltic Sea, as they may attenuate the adverse impact of the mud crab. To understand the ecological impacts of introduced predators in the native communities, we need more research that considers the multiple trophic levels involved in predator-prey dynamics and the key role of prey naivete in predator introductions.

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Key words: biological introduction, introduced predator, Harris mud crab, predator–prey interaction, prey naivete, emergent effect, risk reduction, Baltic Sea

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## 1. Introduction

### 1.1. Species introductions in marine habitats

In recent decades, the diversity and abundance of marine species and their habitats have dramatically declined and degraded across the globe. The world seas and oceans are under an increasing anthropogenic pressure due to climate change, pollution, overharvesting of fish stocks and non-native species introductions (i.e., biological introductions) (Brondizio et al. 2019). These human-mediated biological introductions are now recognized as one of the most serious threats to global biodiversity, as the rate of new introductions seems higher than ever before and shows no signs of slowing down (Brondizio et al. 2019; Pyšek et al. 2020). Past and present transportation of marine species beyond their native ranges have weakened natural barriers to dispersal and facilitated biological introductions across the globe (Elton 1958; Ruiz et al. 1997). Thus, the number of widespread species has increased, while the number of endemic species has decreased, resulting in a phenomenon called “anthropogenic blender”, where previously distinct ecological communities start to resemble each other (Olenin et al. 2017; Brondizio et al. 2019).

Present biological introductions in marine environments are driven by new technical advancements, international trade and the emergence of new introduction pathways due to globalization (Geburzi & McCarthy 2018; Blakeslee et al. 2019; Pyšek et al. 2020). Maritime traffic is the main pathway of biological introductions, and it is predicted to expand in the future (Molnar et al. 2008; Sardain et al. 2019; Pyšek et al. 2020). The ballast water and hulls of ships provide vectors for non-natives species to be transported as hitchhikers over long distances (Geburzi & McCarthy 2018; Sardain et al. 2019; Pyšek et al. 2020). The significance of ships’ ballast water as a vector has increased over time, as the number, size and speed of modern vessels has increased (Sardain et al. 2019). As a result, even more species are being transported outside their native ranges and are more likely to survive over transportation (Geburzi & McCarthy 2018). Other common marine pathways and vectors include canal constructions, aquaculture, aquarium trade and drifting non-biodegradable plastics (Molnar et al. 2008; Olenin et al. 2017; Pyšek et al. 2020).

Anthropogenic activities not only provide new means of transportation and spread for non-native species, but they also affect the environment in ways that favour their

establishment (Geburzi & McCarthy 2018). Coastal seas and estuaries are the most invaded marine ecosystems due to the proximity to shipping routes, ports, inland waterways and human settlement (Ruiz et al. 1997; Olenin et al. 2017; Sardain et al. 2019). They are also exposed to a high level of natural and anthropogenic disturbances such as eutrophication, pollution and aquaculture (Olenin et al. 2017; Brondizio et al. 2019). Suitable conditions, availability of resources and niches favour the establishment and spread of non-natives species to the detriment of native species (Geburzi & McCarthy 2018). Sheltered coasts and warm river estuaries also provide a high retention of propagules and a multitude of artificial structures that can serve as ‘stepping stones’ for the secondary dispersal of non-native species (Landschoff et al. 2013). Consequently, some of the species may manage to establish self-sustaining populations, become abundant and widespread. In addition, the annual warming of seawater is predicted to make temperate regions with low species diversity more vulnerable to a poleward shift of southern species and their permanent establishment (Hulme 2017). Therefore, non-native species are likely to benefit from the synergies of climate change, human-mediated transport and environmental change.

Given the ongoing loss of a global biodiversity, it is increasingly important to understand the ecological impacts and consequences of non-native species in the native communities. Non-native species that enter new ecosystems become integrated into the ecological communities over time (Zavaleta et al. 2001; Papacostas & Freestone 2019). Therefore, the basic ecological research on food webs, predator–prey dynamics and trophic cascades are needed to evaluate and predict the impacts of non-native species (Zavaleta et al. 2001). The impacts of non-native species can vary across species, communities and ecosystems, depending on their abundance and trophic levels (Pyšek et al. 2020). For instance, crustaceans and crabs are recognised as the most dominant, widespread and problematic marine species group (Molnar et al. 2008). They possess life-history traits that promote pre-adaptability into new habitats, including high fecundity, planktonic larval stage and omnivorous feeding habits (Hänfling et al. 2011). These omnivorous species are effective at imposing trophic cascades (i.e., indirect trophic interactions) in the native communities, altering the function and structure of native ecosystems (Hänfling et al. 2011; Liversage et al. 2021). To predict the consequences of biological introductions, more knowledge is required on the functional roles of the species involved and their biotic interactions in the native communities (Jormalainen et al. 2016).

## 1.2. Predator–prey interactions and prey behaviour

Predator–prey interactions influence the structure and function of entire communities and ecosystems, as well as the evolution of species traits. Predation often acts as a strong selective force, as it can directly cause mortality or indirectly limit the growth, fecundity and reproduction of a prey (Lima & Dill 1990). Furthermore, predation risk can induce the prey to change its behaviour, habitat use and other ecological processes at the expense of energetic costs or missed opportunities (Lima & Dill 1990; Carthey & Banks 2014). Predators and prey that share evolutionary history have evolved adaptations and counter-adaptations in a process called coevolutionary ‘arms race’, in which natural selection favours prey to become even better at avoiding predators and predators in turn become better at catching prey (Dawkins & Krebs 1979; Abrams 2000). However, biological introductions often produce novel predator–prey interactions from which this coevolutionary history is lacking (Sih et al. 2010; Carthey & Banks 2014). Thus, native prey could be more susceptible to non-native predators, leading to unpredictable consequences for native prey populations.

In the classic predation sequence, prey is assumed to have an ability to recognise its natural predators to avoid the encounter. This ability is vital for survival, and it may be the result of a long coevolutionary history, or it can be learned overtime (Carthey et al. 2017; Steindler et al. 2020). Recognition of a predator by prey can be based on various cues such as sensory cues (e.g., visual), behavioural cues (e.g., hunting mode) and habitat cues (e.g., habitat domain) (Kats & Dill 1998; Carthey & Banks 2014). Some of the cues are time- and space-dependent such as predation risk in a particular habitat may be higher at certain times of the day (Carthey & Banks 2014). In addition, prey can detect specific cues, general cues or the combination of both (Sih et al. 2010). Specific cues such as certain odours are associated only with a specific predator taxon, whereas general cues such as predatory odours that are produced by predator metabolism (i.e., meat-eater cue), are characteristic for many predator taxa (Carthey & Banks 2014; Carthey et al. 2017). Prey that have co-evolved with specific predators are likely to detect specific cues and avoid over-responding to other irrelevant cues (Sih et al. 2010; Carthey & Banks 2014). In contrast, prey that have experience with multiple predators may detect general cues and even recognise non-native predators (Sih et al. 2010; Carthey & Banks 2014).

Anti-predator responses are induced after a successful recognition of predators by prey. According to Endler (1991), most prey possess a suite of anti-predator responses and the decision of prey to respond to their predators depend on both the ecological context and the current predation risk (Sih et al. 2010; Carthey & Banks 2014; Melotto et al. 2021). As anti-predator responses are energetically costly, prey should only respond to predators that pose a high predation risk (Carthey & Banks 2014). If the predation risk is posed by a particular predator taxon, prey can evolve specific anti-predator responses that are effective against coevolved predators, but only moderate towards other predators. For instance, prey can use a combination of cryptic colouration and immobility, which is effective response against visually searching predators, while it may be ineffective against stationary predators (Schmitz 2007; Carthey & Banks 2014). When the predation risk is low, however, prey should use less costly responses towards predators. Examples of general responses are microhabitat shift, escape behaviour and shelter-seeking (Belgrad & Griffen 2016). Variation in anti-predator responses within species might also occur due to local adaptations (Hooks & Padilla 2021).

In nature, most prey encounter multiple predators and must respond to varying predator densities and identities (Livernois et al. 2019). Recent studies have pointed out that predators can have other than additive effects on a shared prey and have emphasised the great importance of studying them (Palacios et al. 2018; Livernois et al. 2019; Liversage et al. 2021). Both interactions among predators and anti-predator responses of prey may lead to emergent ‘multiple predator effects’, where the combined mortality by multiple predators is non-additive and cannot be predicted from the sum of the mortality caused by single predators (Sih et al. 1998). Facilitation or synergism among predators, may allow them to consume more prey, leading to an enhancement in prey mortality (Sih et al. 1998; Livernois et al. 2019). Alternatively, antagonism may disrupt mutual predation, resulting in a reduction in prey mortality (Vance-Chalcraft & Soluk 2005; Livernois et al. 2019). Functionally different predators and specific anti-predator responses of prey are predicted to cause predation risk enhancement, as the prey is more susceptible to the other predator to which it fails to respond (Sih et al. 1998; Schmitz 2007). However, risk reduction may occur when predators are functionally similar, as they are more likely to encounter frequently, interfere with each other and compete for the shared prey (Palacios et al. 2018; Livernois et al. 2019; Leighton & Tyler 2021). As multiple predators can have unpredictable effects on prey, studying these effects is highly important. Particularly, introductions of non-native predators may increase the predation pressure of native prey.

### 1.3. Introduced predators and novel biotic interactions

As even more species are introduced beyond their native ranges, it inevitably leads to novel species combinations and consequently, to novel biotic interactions between non-native species and native species (Strauss et al. 2006; Geburzi & McCarthy 2018). Verhoeven and others (2009) referred this situation as an ‘ecological mismatch’, where previously allopatric species are brought together and the following outcome is an ecological (i.e., evolutionary) naivete or the failure to recognise and respond to a threat due to the lack of coevolutionary history (Cox & Lima 2006; Carthey & Banks 2014). This ecological naivete can occur in any antagonistic interaction between non-native and native species such as competition, predation and herbivory (Verhoeven et al. 2009). Of all introduced species, however, introduced predators are considered the most destructive species group with the greatest relative impact on native communities (Salo et al. 2007; Paolucci et al. 2013; Anton et al. 2020). Introduced predators have far stronger direct lethal effects on native prey populations than their natural predators and have caused mass extinctions and declines in many local prey species across the globe (Salo et al. 2007; Paolucci et al. 2013; Papacostas & Freestone 2019; Anton et al. 2020).

Previous studies have emphasised the role of prey naivete in biological introductions, as it could explain the serious impacts of introduced predators on native prey and their establishment success (Paolucci et al. 2013; Papacostas & Freestone 2019; Anton et al. 2020). The archetype hypothesis predicts that native prey will be naive towards unfamiliar predators (i.e., novel predator archetypes), which possess distinct morphological and behavioural counter-adaptations than their native predators to catch prey (Cox & Lima 2006; Carthey & Blumstein 2018; Anton et al. 2020). Moreover, distinctions at the taxonomic level of genus or family have been proposed as a proxy to the archetypes (Carthey & Blumstein 2018). Prey naivete is not a static property – it is rather a continuous process, which is influenced by eco-evolutionary experience with the native predator assembly (Cox & Lima 2006; Paolucci et al. 2013; Papacostas & Freestone 2019; Anton et al. 2020). As a result, prey naivete has been recognised to include four levels through which prey may progress with time and experience (Carthey & Blumstein 2018). At the first level of prey naivete, naive prey may not recognise novel predators as a threat and suffer heavy predation. At the second level, naive prey may recognise the threat but the anti-



predator responses are inappropriate. Naive prey at the third level may have appropriate responses, but they are ineffective against the superior hunting tactics of the novel predators. At the fourth level, naive prey experience excessive indirect non-lethal costs of predation by over-responding to the novel predators (Banks & Dickman 2007; Carthey & Banks 2014; Carthey & Blumstein 2018; Anton et al. 2020).

Recently, global trends in prey naivete have been recognized (Salo et al. 2007; Paolucci et al. 2013; Carthey & Blumstein 2018; Anton et al. 2020). Prey are considered most naive towards introduced predators in marine ecosystems, freshwater communities and oceanic island communities compared to terrestrial ecosystems (Cox & Lima 2006; Paolucci et al. 2013; Papacostas & Freestone 2019; Anton et al. 2020). These systems have in common biogeographic isolation, remoteness, species-poor communities and simple food-webs, which make them extremely vulnerable to biological introductions (Lima & Cox 2006; Anton et al. 2020). For instance, species-poor communities are more likely to include less predator archetypes than species-rich communities (Anton et al. 2020). In addition, most introduced predators are from high diversity ecosystems and consequently, are more experienced with wider range of prey (Anton et al. 2020). Thus, the more novel the introduced predators are to the native prey, the more severe impacts they are likely to have in the native communities (Cox & Lima 2006). Furthermore, it has been proposed that in the predator-free and remote communities, prey may lose energetically costly and irrelevant anti-predator adaptations due to a relaxed selection (Lahti et al. 2009; Carthey & Blumstein 2018; Anton et al. 2020).

Introductions of non-native predators can affect the evolution of species traits for the predators themselves and for native prey (Strauss et al. 2006; Blakeslee et al. 2019). Over time, introduced predators may evolve new morphological, physiological and behavioural adaptations to obtain native prey (Blakeslee et al. 2019). Natural selection can also favour native prey to become less naive towards introduced predators via learning and the evolution of anti-predator adaptations such as predator recognition and avoidance behaviour (Strauss et al. 2006; Paolucci et al. 2013; Papacostas & Freestone 2019). Previous studies have proposed that reduction in prey naivete can evolve over several generations or can be achieved via learning within a generation, as prey often apply more adaptive anti-predator behaviour after the initial period of intense predation (Carthey & Banks 2016; Papacostas & Freestone 2019). However, evolutionary adaptations are possible only, if the effect of introduced predators on native prey is not too strong or sudden, the predators

are not present in all local prey populations and native prey have enough genetic variation (Vellend et al. 2007). Under these circumstances they may be able to coexist within the same community.

The community into which the introduced predator enters may also influence the ecological impact of the predator. Recent research has found strong support on the biotic resistance hypothesis (Elton 1958) according to which species-rich communities are more resistant against introduced species than species-poor communities (Levine et al. 2004; Kimbro et al. 2013; Beaury et al. 2020). Thus, native communities that have high species abundance and various functional roles utilize resources efficiently and limit available niches (Kimbrow et al. 2013; Beaury et al. 2020). Native species may also eliminate non-native species by predation, competition and parasitism. As introduced mesopredators are often prey themselves, they may be exposed to a high predation risk from native top-predators and thus be at a disadvantage due to lack of predator recognition and anti-predator behaviour (Sih et al. 2010; Papacostas & Freestone 2019). Nevertheless, it has been recognised that biotic interactions rarely make native communities completely resistant to introduced species but instead can limit their abundance and spread (Levine et al. 2004; Schulz et al. 2019). Furthermore, biotic resistance is a continuous condition, as the ability of native communities to resist introduced species may vary in time and space (Kimbrow et al. 2013; Beaury et al. 2020).

#### 1.4. Aim of the thesis

The Harris mud crab (*Rhithropanopeus harrisii*, hereafter the mud crab) was observed for the first time in the northern Baltic Sea in 2009 (Karhilahti 2010). It is a functionally novel predator in this region, as there are no other native brachyuran crab species (Jormalainen et al. 2016; Forsström et al. 2018; Lokko et al. 2018). During its short invasion history, the mud crab has reduced the native benthic diversity, altered species composition and caused community-level changes of key herbivore species (Jormalainen et al. 2016; Kotta et al. 2018; Lokko et al. 2018). Particularly, local populations of the marine herbivorous isopod (*Idotea balthica*, hereafter the isopod) have rapidly declined, and abundant populations are observed only in the outer archipelago, where there are no mud crabs, yet (Jormalainen et al., manuscript). Previous studies have suggested that the invasion success of the mud crab may be promoted by prey naivete, or the absence of predator

recognition and appropriate anti-predator behaviour of native prey, due to the lack of co-evolution (Forsström et al. 2015; Jormalainen et al. 2016; Kotta et al. 2018). However, it has not been empirically tested. In addition, the mud crab may have synergistic interactions among other predators, which might increase the predation pressure of the native prey populations. Therefore, the primary aim of my thesis was to examine the effects of separate and combined predation by the recently introduced mud crab and the native fish, European perch (*Perca fluviatilis*, hereafter the perch), on the mortality of the isopod. I also observed the anti-predator behaviour of the prey in the presence of the two predators to determine the level of prey naivete. My study questions and hypotheses were the following:

1. *Do the predators separately cause significant mortality on the isopods?*

Hypothesis: The perch and the mud crab are likely to be efficient predators of the isopods and thus will cause separately significant mortality on the isopods. The mud crab may also benefit from the naivete of the isopods.

2. *Does the combined predation cause an emergent effect (i.e., enhancement or reduction) on the mortality of the isopods?*

Hypothesis: When both predators co-occur, they may facilitate each other's predation success by causing conflicting responses in the isopods, resulting in enhancement in predation mortality.

3. *Does the presence of the predators cause a behavioural response in the isopods?*

Hypothesis: The perch will cause a behavioural response in the isopods, whereas the mud crab will not, due to the naivete of the isopods.

## 2. Material and methods

### 2.1. Study area – the Baltic Sea

The Baltic Sea is a semi-enclosed continental brackish-water sea, with a total surface of 370 000 km<sup>2</sup> (Snoeijs-Leijonmalm & Andrén 2017). It is a microtidal and characterised by steep gradients of temperature and salinity, which varies in horizontal and vertical directions (Leppäkoski et al. 2002; Snoeijs-Leijonmalm & Andrén 2017). A high spatial-

temporal variability in environmental gradients influence species distribution and the composition of local flora and fauna (Leidenberg et al. 2012). The Baltic Sea is also geologically young and species-poor ecosystem and thus only some species have fully adapted to its special conditions (Leppäkoski et al. 2002). The communities are structured around a few habitat forming species such as the bladderwrack (*Fucus vesiculosus*), the common eelgrass (*Zostera marina*) and the blue mussel (*Mytilus trossulus*) (Snoeijs-Leijonmalm & Andrén 2017). In these communities, the role of herbivory is considered crucial, as the herbivores can buffer the effects of eutrophication by grazing an opportunistic epiphytic alga (Jormalainen et al. 2016; Östman et al. 2016). The key herbivores include isopods, amphipods and gastropods (Jormalainen et al. 2016).

Among other coastal seas, the Baltic Sea is susceptible to anthropogenic species introductions and disturbance such as eutrophication, pollution, overfishing and shipping (Snoeijs-Leijonmalm & Andrén 2017). There is a continuous spread of introduced species from nearby regions via shipping, canal constructions and aquaculture (Leppäkoski et al. 2002). Thus, the number of introduced species has increased in the Baltic Sea, which currently hosts 135 introduced species (Rius et al. 2022). Of all introduced species, 72% have caused measurable ecological effects in the local ecosystem (Ojaveer & Kotta 2015). These effects include alterations of food webs and habitat structures, translocation of native species and their disruption by competition and predation (Ojaveer et al. 2021). The species considered with so far largest effects are the benthic and demersal species such as the polychaete worm (*Marenzelleria* spp.), the round goby (*Neogobius melanostomus*) and the mud crab (*R. harrisi*) (Ojaveer & Kotta 2015; Ojaveer et al. 2021). In many instances, introduced species have brought novel ecological functions such as filter feeding, bioturbation and omnivory, and subsequently they have formed new functional links in the native food web (Leppäkoski et al. 2002; Forsström et al. 2015; Ojaveer et al. 2021).

## 2.2. Study species

### 2.2.1. Marine isopod *Idotea balthica*

*I. balthica* (Pallas 1772) is a crustacean mesograzer and one of the key herbivores of macrophyte communities in the northern Baltic Sea (Leidenberg et al. 2012). It has a wide distribution range, which extends from the northern Baltic Sea to the Atlantic coasts and

up to the Mediterranean Sea (Leidenberg et al. 2012). The isopod inhabits perennial macrophyte communities and prefers the bladderwrack and the common eelgrass as food and shelter (Vesakoski et al. 2008a). It is a generalist herbivore but prefers the epiphytes growing on macrophytes (Leidenberg et al. 2012). The species has a lifespan of 13–15 months and a reproduction period from June to July (Salemaa 1979; Jormalainen & Tuomi 1989). It has also five genetically determined colour morphs, including the uniform coloured *uniformis*, the white-spotted *albafusca*, the marbled *maculate*, the white-striped *bilineata*, and *bilineata-lineata* (Merilaita 2001). The local populations differ in microhabitat-use, life-history traits and in colour morph frequencies (Jormalainen & Tuomi 1989; Merilaita 2001; Vesakoski et al. 2008a). The main predators of the isopods are fish such as the perch (*P. fluviatilis*), the cod (*Gadus morhua*) and the eelpout (*Zoarces viviparus*) (Leidenberg et al. 2009). They have several anti-predator adaptations such as cryptic colouration, nocturnal activity, immobility and hiding within the vegetation in the presence of predators (Jormalainen & Tuomi 1989; Merilaita & Jormalainen 2000; Vesakoski et al. 2008b).

### 2.2.2. European perch *Perca fluviatilis*

*P. fluviatilis* (L. 1758) is a stenohaline freshwater fish (Christiensen et al. 2021). The perch is most common coastal fish species in the Baltic Sea, and it has spread across Europe (Lappalainen et al. 2001; Hansson et al. 2019). It has an anadromous life cycle, in which the populations spawn in spring, spend the summer months foraging in estuaries and migrate into deltas and streams to overwinter (Hansson et al. 2019; Christiensen et al. 2021). The species undergoes an ontogenetic diet shift during its life cycle, where the juveniles feed on zooplankton and macroinvertebrates and the adult perch feed on mobile macroinvertebrates and other fish species (Lappalainen et al. 2001). According to Lappalainen and others (2001), the diet of the perch under 10 cm (length) consists mostly of crustaceans, whereas the perch over 20 cm (length) prey on fish in the northern Baltic Sea. Previous laboratory experiments have also found that the perch consume readily small crustaceans and rely on vision to hunt (Jormalainen et al. 1995; Merilaita 2001). More recently Puntilla-Dodd and others (2019) found mud crabs in the stomachs of the perch and the mud crabs eaten were approximately 10–12 mm (carapace width). The perch is considered a key species, as it regulates the local community through trophic

cascades (Christiensen et al. 2021). However, the local populations have declined over the last few decades due to overfishing (Christiensen et al. 2021).

### 2.2.3. Harris mud crab *Rhithropanopeus harrisi*

*R. harrisi* (Gould 1841) is a small brachyuran crab (carapace width < 26 mm) originating from the Atlantic coast of North America (Roche & Torchin 2007; Fowler et al. 2013). It has been introduced to Europe most likely via shipping and was first discovered in 1874 in the Netherlands, from where it has spread to the North Sea and the Baltic Sea (Maitland 1874; Schubert 1936; Wolff 2005). The first observation of the mud crab was made in the Baltic Sea in 1936 (Schubert 1936) and in the Finnish Archipelago Sea in 2009 (Karhilahti 2010). Afterwards, it has spread along the southwestern coast of Finland and currently the range reach the southern Bothnia Sea, which is considered the northernmost population (Fowler et al. 2013; Forsström et al. 2015). At present, the mud crab has formed dense self-sustaining populations in the Archipelago Sea and its range is spreading further to the north (Fowler et al. 2013; Forsström et al. 2015; Riipinen et al. 2017).

In the northern Baltic Sea, the mud crab has been found to colonize almost every habitat type present in the region from the sheltered soft sediments to the wind and wave exposed hard bottoms (Fowler et al. 2013; Riipinen et al. 2017). It is also found in vegetated habitats among dense eelgrass and bladderwrack beds and observed to preferer the latter (Gagnon & Boström 2016; Riipinen et al. 2017). Apparently, the habitat choice depends on the structure and shelter, which are preferred over food (Riipinen et al. 2017). The mud crab is an omnivore with a broad diet, and it feeds on algae, detritus and macroinvertebrates (Hegele-Drywa & Normant 2009; Forsström et al. 2015). However, it has shown to be selective in prey use, as it mostly feed on small and medium sized sessile and mobile prey such as isopods, gammarid amphipods and blue mussels (Forsström et al. 2015; Jormalainen et al. 2016). The mud crab has a distinct hunting mode (i.e., sit-and-pursue) and habitat domain (i.e., associated with macroalgae, rocks and sediment) than the native predatory fish (Hegele-Drywa & Normant 2009).

## 2.3. Field collections

In the autumn of 2020, I collected animals for this study from the Archipelago Sea, South-West Finland (Figure 1). In September, I sampled isopods from four sites near the islands

of Boskär (60°01'57.6"N, 21°46'31.9"E), Fårö (59°55'17.9"N, 21°47'39.4"E), Jurmo (59°49'33.5"N, 21°35'04.3"E) and Rihtniemi peninsula (61°04'05.1"N, 21°18'17.1"E). The sites were located outside the current known distribution range of mud crabs based on the active field research in the region. In addition, I utilized the citizen science provided by the Finnish invasive alien species observation database (<https://vieraslajit.fi/>), which is maintained by the Finnish Advisory Board for Invasive Alien Species. In each sampling site, I searched the isopods in sheltered and shallow (1–2.5 m) littoral zone by collecting bladderwrack (*F. vesiculosus*). I then collected the isopods by shaking them off of the bladderwrack thalli until I had at least 200 individuals from each population. As I did not observe any mud crabs during the extensive sampling, other than one individual in Boskär, I considered the isopod populations mostly naive to the mud crab. I then transported the isopods to the Archipelago Sea Research Institute, University of Turku on Seili island (60°12'N 21°60'E), where I stored them in a cold room (13–15°C) in aerated buckets of approximately 50 individuals in each.

Between September and October, I collected the mud crabs using artificial collectors (N = 27) from the depth of 1–3 m in the vicinity of Seili, where the mud crabs were known to be abundant (Figure 1). I used metal-net collectors (19 x 19 x 15 cm with 1–2 cm<sup>2</sup> ranging mesh), which contained either pieces of clay pots, gardening hoses and rocks, or only autoclaved oyster shells (Figure 2). The collectors have been used successfully to monitor and catch mud crabs in previous studies (see Fowler et al. 2013; Outinen et al. 2019). I brought the collected mud crabs to the cold room and maintained them in two large aerated tanks approximately 100 individuals in each. I enriched the tanks with algae, rocks and pieces of plastic pipes, and fed the mud crabs with aquarium pellets and dried *Cladophora*.

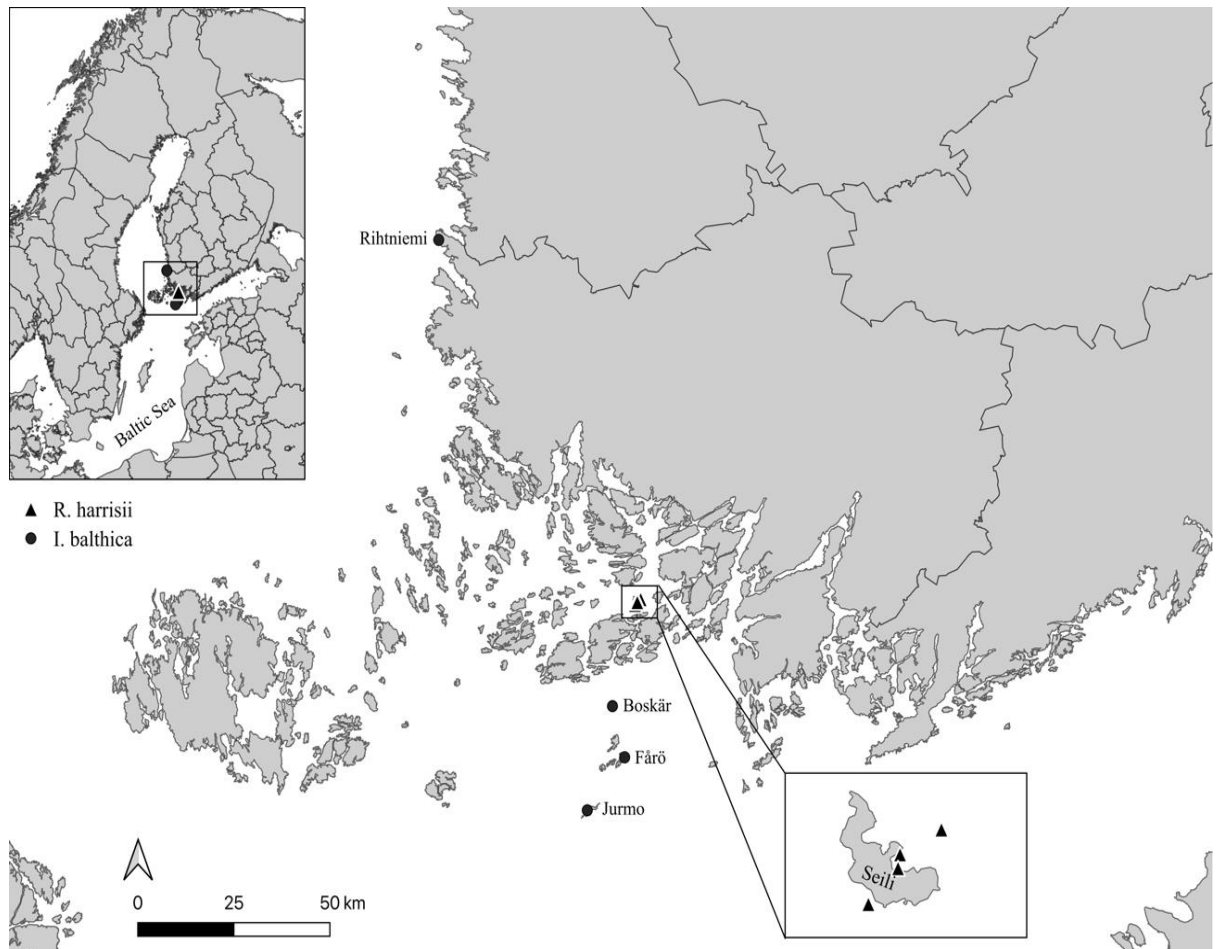


Figure 1. Location of the sampling sites of the isopod populations (black circles) and the mud crabs (black triangles). The perch were caught from several locations near the island of Seili.

In October, I caught perch with seven passive fish traps (50 cm with 19 mm mesh size) from the depth of 2–5 m, and temporarily stored them in a fish chest by a dock. As the catch remained low, I exploited one fishing net (20 mm mesh size) to enhance capture rate. I handled the perch carefully and chose only individuals in good condition to ensure, that they were able to prey on the isopods. I then brought the perch to the laboratory, where I placed them in aeriated tanks ( $N = 2-5$  in each) filled with seawater, algae and rocks. I let the tanks to gradually warm to a room temperature for a day or two and placed the perch to experimental aquaria when the temperature difference between the tanks and the aquaria was not more than two degrees. This was a very important process, as the ectothermic fish are prone to thermal stress, which can increase their rate of oxygen uptake and expose them to diseases and infections. I also treated the perch with water soluble medication against fungal infections (eSHa 2000<sup>®</sup>) prior to the predation experiment. I conducted the predation experiment using a permit granted by the Regional State Administrative Agency for Southern Finland (ESAVI).





Figure 2. The mud crabs were collected using metal net collectors. The collectors provided habitat and shelter to the mud crabs and lured them inside.

#### 2.4. Measuring mortality caused by the predators

To examine the effects of separate and combined predation by the mud crab and the perch on the mortality of the isopods, I conducted a predation experiment in a set of water-circulation aquaria at the Archipelago Sea Research Institute, University of Turku. In the predation experiment, I applied a 2 x 2 factorial design with two factors (crab or fish predation), both with two levels (predator absent or present). It is a standard design to observe simultaneously both the separate and combined predation effects (i.e., enhancement or reduction) on prey mortality in a single experiment (Sih et al. 1998). Specific combinations of factors form four types of treatment groups: (1) the crab predation group, (2) the fish predation group, (3) the combined predation of crab and fish group (hereafter both predation group) and (4) the control group with only isopods present. I used the control group to record the natural mortality of the isopods without the predators.

I conducted the replicates of each treatment group in an individual aquarium rack that consisted of 12 aquaria (35 x 30 x 30 cm, 24 L), four aquaria placed on each shelf and a 300-L water reservoir at the bottom (Figure 3; Appendix 1). In each aquarium rack, I used eight aquaria for the predation experiment and the rest of the aquaria for habituation

of the animals. I filled the aquaria with natural brackish water (6.1–6.2 ppt), which was mechanically and biologically filtered and UV-sterilized. I then adjusted the water temperature to 18°C ( $\pm 1$  °C), and it was maintained with an aquarium chiller. I also placed LED lamps above the aquaria and set a light rhythm for 07:00–20:00 with dawn in the morning and dusk in the evening. To provide a semi-natural habitat to the animals, I furnished the aquaria with three small rocks, three short plastic pipes and three bushes of the bladderwrack (*F. vesiculosus*), which I collected from nearby Seili. Lastly, I installed small plastic nets in the outflow opening of the aquaria to prevent the isopods from escaping.

Prior to the predation experiment, I measured the body length of all prey and predator individuals used in each replicate. First, I measured the length of the isopods from the base of the antenna to the tip of the telson with a millimeter paper (mean  $\pm$  SD; Boskär 17.3  $\pm$  2.7 mm,  $N = 200$ ; Fårö 17.1  $\pm$  2.8 mm,  $N = 200$ ; Jurmo 13.6  $\pm$  2.4 mm,  $N = 200$ ; Rihntiemi 15.6  $\pm$  2.5 mm,  $N = 191$ ). For each treatment group, I divided 200 isopods randomly among eight aquaria, so that I had two replicates from each isopod population within each treatment group. I let the isopods to acclimate overnight without the predators. Thereafter, I measured the carapace width of the mud crabs with a digital caliper and divided them into four size classes with small to medium-sized individuals (mean  $\pm$  SD; I: 11.1  $\pm$  1.4 mm,  $N = 12$ ; II: 14.0  $\pm$  1.0 mm,  $N = 14$ ; III: 15.4  $\pm$  1.1 mm,  $N = 15$ ; IV: 18.3  $\pm$  1.2 mm,  $N = 12$ ). I then took one mud crab from each size class and put them in the habituation aquarium overnight. Lastly, I measured the length of the perch with a ruler (mean  $\pm$  SD; 15.8  $\pm$  1.4 cm,  $N = 32$ ). I placed the perch in empty aquaria for few days and fed them with dried gammarids and fresh mealworms (*Tenebrio molitor*). The perch were starved for 24 hours before the experiment.

I started the predation experiment by adding the predators with the isopods in the same aquaria (except in the control group). For the three predation groups, I used four mud crabs and two perch in the aquarium. I did this to compensate the size difference between the mud crabs and the perch. During the experiment, I recorded the mortality of the isopods every other day by removing the content of the aquaria and carefully counting the remaining individuals. I also replaced any dead mud crab with a similar-sized new individual. I finished a predation replicate when no more than three alive isopods were left in the aquarium. The replicates of the crab predation group and the control group ran 18 days (from 3 to 20 October), while the replicates of the fish predation group 14 days (from

27 October to 4 November) and the replicates of both predation group six days (from 12 to 16 November). After the experiment, I stored the remaining isopods in ethanol, disposed the mud crabs appropriately and released the perch back to the sea.

In addition, I filled the water reservoirs to constant volume with deionized water every other day, as the water evaporated quickly. I also adjusted the salinity, temperature and dissolved oxygen weekly. The water temperature was also measured automatically by Onset's HOBO pendant data loggers, which I placed in eight randomly chosen aquaria.

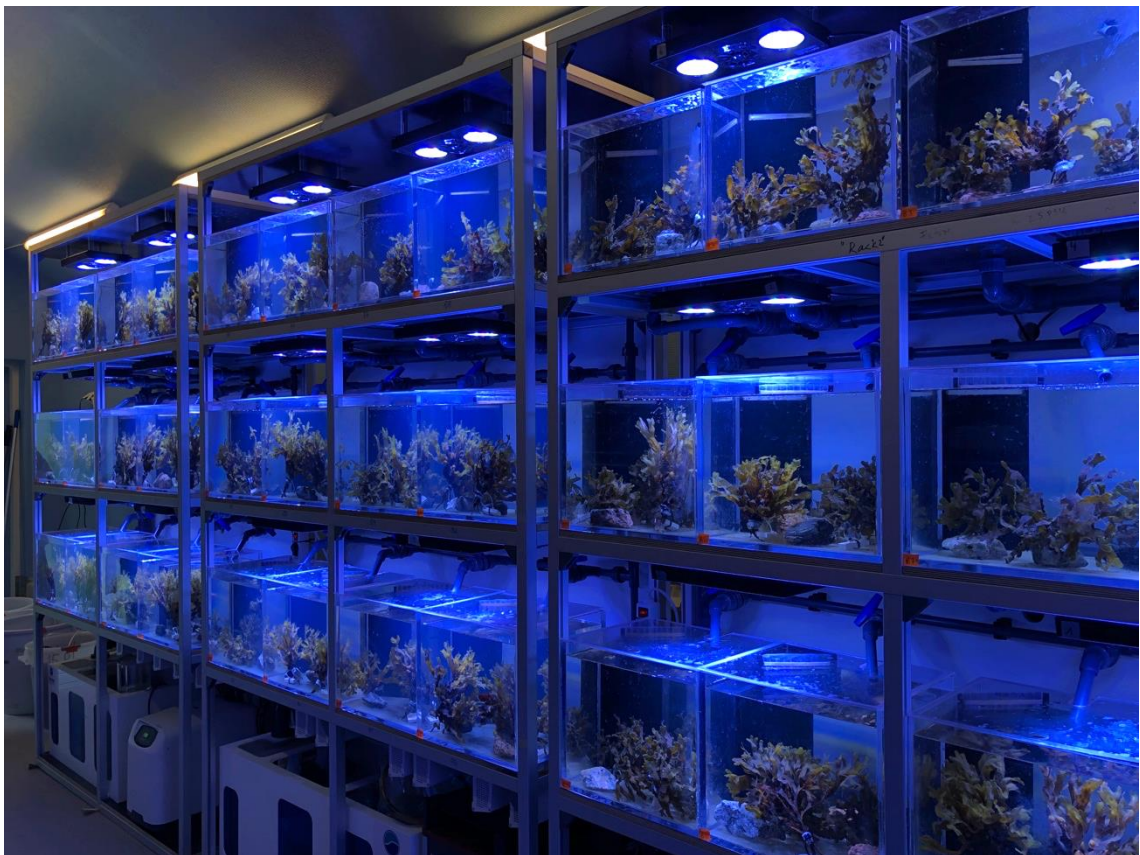


Figure 3. The predation experiment was conducted in a set of water-circulation aquaria at the Archipelago Sea Research Institute, University of Turku. The aquaria were placed on the selves of four aquarium racks and furnished to create a semi-natural habitat to the animals.

## 2.5. Observing behavioural responses to predators

I carried out behavioural observations of prey simultaneously with the predation experiment to observe, whether the presence of the predators would cause a behavioural response in the isopods. I was particularly interested in the anti-predator behaviour of the naive isopods in the presence of the mud crab (Figure 4). As the isopods have several

anti-predator adaptations, I focused only on their level of mobility in the presence of the predators. I observed the isopods for two minutes in each aquarium and counted every mobile isopod irrespective of its location. This was done in the morning (10:00–11:00) before the survival records to avoid disturbing the isopods. I also recorded the mobility once at night (Figure 5), approximately half an hour after the lights went off (at 20:30) using a dim red-light torch to detect whether predation caused variation in the mobility of the nocturnal isopods (Jormalainen & Tuomi 1989; Vesakoski et al. 2008b; Yli-Renko et al. 2015). Within the first few days, I made a total of five observations: (1) an hour before the isopods were exposed to the predators, (2) four hours after adding the predators, (3) at night, (4) two days later and (5) four days later. I focused on the first few days, when most of the predator–prey interactions occur, including the predator recognition by prey, which tend to decrease over time due to predator satiation and prey depletion (Palacios et al. 2018).



Figure 4 & 5. Behavioural observations were conducted to examine the anti-predator behaviour of the isopods in the presence of the predators. In Figure 4, the mud crab is approaching the isopod that is resting on the bladderwrack. One observation was also made at night using a red-light torch (Figure 5).

## 2.6. Statistical analyses

### 2.6.1. Analysis of predation mortality



I conducted statistical analyses using SAS Enterprise Guide 7.1. (SAS version 9.4.). I analysed the data from the predation experiment with the procedure PHREG using the Cox proportional hazard model (Cox 1971) and the Efron approximation method (Allison 2010). The Cox proportional hazard model is a frequently applied survival analysis that incorporates right censored observations (i.e., the isopods that survived until the end of the experiment). The Cox's regression uses a maximum partial likelihood to estimate the effects of covariates (crab predation, fish predation, population) and their two- and three-way interactions on the survival rate (i.e., hazard rate) of the isopods. In addition, I set the aquarium as a random effect, as the survival probability of the isopods within the same aquarium was not independent.

To compare the mortality risk between the predation groups, I computed custom hazard ratios for the covariates by dividing the hazard rate of the predation group by that of the control group. They represent the instantaneous event rate, which means the probability that an individual would experience an event at a particular given point in time after the intervention (Allison 2010). The Cox regression is a semi-parametric test, as it makes no specific assumptions about the nature of the distribution (Cuthill et al. 2006). I confirmed the assumption that hazards were proportional across all treatments with a test on Schoenfeld residuals (Allison 2010). The crab predation covariate did not fully conform with the assumptions, but according to Allison (2010), the Cox's proportional hazards model can be used also with nonproportional hazards. In this case, the interpretation is that the hazard ratio refers to the average risk during the experiment. Thus, I took this into consideration in the final analysis. I then used Akaike's information criteria to choose the best model. Lastly, I made the graphs using Microsoft Excel 2016 -software.

#### 2.6.2. Analysis of behavioural responses

I analysed the behavioural data using generalized linear mixed models (GLMM) with a normal distribution and log link-function. I set the crab predation, the fish predation, time and their two- and three-way interactions as fixed effects in a repeated measures analysis. During the data exploration, I noticed that the mobility of the isopods varied during the course of the experiment being higher in the fish predation group and in both predation group, which were conducted later in the course of the study. Thus, I used the first observation as a reference level, i.e., the analysed mobility during the other four observation times minus mobility during the first observation. In addition, I used the aquarium as a

between-subject factor and time as within-subject factor. I also set population as a random factor. To estimate the covariance between repeated observations, I used an unstructured covariance matrix. It estimated the covariance-variance structure separately between every time-point. In line with the results of previous studies (Jormalainen & Tuomi 1989; Vesakoski et al. 2008b; Yli-Renko et al. 2015), visual inspection of the data suggested a difference in mobility between the day and night. Therefore, I analysed the single night observation and the day observations separately. I then inspected the model residuals visually and using normality tests to confirm that the model met the assumptions of normality and homoscedasticity and chose the best model based on the AIC-value. I also used Tukey's post-hoc test to compare the differences between the predation groups.

### 3. Results

#### 3.1. Results of predation mortality and mortality risk

The presence of crabs or fish in an aquarium resulted in a substantially higher mortality of the isopods, in comparison to the control aquaria where there were no predators (Figure 6). This indicates that the predators consumed the isopods readily. In the presence of predators, survival of the isopods dropped below 60% already on the second day of the experiment.

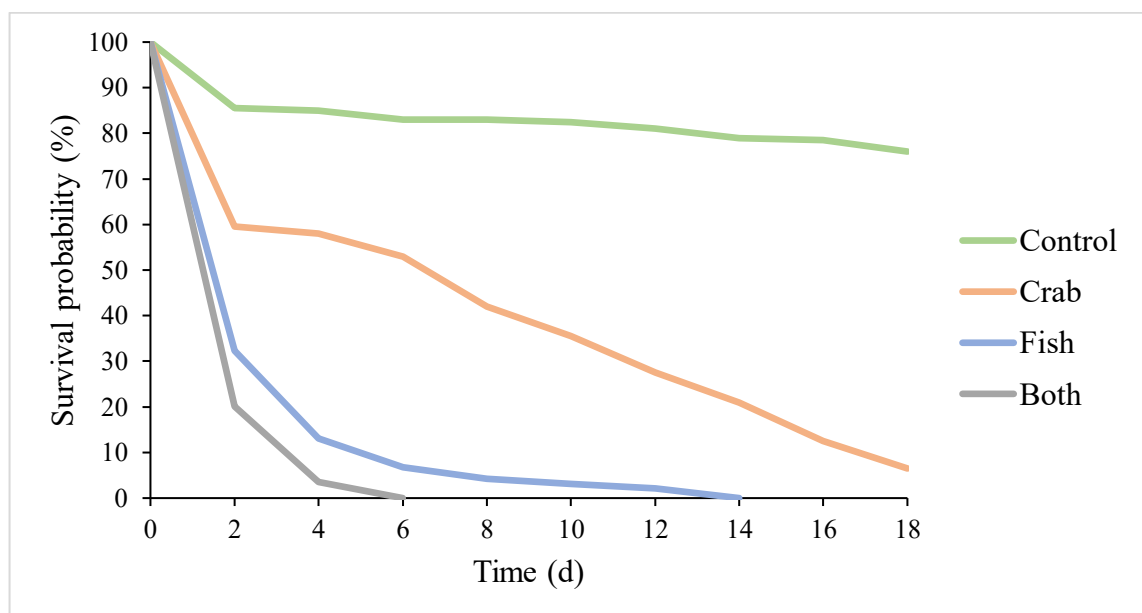


Figure 6. The average survival probability (%) of the isopods in the four experimental groups: Control, Crab, Fish and Both (i.e., crab and fish). The survival of the isopods was observed at two-day intervals for 18 days.

The mud crabs consumed a total of 187 isopods over 18 days, which is, on average, a bit less than six isopods per individual mud crab, or one isopod per mud crab every third day. Therefore, the main effect of crab predation on the mortality of isopods was statistically significant (Cox's regression:  $\chi^2_{(1)} = 96.5$ ,  $p < 0.001$ ; Figure 6), and this effect differed among the isopod populations (crab-by-population -interaction:  $\chi^2_{(3)} = 21.0$ ,  $p < 0.001$ ; Figure 7). The mortality risk due to crabs alone, estimated as the hazard ratio, was relatively small in the population of Boskär in contrast to the other isopod populations. The mortality risk was almost 15-fold in Rihtniemi, 6-fold in Jurmo and 5-fold in Fårö compared to Boskär. Across all the isopod populations, the mortality risk in the crab predation group was 10-fold compared to the control group (average with 95% confidence limits (hereafter CI): 9.9, 5.9 - 16.6).

The fish consumed a total of 192 isopods over 14 days, which is, on average, 12 isopods per individual fish. Thus, the main effect of fish predation on the mortality of isopods was statistically significant ( $\chi^2_{(1)} = 255.6$ ,  $p < 0.001$ ; Figure 6), and this effect did not vary statistically significantly among the isopod populations (fish-by-population -interaction:  $\chi^2_{(3)} = 7.2$ ,  $p = 0.067$ ; Figure 7). Across all the populations the mortality risk in the fish predation group was 30-fold compared to the control group (Average 30.1, CI: 17.5 - 51.7). In addition, the mortality risk was threefold compared to of the risk caused by the mud crabs.

The two predator species together consumed a total of 199 isopods in six days, which is, on average, slightly more than four isopods per individual predator a day. The predators affected the behaviour of each other; when both predator species were present, the combined mortality was lower than the sum of the mortality caused by a single predator species. This was indicated by the significant interactive effect of crab and fish predation (crab-by-fish -interaction:  $\chi^2_{(1)} = 60.4$ ,  $p < 0.001$ ; Figure 6). This interaction effect was also influenced by isopod population (crab-by-fish-by-population -interaction:  $\chi^2_{(3)} = 10.3$ ,  $p = 0.016$ ; Figure 7). The perch caused higher mortality than the mud crabs in the populations of Boskär, Fårö and Rihtniemi, whereas they both caused similar mortality in Jurmo.

In addition, the main effect of population was significant ( $\chi^2_{(3)} = 13.7, p = 0.003$ ). The population of Boskär had a lower mortality risk compared to the other isopod populations.

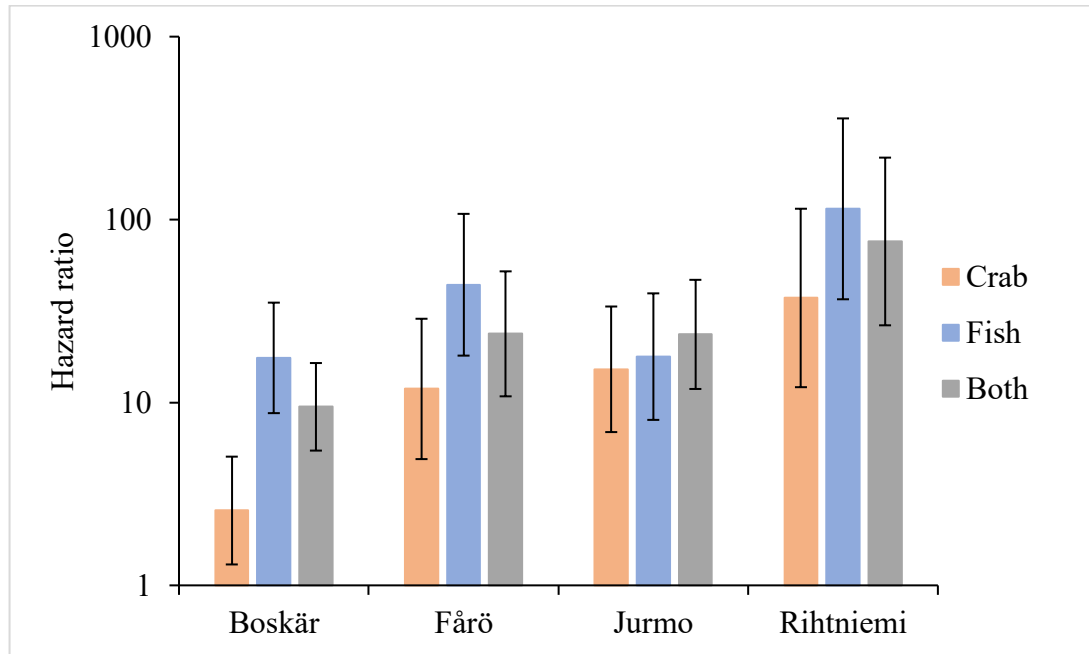


Figure 7. Hazard ratio with 95% confidence intervals, presented on a logarithmic scale, for survival under crab predation (Crab), fish predation (Fish) and crab and fish predation (Both), separately for the four isopod populations. The hazard ratio indicates the ratio of mortality risk between a given predator(s) and the control.

Across the isopod populations, the observed mortality risk posed by the co-occurring predators was only 22-fold (Average 22.1, CI: 13.1 - 37.1) compared to the control group. Thus, it was clearly lower than would have been expected based on the sum of the hazard ratios ( $9.9_{\text{Crab}} + 30.1_{\text{Fish}} = 40.0$ ) when the predators occurred separately. This also applies to every isopod population (Figure 7), indicating that there was an antagonistic interaction between the two predator species. To conclude, the combined effect of crab and fish predation was non-additive and resulted in reduction in predation mortality, as the predators together consumed less isopods than would have been expected based on the consumption by each predator species separately.

### 3.2. Results of anti-predator behaviour

In general, most of the isopods remained immobile on the bladderwrack during the behavioural observations. In the presence of fish, however, the daytime mobility of the isopods was modified by time from the start of the experiment (fish-by-time -interaction:  $F_2$ ,



$_{81} = 4.12, p = 0.020$ ; Figure 8). Tukey's post-hoc test revealed that the presence of the fish did not influence mobility four hours after the isopods were exposed to the fish predation ( $p = 0.464$ ) or two days later ( $p = 0.413$ ), but four days later the isopods were significantly less mobile when the fish were present than when they were absent ( $p = 0.006$ ) (Figure 8). The main effect of the presence of the fish was not significant (the main effect of fish:  $F_{1, 81} = 2.14, p = 0.147$ ; Figure 9).

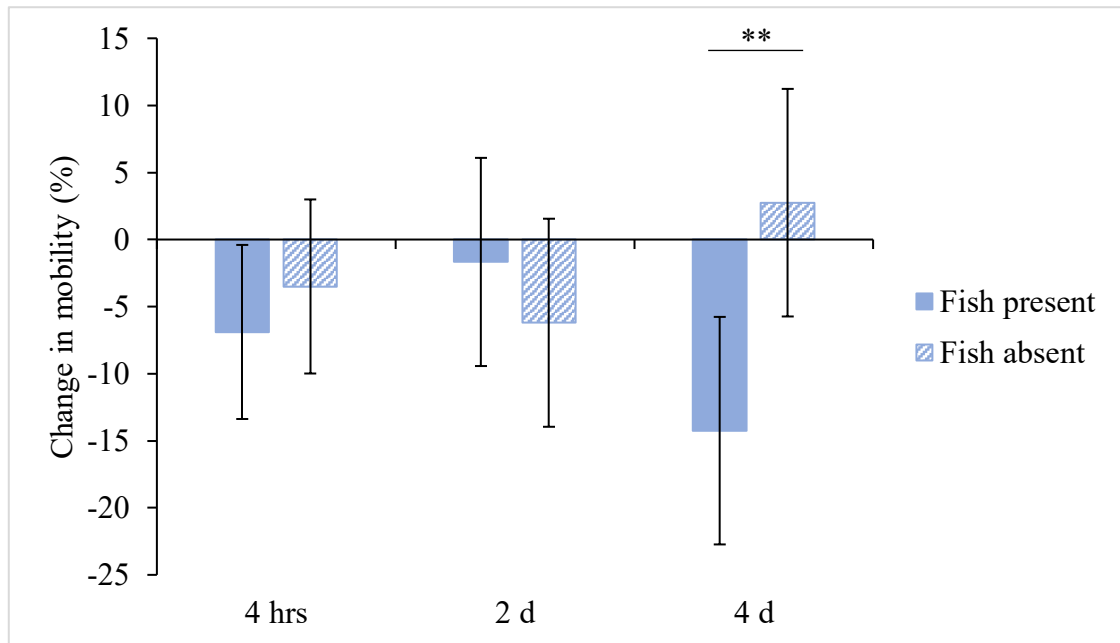


Figure 8. Change in the mobility of the isopods between the daytime observations and the initial observation before the predators were introduced, in the presence and the absence of fish with 95% confidence intervals. The mobility of the isopods was measured as the proportion of mobile isopods of all the isopods. The asterisks indicate a statistically significant difference (\*\* =  $p \leq 0.01$ ).

The interactive effect of crab and fish on the mobility of the isopods was a marginally non-significant but biologically interesting (fish-by-crab -interaction:  $F_{1, 81} = 3.06, p = 0.084$ ; Figure 9) and temporally consistent (fish-by-crab-by-time -interaction:  $F_{2, 81} = 0.43, p = 0.649$ ). This interaction suggests that the effects of crab and fish may depended on each other, as the isopods were marginally less mobile in the presence than the absence of both predators. Tukey's post-hoc test indicated that the isopods were significantly less mobile in the presence of both predators compared to control group ( $p = 0.024$ ), fish predation group ( $p = 0.014$ ) and to crab predation group ( $p = 0.026$ ) (Figure 9).

The effect of the presence of the crab on the mobility of the isopods was marginally non-significant (the main effect of crab:  $F_{1, 81} = 3.23, p = 0.076$ ; Figure 9) and this effect did

not interact with time (crab-by-time -interaction:  $F_{2, 81} = 1.21, p = 0.304$ ). Across the treatments, the influence of time was non-significant on the mobility (the main effect of time:  $F_{2, 81} = 0.12, p = 0.891$ ).

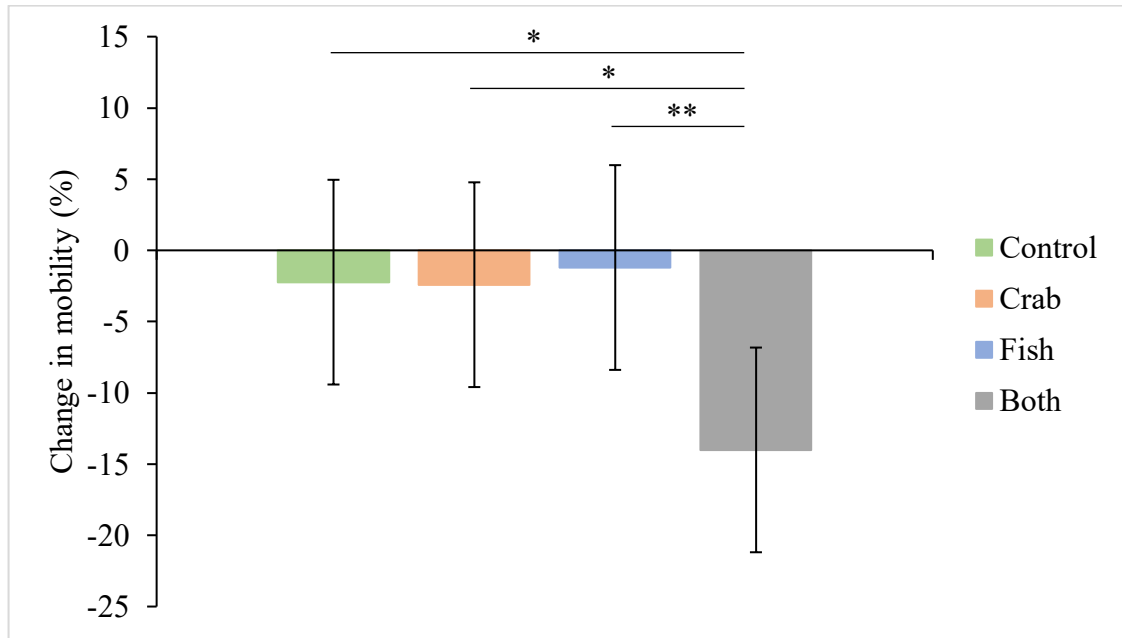


Figure 9. Change in the mobility of the isopods between the daytime observations and the initial observation before the predators were introduced, presented by the experimental groups with 95% confidence intervals. The mobility of the isopods was measured as the proportion of mobile isopods of all the isopods. The asterisks indicate a statistically significant difference (\*\* =  $p \leq 0.01$ , \* =  $p \leq 0.05$ ).

At night, the isopods were less mobile in the presence than in the absence of fish (the main effect of fish:  $F_{1,25} = 6.01, p = 0.022$ ; Figure 10). However, the presence of crab did not affect the mobility of the isopods at night (the main effect of crab:  $F_{1,25} = 0.95, p = 0.340$ ; Figure 10), and the effects of crab and fish did not depend on each other (crab-by-fish -interaction:  $F_{1,25} = 0.09, p = 0.763$ ).

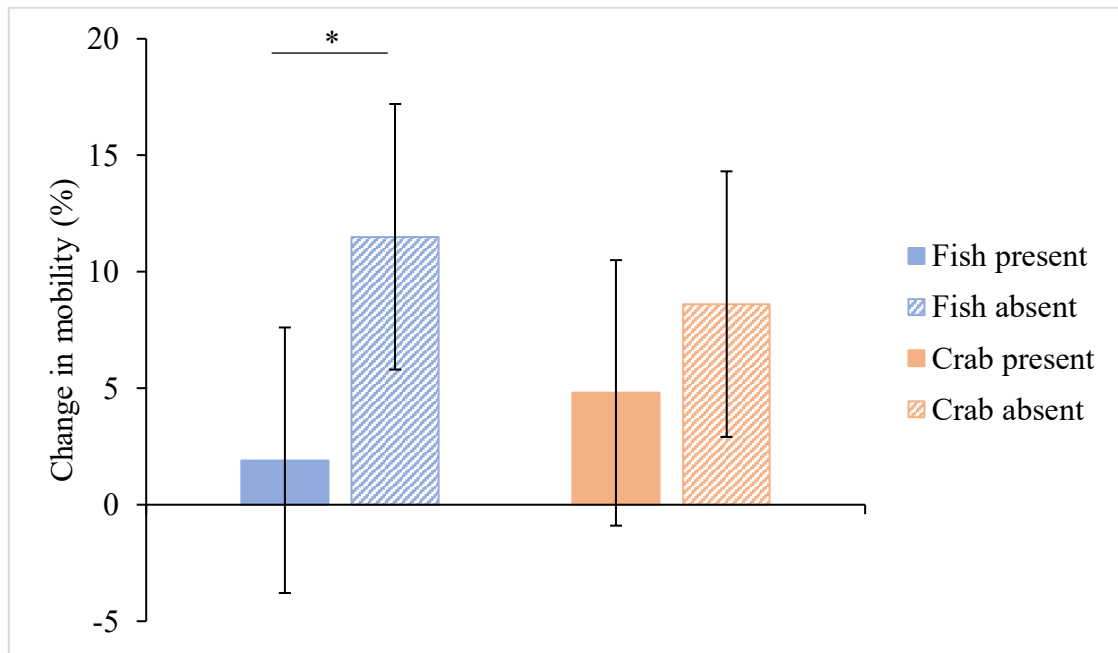


Figure 10. Change in the mobility of the isopods between the night observation and the initial observation before the predators were introduced, in the presence and the absence of fish or crab with 95% confidence intervals. The mobility of the isopods is measured as the proportion of mobile isopods of all the isopods. The asterisks indicate a statistically significant difference ( $* = p \leq 0.05$ ).

## 4. Discussion

### 4.1. Comparing separate effects of non-native and native predator

In line with my first hypothesis, the present predation experiment shows that the predators, the mud crab and the perch, separately cause significant mortality on the isopods. They consumed the isopods readily, as the isopod mortality and consequently, mortality risk was substantially higher in the predation treatment groups than in the control group. This is in accordance with previous studies that have found that the perch is an effective predator of the isopods both in laboratory conditions (Jormalainen et al. 1995; Merilaita 2001) and in the nature (Lappalainen et al. 2001). Previous studies have also suggested that the mud crab could be a potent predator of the isopods (Forsström et al. 2015; Jormalainen et al. 2016). Although ecological conclusions should be done with special caution when they are based on laboratory trials, the recorded predation mortality under the current experiment would probably translate into significant predation pressure on the isopods in the rocky littoral communities of the northern Baltic Sea.

Above all, the experiment indicates that the mud crab is a relevant predator of the isopods. The mud crab is a successful global invader, due to its highly plastic and opportunistic life-history traits (Roche & Torchin et al. 2007; Hänfling et al. 2011; Liversage et al. 2021). Thus, it has likely encountered similar prey species, which perhaps explains its adaptability in the new community. In addition, Forsström and her colleagues (2015) found that the mud crab feeds readily on *I. balthica* and shows a clear preference for this particular species in the laboratory conditions. The authors recorded that their diet consisted up to 90% of the isopods over a 12-hour period, when the mud crabs were provided the isopods simultaneously with the blue mussels (*M. trossulus*) and gammarid amphipods. The mud crabs consumed more isopods than the other prey species, although their prey choice is generally influenced by prey availability. This same trend has also been observed in the field (Jormalainen et al. 2016). Previously the mud crab was considered an ineffective predator of mobile prey (Hegele-Drywa & Normant 2009), but more recent studies have shown that it can readily catch mobile prey (Forsström et al. 2015; Jormalainen et al. 2016). During my study, I evidenced several times mud crabs catching and feeding on isopods, supporting these previous findings. Thus, the mud crab may be a more effective predator than previously thought, and its selective predation may potentially influence the structure and abundance of local prey populations – particularly isopod populations.

Interestingly, the predation mortality caused by the mud crab varied between the isopod populations, in contrast to that of the perch. The population of Boskär had a lower mortality risk than the other isopod populations, which may be due to the invasion status of that area. The population is located closer to the currently known distribution range of the mud crabs than the other populations, and I found one mud crab from Boskär. Before this, no mud crabs have been found from there, as far as I am aware. Therefore, I believe that the invasion of the mud crab is relatively recent and probably not older than a year or two. As the isopods do not have overlapping generations (Jormalainen & Tuomi 1989), this suggests that about one or two generations of the isopods may have been exposed to the mud crabs. Thus, the isopods from Boskär may have adapted their behaviour, reducing their mortality by the novel predator. However, the mortality risk caused by the perch did not vary between the isopod populations, which could be a sign of the long coevolutionary history between the species. In addition, the perch caused a higher mortality risk than the mud crab, except in the population of Jurmo. According to my measurements, the isopods

from Jurmo had a bit smaller body size than the isopods from the other populations, and perhaps they were more attractive prey for the mud crabs than for the perch.

In accordance with my third hypothesis, I observed that the mud crab did not induce as strong behavioural response in the isopods as did the presence of the perch. At night, the isopods clearly reduced their mobility only in response to the perch. As the isopods are nocturnal, reduction in mobility suggests anti-predator behaviour (Jormalainen & Tuomi 1989; Vesakoski et al. 2008b; Yli-Renko et al. 2015). Interestingly, I evidenced that the isopods did not avoid the mud crabs in the aquaria. During the day, the mud crab did not cause a behavioural response and the perch only on the fourth day, when the mobility of the isopods decreased significantly. This reflect a change in behaviour, but it may also be due to a selective predation by two different predators. In other words, because the perch may recognise and catch easier mobile than immobile prey, the proportion of immobile prey have increased in the course of the experiment, resulting in decreased mobility on the fourth day. The mud crab, on the other hand, may select slower or immobile individuals that are easier to catch, meaning that mobility would remain relatively high during the experiment, which is in accordance with my results.

The mud crab did not cause a reduction in the mobility of the isopods at night, but during the day the decrease in mobility due to its presence was close to statistical significance. However, due to the unpredictably fast predation rate, the behavioural data set was quite small. Thus, I cannot completely rule out the possibility that there may have been some change in the mobility of the isopods when the mud crabs were present. Carthey and Banks (2014) suggested that prey that have prior experience of predators and can respond to general cues, are likely to learn to recognise a novel predator over time. Thus, it may be possible that during the experiment, some of the isopods became more aware of the mud crabs, tried to avoid them, and slightly reduced their mobility in response. Nevertheless, current observations show an indication that the isopods may be naive to the recently introduced mud crab due to the lack of coevolutionary history, as predicted by the prey naivete hypothesis (Cox & Lima 2006). In general, the isopods may be at the first level of prey naivete, at which they might lack predator recognition (Banks & Dickman 2007). However, it raises the question of whether the isopods from Boskär are already at the second level of prey naivete, at which they may have learned to recognise the mud crab as a threat but still might lack appropriate anti-predator adaptations against it (Banks & Dickman 2007).

In the species-poor communities of the northern Baltic Sea, the mud crab is a functionally novel predator archetype (Jormalainen et al. 2016; Forsström et al. 2018; Lokko et al. 2018). Phylogenetically different predators tend to emit dissimilar chemical cues (Sih et al. 2010; Carthey et al. 2017). For instance, the mud crab has an omnivorous diet consisting also of detritus and algal matter, and thus it may emit chemical cues that the isopods might not recognise (Papacostas & Freestone 2019). The mud crab is also a ‘sit-and-pursue’ predator that remains at a fixed feeding place and rushes at on the prey when it is close enough (Schmitz 2007). These relatively stationary predators often emit persistent point-source cues, whereas actively searching predators emit diffused cues throughout the microhabitat (Schmitz 2007). On the other hand, Alcock (2009) suggested that the isopods might rely on detecting pressure waves from approaching predators rather than chemical cues. In the present experiment, I evidenced several times mud crabs approaching isopods slowly and then suddenly caught the isopod. This hunting strategy may be efficient, as it may not produce large pressure waves that might otherwise allow the isopods to recognise the mud crabs. As the main predators of the isopods are fish, perhaps the isopods associate predation risk only with large-bodied and fast-moving fish that have large forward-facing eyes (Rehage et al. 2009; Carthey & Blumstein 2018). Jormalainen and his colleagues (2016) also found in the field that the native herbivores, including the isopods, did not avoid the algae that was inhabited by the mud crabs, supporting these findings.

The isopods have several anti-predator adaptations towards their natural predators (Jormalainen & Tuomi 1989; Merilaita & Jormalainen 2000; Vesakoski et al. 2008b), but these adaptations may be somewhat inappropriate against the functionally novel predator. The mud crab has a well-developed chemoreceptor sense, which it uses to detect prey (Kiwada et al. 2004). Thus, it may be able to detect the nocturnal isopods also at night, which I did observe several times in the aquaria. In conclusion, it appears likely that the mud crab benefits from the naivete of the isopods, increasing the predation pressure on them.

#### 4.2. Examining combined effect of the two different predators

I hypothesised that when the mud crab and the perch co-occur, they might facilitate each other's predation success and cause increase of mortality of the isopods. However, my results showed that the predators together caused half of the predation mortality compared to their summed separate effects. Apparently, the predators may have modified each other foraging behaviour, which resulted in reduction in the mortality of the isopods and in the overall predation pressure. These results are in accordance with previous studies that have found that functionally different predators do not necessarily cause enhancement of predation mortality, due to passive interference among predators and alternative prey behaviours (Vance-Chalcraft & Soluk 2005; Nurkse et al. 2018; Leighton & Tyler 2021). In his meta-analysis, Schmitz (2007) found that risk reduction and risk enhancement were equally commonly observed emergent effects in laboratory trials after the additive effects, but in the field, however, risk reduction was observed more frequently (71%) than risk enhancement (7%) or additive effects (22%). In addition, I found that the mortality risk was smaller in the population of Boskär than in the other isopod populations. This may suggest that the isopods from Boskär might have adapted to a higher predation pressure in the nature, and consequently they were better at avoiding the predators than the other isopods.

The behavioural observations showed that simultaneous presence of the mud crab and the perch caused a significant decrease in the mobility of the isopods only during the day. As predator density and diversity was greater, it may have influenced the behaviour of the isopods. Previous studies have proposed that some prey species can sense predatory cues in a concentration-dependent manner that allows them to assess predator proximity and size either as a combined biomass or number of individuals (Smee & Weissburg 2006; Papacostas & Freestone 2019). Thus, I cannot rule out the possibility that the isopods in this experiment may have responded to predator density rather than to predator identity, and their response was somewhat stronger during the day than at night. Perhaps, the predators moved more during the day, which made the isopods remain immobile. Moreover, prey that use general anti-predator responses such as reduced mobility and cryptic colouration, are likely to increase their anti-predator behaviour in the presence of multiple predators (Matsuda et al. 1993; Sih et al. 1998; Vance-Chalcraft & Soluk 2005). This is in accordance with my behavioural results. The mud crab and the perch may have had a synergistic effect on the anti-predator response of the isopods, which in turn could explain their antagonistic effect on the mortality of the isopods. Thus, alteration in the behaviour

of the isopods may have made both predator species less effective in the presence of the other predator.

Previous studies have found that risk reduction is often associated with antagonistic interactions among the predators (Vance-Chalcraft & Soluk 2005; Nurkse et al. 2018; Palacios et al. 2018; Livernois et al. 2019; Leighton & Tyler 2021). Indeed, Nurkse and others (2018) found that the simultaneous presence of the mud crab and the round goby (*N. melanostomus*) caused reduction on predation rates, and the authors suggested that interference competition may have influenced it. They also demonstrated that the round goby reduced the predation rate of the mud crab on their shared prey, although they did not observe intraguild predation or aggressive behaviour among the predators. However, it may be also possible that the confined space of an aquarium in my experiment might have influenced the encounter rate and passive interference between the predators, as they have more distinct habitat domains in the nature. Thus, the results should be treated with some caution. Nevertheless, reduction in prey mortality may be more common in the nature than enhancement, as generalist predators can shift from one prey type to another, releasing predation pressure on particular prey species (Palacios et al. 2018).

In this experiment, the predators may have competed through reciprocal effects on prey availability by affecting prey mobility and microhabitat use (Matsuda et al. 1993; Vance-Chalcraft & Soluk 2005; Leighton & Tyler 2021). In the presence of the perch, the isopods may have decreased mobility and chosen a microhabitat that provides protection from the perch, which might have resulted in reduction in the mud crab predation, and vice versa. Leighton and Tyler (2021) also found that two predator species had a different effect on the microhabitat choice of the mussel (*M. trossulus*), which resulted in risk reduction, as the mussels moved to locations that were more difficult for one predator to access. In addition, Sih and others (1998) proposed that stationary predators may disrupt the predation of actively searching individuals. The mud crab, for instance, may have avoided the perch, causing more pressure waves, which may have increased the vigilance of the isopods. The perch in turn, may have decreased the mobility of the isopods in the water column or in other substrates than the bladderwrack, which may have made the isopods less accessible to the mud crab.

The fear of intraguild predation may have also reduced the combined effect of predators on prey and caused risk reduction (Vance-Chalcraft & Soluk 2005; Grabowski et al. 2008;



Sitvarin & Rypstra 2014). Puntilla-Dodd and others (2019) reported that in the Baltic Sea the mud crab is consumed by several fish species, including the perch. Although, the perch in my experiment did not prey on the mud crabs, perhaps due to a gape-size limitation or availability of other prey, the risk of predation may have influenced the behaviour of the mud crab. Interestingly, Grabowski and others (2008) found that the top predator, oyster toadfish (*Opsanus tau*), suppressed the foraging activity of the intermediate predator, the mud crab, by indirectly modifying its behaviour. The authors observed that the mud crab was seeking refuge deeper within the shell matrix of oysters and moved less frequently when the toadfish was present. I also consider that the mud crabs in my experiment may have recognised the perch as a threat and decreased their foraging during the day. Thus, it would have been interesting to study the behaviour of the predators, for instance, by recording the location of the mud crabs to assess, whether they were seeking shelter or lurking on the prey in the presence of the perch.

All in all, I consider that the observed reduction in the mortality of the isopods in this multiple predator system may have been driven by trait-mediated indirect interactions, particularly behavioural modifications, among predators and prey. Trait-mediated indirect interactions mean that interactions among individuals affect traits such as behaviour, rather than mortality (Golubski & Abrams 2011). Trait-mediated indirect interactions have commonly been observed in multiple predator environments (Vance-Chalcraft & Soluk 2005; Leighton & Tyler 2021). Therefore, the presence of the perch is likely to have a major influence on the predation by the mud crab, as their combined presence results in reduction in predation mortality. Thus, my results may show an indication that the mud crab has already integrated into the native food web of the Baltic Sea, as the native predators have learned to prey on them, causing both direct and indirect effects on the mortality and behaviour of the mud crabs.

#### 4.3. Concluding remarks and future perspectives

In this thesis, I evidenced that both the separate and combined predation by the mud crab and the perch caused significant mortality on the isopods. The mud crab may have benefitted from the naivete of the isopods, as the presence of the novel predator did not cause as strong behavioural response as did the presence of the native predator. Previous studies have only speculated on the possible effect of prey naivete in the native isopods

(Forsström et al. 2015; Jormalainen et al. 2016), but this study is the first to present evidence for it. In addition, I found that the mud crab and the perch may have affected the behaviour of each other; when both predators were present, the combined mortality was lower than the sum of the mortality caused by a single predator species. The predators consumed less isopods together than separately, resulting in reduction on prey mortality. During the day, the isopods were immobile and relied on cryptic colouration, which made both predators less effective in the presence of the other predator. The predators may also have disturbed each other via antagonistic interactions. I thus consider that the risk reduction in this multiple predator system was primarily caused by trait-mediated indirect interactions either between the predators and the prey, between the predators only, or possibly these both.

My results provide valuable insights into the research of the impacts of introduced predators and the key role of prey naivete within the introductions. I found an interesting indication that the isopod population in Boskär, that may have had prior experience with the mud crab, might have recognised the mud crab as a threat. Recent research on introduced species has focused increasingly on the cognitive capabilities of prey such as learned predator recognition (Steindler et al. 2020). The prey may learn to recognise the novel predator over time with a gradual exposure to the predator. For future reference, it would be interesting to compare the behavioural responses of the naive isopods to the responses of experienced isopods. However, this is only possible if the mud crab predation does not eradicate the local isopod populations too fast. The isopods have entered the ecosystem of the Baltic Sea after the last glaciation period from the surrounding seas, where other crab species occur (Leppäkoski et al. 2002). As the mud crab is currently the only brachyuran crab in the Baltic Sea, relaxed selection might have reduced the anti-predator behaviour of the isopods in the past (Lahti et al. 2009). Perhaps, if the isopods were gradually exposed to the mud crab, they might learn or evolve to recognise it as a threat.

This thesis emphasises the importance of studying the emergent effects of multiple predators on prey, as their synergistic or antagonistic interactions can cause unexpected effects in the native community. I found it interesting, that the presence of the mud crab and the perch caused reduction in the mortality of the isopods. This suggest that the perch has a very important top-down effect in the ecosystem of the Baltic Sea. The perch and other fish species can control the population growth of the mud crabs by consuming them and

therefore generate biotic resistance. Predation and interspecific competition among the fish and the mud crabs may explain the observed boom-and-bust pattern in the population growth of the mud crab (Forsström et al. 2018). By regulating the population size of the mud crab, the native fish may attenuate the ecological impact of the mud crab in the native community. In addition, the risk reduction has important community and ecosystem-level implications. As the risk reduction results in lower prey mortality, prey populations may sustain higher population growth than would be predicted by independent predator effects (Vance-Chalcraft & Soluk 2005; Palacios et al. 2018). In the Baltic Sea ecosystem, herbivores have a key role, as they buffer the effects of eutrophication by grazing the epiphytic algae on the macrophytes (Jormalainen et al. 2016; Östman et al. 2016). As the Baltic Sea is threatened by eutrophication, pollution and other anthropogenic disturbances, the importance of herbivory may be greater than ever before.

To understand the actual ecological impacts of introduced predators in the native communities, we need more research that considers the multiple trophic levels involved in predator-prey dynamics and the key role of prey naivete in predator introductions. The predatory effects may cascade throughout the food web and cause alternations in the structure and function of ecosystems. The species do not interact in isolation, as the ecological communities are built on dynamic networks of trophic interactions.

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## Appendices

Appendix I. Attached is the detailed presentation of the predation experiment set-up. All the predation treatment groups and the control group were conducted in separate aquarium racks. Every aquarium rack consisted of twelve aquaria. In each aquarium, the species and their numbers are presented. All the isopod populations Boskär, Fårö, Jurmo and Rihtniemi are bolded. In addition, the perch were habituated in the aquarium rack B after the crab predation group was conducted.









Aquarium rack A: Control group

<b>Fårö</b> <i>I. balthica</i> N = 25 	<b>Fårö</b> <i>I. balthica</i> N = 25 	<b>Jurmo</b> <i>I. balthica</i> N = 25 	<b>Boskär</b> <i>I. balthica</i> N = 25 
<b>Boskär</b> <i>I. balthica</i> N = 25 	<b>Rihtniemi</b> <i>I. balthica</i> N = 25 	<b>Jurmo</b> <i>I. balthica</i> N = 25 	
		<b>Rihtniemi</b> <i>I. balthica</i> N = 25 	








Aquarium rack B: Crab predation group

Crab habituation	Crab habituation	Crab habituation	Crab habituation
<b>Jurmo</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 	<b>Jurmo</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 	<b>Fårö</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 	<b>Rihtniemi</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 
<b>Boskär</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 	<b>Rihtniemi</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 	<b>Fårö</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 	<b>Boskär</b> <i>I. balthica</i> N = 25 <i>R. harrisii</i> N = 4 

Aquarium rack C: Fish predation group

<p><b>Fårö</b>  <i>I. balthica</i> N = 25  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Boskär</b>  <i>I. balthica</i> N = 25  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Jurmo</b>  <i>I. balthica</i> N = 25  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Jurmo</b>  <i>I. balthica</i> N = 25  <i>P. fluviatilis</i> N = 2</p> 
Fish habituation	Fish habituation	Fish habituation	Fish habituation
<p><b>Boskär</b>  <i>I. balthica</i> N = 25  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Rihtniemi</b>  <i>I. balthica</i> N = 21  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Fårö</b>  <i>I. balthica</i> N = 25  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Rihtniemi</b>  <i>I. balthica</i> N = 21  <i>P. fluviatilis</i> N = 2</p> 

Aquarium rack D: Both predation group

Crab habituation	Crab habituation	Crab habituation	Crab habituation
<p><b>Rihtniemi</b>  <i>I. balthica</i> N = 24  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Boskär</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Jurmo</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Fårö</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 
<p><b>Fårö</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Rihtniemi</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Jurmo</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 	<p><b>Boskär</b>  <i>I. balthica</i> N = 25  <i>R. harrisii</i> N = 4  <i>P. fluviatilis</i> N = 2</p> 