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Eco-Evolutionary Dynamics in the Baltic Sea

Distribution and impact of threespine stickleback
phenotypes in a changing environment

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 groningen

Eco-Evolutionary Dynamics in the Baltic Sea

Distribution and impact of threespine stickleback
phenotypes in a changing environment

PhD thesis

to obtain the degree of PhD at the
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on the authority of the
Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

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CHAPTER

01

Introduction

Chapter 1

Across the globe, overfishing, eutrophication, and climate change are rapidly modifying marine ecosystems (Pauly et al. 1998; Worm and Paine 2016). Because many ecosystems provide economic and ecological services to humans, management aims to restore and maintain ecosystem functions which benefit humans (Clewett and Aronson 2006). However, the path to this goal is rarely, if ever, straightforward. Ecosystems' structural ambiguity complicates restoration and maintenance of specific functions. In some cases, multiple species can exhibit functional redundancy (fill the same functional role). In an ecosystem with functional redundancy if one species is removed, other species from the same functional group remain. The persistence of those species means that the functional role is not lost and the effects of the loss of one species do not cascade to other trophic levels in the ecosystem and can go unnoticed (Lawton and Brown 1994). Functional redundancy can influence an ecosystem's adaptive capacity. The adaptive capacity of an ecosystem is its ability to adjust to new environmental conditions with minimal loss of function (Lindner et al. 2010). Greater functional redundancy leads to greater adaptive capacity and less chance that an ecosystem will experience large-scale negative effects of resource exploitation and climate change.

As human activity continues to modify ecosystems more rapidly and intensely, we need to understand the adaptive capacity of our ecosystems. Thus, we need to quantify if there are species available that can adapt to fill the same functional roles as those species that are eliminated (Steffen et al. 2015; Steffen et al 2018). This can be measured, in part, by the adaptive responses of the species utilizing the ecosystem. Inherent adaptive capacity of individual species is the evolutionary ability of a species to adjust to new environmental conditions and is important in predicting a species' response to change (Lindner et al. 2010). Change within the same ecosystem impacts species differently because they interact with the environment differently and can adapt to exploit various resources or tolerate disparate environmental conditions. In some populations, there is not a quick enough adaptive response to deal with new environmental conditions or changes to biological community structure and the population becomes extirpated. Others can meet the demands of an ever-changing ecosystem through adaptation. In some cases, only part of the population will adapt to exploiting a new or empty niche. Adaptation of a subset of a population may arise through standing genetic variation. A rare allele may be beneficial to part of the population and become fixed within that group while the other part of the population retains the ancestral polymorphism. Thus, within a single species, there may be multiple populations which fill different functional roles in an ecosystem (Luck et al. 2003).

Now that it has become widely accepted that evolution can occur on ecological timescales, it is believed that some populations that live in a habitat constantly subjected to environmental modifications undergo ongoing evolution (Meyer 1993; Carroll et al. 2007). This can further complicate our understanding of ecosystem dynamics and function as more changes to the ecosystem can actually result from a species' adaptations if it modifies their functional role and impact on resources. This is the basis of eco-evolutionary theory; ecological and evolutionary processes acting simultaneously and influencing one another within a system (Abrams 2000; Fussman et al. 2007). Understanding eco-evolutionary dynamics can provide crucial insight to how ecosystems may respond to future change, be it further degradation or restoration, through species adaptations and ecosystem modification. Knowledge gained from studying these dynamics can then inform management decisions in ecosystems undergoing constant change. This thesis was designed to uncover the eco-evolutionary dynamics of coastal Baltic Sea threespine stickleback (*Gasterosteus aculeatus*), whose population has expanded dramatically over the past two decades, by studying resource adaptation of different morphological phenotypes and how this possible adaptation influences the ecosystem.

Study Area and Species

The Baltic Sea is one of the world's largest brackish water bodies with a surface area of 420,000 km². It is semi-enclosed by nine countries and connected to the North Sea through the Danish straits. The Danish straits are the only source of marine water exchange while many rivers and streams bring freshwater into the Baltic Sea (Figure 1). Freshwater and marine species which can tolerate brackish water inhabit the Baltic Sea. Brackish water imposes physiological stress on freshwater and marine species resulting in relatively low biodiversity. In recent decades eutrophication, fisheries, and habitat destruction have further reduced biodiversity disturbing the complex interaction network that supplies ecological resources (ICES 2008; HELCOM 2009b; HELCOM 2010). High nutrient loadings between the 1950s and 1980s caused eutrophication (Larsson et al. 1985; Bonsdorf et al. 1997; Gustafsson et al. 2012; Andersen et al. 2017). Despite reductions in discharges, losses, and emissions, and therefore overall loads since the late 1980s, much of the Baltic Sea remains eutrophic (HELCOM 2010; Gustafsson et al. 2012; Carstensen et al. 2014; HELCOM 2014; Andersen et al. 2015). This is thought to be due to the accumulation of nutrients over previous decades and the release of nutrients retained in the sediments because of hypoxic conditions in combination with the slow turnover of water in the Baltic Sea (Mort et al. 2010; Gustafsson et al. 2012; HELCOM 2014). Eutrophication often increases turbidity and sedimentation and benefits phytoplankton and bloom-forming

algae over perennial macrophytes such as bladderwrack (*Fucus vesiculosus* L.) which are important habitat forming algae (Dahlgren and Kautsky 2004; Vos et al. 2004; Heck and Valentine 2007; Casini et al. 2008; Korpinen 2008; Eriksson et al. 2009; HELCOM 2018). The phytoplankton and bloom-forming algae was previously controlled by zooplankton and benthic invertebrates but the invertebrate community faces pressure from predation by mesopredatory fish which have increased in abundance following the decline of piscivorous fish, marine mammals, and birds and can no longer regulate algal growth (Korpinen and Jormalainen 2008; HELCOM 2010). Offshore populations of cod (*Gadus morhua*), as well as nearshore populations of pike (*Esox Lucius*) and perch (*Perca fluviatilis*) have declined in the past few decades (Nilsson et al. 2004; ICES 2009; Lehtonen et al. 2009; Ljunggren et al. 2010). Cod stocks declined due to overfishing in combination with poor recruitment caused by increased water temperature and a period of limited inflow of saline water from the North Sea (ICES 2009). The depletion of cod altered offshore food webs by releasing one of their main prey species, the mesopredatory fish sprat (*Sprattus sprattus*), from predation (Casini et al. 2006; Eriksson et al. 2011). Sprat feed on plankton which is a main food source for larval pike and perch. Increased competition for plankton contributed to recruitment failures of perch and pike over multiple years (Casini et al. 2008; Mollmann et al. 2008; Ljunggren et al. 2010). Subsequent recruitment failure of sprat freed up plankton resources for another small-bodied mesopredatory fish, the threespine stickleback (hereafter referred to as, “stickleback”) which dominates many near- and offshore fish communities today (Ljunggren et al. 2010; Eriksson et al. 2011).

Figure 1. Map of the Baltic Sea showing currents in bold black arrows (shape files and data from Seifert et al. 2001; JRC 2007; Vogt et al. 2007).

Many species in the Baltic Sea are sensitive to environmental change including temperature and salinity (Matthäus and Nausch 2003). Salinity has been decreasing throughout the Baltic Sea due to increased input of freshwater, and temperature is predicted to increase by 3 to 5°C mean annual temperature by the end of the century which may lead to further loss of biodiversity (HELCOM 2007; HELCOM 2018). Over 85 million people live in the catchment area of the Baltic Sea and countless more rely on the area for resources and recreation (HELCOM 2018). Biodiversity loss reduces the capacity of the Baltic Sea to provide resources and services to those people who rely on it (HELCOM 2010). The current status of vegetation and fish stocks are estimated to result in a loss of more than 2 billion euros each year and an additional loss of 2 billion euros per year is estimated from impaired recreation (HELCOM 2018). There is therefore great interest in restoring Baltic Sea ecosystems and to do so, it is crucial to understand how and through what mechanism(s), species respond to changes in this environment.

There are some species in the Baltic Sea which are able to adapt and diversify to meet the demands of the currently changing ecosystem (Johannesson and André 2006). The stickleback is one such species. It is abundant throughout the Baltic Sea and has repeatedly demonstrated rapid evolution to changing food-web and environmental properties in similar ecosystems across the northern hemisphere (DeFaveri and Merilä 2013; DeFaveri et al. 2013). Stickleback are present throughout the Baltic Sea and recent interest has been drawn to the population along the Swedish Baltic coast where adults have increased 45-fold in offshore and 25-fold in nearshore areas since the 1990s (Bergström et al. 2015; Eklöf et al. 2020). Three main stickleback phenotypes have been identified based on number and pattern of armor plating (although others have been identified, see e.g. Ziuganov 1983; Raeymakers et al. 2007; Lucek et al. 2010; Lucek et al. 2012). The completely-plated phenotype has plates that run uninterrupted from immediately behind the head to the end of the caudal peduncle, the partially-plated phenotype has a gap in plates on the mid-section of the body which is the width of two or more plates, and the low-plated phenotype has plates running from immediately behind the head to somewhere on the mid-section of the body and does not have any plates on the caudal peduncle (Münzing 1963; Figure 2). Plating has been proposed to be an adaptation to different ecological factors. Completely-plated stickleback may be better protected against predation by piscivorous fish as compared to partially- and low-plated stickleback (Reimchen 1992, 2000). Partially- and low-plated stickleback may have increased fast-start performance and body flexibility which aids in escaping and hiding from predators in densely vegetated areas (Bergstrom 2002; Leinonen et al. 2011). All three of these phenotypes are present in coastal habitats in the Baltic Sea, albeit in different abundances (Aneer 1974). Since the increase in overall stickleback abundance, the species has been studied extensively along the Swedish Baltic coast, however few studies have taken phenotype into consideration when examining the role that stickleback play in these environments (Sieben et al. 2011; Byström et al. 2015; Jakubavičiūtė et al. 2018).

Figure 2. Stickleback plate phenotypes commonly found in the Baltic Sea: A) Low plated, B) partially plated, and C) completely plated.

At first glance, the phenotypes of stickleback in the Baltic Sea appear to occupy the same niche and ecological role with no apparent spatial or temporal separation. The only thing that distinguishes the phenotypes is the plating pattern, making them morphologically indistinguishable to the untrained eye. In well described populations from other temperate areas such as Canada and the Pacific Northwest United States, there are obvious differences between the phenotypes in terms of body size and shape and resource utilization (reviewed in McKinnon and Rundle 2002; Arnegard et al. 2014). In these populations, plate phenotype is clearly adaptive. Since the phenotypes in shallow coastal bays in the Baltic Sea overlap in distribution, morphometrics, and resource use, this brings into question if plating patterns in this system are adaptive or merely a heritable trait which selection does not act upon. To understand the full impact of stickleback population expansion, we must know if plate phenotype is an adaptive trait leading parts of the population to fill different functional roles and what

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it is developing as a response to. If it is not adaptive then studying the impact of the expanding stickleback population on the ecosystem can be done without regard to phenotype abundances. If plate phenotype is adaptive, then it is important to understand what it is an adaptation to in order to predict how the population will evolve in response to future changes in the ecosystem.

Thesis Outline

The aim of this thesis is to improve our understanding of the eco-evolutionary dynamics of threespine stickleback along the Swedish Baltic coast. Utilizing field data collected over two seasons across approximately 350 km centered around the Stockholm archipelago and data from an experiment conducted with organisms and resources collected within the field study area (Figure 3), I specifically examined: stickleback phenotype distribution in relation to other species and physical habitat characteristics (Chapters 2 and 3) and divergent resource exploitation by stickleback phenotypes and the effect on other components of the biological community, including how these dynamics may be affected by climate change (Chapter 4). Chapters 2 and 3 provide insights into possible adaptations for utilization of different habitat types and food sources as suggested by associations with different biological factors (Figure 4). Data for Chapters 2 and 3 were collected at similar shallow coastal bays but on different scales. Chapter 2 looks at the association of phenotypes with average conditions at each bay while Chapter 3 looks at associations of phenotypes with patches of resources, or microhabitats, within bays. Chapter 4 details a mesocosm experiment designed to help us understand if phenotypes are adapted to utilize different resources, if these differences culminate in changes to the whole food web (Figure 5).

Figure 3. Map of all sampling locations from 2014 (grey diamonds) and 2017 (black circles) and the location where the mesocosm experiment was conducted in 2018 (white star). Axis labels are latitude and longitude.

Chapter 2, “Habitat segregation of plate phenotypes in a rapidly expanding population of three-spines stickleback”, illustrates the change in trophic structure of coastal bays due to the increase in juvenile stickleback abundance and coinciding decrease of juvenile predatory fish abundance. Chapter 2 presents findings from a collaborative field study designed to look at overall stickleback and phenotype abundances in relation to other resources and habitat characteristics. We show that overall abundance and abundance of incompletely-pated stickleback increases with increasing topographical openness of bays and decreases with increasing piscivorous fish abundance, suggesting some degree of habitat divergence between phenotypes. We

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also show dietary divergence with completely-plated stickleback consuming more amphipods than incompletely-plated stickleback.

In Chapter 3, “Predator biomass and vegetation influence the coastal distribution of threespine stickleback morphotypes”, I show that there is habitat divergence between phenotypes at a microhabitat scale. The proportion of completely-plated stickleback increases as coverage of bladderwrack becomes more dense. The proportion of completely-plated stickleback also increases as biomass of piscivorous fish increases, confirming the results of Chapter 2.

Figure 4. A) Snorkeler recording vegetation coverage around traps (B) placed in different habitat patches to catch stickleback (C) during 2014 and 2017 field surveys.

Figure 5. A) Mesocosm setup from 2018 study at Askö Laboratory. Each grey tank (B) contained twenty 50 L buckets (C, D). The buckets contained stickleback, invertebrates, and algae and were used to measure changes in biological community structure over time in the presence of different stickleback phenotypes.

Chapter 4, “Warming modifies the effects of intraspecific variation in stickleback morphology on benthic invertebrates and primary producers in a mesocosm experiment”, describes the effects that stickleback communities of different phenotype compositions have on the food web due to dietary differentiation. As this chapter

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concerns understanding how ecosystem structure will change over time, I also included a climate change treatment to examine how a future climate change scenario may affect the impacts that different stickleback communities have on other trophic levels. I show that different plate phenotypes may differentially affect lower trophic levels, and that these effects depend on water temperature.

In Chapter 5, I summarize the findings of Chapters 2 through 4 and what they may mean for our understanding of adaptation to environmental change in the Baltic Sea and in a global context.

CHAPTER

02

Habitat segregation of plate phenotypes in a rapidly expanding population of three-spined stickleback

Britas Klemens Eriksson, Casey Yanos, Sarah Bourlat, Serena Donadi, Michael C. Fontaine, Joakim P. Hansen, Eglė Jakubavičiūtė, Karine Kiragosyan, Martine E. Maan, Juha Merilä, Åsa N. Austin, Jens Olsson, Katrin Reiss, Göran Sundblad, Ulf Bergström, and Johan S. Eklöf

Declines of large predatory fish due to overexploitation are restructuring food webs across the globe. It is now becoming evident that restoring these altered food webs requires addressing not only ecological but also evolutionary processes, but evolutionary ones as well, because human-induced rapid evolution may in turn affect ecological dynamics. We studied the potential for niche differentiation between different plate armor phenotypes in a rapidly expanding population of a small prey fish, the three-spined stickleback (*Gasterosteus aculeatus*). In the central Baltic Sea, three-spined stickleback abundance has increased dramatically during the past decades. The increase in this typical mesopredator has restructured near-shore food webs, increased filamentous algal blooms, and threatens coastal biodiversity. Time-series data covering 22 years show that the increase coincides with a decline in the number of juvenile perch (*Perca fluviatilis*), the most abundant predator of stickleback along the coast. We investigated the distribution of different stickleback plate armor phenotypes depending on latitude, environmental conditions, predator and prey abundances, nutrients and benthic production; and described the stomach content of the stickleback phenotypes using metabarcoding. We found two distinct lateral armour plate phenotypes of stickleback, incompletely and completely plated. The proportion of incompletely plated individuals increased with increasing benthic production and decreasing abundances of adult perch. Metabarcoding showed that the stomach content of the completely plated individuals more often contained invertebrate herbivores (amphipods) than the incompletely plated ones. Since armour plates are defence structures favoured by natural selection in the presence of fish predators, the phenotype distribution suggests that a novel low-predation regime favours stickleback with less armour. Our results suggest that morphological differentiation of the three-spined stickleback has the potential to affect food web dynamics, and influence the persistence and resilience of the stickleback take-over in the Baltic Sea.

Introduction

Human-induced biodiversity loss strikes particularly hard on higher trophic levels, and many populations of top predators have declined across the globe (Ripple et al. 2014). In marine systems, loss of larger predatory fish has induced changes in predator-prey relations that propagate through food webs, affecting biodiversity, and change ecosystem structure (Jackson et al. 2001, Daskalov 2002, Worm et al. 2006). In response to the severe economic and cultural losses resulting from these changes, various measures have been initiated to restore biodiversity at higher trophic levels (e.g. marine protected areas; Gell and Roberts 2003, Lester et al. 2009). However, these measures may be ineffective because of pervasive but poorly understood feedback mechanisms within the ecosystem (Nyström et al. 2012), as well as interactions between multiple human impacts that delay or prevent recovery (Frank et al. 2005, Eriksson et al. 2010, Frank et al. 2011).

Currently, we have an inadequate understanding of the drivers that structure food webs that are, themselves, changing. One poorly understood factor is rapid evolution. Evolution was traditionally assumed to be slow and unimportant on ecological timescales, and has therefore rarely been included in ecological studies or ecosystem management (Fussman et al. 2007, De Meester et al. 2019, Hendry 2019). Today, however, we know that selection can change population dynamics on short timescales, and that overfishing in particular has the potential to cause exceptionally rapid evolution of target species (Conover and Munch 2002, Kuparinen and Merilä 2007, Hutchings and Fraser 2008, Audzijonyte et al. 2013). Over the past decade, we have also become increasingly aware that rapid evolution can feed back on to ecosystem properties, so called eco-evolutionary feedback (Post and Palkovacs 2009, Schoener 2011, Turcotte et al. 2011, Hendry 2017, Govaert et al. 2019). Still, evolutionary consequences of large-scale predator loss for lower trophic levels are not widely understood.

Declines of large predatory fish are typically followed by dramatic increases in the abundance of their main prey, often smaller fish species; a process known as “mesopredator release” (Prugh et al. 2009). For the prey fish, the strong increase in density intensifies intraspecific competition, and may shift the selection regime from predation defence towards increased individual-level resource specialization and population-level resource use diversity (Svanbäck and Persson 2004, Svanbäck and Bolnick 2005, Svanbäck and Bolnick 2007). Accordingly, there are clear examples of how populations have increased their competitive ability and diversified after escaping control from their native predators (Palkovacs et al. 2011, Mlynarek et al. 2017). Thus, mesopredator release caused by the loss of large predators may lead to a strong selective pressure for different traits among prey fish. This may, in turn, impact other

trophic levels by introducing new predator-prey interactions, ultimately impacting the nature and strength of trophic cascades. Here we explore the potential for niche differentiation in a population of prey fish in the Baltic Sea, which have increased dramatically the past decades at the same times as a number of predator populations have decreased strongly.

In the western parts of the central Baltic Sea (NW Europe), densities of a small mesopredatory fish – the three-spined stickleback (*Gasterosteus aculeatus* L., hereafter stickleback) – have increased up to 45 times during the last decades, following regional and/or local declines of their natural predators; the large-bodied commercially and recreationally harvested fish cod (*Gadus morhua*), pike (*Esox lucius*), and perch (*Perca fluviatilis*; Ljunggren et al. 2010, Eriksson et al. 2011, Bergström et al. 2015, Olsson 2019, Olsson et al. 2019). Suggested mechanisms behind the stickleback population explosion include a combination of human impacts that link drivers of change across large spatial scales. Juvenile stickleback migrate offshore during autumn and, when mature, return to the coast for spawning in spring (Bergström et al. 2015). Offshore, overfishing of cod, increased water temperatures, and increased food availability due to eutrophication may have contributed to their increased survival (Eriksson et al. 2011, Lefebure et al. 2014). Inshore, local decreases in perch and pike populations may have increased recruitment and juvenile survival of stickleback in some areas (Östman et al. 2014, Bergström et al. 2015, Hansson et al. 2018). The local decreases in pike and perch are caused by loss or degradation of reproduction habitats due to wetland drainage, coastal constructions, and boating (Nilsson et al. 2014, Sundblad and Bergstrom 2014, Hansen et al. 2019) and, potentially, increased predation from seals and cormorants (Östman et al. 2014, Hansson et al. 2018, Veneranta et al. 2020). Today, there is a strong inverse relationship between local abundances of stickleback on the one hand and perch and pike on the other (Bergström et al. 2015), and we have indications that in large abundances the stickleback themselves limit the perch population by consuming their eggs and larvae (Byström et al. 2015, Eklöf et al. 2020). Spatially explicit analyses demonstrate that a shift in dominance from perch and pike to stickleback dominated bays has propagated from the outer archipelago towards the coast as a spatially propagating wave through time (Eklöf et al. 2020). The dramatic increase in stickleback abundance, in combination with elevated resource availability from large-scale eutrophication, has restructured near-shore food webs and increased filamentous algal blooms; thereby threatening coastal biodiversity (Eriksson et al. 2009, Sieben et al. 2011a, Byström et al. 2015, Donadi et al. 2017).

Globally, the marine stickleback is known to have rapidly and repeatedly adapted to freshwater habitats with low predation pressure by evolving reduced armour

phenotypes (i.e. lower number of bony lateral plates and loss/reduction of pelvic girdle), apparently in response to relaxed selection on these anti-predator traits (Bell 2001, Huntingford and Coyle 2006). A large body of research supports the hypothesis that fully plated marine individuals are better protected against predation by fish than individuals with reduced plating (e.g. Reimchen 1992, 2000). On the other hand, reduced lateral plate numbers can increase fast-start performance and manoeuvrability, and thereby provide means to avoid predation in habitats where refuge from predators (e.g. vegetation) is present (Bergström 2002, Leinonen et al. 2011b). In addition, adaptation to predation can strongly influence the feeding ecology of stickleback (reviewed in Bell and Foster 1994, Harmon et al. 2009).

Three lateral plate phenotypes are described for the three-spined stickleback in the Baltic Sea: fully plated individuals, which have plates along the sides of the body that run uninterrupted from the head to the tail fin (caudal end); partially plated individuals, which lack plates on the mid-section of the body; and low plated individuals, which only have plates on the head (pectoral) region of the body (Münzing 1963, Reimchen 2000). The plate phenotype is highly heritable and largely controlled by one major gene (Ectodysplasin; Colosimo et al. 2004), and hence, indicative of the underlying genotype (Loehr et al. 2012, DeFaveri et al. 2013). In the Baltic Sea, all three plate phenotypes of the three-spined sticklebacks have co-occurred for at least a century (Aneer 1974, Jakubavičiūtė et al. 2018), and there is evidence for adaptive phenotypic differentiation in number of lateral plates across the Baltic Sea (DeFaveri and Merilä 2013).

The aim of this study was to evaluate the potential for niche differentiation between different plate morphs as a response to changing predation regimes, in the dramatically expanding population of three-spined stickleback in the Baltic Sea. We first assessed temporal trends in the abundance of the three-spined stickleback and juvenile perch, the most common predator, using time series data covering 22 years (1996-2017). Then, we related differences in the spatial distribution of plate phenotypes to the abundance of predatory fish and production of the community. Finally, we documented differences in the diets of the different plate phenotypes, to evaluate the potential for feedbacks on food web structure caused by changes in plate phenotype frequencies. We hypothesised: 1) that in areas with relatively low abundances of predatory fish, reduced selection for anti-predator traits results in an increased proportion of incompletely (low or partially) plated stickleback, compared to completely plated individuals; and 2) that the different stickleback plate phenotypes have different feeding ecologies, as reflected by different prey compositions in their stomach contents.

Material and Methods

Trends in three-spined stickleback and juvenile perch abundances over time

We first analysed temporal trends in the abundance of juvenile three-spined stickleback and perch (the dominant coastal predator on stickleback). Data on temporal trends of juvenile three-spined stickleback and perch abundances from 1996 to 2017 was extracted from the Swedish national database for coastal fish (<http://www.slu.se/kul>). The data combined samplings of juvenile fish from various monitoring programs and research projects that quantified fish recruitment along the Swedish Baltic Sea coast. The available data on juvenile fish are spatially far more comprehensive than those for adults. Therefore, use of juvenile data enabled us to extract a time series that covered the full outer to inner archipelago gradient, which better represents the full range of habitats occupied by different species of fish than the regular coastal fish monitoring programs (see e.g. HELCOM 2018). The database on juvenile fish also includes small species, such as stickleback, which are poorly represented in the regular monitoring programs (as the multimesh gillnets used for monitoring lack mesh sizes small enough to capture stickleback in a representative way; HELCOM 2018).

We extracted abundances of juvenile perch and three-spined stickleback from an area covering a 360 km stretch of the central Baltic Sea coastline (counties of Uppsala, Stockholm, Södermanland and Östergötaland; about from Skutskär to Västervik). This area overlapped with separate sampling of stickleback phenotypes in 2014, presented below. The data on juvenile fish collected in the Swedish national database for coastal fish, were sampled in shallow bays using small underwater detonations; a standard method that catches fish with a swimbladder of lengths up to 20 cm within a blast radius of 2.5-5 m depending on detonation strength (1-10 g; size range of fish 2-10 cm; Snickars et al. 2007). Since fish are heterogeneously distributed at small spatial scales and the detonation method takes a snapshot of a defined area (ca. 20-80 m² depending on detonation strength), the abundance data is highly variable. In addition, bays have been sampled at various intensities (from 5 to 90 detonations per bay, mean $\pm$ SD = 16 $\pm$ 11). We therefore square-root transformed abundance data from each detonation and then used averages for each bay for the statistical analyses. Moreover, we only included the years 1996 to 2017 in the analysis because before 1996 most years were only represented by one single bay; leaving 7553 detonations from 480 bay-year combinations in the dataset. The mean depth of all sampling was 1.7 $\pm$ 0.59m ($\pm$ SD) and nearly all sampling (94%) was conducted during late July to early September. All sampling was performed by certified personnel and with required ethical permits and exemptions from national fishing regulations.

We analysed trends in stickleback and perch abundances by fitting linear models with year as an explanatory variable. We did not perform a formal time series analysis accounting for autocorrelation of sampling stations over time, because most of the bays were sampled only once (184 out of 256 unique bays) and less than 10% were sampled more than four times. In addition, the replication of bays for each year was highly skewed: the first five years had an average replication of ca. 10 bays per year, compared to ca. 30 for the following decades. To balance the influence of the different time-periods on the model fit, we therefore used the averages for each year as response variable and treated each year average as a true replicate. However, this reduces the variability of the data, meaning that the R^2 of the model fit only is related to the variation in yearly means; not to the variation between bays within or across years. Since the sub-set of bays were different each year, we included a spatial structure in the model to make sure trends were not caused by differences in sampled regions or characteristics of bays. The spatial structure consisted of adding average sampling depth, latitude, and longitude as explanatory factors to the model. We selected the best model for perch and stickleback abundances based on AICc (AIC for small samples); using the MuMIn package in R 3.5.2 (version 1.43.15; R Core Team, 2018. Two years (1996 and 2000) were excluded from the model selection because they caused strong collinearity between latitude and longitude; sampling predominantly occurred in the southern and innermost part of archipelago. After the selection procedure, the omitted years were reintroduced into the final model. In the reduced dataset there was no correlation between the explanatory variables and we did not detect any problems with collinearity in the candidate models (the variance inflation factor (VIF): year=1.10, latitude=1.19, longitude=1.22, depth=1.06), or the final models (VIF: year =1.00, depth=1.00). If necessary, variables were normalized using square root transformations.

Characterising the coastal food web and stickleback phenotype composition

Since the time series data described above mainly contain juvenile fish abundances, we tested which factors that best described stickleback abundances and plate phenotype distribution by characterizing the benthic food web in 32 shallow coastal bays in spring 2014 (max sampling depth between 3 and 6 m). The area covered was the same as where the time-series data was extracted from (Appendix S1: Table S1, Fig. S1). The bays represented a gradient from inner to outer archipelago, characterized by gradients in wave exposure and bay morphology; factors that regulate the composition of the biological communities in the Baltic Sea archipelago (see Appendix S1 for detailed explanation of selection criteria and calculations). Some of the field data was included in a structural equation model published in Donadi et al. (2017),

which specifically analysed top-down effects of sticklebacks on lower trophic levels. Here we focus on explaining the distribution of the different plate phenotypes of stickleback – which is previously un-published data.

The fish community was sampled in spring (from May to early June) using three to six Nordic survey gillnets set out overnight in each bay (mesh size 5–55 mm, details in supplementary information in Appendix S1). All fish caught were identified to species, measured for total length, and counted. Bleak (*Alburnus alburnus*), ruffe (*Gymnocephalus cernuus*), three-spined stickleback, perch and roach (*Rutilus rutilus*) dominated the fish fauna (Appendix S1: Table S2). Bleak, perch, ruffe, roach and three-spined stickleback all eat a wide variety of food items, including plant material, invertebrates, and zooplankton; but larger perch also more commonly include medium sized fish in its diet (Appendix S1: Table S2; Jacobson et al. 2019). The number of individuals of each fish species caught were pooled for each bay and catch per unit effort (CPUE) was expressed as number of individuals per net and night of each species. In each bay, we estimated vegetation and filamentous algae cover, collected zooplankton, and measured depth, fluorescence, temperature and turbidity, at three to six randomly distributed stations (Appendix S1: Fig. S2a). Mesograzers (benthic macro-herbivores) biomass was quantified at each station by sampling epifauna in a randomly placed 0.2×0.2 m frame connected to a 1 mm-mesh bag (Appendix S1: Fig. S2b,c). After identification of mesograzers in the laboratory (i.e. shredders and gatherers, whose diet is dominated by macrophytes: see Donadi et al. 2017), the biomass of mesograzers was estimated as gram ash-free dry-mass (AFDM) using taxon-specific correlations between length and weight (Eklöf et al. 2017). The number of both stations and nets depended on the size of the bay. They were randomized within depth strata from a topological map to be representative of the depth profile in each bay, but restricted to a depth between 0.5 and 3 m (Appendix S1; also see Donadi et al. 2017 for a detailed description of sampling procedures).

In each bay, we sub-sampled 30 individuals of the sticklebacks caught in the nets for morphological and diet analyses. However, in 16 of the bays we caught less than 30 individuals (mean=5.5, SD=5.3, min=0, max=22; CPUE). In the other 16 bays we caught high abundances of stickleback and the fish community was often completely dominated by three-spine stickleback individuals (stickleback bays: mean=860.3, SD=662.4, min=32, max=1799; CPUE). Earlier studies show an inverse relationship between stickleback and perch abundances (Bergström et al. 2015, Eklöf et al. 2020). Accordingly, the average abundance of perch was three times higher in the 16 bays where we found less than 30 stickleback (mean=87.1, SD=65.0; CPUE), in comparison to the 16 bays where we caught more than 30 stickleback (mean=28.3, SD=21.6; CPUE). To classify the bays, we calculated a mesopredator index as the total number

of sticklebacks in each bay divided by the total number of perch and stickleback together. Thus, if the bay had stickleback present but no perch, the index equalled 1; if the bay had perch present but no stickleback, the index equalled 0. The index confirmed the negative relationship between perch and stickleback (Appendix S1: Fig. S3): the 16 bays where we found less than 30 stickleback had an index of less than 0.4 (perch-dominated bays) and the 16 bays where we caught more than 30 stickleback had an index of more than 0.6 (stickleback-dominated). To get a reliable and comparable estimate of the fraction of plate phenotypes, we only included the 16 stickleback dominated bays where we could collect ≥ 30 individuals in the analyses of plate phenotype distributions (Appendix S1: Table S1). To determine body shape variation, eleven length parameters were documented from all individuals in the stickleback dominated bays (following Jones et al. 2012, Appendix S2). The number of lateral plates were counted on both the left and right sides of the body and the average number of lateral plates for each individual was calculated. Plates were counted starting immediately after the operculum to the end of the caudal peduncle, following Aneer (1974). Plate phenotypes were identified by looking at gaps in the plate armour: low plated fish lacked caudal plates; partially plated fish had a clear gap between the main body plates and the caudal plates (the width of at least 2 body plates); fully plated fish had no clear gap in the plating. However, there were a number of phenotypes in the transition phase between low and partially plated individuals; for example, individuals with only a few clear body plates followed by a clear gap and then a keeled tail consisting of reduced plates. For the analyses, we therefore divided the phenotypes into completely (fully) or incompletely (low and partially) plated sticklebacks. In total, we morphotyped 492 individuals from the 16 stickleback dominated bays. Since we only defined the stickleback as completely or incompletely plated, their relative proportions of the phenotypes are the inverse of each other. In the analyses, we therefore only modelled the proportion of incompletely plated three-spined stickleback individuals.

Algal production was estimated for each bay as percent cover of filamentous algae on settling plates following three months in the water (May to August). Each plate consisted of four 5×5 cm unglazed ceramic tiles glued in pairs to two bricks, which were placed flat on the bottom at ca 1.5 m depth (Appendix S1: Fig. S2d). Algal cover on settling plates is a good measure of relative net primary production in the Baltic Sea (e.g. Eriksson et al. 2006, Eriksson et al. 2009, Eriksson et al. 2012), as filamentous algae respond to nutrient enrichment by fast accumulation of biomass, but are simultaneously grazed by invertebrate mesograzers (Worm et al. 2002, Råberg and Kautsky 2007). Mesograzers are in turn eaten by fish, and thereby play a key role in trophic cascades (Eriksson et al. 2009, Donadi et al. 2017). In eight of the 32 bays,

individual tiles were either lost or completely/partially covered with sediment. These bays were therefore not included in the analysis of algae cover development.

We characterized the physical environment of each bay by estimating depth, wave exposure and topographic openness (Ea). Depth was estimated as the average depth of all sampling points in each bay. Wave exposure was estimated for each bay using a simplified wave model based on fetch, topography, and wind conditions using Geographical Information System (GIS) methods (Sundblad et al. 2014). Ea is defined as $100 \times A_t/a$: where A_t is the cross sectional area of the smallest section of the opening of the bay towards the sea and a is the bay surface area. Topographic openness is one of the most important factors structuring the coastal communities of Baltic Sea bays (Hansen et al. 2012, Hansen 2013). We used topographic openness as a proxy for production in the bays, because we wanted a factor in the model that is independent of stickleback dynamics. The more direct measures, the development of algae and vegetation, are influenced by indirect top-down and direct fertilization effects of the stickleback themselves (Sieben et al. 2011a,b, Donadi et al. 2017). With increasing openness the influence of seawater and the influx of nutrients from surrounding water bodies increases. This promotes the benthic community (Donadi et al. 2017), which is reflected in higher biomass of marine and brackish water algae and crustacean macro-herbivores (mainly benthic amphipods) in more open bays (Hansen et al. 2012, Hansen 2013). We tested our assumption that topographic openness determine production by modelling the relation with vegetation cover and algae development on the settlement tiles (linear models; `lm` function in R, version 3.6.1).

We tested which factors that best described stickleback abundances (# CPUE) and plate phenotype distribution (proportion of partially plated) by comparing General Linear Models including explanatory factors related to: spatial structure (latitude, longitude), physical characteristics (wave exposure; depth [average depth of sampling points in bay]; and topographic openness [Ea]), prey availability (mesograzers and zooplankton), water temperature (at sampling), nutrient availability (the limiting nutrient phosphorus), and predation pressure (cpue perch >25 cm). We used perch abundance as an indicator of predation pressure because perch is by far the most common stickleback predator in the coastal fish community. We only included perch >25 cm, as stickleback then constitute their most common prey item (>40 % of the diet in spring; Jacobson et al. 2019). The other common coastal fish predator, pike, is poorly represented by the gill-net catches because of their sedentary life-style, and was therefore not included in the analyses. We selected the best linear model (`lm` function) for stickleback abundances and plate distribution based on AICc (AIC for small samples); using the MuMIn package in R 3.5.2 (version 1.43.15; R Core Team, 2018). Data was square root (# stickleback; Ea and # perch > 25 cm), log10 (# zooplankton,

mesograzers; temperature) or logit (proportion partially plated stickleback) transformed, to fit assumptions of normality. The logit transformation of plate phenotype was calculated as the natural logarithm of the proportion of partially plated individuals divided by the proportion of fully plated individuals ($\text{LN}[\text{proportion partially plated}/\text{proportion fully plated}]$).

Stickleback diet analyses

We used a metabarcoding approach to analyse diet differentiation between sympatric stickleback plate phenotypes. In many of the bays with few sticklebacks, we only found one of the two plate phenotypes. To avoid bias in stomach content analyses caused by the food availability in those bays with only one phenotype present, we randomly selected 177 individuals from 10 bays where both plate phenotypes were present (Appendix S1: Table S1). In the laboratory, the stomachs were dissected and flushed with 80% EtOH to remove all stomach contents and stored at -20°C in 80% EtOH. DNA was then extracted from the stomach contents using the UltraClean Tissue and Cells DNA Isolation Kit (MO BIO Laboratories). The dual PCR amplification method was then used for Illumina MiSeq library preparation (Bourlat et al. 2016). The amplicon primers were based on Leray et al. (2013) yielding a 313 bp fragment targeting the cytochrome c oxidase subunit 1 mitochondrial gene (CO1), and a blocking primer for *G. aculeatus* was also used to prevent amplification of predator DNA (all primer sequences can be found in Jakubavičiūtė et al. (2017)). Illumina MiSeq produced 30,103,790 paired-end reads of 300 bp in length. The processing steps were performed using Qiime (Quantitative Insights into Microbial Ecology) version 1.9.1 (Caporaso et al. 2010) and custom python scripts. Paired-end joining was done using the Qiime fastq-join tool. Primer sequences were removed using a custom python script, and remaining chimeric reads were excluded using UCHIME (Edgar et al. 2011). The Bayesian clustering algorithm CROP was used to cluster the sequences into operational taxonomic units (OTUs; Hao et al. 2011). Taxonomic assignment was performed with the Uclust software implemented in Qiime (Edgar 2010), using a 97 % similarity limit when comparing the CO1 sequences with our own reference database of Chironomidae, Nemertea, Xenacoelomorpha and Oligochaeta, combined with barcodes of Swedish Echinodermata, Mollusca, Cnidaria and Arthropoda from the Swedish Barcode of Life database (SweBol). Detailed methods for DNA extraction, amplicon library preparation, and bioinformatic analyses of the current samples can be found in Jakubavičiūtė et al. (2017). The raw sequence data are available as fastq files from the NCBI sequence read archive under accession number SRP101702, BioProject number PRJNA378633.

For analysis, the OTU tables were converted to a presence/absence matrix. The prey species were divided into broad taxonomic groups relevant for the ecology of the system: crustacean mesograzers (amphipods, isopods, and mysids), molluscs (gastropods and bivalves), benthic worms (polychaetes and annelids), insects (chironomids and others), and zooplankton/meiofauna (cladocerans, copepods, and ostracods). For the complete list of prey taxa found in this study, see table S1 in Jakubavičiūtė et al. (2017). A table of presence or absence of prey groups in the stomach of each fish was compiled. Differences in the occurrence of prey in the stomachs of completely compared to incompletely plated phenotypes, was analysed using the multivariate statistics package Vegan (version 2.5-6) in R. First, we performed an Analysis of Similarities (ANOSIM) to test if there was a difference in the composition of prey in the stomachs of completely and incompletely plated sticklebacks (model: species composition of prey ~ plate phenotype). Then we plotted prey groups and the centroids of plate phenotypes using the information from an unconstrained NMDS ordination, to identify which prey groups contributed most to the differences between plate phenotypes. For each of the prey groups that was identified by the visual inspection (amphipods, mysids, bivalves and polychaetes; see results) we analysed the difference in stomach content between completely and incompletely plated fish, using a generalized mixed-effects model using the lme4 package in R (version 3.5.2; Bates et al. 2015). The model was based on the binomial error distribution with a logit link function (logistic regression), and included plate phenotype as a categorical explanatory variable, and bay as random factor.

Results

Changes in predator-prey relations along the coast

Juvenile three-spined stickleback abundances increased strongly along the western coast of the central Baltic Sea between 1996 and 2017 (Fig. 1). The best fitting model only included year as an explanatory factor (exponential fit: $F_{1,20}=42.2$, $R^2_{adj}=0.66$, $p<0.001$). In contrast, juveniles of the dominating coastal predator perch decreased linearly between 1996 and 2017 (linear fit 1996-2017: $t=-2.3$, $p=0.034$), and sampling depth was included as a very important factor in the best fitting model (abundance increased with sampling depth: $t=4.1$, $p<0.001$; Full model: $F_{2,17}=12.2$, $R^2_{adj}=0.54$, $p<0.001$; Fig. 1). Thus, the relative abundances of juveniles of top- and mesopredators have changed dramatically over the past decades, likely reflecting a changed trophic structure along the coast.

Figure 1. Temporal trends in three-spined stickleback (orange solid line) and perch (blue solid line) juvenile abundances (averages of square rooted numbers); between 1996 and 2017. For perch, the values are adjusted to account for differences in sampling depth. The solid lines show significant models describing the development over time, for perch it is partial residuals from a models accounting for sampling differences in depth. Dotted lines show 95% confidence interval around the predicted values.

Stickleback abundances and the distribution of plate phenotypes along the coast

The food web field survey indicated the importance of production and predation in regulating the three-spined stickleback population. Stickleback abundance was best described by a model including topographic openness and the number of large predatory perch (model recommended by AICc: $R^2_{adj}=0.43$, $F_{1,29}=12.7$, $p<0.001$; Appendix S3: Table S1), where stickleback abundance increased with increasing topographic openness (Ea: $df=29$, $t=4.2$, $p<0.001$) and decreased with increasing abundance of large predatory fish (# perch > 25 cm: $df=29$, $t=-1.9$, $p=0.065$). There was no correlation between the explanatory variables topographic openness (Ea) and CPUE of large perch (Pearson's product moment correlation: $r=-0.19$, $t = -1.03$, $df = 30$, $p=0.309$), and we did not detect any problems with collinearity in the model (VIF=1.04). However, the individual effect of perch was only a statistical trend and the fit of the residual after correcting for topographic openness was poor (residual # stickleback [sqrt CPUE ~ Ea] depending on # perch > 25 cm: $R^2_{adj}=0.08$).

Considering the bimodal distribution of bays, where half were dominated by perch and the other half by stickleback, we further explored the data by allowing for an interaction between topographic openness and bay type (perch or stickleback dominated) instead of perch numbers. This model improved the fit considerably and showed that stickleback increased strongly with topographic openness in stickleback-dominated bays, but not in perch-dominated bays (Full model: $R^2_{adj}=0.77$, $F_{3,28}=12.7$, $p<0.001$; significant interaction effect: $df=28$, $t=2.3$, $p=0.028$; Fig. 2). Topographic openness significantly explained estimates of net production in the bays; both general vegetation cover ($R^2_{adj}=0.22$, $F_{1,30}=9.6$, $p=0.004$, Fig. 3a) and algal cover development on the settlement tiles ($R^2_{adj}=0.25$, $F_{1,23}=9.1$, $p=0.006$; Fig. 3b) increased with openness.

Figure 2. The relationships between the density of adult three-spined stickleback and topographic openness, in bays dominated by stickleback (a) or perch (b). The solid trend line shows a significant relationship between the number of stickleback and topographic openness in the bays dominated by stickleback determined by linear regression ($df=14$, $r^2=0.37$, $t=2.9$, $p=0.013$).

Figure 3. The relationship between a) topographic openness and vegetation cover, and b) topographic openness and algal cover development on settlement tiles; indicating that topographic openness is a proxy for general community production. The trend lines show significant relationships determined by linear regressions.

We observed two distinct stickleback phenotypes corresponding to completely (fully) and incompletely (partially/low) plated phenotypes and very few intermediates (Fig. 4). The plate phenotype distribution was best described by a model including topographic openness and the number of large predatory perch (# perch > 25 cm)(model recommended by AICc: $R_{adj} = 0.39$, $F_{2,13}=5.9$, $p=0.015$; Fig. 5; Appendix S3: Table S2), where the proportion of incompletely plated individuals increased with topographic openness ($t=2.7$, $n=16$, $p=0.019$) and decreased with the abundance of large perch ($t=-2.5$, $n=16$, $p=0.026$). This indicates a potential difference in selective regime on plate phenotypes depending on bay characteristics and predation. The incompletely plated phenotype was five times more common than the completely plated phenotype, and their relative abundance increased from 75% in the most enclosed bays to 90% in most open bays (Fig. 5a). At the same time, bays where perch was more abundant had a lower proportion of incompletely plated stickleback than expected from the topographic openness of the bay (Fig. 5b).

Figure 1. Frequency distribution of the average number of lateral plates, indicating the completely (fully) and incompletely (partial and low) plated three-spined stickleback phenotypes.

Figure 2. The residual variation of the proportion of incompletely plated adult sticklebacks (logit transformed data) explained by: a) topographic openness, after taking the abundance of large perch (>25 cm) into account; and b) the abundance of large perch after taking the topographic openness into account. Trend lines show significant relationships determined by linear regressions.

Diet of the plate phenotypes

Metabarcoding of stickleback stomach content revealed significant differences in the diet of the two plate phenotypes (ANOSIM: $R=0.075$, $p=0.037$). The overall difference was small, because almost all sticklebacks had some insect ($98.3 \pm 1.0\%$; chironomids; others) and/or zooplankton/meiofauna prey in their stomachs ($99.4 \pm 0.5\%$; ostracods: $79.7 \pm 3.0\%$; cladocerans: $97.2 \pm 1.2\%$; copepods: $93.2 \pm 1.9\%$; mean $\pm$ SE). However, the NDMS ordination highlighted four prey groups that contributed most strongly to variation along the ordination axes: amphipods, bivalves, mysids and polychaetes (Appendix S4: Fig. S1). Analysing these groups separately, it was clear that the completely plated sticklebacks included amphipods more often in their diet compared to the incompletely plated ones (logistic regression: $n=172$, $\chi^2=7.7$, $p=0.006$; Benjamini Hochberg corrected $p = 0.023$). Amphipods are the dominant benthic herbivores in the area (Eriksson et al. 2009, Sieben et al. 2011b) and $56.1 \pm 7.8\%$ of the fully plated individuals had amphipods in their stomachs, compared to $30.1 \pm 4.0\%$ of the incompletely plated individuals. Meanwhile, the proportion of occurrence of bivalves, mysids and polychaetes did not differ between stickleback phenotypes (logistic regression, $n=172$; bivalves: $\chi^2=0.66$, $p=0.418$; mysids $\chi^2=0.19$, $p=0.662$; polychaetes: $\chi^2=0.17$, $p=0.683$).

Discussion

Our results suggest that decreasing predator populations influence the selection regime of three-spined stickleback across the western Baltic Sea. Coastal time-series data show that a dramatic increase in stickleback abundance coincided with a decline in the abundance of juveniles of its main predator, perch, between 1995 and 2015. Suggested causes for the decline of perch include loss of recruitment habitats (Sundblad and Bergström 2014), but recent studies indicate that sticklebacks can also control the recruitment of perch by feeding on its juvenile stages (Byström et al. 2015, Eklöf et al. 2020). In 2014, there was a clear division of bays along the coast, where half of the 32 investigated bays were dominated by perch and the other half by large numbers of stickleback, supporting recent results by Eklöf et al. (2020). In the stickleback-dominated bays, the distribution of plate armour phenotypes was best explained by bay production and predator presence: the ratio of incompletely vs completely plated armour phenotypes increased with topographic openness and decreased with the abundance of large predatory perch. The relation between the distribution of plate armour phenotypes and predatory perch, indicates that declining predator populations have the potential to enable a shift in selection pressures for the increasing stickleback population: from high predation pressure to high intra-specific competition. Moreover,

the two plate armour phenotypes had small but significant differences in their diets. This indicates that niche differentiation between the plate phenotypes may even alter the effects of the stickleback population on food web dynamics; but this is a hypothesis that needs to be further explored.

The frequency of incompletely plated stickleback phenotypes increased with increasing topographic openness and decreasing abundances of large predators. The relation with topographic openness may depend on the general increase in production with openness in this system (Hansen et al. 2012, Hansen 2013, Donadi et al. 2017, this paper); meaning increasing abundances of invertebrate and plankton prey reducing intra specific competition, but also increasing vegetation cover (see Fig. 4a). Vegetation cover may favour fewer armour plates since unarmored sticklebacks may move through vegetation cover more effectively (Bergström 2002, Leinonen et al. 2011b). The relation between plate phenotype and predation is interesting because the three-spined stickleback has, since the last glaciation, repeatedly colonized and adapted to predator-free freshwater systems, by evolving a low plated phenotype characterised by a reduced number of bony lateral plates (Bell 2001, Leinonen et al. 2011a). This striking example of repeated evolution is characterized by a consistent reuse of globally shared standing genetic variation (Schluter and Conte 2009, Jones et al. 2012). The lateral plate number is a trait under selection (Barrett et al. 2008) and ca 75 % of the variation is determined by a major locus, ectodysplasin (EDA; Colosimo et al. 2004, Le Rouzic et al. 2011). Plates act as armour against gape limited toothed predators (Reimchen 1992, Bergström 2002) and there is both observational and experimental evidence to support the notion that the low plate phenotype is favoured in freshwater populations due to reduced predation by fish (Bell et al. 2004, Cresko et al. 2004, Kitano et al. 2008, Leinonen et al. 2012). Therefore, it is possible that the observed differences in distribution of the different plate phenotypes along the coast in fact reflects a temporal change in predation pressure due to declining predatory fish abundances (see also Jakubavičiūtė et al. 2018).

Although the different stickleback phenotypes co-occur in the Baltic Sea (Jakubavičiūtė et al. 2018), our results indicate that they differ in their diets at least in coastal areas. The diet differences were caused by one prey group only: completely plated sticklebacks more often consumed amphipods. Amphipods are important benthic herbivores in the area (Eriksson et al. 2009, Sieben et al. 2011b), and they form a key grazer group that mediate cascading ecosystem effects in coastal areas across the world (Duffy and Hay 2000, Råberg and Kautsky 2007, Moksnes et al. 2008, Duffy et al. 2015, Donadi et al. 2017). We have no indication as to why completely plated stickleback ate more amphipods than the partially plated individuals. Our expectations were that the partially plated stickleback would seek shelter from predators in the

vegetation to a larger degree than the completely plated ones, since they have less armour. We therefore assumed that partially plated stickleback would feed more on those mesograzers that themselves seek shelter and feed in the vegetation, such as amphipods. The opposite may be true if the completely plated stickleback in general are better adapted to habitats with predators; combining better plate armour with being more cautious and seeking shelter to a larger degree. Experimental testing or more controlled sampling of different phenotypes, while controlling for prey availability, is needed both to understand what drives the diet difference and to evaluate how important this diet difference is for food web dynamics. However, we already know that changes in the distribution of stickleback plate phenotypes can have strong effects on ecosystem function in a number of ways (Harmon et al. 2009). Effects of rapid diversification of partially plated stickleback include changes in prey community structure, biomass and abundance (Matthews et al. 2016, Rudman and Schluter 2016, Schmid et al. 2019); elemental stoichiometry (El-Sabaawi et al. 2016), phosphorus excretion (Paccard et al. 2018) and phosphorus concentrations in the water (Matthews et al. 2016). In addition, adaptive shifts in mean trait values may lead to increased stickleback abundances, which in turn should change their ecological effects (indirect eco-evolutionary effects: Hendry 2017). To understand if similar effects are occurring in the Baltic Sea, potentially causing eco-evolutionary feedbacks that change the effects of the mesopredator release, experimental studies are needed.

The relative importance of different factors for the increase in three-spine stickleback abundance in the Baltic Sea are currently not known. Our results show that juveniles of the main coastal predator, perch, have decreased over the past decades, and that the selection for anti-predator traits (plating) may have become relaxed as the incompletely plated phenotype dominates the studied stickleback population. This suggests that decreasing predation from declining abundances of coastal predators has been important for the expansion of the stickleback population in the past decade. Yet, the causality needs to be established in future studies, as there are many other factors that may contribute to the increased stickleback abundance; such as increasing temperatures and habitat change (Eriksson et al. 2011, Lefebure et al. 2014, Nilsson et al. 2014, Sundblad and Bergström 2014, Hansen et al. 2019). In fact, similar to the Baltic Sea, the White Sea has shown an increase in three-spined stickleback abundance since the late 1990's (Lajus et al. 2020). The increase in the White Sea has been attributed mainly to increased water temperatures and subsequent changes in habitat availability (Rybkina et al. 2017, Lajus et al. 2020). The migrating behaviour of stickleback in the Baltic Sea complicates the analysis of causal factors further by coupling pelagic and coastal processes across scales. The change in the fish community along the coast may therefore be caused by changes both in local and/or pelagic interactions and conditions.

Our results, together with evidence for that changes in the trait distribution in three-spined sticklebacks can have variety of community and ecosystem effects (e.g. Matthews et al. 2016, Rudman and Schluter 2016, El-Sabaawi et al. 2016, Schmid et al. 2019, Paccard et al. 2018), indicate the potential for eco-evolutionary effects in the broad sense (de Meester et al. 2019). We therefore hypothesise that the changes in the relation between larger predatory fish and their prey, mesopredators, have generate changes in selection regimes, which in turn may feedback on a variety of ecological processes (Fig. 6). Models of trophic dynamics suggest that predator effects on lower trophic levels interact with resource availability, such that the abundance of the highest (apex) trophic level increases with primary production (Fretwell 1977, Oksanen et al. 1981, Nisbet et al. 1997, Oksanen and Oksanen 2000, Eriksson et al. 2012). Thus, in a system where larger predatory fish control trophic dynamics, the larger predator community accumulates biomass. For their mesopredator prey there should be an increase in selection for antipredator traits, but no increase in density with increasing productivity (Oksanen and Oksanen 2000, Eriksson et al. 2012). However, in a system where the large predators have declined strongly, energy (biomass) accumulates at the mesopredator level, while at the same time the absence of top predators result in a relaxed selection for antipredator traits. Here, increasing productivity would lead to an increase in mesopredator density, and consequently, density-dependent competition (Oksanen and Oksanen 2000, Svanbäck and Bolnick 2007, Eriksson et al. 2012). Thus, the abundance of larger predators and the productivity of the ecosystem should together determine mesopredator abundance as well as trait distribution (Fig. 6a), which may in turn influence trophic dynamics through changes in, for example, diet composition, behaviour, and/or elemental stoichiometry; and thereby generate an eco-evolutionary feedback on food web structure (broad-sense eco-evolutionary feedback; Hendry 2017, de Meester et al. 2019)(Fig. 6b).

Figure 3. Conceptual model illustrating how the density development and trait distribution of a mesopredator population depend on the presence of a higher trophic level of predators and productivity of the system (according to the exploitation ecosystems hypothesis by Oksanen et al. 2000). With top predators, the system has four trophic levels (dotted blue line). Without top predators, the system has three effective trophic levels and mesopredators dominate the predator community (solid red line). a) In a system with three trophic levels, the mesopredator community accumulates biomass. Ecological effects: Increase in mesopredator density with increasing productivity (Oksanen and Oksanen 2000, Eriksson et al. 2012). Evolutionary effects: the absence of top-predators results in a relaxed selection for antipredator traits; and increased selection for traits favourable under density-dependent competition for food (Svanbäck and Bolnick 2007). b) Conceptual model illustrating how increased mesopredator density and resulting changes in trait distribution (caused by the combination of predator loss and increased productivity) may influence trophic dynamics by changing in energy flow (e.g. changes in diet, elemental stoichiometry, behaviour), thereby leading to a broad-sense eco-evolutionary feedback on food web structure (Hendry 2017, de Meester et al. 2019).

The hypothesis of an eco-evolutionary feedback in the Baltic Sea is partly supported by our results: the number and proportion of incompletely plated stickleback increased with production in bays with low abundances of perch, indicating a reduced selection for anti-predator traits and increased density dependent selection when larger predators of stickleback decrease. We also found small but significant differences in diets between the phenotypes, where the incompletely plated individuals consumed less amphipods; which is a key benthic herbivore regulating trophic transfer between predators and algae in many shallow marine systems across the world (e.g. Duffy and Hay 2000, Moksnes et al. 2008, Duffy et al. 2015, Donadi et al. 2017). However, to provide solid empirical predictions of eco-evolutionary consequences of the shift in selection pressures, we need experimental testing of the ecological effects of different phenotypes, behavioural studies to assess morphology-dependent mating patterns, and genetic studies to identify signatures of selection. Our current results emphasize the need to better understand how ecological feedbacks can change selective regimes,

potentially driving rapid adaptation, and the need to take these effects into account in ecosystem management strategies (de Meester et al. 2019, Hendry 2019).

Supplementary Information

Appendix S1: Extended methods and results for sampling the food-web structure in bays along the west coast of the Baltic Sea

The distribution of three-spined stickleback ecotypes and the structure of the coastal food-web was analyzed by sampling the shallow-water fish community, invertebrates, vegetation and macroalgae in 32 bays along a 360 km stretch of the central Baltic Sea coast, in spring 2014 (Fig. S1, Table S1 and S2). The sites were distributed throughout the area to achieve variation in both abiotic and biotic factors, such as salinity, exposure, fish community structure and densities of different species. The investigated bays were chosen using the following criteria:

- Distance between bays was set to a minimum of 10 km unless they were separated by naturally occurring barriers (e.g. large bodies of open water or land areas) to assure sampling fish from different populations.
- Gradient in wave exposure (moderately exposed to extremely sheltered)
- Gradient in topographic openness (open to semi-enclosed bays)
- Gradient in salinity (4-7 practical salinity units (PSU))
- Sufficient areas with a depth between 0.5-3m
- Suitable nursery areas for three-spine stickleback and pike and perch (Sundblad et al. 2011, Nyström Sandman A. et al. 2013)

Figure S1. The 32 bays sampled in 2014 along the extensive archipelago in the central part of the western Baltic Sea, Sweden.

Chapter 2

Table S1. Location of 32 bays sampled across the extensive archipelago in the central part of the western Baltic Sea, Sweden, and the selection of bays for the different analyses. Plate form analyses only included stickleback dominated bays where 30 or more 3-spine sticklebacks were found and morphotyped. Diet analyses only included bays where we had metabarcoding data from both plate phenotypes (fully or partially plated phenotypes).

Bay abbreviation	Bay name	Latitude	Longitude	Stickleback or Perch dominated	Plate-form analyses	Diet analyses
HATT	Hatten	60.382	18.249	perch	NO	NO
SAHO	Sandå Holme	60.360	18.560	perch	NO	NO
MAST	Lilla Måstensfjärden	60.36	18.56	perch	NO	NO
JARS	Järsön	60.309	18.473	stickleback	YES	NO
SFJA	Söderfjärden	60.27	18.628	perch	NO	NO
ENHO	Enholmarna - sundet Saltö	60.270	18.628	perch	NO	NO
BRAN	Brännskär	59.693	19.148	stickleback	YES	NO
SJAL	Långviken, Själbottna	59.565	18.804	stickleback	YES	YES
BYVI	Byviken	59.392	18.657	perch	NO	NO
ALGK	Älgkilen	59.388	18.937	perch	NO	NO
BOLV	Bolviken	59.383	18.53	perch	NO	YES
LOVO	Lövösundet	59.272	18.675	perch	NO	NO
KRYS	Kryssviken	59.272	18.675	stickleback	YES	YES
TORV	Torviken	59.113	18.479	perch	NO	YES
HASL	Hasslinge-Skäret	59.105	18.243	stickleback	YES	NO
HANS	Hansviken	59.028	18.023	perch	NO	YES
VARN	Värnöfladen	59.015	18.384	stickleback	YES	YES
SORV	Sörviken, Fifång	58.841	17.716	perch	NO	NO
BOCK	Bockholms-sundet	58.829	17.616	stickleback	YES	NO
LACK	Hamnhamn, Lacka	58.747	17.572	stickleback	YES	NO
OLER	Östra Lermaren	58.747	17.572	perch	NO	NO
OSVI	Östra viken	58.729	17.19	stickleback	YES	YES
KUGG	Kuggviken	58.728	17.444	perch	NO	NO
BETN	Beten	58.645	17.155	stickleback	YES	YES
LBRE	Lilla Brevik	58.644	17.021	stickleback	YES	YES
OSTA	Östantillsviken	58.409	16.892	perch	NO	NO
BROT	Brottskären	58.347	16.94	stickleback	YES	NO
SORS	Sörsundsviken	58.252	16.88	stickleback	YES	NO
SKRA	Skräckskärs-klubben	58.167	16.978	stickleback	YES	NO
BANK	Bankeböte	58.107	16.733	stickleback	YES	YES
HEMV	Hemviken Ångsö	58.004	16.719	perch	NO	NO
HUML	Norra Hummeldalen	57.949	16.79	stickleback	YES	YES

Table S2. All fish collected with gillnets in 32 bays sampled across the extensive archipelago in the central part of the western Baltic Sea, Sweden, in spring 2014. Total number of individuals is given for all species. A number of the dominating larger fish species: Perch, Pike, and Roach were dissected for stickleback in their stomachs; to evaluate their contribution to stickleback predation. See Appendix A in Donadi et al. 2017 for biomass conversions.

Scientific name	Common name	Abundance (n)	Stickleback regularly documented in stomach
<i>Abramis brama</i>	Bream	57	
<i>Alburnus alburnus</i>	Bleak	2946	
<i>Ammodytes tobianus</i>	Lesser sandeel	4	
<i>Blicca bjoerkna</i>	White bream	283	
<i>Carassius carassius</i>	Crucian carp	1	
<i>Clupea harengus</i>	Baltic herring	233	
<i>Coregonus lavaretus</i>	Whitefish	1	
<i>Esox lucius</i>	Pike	40	YES (15 out of 34 dissected individuals)
<i>Gasterosteus aculeatus</i>	Three-spined stickleback	13854	
<i>Gobius niger</i>	Black goby	7	
<i>Gymnocephalus cernuus</i>	Ruffe	910	
<i>Hyperoplus lanceolatus</i>	Great sandeel	9	
<i>Leuciscus idus</i>	Ide	16	
<i>Myoxocephalus scorpius</i>	Shorthorn sculpin	10	
<i>Nerophis ophidion</i>	Straightnose pipefish	34	
<i>Osmerus eperlanus</i>	Smelt	121	
<i>Perca fluviatilis</i>	Perch	1847	YES (124 out of 643 dissected individuals)
<i>Phoxinus phoxinus</i>	Minnow	46	
<i>Platichthys flesus</i>	Flounder	2	
<i>Pomatoschistus minutus</i> , <i>P. microps</i>	Sand goby, Common goby	55	
<i>Pungitius pungitius</i>	Ninespined stickleback	447	
<i>Rutilus rutilus</i>	Roach	3498	NO (0 out of 55 dissected individuals)
<i>Salmo trutta</i>	Trout	2	
<i>Sander lucioperca</i>	Pike-perch	1	
<i>Scardinius erythrophthalmus</i>	Rudd	25	
<i>Spinachia spinachia</i>	Fifteen-spined stickleback	1	
<i>Sprattus sprattus</i>	Sprat	27	
<i>Syngnathus typhle</i>	Broadnosed pipefish	3	
<i>Tinca tinca</i>	Tench	15	
<i>Zoarces viviparus</i>	Eelpout	9	

Fish were caught using Nordic multimesh gillnets. There is a standardized survey method in the European Union for this gear type (EN 14757:2005), which gives an estimation of the relative fish abundance and fish species distribution of the entire fish community in an area. The method is also used in the Swedish national fish monitoring program (Havs och vattenmyndigheten 2014). The Nordic gillnets were 30 meters in length, 1.5 meters in height and consists of 12 panels, each 2.5 meter in length with different mesh sizes in the following order: 43, 19.5, 6.25, 10, 55, 8, 12.5, 24, 15.5, 5, 35 and 29 mm knot-to-knot. Four nets were set in each bay at a depth between 1.5-3 meters >30 meters from each other, and >10 meters from both land and bay opening. The nets were placed between 16:00-19:00 and lifted between 07:00-09:00 the next morning. Depth of the net placement was measured at the two ends of each net when setting the net, while salinity, turbidity and time were recorded both when setting and lifting the nets. Turbidity was measured with a fluorometer (Aquafluor), salinity and temperature with a Multi 340i voltmeter (WTW, Germany).

All fish caught were identified to species, measured (total length, closest cm) and counted. The number of individuals of each fish species caught in all four nets were pooled and expressed as catch per unit effort (CPUE). CPUE was then used as an estimate of the fish densities within each bay. In each bay, we randomly collected 30 three-spined sticklebacks from the total amount of stickleback caught in the nets and stored them in plastic vials with ethanol (analytical grade 95 %). If less than 30 was caught in a bay, all individuals were collected.

Vegetation cover, the biomass of epifauna, zooplankton abundance, temperature and salinity, were measured at three to six randomly distributed stations; the number of stations depended on the size of the bay (Donadi et al. 2017). A station was defined as a 5 m-diameter area around a pre-selected way-point (marked with a buoy) at a depth of 0.5–3 m. Each station was placed at least 30 m apart. The percentage cover of macrophytes on the bottom (vegetation cover) was visually estimated by a snorkeler in three randomly placed 0.5 x 0.5 m frames, within the large area Fig. S2a). Epifauna was collected by a snorkeler in a randomly placed 0.2 x 0.2 m frame connected to a 1 mm-mesh bag (Fig. S2b). The randomization of frames was accomplished by the snorkeler, by closing her/his eyes, diving down in the water column and placing the frame blindly on the bottom (Fig. S2c). After identification in the laboratory, the biomass of the epifauna was estimated as gram ash-free dry mass using taxon-specific length : AFDM correlations. Zooplankton was collected using Apstein nets: a conical net with a mesh size of 80 μ m connected to a circular frame with an opening of 25 cm. 3-5 vertical hauls were taken at each station: in deeper water 3 vertical hauls were taken and in shallower water 5, to make water volume more comparable. The collected samples were immediately fixed in formaline. Zooplankton was later identified to

taxon level in the in the laboratory. Temperature and salinity of the water was measured using a multi-measurer (Multi 340i voltmeter, WTW, Germany).

Figure S2. Sampling a) vegetation cover and b) biomass of vegetation and epifauna, using quadrats randomly placed on the bottom at 0.5–3 m depth, within a 5 m-diameter area around each pre-selected way-point. c) snorkeler closing her eyes and diving to randomly place quantitative quadrat. d) settling plates glued a brick, placed on the bottom on a net to minimize overgrowing vegetation. Temperature logger is attached to the brick.

The development of macroalgal cover was estimated using algal settling plates. A settlement plate consisted of a 5 x 5 cm unglazed ceramic tile. In each bay we placed two clay bricks with two tiles each (total area 1 dm²) glued to the surface, at ca 1.5 m depth. To avoid shading and over-sedimentation, we placed the bricks in areas devoid of vegetation and on as hard substrate as possible (Fig. S2d). The bricks were placed in the water by snorkeling during the fish sampling, and extracted from each bay three months later in August 2014. The tiles were brought to the lab and total accumulation of filamentous macroalgal cover over the summer was determined by overlaying a 5 x 5 cm plexiglas sheet with 25 random dots and counting how many dots that covered

an algae. Determining algal cover accumulation over a longer time period on settlement plates, is a good way to compare net production of algae between different experimental units in the Baltic Sea (e.g. Eriksson et al. 2006, Eriksson et al. 2009, Eriksson et al. 2012). However, it is a relative measure that can only be compared within a study. Filamentous macroalgae respond to nutrient enrichment by fast accumulation of biomass and are grazed on by small invertebrate mesograzers (Worm et al. 2002, Råberg and Kautsky 2007). Mesograzers are in turn eaten by fish and play a key role in the trophic cascades earlier demonstrated in the system (Eriksson et al. 2009, Donadi et al. 2017).

We characterized the physical environment of the bays by estimating topographic openness, wave exposure and distance to the open sea. Bay topographic openness (E_a) is defined as $E_a = 100 \times A_t/a$: where A_t is the cross sectional area of the bay opening, calculated by measuring depth and width of the smallest opening of the bay towards the sea (depth was measured in the field, width using satellite images and GIS methods); and a is the bay surface area estimated from satellite images using GIS methods. Wave exposure was estimated for each bay using a standard wave model based on fetch, topography and wind conditions (Buschbaum et al. 2016). The baseline is calculated by drawing the shortest distance in a straight line between the outermost islands in a nautical chart.

Other explanatory variables related to nutrient availability and plankton production (N and P concentrations, turbidity and fluorescence of the water) was also recorded at each station.

Figure 4. Histogram showing the number of bays with different frequency of three-spined stickleback compared to perch. If the bay had stickleback present but no perch, the frequency had the value 1; and if the bay had perch present but no stickleback, the frequency had the value of 0.

Appendix S2: Morphological determination of 3-spined stickleback

In total, we determined the plate genotype (partial or full plating) for 560 three-spined stickleback individuals by measuring morphological characteristics. Eleven length parameters were documented from all individuals to the nearest millimeter using a Vernier caliper, according to Jones et al. (Jones et al. 2012): (1) total length; (2) standard length; (3) length of first spine; (4) length of second spine; (5) length of third spine; (6) length of caudal peduncle; (7) depth of caudal peduncle; (8) length of pelvic girdle; (9) head length; (10) width of pectoral fin base; (11) length of upper lip. Then the number of lateral plates were counted on both left and right sides to produce average number of later plates for each individual. The method for counting plates was that used by Aneer (1974). Plates were counted starting immediately after the operculum to the end of the caudal peduncle. Three-spined stickleback have three types of plates: plates near the operculum are typically circular, dermal formations which are not attached to any other body structure; plates along the sides of the body are typically oval or rectangular and are also not attached to other body structures; and 3) caudal plates vary in shape from circular to square to diamond-shaped and can be unattached to other body structures or can be the base of caudal scutes. We determined the plate phenotype by looking at gaps in the plate armour: partially plated fish had a clear gap between the main body plates and the caudal plates (the width of at least 2 body plates); fully plated fish had no clear gap in the plating. The entire length of the body of the fish is examined for presence of plates even if a gap is present to ensure plates are not missed. In total we morphotypes 560 individuals. The distribution of number of lateral plates overlapped slightly between the fully and partially plate phenotypes, but showed a clearly bimodal distribution with peaks at 15 and 29 plates for partially and fully plated individuals, respectively. We found no low plated individuals.

Appendix S3: Results from model selections of three-spined stickleback numbers and plate phenotypes

We tested which factors that best described stickleback abundances (# CPUE) and plate phenotype distribution (proportion of partially plated) by comparing General Linear Models including explanatory factors related to: spatial structure (latitude, longitude), physical characteristics (wave exposure; depth [average depth of sampling points in bay]; and topographic openness [Ea]), prey availability (mesograzers and zooplankton), water temperature (at sampling), nutrient availability (the limiting nutrient phosphorus), and predation pressure (cpue perch >25 cm). We selected the best linear model (lm function) for stickleback abundances and plate distribution based on AICc (AIC for small samples); using the MuMIn package in R 3.5.2 (version 1.43.15; R Core Team, 2018). Data was square root (# stickleback; Ea and # perch > 25 cm), log10 (# zooplankton, # mesograzers; temperature) or logit (plate phenotype) transformed, to fit assumptions of normality. The logit transformation of plate phenotype was calculated as the natural logarithm of the proportion of partially plated individuals divided by the proportion of fully plated individuals (LN[proportion partially plated/proportion fully plated]).

Table 3 Table S1. Model building results for the number of three-spined stickleback in different bays (#cpue). Normally distributed data with identity link function. The ranking is based on AICc. Only the 20 best models of 200 iterations are showed. Nutrient availability (phosphorus) did not enter any of the 20 best models.

rank	model	depth	latitude	temperature	zooplankton #	longitude	grazer biomass	topographic openness (Ea)	perch >25 cm #cpue	wave exposure	df	AICc	delta	weight
1		-	-	-	-	-	-	19.47	-2.26	-	4	253.19	0.00	0.070
2		-	-	-	-4.42	-	-	20.14	-2.18	-	5	253.44	0.25	0.062
3		-	-	-	-	-	-	21.11	-	-	3	254.38	1.20	0.039
4		-	-	-	-4.66	-	-	21.76	-	-	4	254.46	1.27	0.037
5		0.49	-	-	-	-	-	17.95	-2.55	-	5	254.90	1.71	0.030
6		-	-	-	-	-3.38	-	20.82	-	-	4	255.21	2.03	0.025
7		-	-3.83	-	-	-	-	20.31	-	-	4	255.28	2.10	0.025
8		-	-	-	-4.59	-3.31	-	21.47	-	-	5	255.43	2.24	0.023
9		-	-2.21	-	-	-	-	19.22	-1.97	-	5	255.45	2.26	0.023
10		-	-	-	-	-1.85	-	19.54	-1.95	-	5	255.50	2.32	0.022
11		-	-3.59	-	-4.47	-	-	20.98	-	-	5	255.65	2.46	0.021
12		0.37	-	-	-4.01	-	-	18.95	-2.40	-	6	255.85	2.66	0.019
13		-	-	-	-4.41	-1.83	-	20.21	-1.87	-	6	255.94	2.76	0.018
14		-	-2.02	-	-4.34	-	-	19.90	-1.91	-	6	255.98	2.79	0.017
15		-	-	-	-	-	-	19.35	-2.22	0.24	5	255.99	2.81	0.017
16		-	-	-	-	-	0.32	19.34	-2.25	-	5	256.01	2.82	0.017
17		-	-	0.52	-	-	-	19.52	-2.30	-	5	256.01	2.82	0.017
18		-	-	-8.35	-	-	-	20.04	-	-	4	256.17	2.98	0.016
19		-	-	-8.12	-4.62	-	-	20.71	-	-	5	256.42	3.24	0.014
20		-	-	-	-4.45	-	-	19.96	-2.12	0.38	6	256.44	3.25	0.014

Table S2. Model building results for the proportion of the plate phenotype distribution of three-spined stickleback in different bays (proportion of partially plated / proportion of fully plated). Normally distributed data with identity link function. The ranking is based on AICc. Only the 20 best models of 200 iterations are showed. Wave exposure did not enter any of the 20 best models.

rank	depth	latitude	temperature	zooplankton #	longitude	nutrient availability (P)	grazer biomass	topographic openness (Ea)	perch > 25 cm #cpue	df	AICc	delta	wei
1	-	-	-	-	-	-	-	0.75	-0.20	4	29.95	0.00	0.1
2	0.07	-	-	-	-	-	-	-	-0.22	4	31.04	1.09	0.0
3	0.05	-	-	-	-	-	-	0.55	-0.23	5	31.35	1.40	0.0
4	-	-	-	-	-	0.05	-	1.03	-0.19	5	32.38	2.43	0.0
5	-	-	-	-	-	-	-	0.64	-	3	32.64	2.69	0.0
6	-	-	-	-	-	-	0.29	0.75	-0.20	5	32.75	2.80	0.0
7	-	-	-	-	-	-	-	-	-0.17	3	33.38	3.43	0.0
8	0.08	-	-	0.21	-	-	-	-	-0.23	5	33.39	3.44	0.0
9	-	-	-	-	-	-	-	-	-	2	33.54	3.59	0.0
10	-	-	-1.30	-	-	-	-	-	-	3	34.05	4.10	0.0
11	0.06	-	-1.44	-	-	-	-	-	-	4	34.12	4.17	0.0
12	-	-	-	-	-0.24	-	-	0.71	-	4	34.13	4.18	0.0
13	-	-	-	-	-	0.06	-	1.00	-	4	34.16	4.21	0.0
14	-	-	-1.07	-	-	-	-	0.57	-	4	34.16	4.21	0.0
15	0.07	-	-	-	-	-	0.27	-	-0.22	5	34.18	4.23	0.0
16	0.05	-	-	-	-	0.05	-	0.83	-0.21	6	34.20	4.25	0.0
17	0.05	-	-	-	-	-	-	-	-	3	34.21	4.26	0.0
18	-	-	-	-	-0.05	-	-	0.75	-0.19	5	34.21	4.27	0.0
19	-	-	-	-0.03	-	-	-	0.76	-0.20	5	34.26	4.31	0.0
20	-	0.02	-	-	-	-	-	0.75	-0.20	5	34.30	4.35	0.0

Appendix S4: NDMS ordination results of diet composition

Figure S1. Ordination diagram showing NDMS plot of diet composition in the stomachs of 172 three-spined stickleback from the Baltic Sea. The blue arrow shows the direction of individuals with a higher number of lateral plates. The circles show individuals that are fully plated. Triangles show individuals that are partially plated. The diagram shows that amphipods, bivalves, mysids and polychaetes contribute most to the variation in diet along the ordination axes.

CHAPTER

03

Predator biomass and vegetation influence the coastal distribution of threespine stickleback morphotypes

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Intraspecific niche differentiation can contribute to population persistence in changing environments. Following declines in large predatory fish, eutrophication, and climate change, there has been a major increase in the abundance of threespine stickleback (*Gasterosteus aculeatus*) in the Baltic Sea. Two morphotype groups with different levels of body armor - completely plated and incompletely plated - are common in coastal Baltic Sea habitats. The morphotypes are similar in shape, size, and other morphological characteristics and live as one apparently intermixed population. Variation in resource use between the groups could indicate a degree of niche segregation that could aid population persistence in the face of further environmental change. To assess whether morphotypes exhibit niche segregation associated with resource and/or habitat exploitation and predator avoidance, we conducted a field survey of stickleback morphotypes, and biotic and abiotic ecosystem structure, in two habitat types within shallow coastal bays in the Baltic Sea: deeper central waters and shallow near-shore waters. In the deeper waters, the proportion of completely-plated stickleback was greater in habitats with greater biomass of two piscivorous fish: perch (*Perca fluviatilis*) and pike (*Esox lucius*). In the shallow waters, the proportion of completely-plated stickleback was greater in habitats with greater coverage of habitat-forming vegetation. Our results suggest niche segregation between morphotypes, which may contribute to the continued success of stickleback in coastal Baltic Sea habitats.

Introduction

Large-scale changes to terrestrial and aquatic ecosystems are increasing worldwide as a consequence of human activity. The acceleration of climate change as well as overfishing and eutrophication have changed many marine biological communities and impacted marine food web functions (Pauly et al. 1998, Worm and Paine 2016). The probability of population persistence in the face of environmental change is often related to niche variation at individual or group level within the population (Durell 2000). For example, when predation on a population is relaxed as a consequence of top predator loss, the resulting increased intraspecific competition for food between phenotypically similar individuals can promote divergence in trophic resource exploitation (Zandonà et al. 2017). For example, when predation on a population is relaxed as a consequence of top predator loss, the resulting increased intraspecific competition for food between phenotypically similar individuals can promote divergence in trophic resource exploitation (Zandonà et al. 2017). Regardless of the cause of increased intraspecific competition, individual niche segregation can reduce intraspecific competition (e.g. in polymorphic cichlid populations: Swanson et al. 2003). Different selective forces are present in different niches. Thus, the continued use of separate niches exposes different sub populations to different selective pressures which favor expression of polymorphic traits related to resource exploitation for which each group of individuals will occupy a different adaptive peak. This can be followed by reduced gene flow between groups, reproductive isolation, and, if persisting for a long enough time, ecological speciation (Smith 1966, Bush 1994, Rice and Hostert 1993). Niche expansion in response to environmental challenges is evidence that individuals can utilize resources beyond their typical ancestral range and can be indicative of populations' evolutionary potential (Bolnick et al. 2003). Therefore, understanding niche variation in a population gives clues as to whether or not the population can persist through predicted environmental change.

In this study, we investigate niche segregation of threespine stickleback (*Gasterosteus aculeatus*) morphotypes that co-occur in coastal bays in the Baltic Sea. The threespine stickleback (hereafter referred to as “stickleback”) is a small mesopredatory fish that shows rapid convergent evolution of functionally similar morphotypes across the northern hemisphere in response to environmental variation (McKinnon and Rundle 2002, McGee and Wainwright 2013). Populations are known to repeatedly diverge into morphotype groups that inhabit distinctly different habitats (Schluter and McPhail 1992, Bentzen and McPhail 1984, Bentzen et al. 1984). However, within a single population, sympatric morphological groups with different ecological behavior may also emerge (Harmon et al. 2009). Stickleback have a broad distribution in freshwater, brackish, and marine habitats (Bell and Foster 1994). Their success, particularly in

areas with depleted predator populations, often leads to high intraspecific competition, which may generate disruptive or divergent selection and favor niche segregation (Schluter and McPhail 1992, Schluter 1994, Bolnick 2004, Schluter 2010). Divergent adaptation in stickleback is largely mediated by standing genetic variation that enables the repeated divergence in morphology that culminates in different morphotypes (Colosimo et al. 2005, Schluter and Conte 2009, Jones et al. 2012). The morphotypes are characterized by differences in feeding behavior, body size and shape, and the extent and pattern of body plating (reviewed in McKinnon and Rundle 2002). Lateral bony armor plates replace scales on stickleback, and the extent to which they cover the entire body defines the plate morphotype of the individual (Hagen and Gilbertson 1973). Different morphotypes often occupy separate niches in terms of prey and habitat type, especially in relationship to the predator landscape (Bentzen et al. 1984, Schluter 2010, Des Roches et al. 2013). Stickleback with greater plate numbers, especially those that have no gaps in their plate coverage, are thought to be better adapted to environments with higher predator density (Reimchen et al. 2002). Stickleback with fewer plates have greater maneuverability in the form of maximum velocity and displacement during fast-start movement, which may give them an advantage when avoiding predators such as birds and piscivorous fish in densely vegetated areas - predators that attack with fast strikes but do not pursue their prey (Reimchen 1992, Bergstrom 2002). In addition, high population densities may favor the evolution of adaptations that increase foraging efficiency (as seen in e.g. guppies, Zandonà et al. 2017). Fast-starts can facilitate catching evasive prey and could be particularly advantageous in areas with high intraspecific competition, implying that stickleback with fewer plates and better fast-starts might be favored in habitats with high intraspecific competition (Rand and Lauder 1981, Harper and Blake 1988).

In the Baltic Sea, a combination of overfishing, eutrophication, and climate change has restructured offshore and coastal food webs which includes a dramatic increase in stickleback abundance (Alheit et al. 2005, Casini et al. 2008, ICES 2010, Ljunggren et al. 2010, Eriksson et al. 2011, Bergström et al. 2015). Since the 1990s, adult stickleback have increased up to 45-fold in offshore areas and up to 25-fold in nearshore areas along the Swedish coast (Bergström et al. 2015). In shallow bay habitats along the Swedish coast in the central Baltic Sea, stickleback populations show a bimodal distribution in the number of lateral plates, with peaks at 15 and 29 plates, suggesting the presence of at least two different morphotypes (Aneer 1974, Eriksson et al. 2021, Fig. 1b). Plates of the individuals that fall around the first peak (hereafter referred to as “incompletely plated”) are interrupted by one or more gaps, while plates of the individuals that fall around the second peak (hereafter referred to as “completely plated”) run uninterrupted from immediately behind the operculum to the end of the caudal peduncle. Incompletely-plated stickleback are typically separated

into two morphotypes based on the presence or absence of caudal plates which form a keel. Those with caudal plates are designated as partially plated and those without as low plated. In the Swedish coastal populations, individuals without a keel are extremely rare. In two sampling seasons, Aneer (1974) found only two low-plated stickleback (out of 1791 individuals) and excluded them from analysis. In a sample of 560 stickleback caught during one season, Eriksson et al. (2021) did not observe any low-plated individuals, but found a few intermediate phenotypes with a keel on only one side of their body. They therefore categorized all individuals with gaps in plating the width of two or more plates as incompletely plated, as we do here.

In the Baltic Sea, completely- and incompletely-plated stickleback live intermixed in an apparently single population; apart from the differences in the number of plates, they are morphologically similar. These plating patterns correspond to those exhibited by stickleback in other geographic regions, such as the Pacific northwest of America and brackish water habitats in Norway (Hagen and Gilbertson 1973, Østbye et al. 2018). While stickleback have been extensively studied in coastal Baltic Sea habitats for decades, few studies have considered the morphotypes in these populations and little is known about whether they exhibit niche segregation (Candolin and Voigt 1998, Sieben et al. 2011, Byström et al. 2015, Jakubavičiūtė et al. 2016, Olsson et al. 2019, Saarinen and Candolin 2020). Recent evidence does indicate that while both morphotypes consume insects, zooplankton, and benthic invertebrates, completely-plated stickleback consume more amphipods than incompletely-plated stickleback. In addition, incompletely-plated individuals make up a greater proportion of the stickleback population in areas of lower piscivorous fish biomass and greater benthic production, suggesting a trade-off between predator defense and resource utilization (Eriksson et al. 2021). Exploitation of different resources by morphotypes could have substantial impacts on coastal food webs in the Baltic, as stickleback populations dominate fish communities in many areas (Sieben et al. 2011, Staveley et al. 2020). For example, different relative abundances of Canadian lake benthic and limnetic stickleback used in a mesocosm experiment had different impacts on the biological community by modifying zooplankton species composition, algal biomass, primary productivity, dissolved organic content composition, and light transmission (Harmon et al. 2009).

Figure 5. Plate patterns and frequencies of stickleback morphotypes commonly found in the Baltic Sea. a) The three most common morphotypes in the Baltic Sea: 1) completely plated, 2) partially plated, and 3) low plated, b) the frequency of the morphotypes, partially plated and low plated are grouped into an incompletely plated category, overlap represents plate counts that were present in both morphotype groups, and c) Parameters measured for morphological analysis. (1) total length, (2) standard length, (3) length of first dorsal spine, (4) length of second dorsal spine, (5) length of third dorsal spine, (6) length of caudal peduncle, (7) depth of caudal peduncle, (8) length of pelvic spine, (9) head length, (10) width of pectoral fin base, (11) length of upper lip.

The present study was designed to determine if sympatric stickleback morphotypes exhibit niche segregation by occupying different microhabitats. We measured the abundance of completely- and incompletely-plated stickleback in different microhabitats in coastal bays along the Swedish coast to document potential microhabitat segregation. We took samples from two habitat types, assessing i) variation in fish, stickleback, and vegetation communities and water quality in deeper (≥ 1.0 m) central waters, and ii) variation in stickleback and vegetation communities and water quality in shallow waters (≤ 1.0 m) along the shoreline. In the Baltic Sea, shallow bays with high vegetation cover are characterized by a high biomass of stickleback compared to nearby bays with more areas of bare substrate, likely owing to the stickleback's preference for the protection, and breeding and feeding ground provided by vegetation (Candolin and Selin 2012, Staveley et al. 2017, Saarinen and Candolin 2020, Staveley et al. 2020). Within bays, stickleback are more abundant in

macroalgal beds of the habitat-forming brown algae *Fucus vesiculosus* (hereafter referred to as “*Fucus*”) compared to meadows of rooted, submerged vegetation and bare substrate (Gagnon et al. 2019). In those *Fucus* patches, the high density of stickleback may increase intraspecific competition for food or space, possibly leading to selection for competitive ability (foraging or territory defense), rather than antipredator traits. We therefore hypothesized that: 1) completely-plated stickleback make up a greater proportion of the stickleback community in bays where piscivorous fish are more abundant, while 2) completely-plated stickleback make up a smaller proportion of the stickleback community in vegetated patches along the shoreline.

Material and Methods

Study area

The Baltic Sea is the world’s second largest body of brackish water. It is almost completely enclosed by land, with the Danish Straits and Øresund being the only connections to the open waters of the North Sea. A strong salinity gradient is created by the input of freshwater from rivers and streams mainly in the north and saltwater from the Danish Straits in the southwest. Gradients in physical and chemical characteristics generate a diversity of habitats and biological communities (Rönnberg and Bonsdorff 2004, Zettler et al. 2007). Diversity of habitat characteristics and biological communities can be very pronounced even at small spatial scales in the Baltic Sea due to the great topographic complexity of the coastal zone. For example, the archipelago region of the central Swedish Baltic Sea contains innumerable (average depth ranges from less than 1 m to 5 m) bays created by post-glacial land uplift (Appelgren and Mattila 2005, Hansen et al. 2008). The bays vary in the amount of water exchange and wave exposure from the open sea, creating a gradient in physical isolation (Hansen et al. 2008). However, the system is relatively open with fish migrating from the offshore open water of the Baltic Sea in the spring to mate in the bays before migrating back in the autumn (reviewed in Aro 2002). Salinity in the bays ranges from around 1 to 7 practical salinity units (PSU). Vegetation composition consists of a mix of species with contrasting life histories, of marine or freshwater origin inhabiting hard or soft substrates. Open and more saline bays show higher proportions of hard substrates and perennial marine macroalgae (e.g. *Fucus*), while the most enclosed bays with organic rich sediments are dominated by rooted annual species of freshwater origin (e.g. *Chara* spp. and *Najas marina*) (Appelgren and Mattila 2005, Hansen et al. 2008). Other common and widely distributed species in the bays are the freshwater species *Stuckenia pectinata*, *Potamogeton perfoliatus*, *Myriophyllum spicatum*, *Zannichellia palustris* and the marine *Ruppia cirrhosa*. Some of these species grow drastically during the spring and summer and create variation in the habitats available for fish that migrate in during spring and summer (Berglund et

al. 2003). The invertebrate community is mainly composed of gastropods, bivalves, insects, and crustaceans (Hansen et al. 2008).

Survey

We conducted field surveys from May to July 2017 at 27 bays along the central Swedish Baltic Sea coast (Fig. 2). Sites were selected (in collaboration with the County Administrative Board of Stockholm) to represent a range of physical and biological characteristics, including habitats typical for both larger predatory fish and stickleback (Supplementary material Appendix 1 Table A1). Twenty-four of these sites were paired, with each pair containing two sites of similar physical characteristics, but one of the two was an MPA (Marine Protected Area) with a seasonal fishing ban from 1 April to 15 June, and the other was a reference site open to fishing (Supplementary material Appendix 1 Table A1). We sampled two parts of each bay, the deeper water (≥ 1 m) in the center of the bay (hereafter referred to as “center-of-bay sampling”) and the shallow waters (≤ 1 m) along the shoreline (hereafter referred to as “shallow sampling”), for stickleback morphotype abundance and environmental properties. We chose these areas because they allowed us to get a snapshot of different biological communities within the bays. The fish communities were sampled in the center-of-bay sampling using gillnets, to get a broad representation of species and size classes. The shallow sampling, on the other hand, allowed us to sample more diverse vegetation as in these areas we find rocks with algae interspersed with reed and flowering vegetation, while in the deeper parts of the bay the vegetation is often more homogenous and the substrate is dominated by rock or mud.

Figure 6. Map of sampling locations. Axis labels are latitude and longitude.

Center-of-bay sampling

To analyze variation in stickleback morphotype distribution in the central parts of the bays, we collected data on fish, vegetation, and substrate, as well as water temperature, salinity, and turbidity at 3-5 stations within each bay. Fish were sampled with 30 m long by 1.5 m high Nordic survey gillnets consisting of twelve 2.5 m long panels, each with a different mesh size: 43, 19.5, 6.25, 10, 55, 8, 12.5, 24, 15.5, 5, 35, and 29 mm knot-to-knot (European Union standardized method EN 14757:2005). Nets were placed at 1.5 to 3 m depth, following a stratified randomization. Three to five nets were set in each bay, depending on the surface area of the bay, for a total of 102 nets across 27 bays. The nets were set in the afternoon and retrieved the following morning. Fish were identified to species, measured for total length (cm), and counted. Stickleback were counted, but when more than 30 were caught only a haphazardly chosen sample of 30 individuals were measured (total length, mm) and preserved in 95% ethanol for later morphometric analysis. Total length was used to calculate biomass for all species following length/weight conversion factors from the Swedish National Database for Coastal Fish (<http://www.slu.se/kul>). Catch per unit effort (CPUE) was calculated as biomass per net night. At both ends of each net, vegetation surveys were conducted by a snorkeler who estimated the percent cover of all coarsely structured algae and plants, coverage of filamentous algae, average and maximum vegetation height, and substrate composition within a 5 m radius. Algae and plants were identified to the lowest taxonomic level possible. Substrate was categorized based on grain size of the sediment: mud, sand, gravel (2 – 20 mm), stone (20 – 200 mm), boulder (200 – 600 mm), large boulder (> 600 mm), and bedrock. We also recorded water temperature and salinity with a multimeter and fluorescence and turbidity with an *Aquaflor*® fluorometer/turbidimeter (Turner Designs, California, USA) at each net.

Shallow sampling

To analyze variation in shallow water along the shoreline, we collected data on fish, vegetation, and substrate. Fish were caught with 45 x 24 x 24 cm (length x width x height) minnow traps (KAYOBA, Skara, Sweden) with a mesh size of 3 mm and two 5.5 cm diameter round holes on each short side. To standardize the vegetation measurements around the trap, so that one measurement was always in front of the opening and one to the left and right, we closed off one hole on each trap with a cable tie. We set five traps for a total of 135 traps across 27 bays at 0.5 to 1 m depth. Traps were used instead of gillnets because the dense vegetation in some areas and the shallow depth throughout made the use of nets impossible. The traps were deployed in the afternoon and retrieved the following morning. Traps were distributed such that they covered a gradient of vegetation cover. All fish caught were identified to species, measured for total length (mm), and counted. If more than 30 stickleback were caught in a trap they were all counted, but only a haphazardly chosen sample of 30 individuals

were measured for total length (mm) and preserved in 95% ethanol for later morphometric analysis. At each trap, a vegetation survey was conducted by placing three 0.5 x 0.5 m frames within an estimated 5 m radius: one in front of the trap entrance and one on each long side of the trap. In each frame, we visually estimated the percent cover of all coarsely structured algae and plants, coverage of filamentous algae, average and maximum vegetation height, and substrate composition within the frame area. We chose to use a different method for the shallow vegetation survey than for the center-of-bay vegetation survey, because the vegetation and substrate is more diverse in the shallow areas and using this method gave us a more accurate picture of how the vegetation varied around the trap as it was rarely homogenous on all sides.

Morphometric Analysis

A total of 2085 stickleback were collected for morphometric analysis, 1672 from the center-of-bay sampling and 413 from the shallow sampling. Following the methods of Jones et al. (2012) we measured 11 length parameters using digital Vernier calipers. To account for effects of body size, especially caused by age differences between individuals, we standardized all measurements to a standard length of 50mm (Thorpe 1976, Lavin and McPhail 1985, Hagen and Gilbertson 1992). We compared morphometrics between morphotypes using Mann-Whitney U-tests. We counted the number of lateral body plates on each individual (Fig. 1c). All plates on both sides of the fish were counted, from immediately after the operculum to the end of the caudal peduncle, and morphotypes were categorized based on plate distribution: individuals with plates across the entire length of their body were categorized as completely plated, individuals with a gap in plates greater than or equal to the width of two body plates between the anterior plates and plates along the caudal peduncle were categorized as partially plated, and individuals with few anterior plates and no plates on the caudal peduncle were categorized as low plated (Fig. 1a). In our sample, two fish were low plated on both sides of their body and four individuals were categorized as partially plated on one side of the body and low plated on the other side. We therefore pooled all partially- and low-plated stickleback into the single category of incompletely plated.

Data analysis

We analyzed the distribution of stickleback morphotypes using mixed effects logistic regression models with a binomial distribution and logit link function. We ran separate models for the center-of-bay and shallow data. In both cases, the dependent variable was a two-column matrix where the first column was the number of completely-plated stickleback and the second column was the number of incompletely-plated stickleback in a sample. When analyzing the center-of-bay data, we included net nested within bay

as a random factor. Based on previous knowledge, we included the following predictor variables as fixed factors: coverage of habitat forming vegetation, piscivorous fish CPUE, maximum depth of the bay, wave exposure, bay location (inner, mid, or outer archipelago), relative latitudinal bay position (north, middle, or south), and bay topographic openness. We evaluated models with all possible combinations of predictor variables except variables which were collinear. Wave exposure was estimated from fetch and long-term wind data using digital nautical charts and GIS methods, where refraction/diffraction effects are simulated by a spreading algorithm (Isæus 2004, Sundblad et al. 2014). Bay location was categorized based on the relative shortest water distance to the open sea estimated by GIS methods (e.g. Eklöf et al. 2020). Bay topographic openness (Ea) was calculated using the formula

$$Ea = 100 At/a,$$

where At is the cross-sectional area of the smallest connection to the sea, and a is bay surface area (e.g. Håkanson 2008).

For the center-of-bay data, we only included sampling events where we caught at least 20 stickleback in the net which left us with 39 of the 101 nets that we sampled. This threshold was chosen by first visually examining the proportion of completely plated individuals caught in the net plotted against the total number of stickleback caught in the bay, and then estimating where the variation in the proportion of completely-plated individuals became consistent (Supplementary material Appendix 1 Fig. A1a). Continuous variables were on different scales so all were centered and scaled before models were run. We checked models for collinearity by calculating variance inflation factors (VIFs) of each predictor variable. Maximum depth of the bay, bay location, and relative bay position were collinear ($VIF > 2$) so they were included in separate models. When analyzing the shallow data, we included trap nested in bay as a random factor and coverage of habitat forming vegetation, *Fucus* presence, maximum depth of the bay, wave exposure, bay location, relative bay position, and bay topographic openness as fixed factors. We chose to include *Fucus* presence in the shallow data analysis as it is a perennial habitat-forming species and hence often is the dominant vegetation habitat in the spring before annual or semi-annual species have gained substantial biomass. All continuous variables were scaled and centered. Maximum depth of the bay, bay location, and relative bay position were collinear ($VIF > 2$) so they were included in separate models. For the shallow data, we ran two models, one where we only included data from traps where we caught at least three stickleback ($n=36$ traps out of the 135 traps in total) and another that only included data from traps where we caught at least 10 stickleback ($n=14$ traps). The thresholds were again chosen by visually examining the proportion of completely plated individuals caught in the trap plotted against the total number of stickleback caught and estimating where the variation in the proportion of completely plated individuals became consistent (Supplementary material Appendix 1 Fig. A1b). The thresholds were again chosen by

visually examining the proportion of completely plated individuals caught in the trap plotted against the total number of stickleback caught and estimating where the variation in the proportion of completely plated individuals became consistent (Supplementary material Appendix 1 Fig. A1b). All analyses were conducted in R v. 3.5.1 (R Core Team 2019). The models were run using the *lme4* package (Bates et al. 2015), and model selection was run based on the Akaike's Information Criterion corrected for small sample size (AICc) with the *MuMIn* package (Barton 2019). Model assumptions were checked using functions in base R as well as the *DHARMA* package (Hartig 2020).

Results

We observed a wide range of variation in piscivore biomass, vegetation coverage, stickleback abundance, and stickleback morphotype relative abundance (Supplementary material Appendix 1 Table A2). Of the 2085 stickleback collected, we morphotyped 2038 individuals (the rest were damaged during storage and transportation). We categorized 333 (16%) as completely plated and 1705 (84%) as incompletely plated (two of which were low plated). Plate counts displayed a binomial distribution, corresponding to the completely-plated and incompletely-plated morphotype groups (Fig. 1b). Most morphometric parameters, including standard length, did not differ between morphotype groups ($p > 0.05$, Supplementary material Appendix 1 Table A3). The exceptions were length of the second and third dorsal spines and caudal peduncle depth which were all greater for incompletely than completely-plated stickleback ($p < 0.05$, Supplementary material Appendix 1 Table A3).

Center-of-bay microhabitat segregation

All models had similar AICc values (AICc = 186.1, 186.6, and 188.5 for the models constructed with maximum depth of the bay, relative latitudinal bay position, and bay location, respectively) and showed the same relationship between stickleback morphotype abundance and predictor variables. Thus we focus on the model that includes maximum depth of the bay as it has the lowest AICc. Morphotype relative abundance was best predicted by piscivore biomass: bays with greater piscivore biomass had higher proportions of completely-plated stickleback ($R^2 = 0.21$, $p < 0.01$, Fig. 3, Supplementary material Appendix 1 Table A4). At low piscivore biomass, the entire range of proportions of completely-plated stickleback was present. With higher piscivore biomass, the lower range of proportions of completely-plated stickleback disappeared, resulting in a narrow range of relatively high proportions of completely-plated stickleback (Fig. 3).

Figure 7. Relationship between piscivore biomass and proportion of completely plated stickleback from center-of-bay sampling from nets with at least 20 stickleback. Symbol sizes represent the total number of stickleback caught in each net.

Shallow-water microhabitat segregation

All models had similar AICc values (AICc = 104.4, 104.6, and 104.8 for the models constructed with bay location, maximum depth of the bay, and relative bay position, respectively). All models showed the same relationship between stickleback morphotype abundance and predictor variables. Thus we focus on the model that includes bay location as it has the lowest AICc. Morphotype relative abundance from the shallow-water sampling was best predicted by vegetation coverage around the traps, at both thresholds (traps with at least three and 10 stickleback; Supplementary material Appendix 1 Table A5 and A6, respectively). In both models there was a positive relationship between vegetation coverage and proportion of completely-plated individuals ($R^2=0.23$, $p<0.01$ and $R^2=0.41$, $p<0.01$, respectively; Fig. 4).

Figure 8. Relationship between percent coverage of habitat forming vegetation and proportion of completely plated stickleback from shallow sampling. Symbol sizes represent the total number of stickleback caught in each trap.

Discussion

We investigated habitat use of two stickleback morphotypes in coastal Baltic Sea populations in order to determine whether or not they exhibit intraspecific niche partitioning. Within the Baltic Sea stickleback population there is variation in plate morphology (Aneer 1974). This study documents that this variation is associated with ecological differentiation along two axes of habitat variation. First, comparison of morphotype relative abundance in deeper central waters of the bays, confirmed our hypothesis and earlier findings (Eriksson et al. 2021) that the proportion of completely-plated stickleback morphotypes increases with increasing abundances of fish predators. Second, comparison of morphotype relative abundances in shallow waters along the shoreline shows that the completely-plated morphotype is associated with a higher vegetation cover.

Disruptive selection which favors two morphotypes – one completely and one incompletely plated – is a recurrent pattern in freshwater stickleback populations (Zeller et al. 2012a, Marchinko et al. 2014). In these populations, the completely-plated morphotype is morphologically similar to the marine ancestor; it likely invaded from open water where it preferentially feeds on zooplankton and occupies the same habitat as large piscivorous fish (Taylor and McPhail 2000). Body plating has been shown to serve as protection against predation and completely-plated stickleback typically

occupy niches dominated by predators (Reimchen 1992, Reimchen 1994, Reimchen 1995). While full plating offers better protection against predation, low plating gives a higher body flexibility and possibly protection against insect predators, which may explain why less plated stickleback typically dominate in habitats with low piscivorous fish abundances (Reimchen 1994, Zeller et al. 2012a). This is also attributed to variation in other morphological features. Comparisons of other stickleback populations show that dorsal spine length is positively correlated with piscivorous fish abundance and body plate number (Hagen and Gilbertson 1972). It has also been suggested the dorsal spines and other bony structures are used by predatory insects to grasp onto stickleback (Reimchen 1980). However, this spine reduction is in the number of dorsal spines which did not occur in our population (no individual had <3 dorsal spines) and an experimental study by Zeller et al. (2012b) showed no association between dorsal spine length and predation by insects. We did not collect data on predatory insects so we cannot speak to this relationship. Despite a lack of reduction in number of dorsal spines, it still follows that the incompletely-plated stickleback in our samples would have shorter spines as they are associated with lower abundances of piscivorous fish. However, length of the second and third dorsal spine are longer in the incompletely- than completely-plated stickleback and therefore do not follow the expected pattern. Caudal peduncle depth has also been shown to vary between morphotypes possibly because of use of different habitats. In a study of swimming performance in anadromous and freshwater stickleback, Taylor and McPhail (1986) showed the freshwater stickleback had deeper caudal peduncles and related this to a greater fast-start performance. Freshwater stickleback typically have fewer plates than anadromous stickleback and stickleback with less plates have been shown to have better fast-start performance (Bergstrom 2002). Freshwater stickleback occupy densely vegetated areas where their predators mainly strike but do not pursue them which makes fast-start important to survival. We found caudal peduncles to be deeper in incompletely- than completely-plated stickleback following the idea that fish which occupy areas with less piscivorous fish predators which hunt by pursuit have better fast-start performance, although this needs to be explicitly tested in our population. Our results appear consistent with the overall pattern of greater abundances of stickleback with fewer plates in areas with less piscivorous fish predators however, incompletely-plated stickleback dominated all bays; the percentage of completely-plated stickleback in the 27 bays never surpassed 40%, even at high piscivorous fish biomass (Supplementary material Appendix 1 Table A2). This may be due to the low overall piscivorous fish biomass across the bays that we sampled and the dichotomy of piscivore- or stickleback-dominated bays; in bays with piscivorous fish biomass above 2 kg there were often no stickleback at all, or too few to include in analysis (Supplementary material Appendix 1 Fig. A2). This study therefore may not have included the threshold of high piscivore biomass where completely-plated stickleback would dominate (i.e. make up $\geq 50\%$ of the stickleback community). The morphotype composition could also be influenced by the open sea fish community. Predation pressure is much lower in the open sea where stickleback spend the late autumn to early spring months and this may influence the morphotype composition of the entire population (Eriksson et al. 2011, Bergström et al. 2015). This could mean that

regardless of the fish community in the coastal bays, completely-plated stickleback will always make up a relatively low percentage of the stickleback community. Nevertheless, we observed an association between higher piscivore biomass and the relative abundance of completely-plated stickleback in the range of data that we did have, suggesting that there is some association between piscivore and stickleback morphotype abundance. In addition to piscivore abundance, or as an alternative explanation for the observed pattern, morphotype distribution may be influenced by abiotic environmental variables. Previous studies have suggested that completely-plated stickleback tolerate higher salinities than incompletely-plated stickleback and that across the stickleback's European range, higher abundances of completely-plated individuals are observed in areas with higher salinity (Heuts 1947). Salinity was never indicated as an important variable in any of the model selection analyses which may be due to the low variation in salinity between our sampling locations (4.8 to 6.3 PSU; Supplementary material Appendix 1 Table A1). Winter temperature has also been shown to influence morphotype distribution, with completely-plated individuals tolerating lower temperatures than incompletely-plated individuals (Smith et al. 2020). We did not include winter temperature in our analysis, but the temperature at the time of sampling was not selected in any of the model selection analyses as an important variable. Furthermore, the variation in temperature between our sites was mostly due to temporal variation throughout the sampling period (when water temperatures were low during early spring sampling, there were typically too few stickleback present to include in analysis) while spatial variation in temperature at any given time was minimal (Supplementary material Appendix 1 Table A1, A2). Lower pH and ion concentrations have also been associated with lower plate numbers (Bourgeois et al. 1994, Spence et al. 2013, Smith et al. 2020). However, this needs to be explicitly tested in our study area as we were unable to measure these variables.

In shallow-water sampling sites, we found a higher proportion of completely-plated stickleback in microhabitats with high densities of habitat-forming vegetation, which supports the idea that stickleback morphotypes segregate across microhabitats. However, this result goes against a well-established pattern in stickleback ecology: that incompletely-plated stickleback are more flexible in their movements and therefore better able to maneuver through dense vegetation. The finding is hence in contrast to our hypothesis that incompletely-plated stickleback would be more successful in patches with dense vegetation (Reimchen 1992, Leinonen et al. 2011). We based this hypothesis on other polymorphic stickleback populations where individuals with fewer plates are better adapted to benthic habitats and individuals with more plates are better adapted to pelagic habitats (Bjærke et al. 2010). However, Bjærke et al. (2010) found that differences in spatial distribution may be related not just to plating but to overall body shape, and showed that the largest differences in adaptation to different habitats were between low- and completely-plated stickleback while partially- and completely-plated stickleback were very similar. Our samples of incompletely-plated stickleback mainly consist of partially-plated individuals and, based on the body morphometrics that we measured, overall body shape varies little between our incompletely- and completely-plated stickleback which could minimize differences in their distribution. Spatial distribution can also be related to foraging

behavior. Stickleback with fewer plates have been shown to consume more benthic invertebrates while those with more plates have been shown to consume more zooplankton (Larson and McIntire 1993, Bjærke et al. 2010). Again, Bjærke et al. (2010) found the most pronounced differences in consumption of different resources between low and completely-plated stickleback but Larson and McIntire (1993) studied a population composed of morphotypes with no to few (<5) plates and still found pronounced differences in consumption of benthic and limnetic prey between individuals with different numbers of plates. In Swedish coastal bays similar to those in this study, completely-plated stickleback were found to consume more amphipods than incompletely-plated stickleback (Eriksson et al. 2021). The results of our study may therefore suggest that microhabitat segregation in shallow waters is based on feeding preference. In morphotype pairs outside of the Baltic Sea, a tendency to eat more amphipods is associated with stickleback inhabiting more benthic areas which are densely vegetated (Bentzen and McPhail 1984, Harmon et al. 2009, Des Roches et al. 2013). In coastal bays, amphipods are found in high densities in dense vegetation, particularly where there is *Fucus* (Schagerström et al. 2014). Our results therefore suggest that feeding preference may drive the association with dense vegetation as most patches of vegetation had some *Fucus* present. While we think that differences in feeding preferences are linked to associations with different microhabitats, we do not know to what extent it drives evolution. In other study systems, incompletely-plated morphotypes have evolved from the marine fully plated ancestor (Taylor and McPhail 2000). The partially plated morphotype may therefore be adapted to lower salinity habitats which could lead them to being in higher abundances in areas with more freshwater plants than *Fucus* and to feeding on insects more than marine amphipods (Heuts 1947, Smith et al. 2020). This is, however, conjecture and cannot be tested with our data. To begin testing these hypotheses, feeding preferences must first be examined. For this, morphometrics of feeding structures and stomach content data of the different morphotypes are needed. Gape size, suction strength, and gill raker length and number have all been shown to differ between stickleback with different diets (Schluter and McPhail 1992, Robinson 2000, McGee and Wainwright 2013). We did not collect this information from our samples, with the exception of jaw length which did not differ between our morphotypes. Alternatively, our results could be driven, similar to the center-of-bay results, by the piscivorous fish community as there is typically higher piscivorous fish biomass in densely vegetated areas (Eklöv 1997). However, sampling with minnow traps does not give us information on the piscivorous fish community (we caught a single perch and no pike in the traps). Perch are caught more effectively with gillnets and pike with rod and reel fishing. In the shallow sampling we chose to use traps because they allowed fine-scale microhabitat sampling, which would not be possible with the other methods.

Already before the recent increase in stickleback numbers, multiple plate morphotypes were present in coastal Baltic Sea habitats (Aneer 1974). Thus, the presence of multiple morphotypes is not a recent phenomenon. However, there are no historical data on morphotype frequencies, resource preference, or spatial segregation of morphotypes.

One potential caveat of our study is that we collected stickleback during the mating season (Snickars et al. 2009). In some areas of the world, stickleback morphotypes have different nesting site preferences, where one morphotype prefers to nest in dense vegetation and the other on sand and gravel; however, no previous studies have examined nesting site preference in the Baltic Sea morphotypes (Candolin 2004, Southcott et al. 2013). The segregation we see may therefore reflect not only the differences in predator avoidance and/or foraging strategy, but also nesting site preference. Thus, it may over or underestimate the effects of predators and vegetation. Future studies should look at nest site preference, and characterize (micro)habitat segregation throughout the year in the Baltic Sea as this may change depending on the season.

High abundance of stickleback has been shown to strongly impact lower trophic levels, generating trophic cascades that benefit filamentous algae, in both controlled experiments and comparative field surveys (Candolin et al. 2016, Donadi et al. 2017). The habitat segregation between the two morphotypes we observed could intensify these ecosystem effects of the stickleback increase, in at least two ways. First, it may simply allow more sticklebacks to coexist. Second, it may broaden their impact by affecting a wider range of prey groups. For example, completely-plated stickleback may deplete algal grazer populations while incompletely-plated stickleback deplete zooplankton populations. These hypotheses require further study, with possible implications for ecosystem management and restoration.

Here we have presented a snapshot of habitat use during spring by two stickleback morphotypes in the Baltic Sea. The morphotypes are unevenly distributed across microhabitats. Combined with previously documented differences in diet (Eriksson et al. 2021), this suggests niche partitioning. In other ecosystems, stickleback are well known for rapid adaptive evolution (Colosimo et al. 2005, Kitano et al. 2008). Our study is notable because coastal bays in the Baltic Sea are highly connected to the open sea, and the different morphotypes seem to be fully overlapping in geographic distribution. Other sympatric morphotypes differ noticeably in morphology and utilize different spawning sites (i.e. different parts of a watershed or river) and thus have some restrictions to gene flow (Kume et al. 2010, Marques et al. 2016). Here we show that even in an open system with constant multidirectional migration where morphotypes do not exhibit strong morphological differentiation other than plate number, there is affinity for different microhabitats. The slight habitat segregation may indicate ongoing directional evolutionary change (Bolnick et al. 2003, Smith 1966, Rice and Hostert 1993). Future studies should place more emphasis on understanding the mechanisms driving this habitat segregation as it is important to more fully understand how this species responds to change. This is crucial information as understanding species' responses to environmental change will help elucidate the overall response of the Baltic Sea ecosystem to ongoing and projected change.

Supplementary Information

Figure A1. Proportion of completely plated stickleback in the catches for a) center-of-bay sampling and b) shallow sampling, against the total number of stickleback in each catch.

Figure A2. Relationship between piscivore biomass (in kg) and total stickleback count from center-of-bay sampling.

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Table A1. Locations and physical characteristics of sites sampled in 2017. The table includes Site Number which corresponds to Figure 2 of the main text, the Swedish name of the site (Site Name), whether the site is a marine protected area (MPA) or not (Ref) (Status), which two bays were paired up (Bay Pair), relative latitudinal bay position within the archipelago (Position), location within the archipelago relative to the mainland (Location), the deepest part of the bay in meters (Maximum Depth), the distance in meters from the baseline which is defined as the start of territorial waters at the outermost islands of the archipelago (Distance From Baseline), surface wave exposure calculated with a 25 m resolution wave model (Wave Exposure), surface area (Area), the smallest opening connected to the sea (Opening Area), a dimensionless measure of the bay topographic openness (Openness), mean time water spends in the bay (Retention Time), mean salinity (Salinity), mean water temperature (Temperature), and the days the bay was sampled (Sampling Days).

Site Number	Site Name	Latitude	Longitude	Status	Bay Pair	Position	Location	Maximum Depth (m)	Distance From Baseline (m)
1	Svalhagsviken	58.92144	17.66088	MPA	1	South	Inner	5.7	30576
2	Hägnäsviken	58.95439	17.59264	Ref	1	South	Inner	5.2	35789
3	Ryss-sundet/Byviken	58.93603	18.22834	MPA	2	South	Middle	3.5	11188
4	Kyrkviken/Utö	58.9629	18.30283	Ref	2	South	Middle	6	14399
5	Ängsöfladen	59.02605	18.53844	MPA	4	South	Outer	6	7497
6	Mörkviken/ Fjärdlång	59.0504	18.52254	Ref	4	South	Outer	6	12409
7	Hansviken	59.02753	18.02307	MPA	5	South	Inner	2.4	27752
8	Söderängsviken/ Herrön	58.9719	18.00424	Ref	5	South	Inner	4.8	25670
9	Norra fladen/Villinge	59.09632	18.61324	MPA	6	Middle	Outer	5	10511
10	Fladorna på Östra Jungfruskär	59.13937	18.68248	Ref	6	Middle	Outer	2.4	12338
11	Söderfladen	59.66354	18.86633	MPA	8	North	Inner	2.7	32473
12	Eknövik	59.66731	18.88962	Ref	8	North	Inner	3	29600
13	Gisslingöfladen	59.77396	19.16514	MPA	9	North	Middle	2.2	9877
14	Tofladen/ Gropaviken	59.83884	19.04144	Ref	9	North	Middle	6	12909
15	Tranviksfjärden	60.16216	18.78119	MPA	10	North	Outer	3	6477
16	Söderöfjärden/ Sladdarön	60.26954	18.62787	Ref	10	North	Outer	3.9	17139
17	Sunden N Sundskär	59.69214	19.14541	MPA	11	North	Outer	1	16819
18	Rödlöga/Högskär	59.59636	19.16603	Ref	11	North	Outer	3	22247
19	Dalviken/ Bodafjärden	60.20238	18.72852	MPA	7	North	Middle	6	13992
20	Rotholmaviken	60.1162	18.69427	Ref	7	North	Outer	3	11840
21	Torpe-Infjärden, Björnöfjärden	59.22687	18.54536	MPA	12	Middle	Inner	8.2	27154
22	Djurövik	59.31725	18.71796	Ref	12	Middle	Inner	5.1	31450
23	Lännäkersviken	59.11518	18.24139	MPA	13	Middle	Inner	4.5	30628
24	Askviken	59.09716	18.27829	Ref	13	Middle	Inner	4.7	28306
25	Idkroken	58.81273	17.64653	Ref	NA	South	Middle	4.1	18959
26	Skutviken	58.80534	17.67558	Ref	NA	South	Middle	3.9	17533
27	Hästdammsviken	58.8224	17.65245	Ref	NA	South	Middle	3.2	20399

Table A1. Continued

Site Number	Wave Exposure (m ² /s)	Area (km ²)	Opening Area (m ²)	Openness	Retention Time (days)	Salinity (PSU)	Temperature (°C)	Sampling Days
1	3644	1.462	2704	0.185	5.1	6.2	15.4	26/05/2017 : 27/05/2017
2	6839	0.748	2181	0.291	3.2	6.3	12.6	25/05/2017 : 26/05/2017
3	1899	0.443	509	0.115	7.6	5.9	17.8	29/05/2019 : 30/05/2017
4	2600	0.360	1183	0.328	2.7	5.9	13.1	30/05/2019 : 31/05/2017
5	7953	0.115	271	0.237	4.0	5.8	6.8	08/05/2017 : 09/05/2017
6	2211	0.087	310	0.358	2.5	5.8	6.4	09/05/2017 : 10/05/2017
7	2445	0.035	117	0.331	2.7	5.9	7.9	10/05/2017 : 11/05/2017
8	3053	0.073	217	0.297	3.1	5.9	8.1	11/05/2017 : 12/05/2017
9	3449	0.433	169	0.039	13.9	5.9	9.2	11/05/2017 : 12/05/2017
10	1820	0.259	176	0.068	10.6	5.9	7.6	10/05/2017 : 11/05/2017
11	1429	0.072	12	0.017	18.7	5.5	16.2	19/05/2017 : 20/05/2017
12	1259	0.079	1	0.001	29.2	5.5	17.4	20/05/2017 : 21/05/2017
13	1482	0.092	39	0.042	13.4	5.4	10.5	17/05/2017 : 18/05/2017
14	1547	0.129	354	0.27	3.4	5.5	11.6	18/05/2017 : 19/05/2017
15	1432	0.156	10	0.006	23.3	5.1	15.1	22/05/2017 : 23/05/2017
16	1370	0.246	258	0.105	8.1	5.1	12.6	23/05/2017 : 24/05/2017
17	1080	0.234	484	0.207	4.6	5.5	8.7	16/05/2017 : 17/05/2017
18	1568	0.068	445	0.652	1.0	5.7	6.3	15/05/2017 : 16/05/2017
19	2372	0.254	676	0.266	3.5	5.0	11.5	23/05/2017 : 24/05/2017
20	3461	0.437	531	0.121	7.2	5.0	14.4	24/05/2017 : 25/05/2017
21	2545	1.445	40	0.003	26.1	5.1	9.5	08/05/2017 : 09/05/2017
22	1841	0.182	13	0.007	22.8	4.8	9.0	09/05/2017 : 10/05/2017
23	3291	0.444	134	0.030	15.4	5.9	14.2	01/06/2017 : 02/06/2017
24	2673	0.339	39	0.012	20.6	5.8	14.8	31/05/2017 : 01/06/2017
25	42234	0.089	258	0.288	3.2	6.2	19.7	19/06/2017 : 20/06/2017
26	21772	0.035	213	0.612	1.1	6.1	14.6	21/06/2017 : 22/06/2017
27	8247	0.015	517	3.390	0.0	6.2	16.5	23/06/2017 : 24/06/2017

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Table A2. Stickleback abundance and resources surveyed which came out as significant predictors of morphotype relative abundance.

Bay	Total Stickleback (Count)				Morphotyped Stickleback (Count)				Proportion Complete Morphs			
	Nets		Traps		Nets		Traps		Nets		Traps	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
1	7.80	1 - 15	0.00	0 - 0	7.80	1 - 15	0.00	0 - 0	0.13	0.00 - 0.30	-	-
2	292.20	56 - 684	0.00	0 - 0	30.80	30 - 32	0.00	0 - 0	0.13	0.03 - 0.22	-	-
3	97.50	0 - 390	7.20	1 - 18	7.75	0 - 30	6.60	1 - 17	0.18	0.00 - 0.37	0.04	0.00 - 0.18
4	777.25	657 - 966	13.40	4 - 23	30.00	30 - 30	13.40	4 - 23	0.14	0.10 - 0.17	0.07	0.00 - 0.17
5	207.00	53 - 506	0.20	0 - 1	30.33	30 - 31	0.20	0 - 1	0.10	0.07 - 0.13	0.00	0.00 - 0.00
6	63.33	58 - 69	4.20	1 - 15	31.00	30 - 32	3.80	1 - 14	0.16	0.13 - 0.21	0.11	0.00 - 0.50
7	2.67	0 - 7	0.00	0 - 0	2.67	0 - 7	0.00	0 - 0	0.71	0.43 - 1.00	-	-
8	0.33	0 - 1	0.00	0 - 0	0.33	0 - 1	0.00	0 - 0	0.00	0.00 - 0.00	-	-
9	208.20	159 - 268	2.40	1 - 5	30.40	29 - 33	2.20	1 - 5	0.12	0.00 - 0.19	0.00	0.00 - 0.00
10	58.00	0 - 258	1.40	0 - 4	12.00	0 - 30	1.40	0 - 4	0.12	0.03 - 0.20	0.38	0.00 - 1.00
11	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	-	-	-	-
12	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	-	-	-	-
13	0.33	0 - 1	0.00	0 - 0	0.33	0 - 1	0.00	0 - 0	0.00	0.00 - 0.00	-	-
14	2.33	0 - 6	0.00	0 - 0	3.67	0 - 10	0.00	0 - 0	0.65	0.30 - 1.00	-	-
15	5.00	0 - 13	2.40	0 - 4	5.00	0 - 13	2.40	0 - 4	0.00	0.00 - 0.00	0.06	0.00 - 0.25
16	33.67	0 - 94	2.60	1 - 5	12.33	0 - 31	2.60	1 - 5	0.23	0.13 - 0.33	0.07	0.00 - 0.33
17	112.25	78 - 132	3.40	1 - 7	31.75	30 - 36	3.40	1 - 7	0.14	0.07 - 0.20	0.14	0.00 - 0.50
18	116.50	59 - 224	6.80	2 - 18	29.75	28 - 31	6.80	2 - 18	0.20	0.13 - 0.26	0.17	0.00 - 0.50
19	353.75	87 - 555	26.80	4 - 50	30.75	30 - 33	22.20	4 - 30	0.16	0.13 - 0.21	0.24	0.10 - 0.33
20	34.75	10 - 107	1.00	0 - 4	15.75	10 - 31	1.00	0 - 4	0.26	0.10 - 0.33	0.00	0.00 - 0.00
21	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	-	-	-	-
22	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	0.00	0 - 0	-	-	-	-
23	6.20	0 - 16	0.00	0 - 0	6.20	0 - 16	0.00	0 - 0	0.12	0.00 - 0.22	-	-
24	117.40	8 - 198	2.20	0 - 5	25.20	8 - 31	2.20	0 - 5	0.26	0.15 - 0.38	0.07	0.00 - 0.20
25	810.33	34 - 1288	7.60	3 - 15	30.00	30 - 30	7.60	3 - 15	0.20	0.17 - 0.27	0.04	0.00 - 0.13
26	1068.33	947 - 1281	4.00	1 - 14	30.00	28 - 32	4.00	1 - 14	0.15	0.07 - 0.29	0.14	0.00 - 0.50
27	951.00	842 - 1164	1.40	0 - 5	30.00	30 - 30	14.00	0 - 5	0.16	0.13 - 0.20	0.13	0.00 - 0.40

Table A2. Continued

Coverage of Habitat Forming Vegetation (%)					Fucus Presence	Piscivore Biomass (kg)	
Nets			Traps		Traps	Nets	
Bay	Average	Range	Average	Range	# Traps	Average	Range
1	13.10	5.00 - 42.50	98.67	80.00 - 116.67	5	1.39	0.56 - 2.76
2	65.00	33.00 - 95.00	87.40	67.67 - 104.67	5	0.34	0.19 - 0.55
3	22.13	8.00 - 56.00	62.07	18.00 - 106.67	3	9.43	5.55 - 12.50
4	82.88	78.00 - 86.50	45.20	26.00 - 78.33	5	0.75	0.00 - 1.55
5	15.33	5.50 - 28.00	3.27	0.00 - 7.33	2	0.24	0.00 - 0.44
6	13.00	2.00 - 32.50	9.47	0.33 - 36.67	5	0.42	0.00 - 1.00
7	8.67	5.50 - 10.50	5.47	0.00 - 20.00	0	0.37	0.09 - 0.65
8	2.17	0.50 - 5.00	14.33	0.00 - 30.00	1	1.05	0.17 - 2.58
9	50.40	3.00 - 97.00	83.73	11.00 - 111.67	5	0.73	0.00 - 3.02
10	12.30	4.50 - 21.5	0.47	0.00 - 1.33	0	5.40	0.11 - 12.82
11	83.83	65.00 - 98.00	3.67	0.00 - 10.00	0	3.26	0.51 - 5.70
12	1.67	0.00 - 2.50	10.13	0.00 - 46.67	0	2.68	1.09 - 5.04
13	35.33	8.50 - 60.00	9.00	0.00 - 25.00	1	3.66	1.13 - 5.59
14	2.33	1.50 - 3.50	30.07	11.67 - 50.00	2	3.13	1.08 - 6.54
15	65.33	30.50 - 89.50	90.17	65.33 - 111.50	5	2.06	1.50 - 2.85
16	22.17	1.50 - 61.5	34.47	0.00 - 73.33	0	1.93	0.60 - 3.59
17	47.38	12.00 - 92.00	98.47	25.00 - 165.00	5	0.63	0.42 - 1.05
18	18.75	2.00 - 45.00	136.07	31.00 - 192.33	5	0.00	0.00 - 0.02
19	21.00	2.00 - 50.00	83.43	48.33 - 98.33	5	0.69	0.10 - 0.96
20	17.75	0.50 - 35.50	39.00	20.00 - 66.67	0	1.12	0.57 - 1.66
21	23.75	5.00 - 77.5	21.33	3.33 - 36.67	0	3.45	1.70 - 7.99
22	13.88	10.00 - 18.00	5.33	0.00 - 26.67	0	4.61	0.85 - 8.91
23	28.00	2.00 - 67.00	29.93	9.33 - 53.33	2	1.45	0.53 - 4.17
24	23.10	11.50 - 39.50	43.87	4.00 - 100.67	4	2.21	1.20 - 4.64
25	33.50	27.50 - 43.00	62.13	40.67 - 80.00	5	0.22	0.03 - 0.41
26	56.50	33.50 - 82.50	82.20	33.33 - 101.67	5	0.31	0.22 - 0.43
27	38.17	16.50 - 65.50	113.13	106.33 - 122.67	5	0.40	0.15 - 0.70

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Table A3. Comparison of morphometric characteristics between completely and incompletely-plated stickleback measured by Mann-Whitney U-tests; *, $p < 0.05$.

	p
Total Length	0.706
First Dorsal Spine Length	0.268
Second Dorsal Spine Length	0.034*
Third Dorsal Spine Length	0.016*
Caudal Peduncle Length	0.405
Caudal Peduncle Depth	0.017*
Pelvic Spine Length	0.981
Head Length	0.462
Pectoral Fin Base Width	0.212
Upper Lip/Jaw Length	0.749

Table A4. Model selection for mixed effects logistic regression model predicting morphotype relative abundance of center-of-bay sampling showing the 10 best models as ranked by AICc. Model formula: Percent Completely Plated ~ Cover of Habitat Forming Vegetation + Openness + Maximum Depth + Piscivores + Wave Exposure + (1|Bay Number/Net).

Model	AICc	Delta	Weight
Piscivores	176.4	0.00	0.300
Piscivores + Maximum Depth	178.2	1.84	0.120
Piscivores + Vegetation	178.4	2.07	0.106
Piscivores + Wave Exposure	178.6	2.24	0.098
Piscivores + Openness	179.0	2.64	0.080
Piscivores + Maximum Depth + Vegetation	180.4	4.03	0.040
Piscivores + Maximum Depth + Wave Exposure	180.7	4.28	0.035
Piscivores + Vegetation + Wave Exposure	180.8	4.40	0.033
Piscivores + Maximum Depth + Openness	181.0	4.58	0.030
Piscivores + Vegetation + Openness	181.3	4.88	0.026

Table A5. Model selection for mixed effects logistic regression model predicting morphotype relative abundance of shallow sampling for traps with a cutoff of at least three stickleback per trap showing the 10 best models as ranked by AICc. Model Formula: Percent Completely Plated ~ Cover of Habitat Forming Vegetation + Fucus Presence + Openness + Wave Exposure + Bay Location + (1|Bay Number/Trap).

Model	AICc	Delta	Weight
Vegetation	94.9	0.00	0.296
Vegetation + Openness	96.4	1.52	0.138
Vegetation + Fucus Presence	97.3	2.42	0.088
Vegetation + Bay Location	97.5	2.64	0.079
Vegetation + Wave Exposure	97.6	2.69	0.077
Vegetation + Openness + Fucus Presence	98.3	3.47	0.052
Vegetation + Openness + Wave Exposure	99.2	4.29	0.035
Vegetation + Openness + Bay Location	99.2	4.36	0.033
Intercept Only	99.8	4.94	0.025
Vegetation + Fucus Presence + Bay Location	100.0	5.16	0.022

Table A4. Model selection for mixed effects logistic regression model predicting morphotype relative abundance of shallow sampling for traps with a cutoff of at least 10 stickleback per trap showing the 10 best models as ranked by AICc. Model Formula: Percent Completely Plated ~ Cover of Habitat Forming Vegetation + Fucus Presence + Openness + Wave Exposure + Bay Location + (1|Bay Number/Trap).

Model	AICc	Delta	Weight
Vegetation	53.4	0.00	0.608
Intercept Only	55.8	2.39	0.184
Vegetation + Wave Exposure	58.7	5.33	0.042
Vegetation + Openness	58.9	5.47	0.039
Vegetation + Bay Location	59.0	5.57	0.038
Bay Location	59.4	6.03	0.030
Wave Exposure	59.6	6.23	0.027
Openness	60.0	6.62	0.022
Wave Exposure + Bay Location	64.2	10.77	0.003
Openness + Bay Location	65.0	11.60	0.002

CHAPTER

04

Warming modifies the effects of intraspecific variation in stickleback morphology on benthic invertebrates and primary producers in a mesocosm experiment

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Variation within species affects ecosystem structure and functioning. The nature and strength of these effects may be influenced by climate change. To assess how temperature variation affects the ecological impact of intraspecific morphological diversity, we conducted a mesocosm experiment with threespine stickleback (*Gasterosteus aculeatus*), a model species for adaptive radiation that inhabits various habitats that are undergoing environmental change. We introduced different combinations of stickleback plate phenotypes (completely plated, incompletely plated or mixed) at two temperature treatments (ambient and + 4°C) and assessed community composition at lower trophic levels. Under elevated temperature, completely-plated stickleback consumed more amphipods, while incompletely-plated stickleback consumed fewer amphipods under increased temperature. There was an interaction effect between temperature and plate phenotype on diatom biomass: temperature significantly increased diatom biomass in mesocosms with completely-plated stickleback but not in mesocosms with incompletely-plated stickleback. We also found indications of character displacement, as individual completely-plated stickleback consumed less insects when they were paired with an incompletely-plated individual compared to when they were paired with a completely-plated individual. Our results suggest that the different plate phenotypes of the Baltic Sea stickleback may differentially affect lower trophic levels, and that these effects depend on water temperature.

Introduction

Different species have different niches and hence different impacts on ecosystem functioning. In addition, variation within species influences ecosystem structure and functioning. For example, the evolution of different morphotypes or ecotypes affects predation pressure on prey species, adding links to a food web and/or modifying energy flow if the new forms or species have distinct ecological roles (Yonekura et al. 2007; Harmon et al. 2009; Bassar et al. 2010). Changes in selective pressures promoting the diversification of one species can therefore drive the success, failure, and evolutionary adaptation of other species, depending on how they respond to a new ecosystem structure. An example from aquatic food webs is the intraspecific divergence in feeding morphology between anadromous and landlocked populations of alewife (a member of the herring family; *Alosa pseudoharengus*), which cause the different phenotypes to generate differently structured zooplankton communities (Palkovacs and Post 2009; Walsh and Post 2011). Anadromous alewives have a larger gape and target large zooplankton prey species. Consequently the zooplankton communities exposed to anadromous alewives are dominated by zooplankton with life history characteristics such as faster growth and maturation to reproduce early in the spring (Palkovacs and Post 2009; Walsh and Post 2011). In turn, the changes in zooplankton community composition impact phytoplankton abundance and primary productivity. Thus, alewife diversification modifies community structure and functioning across multiple trophic levels (Post et al. 2008; Walsh et al 2012).

Climate change is predicted to influence ecological interactions across the globe, because higher temperatures increase predation pressure (Blois et al. 2013; Romero et al. 2019). Accordingly, in several species of fish, increased water temperature leads to increased food intake and specifically, greater protein intake (Yamamoto et al. 2003; Rowe et al. 2018; Volkoff and Rønnestad 2020). Prey switching also occurs as predators adjust to exploit different species when increased temperatures lead to temporal range mismatches with their typical prey species (Rockwell et al. 2011). These modifications in feeding behavior can cascade through the food web to affect productivity and ecosystem functioning. For example, if populations converge in resource use, this can have a homogenizing effect on the functional biodiversity of the ecosystem, potentially reducing ecosystem resilience (Eklöf et al. 2012). Hence, predicting how climate change influences ecological (or trophic) interactions within and among species is an urgent concern to understand changing ecosystems across the globe.

The threespine stickleback is a small-bodied mesopredatory fish (*Gasterosteus aculeatus* L., hereafter referred to as, “stickleback”) that is common across the northern temperate hemisphere. It is a model species for studying adaptive radiation as it has repeatedly evolved different plate phenotypes in response to heterogeneity in ecological interactions (Bell and Foster 1994). In the Baltic Sea, the abundance of

stickleback has increased drastically in the past few decades (Ljunggren et al. 2010; Eriksson et al. 2011; Bergström et al. 2015; Eklöf et al. 2020), with major impacts on ecosystem structure and functioning. Areas with high stickleback abundance have more algae than areas with low stickleback abundance (Eriksson et al. 2009). Experiments indicate that this is partly caused by increased predation by stickleback on invertebrate grazers such as gammarid amphipods (Eriksson et al. 2009; Sieben et al. 2011; Reiss et al. 2014). Stickleback have also been shown to predate on the eggs and larval stages of their predators including pike and perch, preventing their stocks from recovering (Byström et al. 2015). Currently, our understanding of the ecological impact of Baltic Sea stickleback is based on the implicit assumption that there is one demographically and ecologically homogeneous population (Eriksson et al. 2009; Sieben et al. 2011; Reiss et al. 2014; Donadi et al. 2017; Jakubavičiūtė et al. 2017b). However, in the Baltic Sea there are two dominant morphotype groups based on the pattern of body armor plates: completely plated with plates running uninterrupted from the head to the tail; and incompletely plated with a large gap between a set of plates running from the head, and a set of plates running from the tail (Aneer 1974). Divergence of stickleback plate morphotypes has been shown to impact productivity and prey abundance in other geographic regions, where different plate morphotypes feed on different prey sources, compared to each other and/or compared to their ancestral phenotype (Harmon et al. 2009). Little is known about the role of stickleback morphotype diversity in the Baltic Sea ecosystem although there is some evidence of ecological differentiation based on diet analysis and habitat segregation (Eriksson et al. 2021; Yanos et al. in press). If the morphological differentiation in plating indeed corresponds to ecological differentiation, this likely influences the ecological impact of the dramatic increase in stickleback (McKinnon and Rundle 2002; McGee and Wainwright 2013).

The Baltic Sea is warming quickly, with temperatures predicted to increase roughly 50% more than the global mean temperature increase (HELCOM 2007). This corresponds to an increase of 3 to 5°C mean annual temperature by the end of the century (HELCOM 2007). There is some indication that different plate phenotypes may respond differently to climate change due to differences in heat tolerance. Dammark et al (2018) characterized Danish lake stickleback populations by relative abundance of plate phenotype and observed that a population dominated by completely-plated stickleback had the highest heat tolerance of the populations studied. However, variation in tolerance was slight and the study populations had different relative abundances of plate phenotypes. Population differences in heat tolerance may have been influenced by differences in phenotype relative abundance if each phenotype within a population has different heat tolerance. To our knowledge, within-population differences in heat stress response has not been tested in a polymorphic stickleback population, nor have their possible effects on the ecosystem as a whole. Here we conduct a mesocosm experiment to test the joint effects of temperature and morphotype on ecological interactions, by testing how different plate

phenotypes influence the structure of lower trophic levels at both natural and elevated temperatures. We predict that 1) dietary divergence between morphotypes cascades through the food web resulting in different invertebrate and algal communities, 2) temperature impacts stickleback dietary needs which cascades through the food web, and 3) effects of increasing temperature differ between morphotypes.

Materials and Methods

Experimental set-up

We ran a mesocosm experiment from 13 June to 4 July 2018 at Askö Laboratory in the archipelago of Trosa, Sweden as part of a larger experiment testing effects of stickleback on the food web. We constructed a flow-through system of 60 50-liter buckets with two temperature treatments: ambient seawater temperature (hereafter referred to as, “ambient”) and seawater heated approximately 4°C above the ambient treatment (hereafter referred to as, “heated”). Each temperature treatment was constructed as a stand-alone system where sea water was pumped directly from a shallow bay near the mesocosm site into a holding tank. From here water was directed separately to each bucket via a 1 cm diameter plastic tube. All water was filtered through a 1.4 by 1.6 mm mesh that removed all larger organisms. The water for the heated treatment was heated in the holding tank using a cow milk heater, supplemented with aquaria heaters. Detailed descriptions of methods for mesocosm construction and maintenance can be found in Supplement 1. The study was carried out with the approval of the Linköping Animal Testing Ethics Committee, Sweden (permit ID 1546). We added two stickleback individuals to each mesocosm in three different morphotype combinations: two completely-plated stickleback, two incompletely-plated stickleback (jointly referred to as ‘single-morphotype treatments’), and mixed morphotype with one completely- and one incompletely-plated stickleback. Stickleback ranged in size from 5.4 to 6.9 cm (average 5.9) in total length, in each bucket we tried to use fish as similar in size as possible to help prevent aggressive behavior. Each morphotype treatment was replicated ten times in each temperature treatment. To each mesocosm we added *Fucus vesiculosus* and *F. radicans* (hereafter referred to collectively as, “*Fucus*”; average mass 22.76 g ($\pm$ 4.28)), gastropods (*Theodoxus fluviatilis*; n=150; size range from 3 to 8 mm), isopods (n=1 each of length 12, 13, 14, and 15 mm), and amphipods (*Gammarus* spp.; n=11 to 19 of 4 to 21 mm length, sometimes including one or two females with eggs and one or two mating pairs) and measured biomass at the end of the experiment (Supplement 1). All biological material, including stickleback, was collected from nearby shallow bays. Stickleback were collected with a beach seine; algae and invertebrates were collected by hand. We did not add or remove any material during the experiment with the exception of fish that died during the experiment which were removed as soon as possible and replaced with a fish of the same morphotype and similar size. Fish that exhibited abnormal or

aggressive behavior were also removed as soon as possible and killed with a buffered overdose of MS-222 in seawater and likewise replaced with a fish of the same morphotype and similar size. Twenty-one fish had to be replaced throughout the experiment (out of 120 fish in the experiment).

End of experiment

At the end of the experiment all biological material was removed, separated, identified, and quantified. All invertebrates were sorted by taxa, measured, placed in a drying oven for 48 hours, and weighed for dry weight. Since it was a flow-through and open system, macroscopic algae and invertebrates entered the mesocosms as gametes, larvae, or small juveniles via the water or the air (insects). Diatoms that had accumulated in the water were collected by sieving over a plankton sieve. The diatoms were rinsed from the sieve with a small quantity of water. The resulting diatom-water slush was stored in plastic bags and frozen for later analysis. In the lab, the bags of diatoms were defrosted then left to separate from the water and settle to the bottom of a bucket. The top layer of water was carefully poured off and the diatom mass that was left in the jar was then poured to a pre-weighed foil. The jar was rinsed in the water that had been poured off the top. The water was then filtered through pre-weighed Whatman GF/F filters using a vacuum pump. Everything was then dried to a constant weight at 60°C for 48 hours. We removed all epiphytic algae from the *Fucus* thalli by hand. *Fucus* was placed in a drying oven and dried to a constant weight at 60°C for 48 hours. Epiphytic algae was also dried to constant weight in a drying oven at 60°C for 48 hours.

Fish were removed and killed by being placed in a bucket with brackish water and an overdose of buffered MS-222. After all opercular movement had ceased for three minutes, we severed the spine and destroyed the brain to ensure death. We then measured total length and photographed the fish from the left side. The stomach was then removed and transferred to 70% ethanol and the body was transferred to 95% ethanol for later morphological analysis. In the lab, we measured 11 length parameters using digital Vernier calipers following Jones et al. (2012) and counted the number of lateral plates on both sides of the body (Figure S1). To account for differences in length of morphometric measures due to different body sizes, we standardized all measurements to a standard length of 50mm (Thorpe 1976, Lavin and McPhail 1985, Hagen and Gilbertson 1992).

We analyzed stomach contents by rinsing the stomach with fresh water to remove all contents and then identified them under a stereomicroscope. Contents were sorted by taxa and the stomach and contents were placed in a freeze drier at 60°C degrees for 48 hours after which dry weight was recorded. We identified six categories of stomach contents: algae, amphipods, insects, isopods, unidentifiable material, and plastic which

mainly came from materials in the mesocosms. Stomach contents were generally too digested to identify, hence the majority of the material was classified as unidentifiable.

Data analysis

All analysis was conducted in R 3.6.3 (R Core Team 2020) using base R and the car package (Fox and Weisberg 2019). Graphics were created with base R, and the dplyr (Wickham et al. 2020) and ggplot2 (Wickham 2016) packages. Variables that did not have an approximately normal distribution were transformed by being raised to a 0.25 exponent. We tested differences between treatments in final biomass of amphipods, isopods, gastropods and diatoms in the mesocosms, and stickleback stomach contents, by conducting two-way analysis of variance (ANOVAs). We followed up with Tukey's HSD post-hoc analysis for pairwise comparisons when appropriate. For the stomach contents results, we ran three separate analyses: 1) To determine if plate phenotypes consume different food items, we used individual fish as data points and excluded individuals from mixed morphotype mesocosms to remove any possible confounding effect of character displacement. 2) To determine if populations with different phenotype compositions have different consumption of resources we used mesocosm averages. 3) To assess the occurrence of character displacement in the mixed phenotype mesocosms, we used individuals as data points and included the phenotype of the other fish in the mesocosm as a predictor variable. To test for correlations between the abundances of different taxa in the mesocosms, we ran Pearson's correlations. To test for differences in morphometrics between completely- and incompletely-plated individuals we used Mann-Whitney U-tests.

Results

Community composition

At the end of the experiment, we identified the following invertebrate taxa/organism groups in the mesocosms: bivalves (*Mytilus edulis*), bryozoans (*Electra* spp.), crustacean amphipods (*Gammarus* spp.), crustacean isopods (*Idotea* spp and *Jaera* spp), gastropods (*Hydrobia* spp., *Lymnaea* spp., *Teodoxus fluviatilis* spp.), and insects (beetles and chironomids). However, we restricted the analysis to the three mesograzers taxa/organism groups added to the experiment by us (amphipods, isopods, and the gastropod *T. fluviatilis*). Both stickleback plate phenotype and temperature treatment affected the final biomass of the crustacean mesograzers community (amphipods and isopods) in the mesocosms, but had no effect on gastropods. Plate phenotype and temperature had an interactive effect on the biomass of amphipods (Two-way ANOVA: phenotype, $F_{2,28}=1.280$, $p=0.294$; temperature, $F_{1,28}=5.667$, $p=0.024$; interaction effect, $F_{2,28}=3.892$, $p=0.032$; Figure 1a). Specifically, amphipod biomass was higher in ambient-temperature mesocosms with completely-plated stickleback than it was in heated mesocosms with either completely-plated or incompletely-plated

stickleback (Tukey's HSD; $p=0.021$ and $p=0.020$, respectively). Isopod biomass was lower in the heated than in the ambient-temperature mesocosms, but it was not affected by plate phenotype (Two-way ANOVA; temperature, $F_{1,28}=6.809$, $p=0.014$; phenotype, $F_{2,28}=0.154$, $p=0.858$; interaction, $F_{2,28}=0.394$, $p=0.678$; Figure 1b). *Theodoxus fluviatilis* biomass did not differ significantly between any of the treatments (Two-way ANOVA; phenotype, $F_{2,28}=0.401$, $p=0.673$; temperature, $F_{1,28}=1.765$, $p=0.195$; interaction, $F_{2,28}=1.343$, $p=0.278$; Figure 1c). To be sure that there were no differences between single phenotype treatments that were masked by the inclusion of the mixed phenotype treatments, we repeated the analysis with just the single-phenotype mesocosms. Removing the mixed phenotype mesocosms confirmed the results for all three mesograzer groups: there was a significant interaction effect of phenotype and temperature on amphipod biomass (Two-way ANOVA: phenotype, $F_{1,28}=8.495$, $p=0.009$; temperature, $F_{1,28}=35.411$, $p=0.000$; interaction effect, $F_{1,28}=8.413$, $p=0.010$). For isopods, the effect of temperature remained, although only marginally significant, and phenotype still had no effect on final isopod biomass (Two-way ANOVA; phenotype, $F_{1,28}=0.326$, $p=0.575$; temperature, $F_{1,28}=4.197$, $p=0.055$; interaction effect, $F_{1,28}=0.756$, $p=0.396$). There was still no effect of phenotype or temperature on *T. fluviatilis* (Two-way ANOVA; phenotype, $F_{1,28}=0.077$, $p=0.784$; temperature, $F_{1,28}=0.227$, $p=0.640$; interaction effect, $F_{1,28}=1.583$, $p=0.224$).

Diatom biomass was in general higher in the heated than in the ambient treatment, but we found no significant effects of the plate phenotypes (Two-way ANOVA: phenotype, $F_{2,28}=0.570$, $p=0.572$; temperature, $F_{1,28}=10.343$, $p=0.003$; interaction, $F_{2,28}=2.387$, $p=0.110$; Figure 1d). However, when repeating the analysis excluding the mixed phenotype mesocosms, there was a marginally significant interaction effect between temperature and phenotype (Two-way ANOVA: phenotype, $F_{1,28}=0.958$, $p=0.341$; temperature, $F_{1,28}=6.700$, $p=0.019$; interaction, $F_{1,28}=3.943$, $p=0.062$). Specifically, in the mesocosms with completely-plated stickleback, the diatom biomass was two times higher in the heated compared to the ambient temperature treatment, while there was no difference between the temperature treatments in the mesocosms with incompletely-plated stickleback (Figure 1d).

Figure 1. Biomass of a) amphipods, b) isopods, c) *T. fluviatilis*, and d) diatoms in the mesocosms at the end of the experiment, grouped by stickleback phenotype and temperature treatment. Shaded bars represent mean with error bars showing standard error. *, $p < 0.05$.

Stomach contents

There was no effect of phenotype or temperature on total biomass of stomach contents (Two-way ANOVA: phenotype $F_{2,37}=0.332$, $p=0.719$; temperature $F_{1,37}=0.000$, $p=0.996$; interaction $F_{2,37}=1.376$, $p=0.265$). Removing the mixed phenotype mesocosms confirmed this result (Two-way ANOVA: phenotype $F_{1,24}=0.231$, $p=0.635$; temperature $F_{1,24}=0.873$, $p=0.360$; interaction $F_{1,24}=0.367$, $p=0.551$). Stickleback plate phenotype and temperature had an effect on biomass of the two main identifiable items found in our stickleback stomachs - amphipods and insects - but had no effect on any other item (Table S1). There was no effect on amphipod biomass when including all plate phenotypes (Two-way ANOVA; phenotype $F_{2,37}=0.169$, $p=0.846$; temperature $F_{1,37}=0.062$, $p=0.805$; interaction $F_{2,37}=1.903$, $p=0.164$). However, after removing the mixed phenotype mesocosms, there was a marginally significant interaction between plate phenotype and temperature (Two-way ANOVA; phenotype $F_{1,24}=0.148$, $p=0.704$; temperature $F_{1,24}=0.077$, $p=0.784$; interaction $F_{1,24}=4.117$, $p=0.054$; Figure 2a): completely-plated stickleback consumed more amphipods in the heated treatment than they did in the ambient treatment, while incompletely-plated stickleback consumed more amphipods in the ambient treatment than the heated treatment, although these pairwise comparisons were not significant (Tukey's HSD: $p=0.419$ and $p=0.595$, respectively). Stomach content insect biomass differed between fish in the completely-plated mesocosms and the mixed phenotype

mesocosms, but did not differ in other comparisons (Two-way ANOVA: phenotype $F_{2,37}=6.355$, $p=0.004$; temperature $F_{1,37}=1.361$, $p=0.251$; interaction $F_{2,37}=0.071$, $p=0.931$; Figure 2b). Completely-plated pairs consumed more insects than mixed pairs (Tukey's HSD: $p=0.002$). Individual stomach contents of completely-plated stickleback were significantly different between single- vs. mixed-morphotype treatments (Two-way ANOVA: Partner*Phenotype $F_{1,91}=8.731$, $p=0.004$; Table S2). Completely-plated stickleback with an incompletely-plated partner had less insect biomass in their stomachs than those with a completely-plated partner (Tukey's HSD, $p=0.009$), indicating that there might be character displacement in the mixed morphotype mesocosms (Figure 3).

Figure 29. Stomach content biomass of a) amphipods and b) insects from individuals grouped by phenotype and temperature treatment. Shaded bars represent mean with error bars showing standard error.

Figure 10. Stomach content biomass of insects for completely-plated stickleback with a completely-plated (single phenotype mesocosm) or incompletely-plated (mixed phenotype mesocosm) partner. Shaded bars represent mean with error bars showing standard error.

Stickleback morphometrics

Confirming the morphotype assignments, completely-plated stickleback had more plates than incompletely-plated stickleback (completely plated 31; 27-34 (median;

range) vs incompletely plated 16; 9-25; Mann-Whitney U-test, $p=0.000$). We found a significant difference in jaw length between phenotypes, with incompletely-plated stickleback having longer jaws (Mann-Whitney U-test, $p=0.000$). No other morphometric measurements differed (Mann-Whitney U-test, $p>0.05$, Table S3).

Discussion

In this study, we tested how temperature increase and mesopredator population differentiation influence ecological interactions. In a mesocosm experiment with different plate phenotypes of Baltic Sea stickleback and two temperature treatments, we observed that stickleback phenotype affects ecosystem structure and that these effects depend on water temperature. We also found indications of character displacement under a climate change scenario.

We observed dietary differentiation between the plate phenotypes, suggesting that the documented impact of phenotype composition on lower trophic levels may depend on differences in predation rates between the phenotypes. However, high variation in stomach content between individual stickleback, and overlap between phenotypes, meant that the results were marginally significant. This may be due to weak morphological differentiation of the phenotypes. Baltic Sea stickleback consume a wide range of prey items and while one recent study shows that completely-plated stickleback consume more amphipods than incompletely-plated stickleback, both are able to, and do, consume amphipods, zooplankton, and insects, amongst other food sources (Jakubavičiūtė et al. 2007; Eriksson et al. in 2021). Intraspecific morphological variation of head shape, jaw length, and gill raker architecture is associated with feeding differences in a large number of fish species (e.g. stickleback, Lavin and McPhail 1986; bluegill sunfish *Lepomis macrochirus*, Yonekura et al. 2007; Trinidadian guppies *Poecilia reticulata*, Bassar et al. 2010). In the individuals used in our study, we found that jaw length differed between phenotypes (Table S3). Fish with longer jaws have been shown to consume larger prey items than fish with shorter jaws (Lavin and McPhail 1986). In our study, incompletely-plated stickleback had longer jaws than completely-plated stickleback but under heated conditions, completely-plated stickleback consumed more amphipods than incompletely-plated stickleback suggesting that jaw morphology is not the only, or strongest, factor determining diet. We did not perform morphometric analysis of head shape or gill raker architecture but these features could provide more insight into trophic differentiation between phenotypes of Baltic Sea stickleback. The dietary divergence that we did see in our study may also be an underestimation, as we collected our fish after they migrated into shallow bays to spawn. In some other populations of stickleback that display migratory behavior during spawning, there is a temporary diet shift to benthic prey during breeding season (Allen and Wootton 1984, Gross and Anderson 1984). Baltic Sea stickleback overwinter in offshore, open-water habitats and migrate into coastal bays only for spawning where they may all predominantly consume benthic prey (Bergström et al. 2015). If this is the case, then seasonal changes in the morphotype

composition of Baltic Sea stickleback populations will have only weak effects on coastal bay ecosystems, while differences in diet during other times of the year may affect the biological community in overwintering habitats more strongly.

From analysis of individual stomach contents, we found some indications of character displacement. Completely-plated stickleback consumed less insects in the presence of an incompletely-plated individual (i.e. in the mixed phenotype mesocosms) than in the presence of another completely-plated individual (i.e. in the completely-plated mesocosms). This finding supports the idea that there are differences between the plate phenotypes in ecological interactions. Direct competition brings out these differences more clearly, which may be important in predicting the future role of stickleback in Baltic Sea habitats as stickleback populations, and therefore competition, increases. As mentioned above, these changes may be more apparent in overwintering habitats if dietary differentiation is stronger in non-breeding seasons. Future studies should therefore look into feeding behavior Baltic Sea stickleback in overwintering habitats.

We found an interactive effect of temperature and stickleback phenotype on final amphipod biomass, caused partly by differences in predation by stickleback phenotypes at different temperatures. The difference in amphipod biomass cascaded down to inversely affect diatom biomass. This cascade is seen in the completely-plated mesocosms where stickleback ate more amphipods in the heated condition. Conversely, incompletely-plated stickleback consumed less amphipods in heated mesocosms compared to ambient mesocosms, and this decrease in consumption did not cascade through the food web. Diatom growth typically increases with increasing temperature (Montagnes and Franklin 2001). Our results additionally show that indirect cascading effects of stickleback phenotype-specific predation interact with the effect of water temperature on diatom growth. Heated mesocosms with two completely-plated stickleback generated the highest final diatom biomass across all treatment combinations, indicating that the combination of climate change and different stickleback phenotype compositions may generate different biological conditions.

We did not observe a general increase in stickleback feeding rates from increasing temperatures alone, neither for the biomass of prey items in the stomachs nor the mesocosms, as expected from the literature (Yamamoto et al. 2003; Rowe et al. 2018; Volkoff and Rønnestad 2020). For stickleback from the Gulf of Bothnia off the coast of Sweden, Lefébure et al. (2011) estimated that stickleback can grow in water temperatures from 5 to 29°C with the optimal temperature for growth at 21°C. A study of polymorphic populations from Danish Lakes showed that populations with different compositions of low, partial, and completely-plated stickleback had slight differences in heat tolerance but that all had heat tolerance above 31°C (Dammarck et al. 2018). The highest temperature reached in any of our mesocosms was 20.5°C which was approximately 4°C warmer than the maximum temperature reached in the ambient mesocosms which were kept at natural temperature conditions (Figure S2). If these models can be applied to our stickleback population from the Stockholm Archipelago, then our mesocosms never reached the temperature for optimal growth, let alone a

temperature high enough for our fish to experience heat stress or differential heat stress between phenotypes. Food sources also appear to tolerate temperatures within climate change scenarios well. Native gammarid amphipods, such as those used in our mesocosms, have low mortality at temperatures similar to those reached in our heated mesocosms (Casties et al. 2019). Additionally, non-native gammarid amphipods are more tolerant to constant high temperatures as well as heat waves and their populations are predicted to expand in the Baltic Sea as temperatures warm, thus ensuring a constant food source for species that prey on amphipods (Sareyka et al. 2011; Casties et al. 2019; Torn et al. 2020). Zooplankton abundance also increases with increasing water temperatures in the Baltic Sea (Aleksandrov et al. 2009) and while we were unable to measure zooplankton abundance in our mesocosms or identify them in our stomach content analysis, there are indications that they are an important food source for stickleback in the Baltic (Eriksson et al. 2021). Thus, we may predict that two of the most important food resources for Baltic stickleback will not experience negative impacts of climate change. As a consequence, we have no reason to think that increasing temperatures will lead to food limitation and thereby increased intraspecific competition and dietary differentiation among stickleback phenotypes. Food limitation may arise if climate change extends the duration of the stickleback growing and breeding seasons, thereby increasing both body size and population size. How this would develop in coastal Baltic habitats has not yet been tested but may be important for projecting how climate change may impact coastal Baltic ecosystems (Lefébure et al. 2014; Hovel et al. 2017).

Our results demonstrate that intraspecific phenotypic divergence can lead to environmental modifications through divergent feeding and other behaviors. This impacts the selective pressures acting on many other organisms and thereby the structure and functioning of ecosystems (Bassar et al. 2010, Walsh et al. 2012). Diversification in a single species can even cause evolutionary diversification in other species (Odling-Smee et al. 2013). Abiotic changes such as increasing temperature can further change a population's (feeding) behavior. Here we have shown that there is an interactive effect of increased temperature and stickleback phenotype on biological community composition. If our results reflect what is to be expected in coastal Baltic Sea habitats, climate change will increase primary production especially in bays with greater numbers of completely-plated stickleback. Additional information on temperature tolerance, growth, and metabolic demand of plate phenotypes specifically from the Baltic Sea should be quantified to better understand how further climate change may impact their survival and resource demand and how this will affect coastal Baltic Sea habitats.

Supplementary Information

Supplement 1: Extended methods

Set-up

We constructed a flow-through mesocosm system of 80 50-liter buckets distributed across four 2866.5 L tanks and eight treatments: ambient temperature with no fish, ambient temperature with completely-plated stickleback, ambient temperature with incompletely-plated stickleback, ambient temperature with one completely and one incompletely-plated stickleback, heated temperature with no fish, heated temperature with completely-plated stickleback, heated temperature with incompletely-plated stickleback, and heated temperature with one completely and one incompletely-plated stickleback. Forty buckets in two tanks were used in each temperature treatment and each tank had five buckets with each stickleback treatment. Each temperature treatment was constructed as a stand-alone system where sea water was pumped directly from a shallow bay near the mesocosm site into a 1000 L holding tank which fed the water to a shallow trough above the large tanks. From here water was directed separately to each bucket via a 1 cm diameter plastic tube. We placed 1.4 by 1.6 mm mesh over the end of the tubes to prevent uncontrolled introduction of fauna and algae. Flow rate was controlled using plastic flow control valves and was maintained at 13 L / hour (± 6 L) for all buckets for the duration of the experiment. A 2.5 cm diameter hole was drilled in the side of each bucket 10 cm from the top for water to constantly flow out of the bucket into the space surrounding the buckets in the tank. We covered the outflow holes with 0.5 mm mesh to prevent the loss of algae and animals. Each bucket was covered with 1.4 by 1.6 mm mesh to exclude birds and large insects and to limit the amount of light going into the buckets to more natural levels (personal observation BK Eriksson). The water flowing out of the buckets was circulated around the tank using two aquarium pumps and served as a water bath to stabilize and homogenize the water temperature within the buckets. To regulate the water temperature of the heated treatment, we placed a 3000-watt cow milk heater in the holding tank which raised the temperature 1 to 2°C above the ambient temperature and aquarium heaters in the trough and water tanks which were added and removed as necessary to reach the desired temperature (up to two 200-watt and three 100-watt heaters in the trough and up to four 300-watt heaters and one 100-watt heater per tank).

Fucus vesiculosus and *F. radicans* (hereafter referred to collectively as, “fucus”) were collected from Öregrund, Sweden. Additional *F. vesiculosus* was collected from shallow bays around Askö. Fucus was cleaned of all epiphytes then spun in a salad spinner for 20 rotations to remove excess water, the weight was recorded and a photograph was taken (camera). Each piece was then fixed to a rock using a rubber band to keep it from floating so it could serve as refuge for the stickleback and invertebrates. Each bucket had an average of 40.06 g (± 4.04) of *F. vesiculosus* from Öregrund, 9.82 g (± 0.57) of *F. radicans*, and 150.88 g (± 1.22) *F. vesiculosus* from Askö for an average fucus mass of 200.76 g (± 4.28). We added 150 gastropods that ranged in size from 3 to 8 mm, one isopod with length of 12, 13, 14, or 15 mm, and between 11 and 19 amphipods that ranged in size from 4 to 21 mm which sometimes

included one or two females with eggs and one or two mating pairs, all collected from a nearby shallow bay. Fish were collected from Idkroken, a nearby shallow bay on the island where both phenotypes have been found in high densities (personal observation CL Yanos). To each bucket we added either: no stickleback, two completely-plated stickleback, two incompletely-plated stickleback, or one completely- and one incompletely-plated stickleback. We excluded breeding males, identified by their red coloration and blue eyes, to avoid territoriality/aggression and any impact nesting and breeding could have on feeding behavior. Bloated fish were also excluded as they were likely either females full of eggs or fish with large internal *Schistocephalus solidus* parasites. We chose to exclude females with eggs as stickleback have been shown to cannibalize their eggs and we wanted to exclude this as a possible confounding food source and to avoid the effects breeding could have on feeding behavior. We excluded fish with parasites as the parasites could have affected the health and therefore feeding behavior of the fish.

Running and Maintenance

The experiment ran from 13 June to 4 July 2018. We checked salinity and water temperature daily and made adjustments to the heating elements when needed to maintain the heated treatment at approximately 4°C above the ambient treatment. Algae built up frequently in the troughs and tubes. To ensure a continuous and consistent flow we cleaned the tubes up to two times per day and the troughs when needed. Algae also accumulated on the mesh covering the outflow hole of the buckets. To ensure a continuous flow out of the buckets we cleaned these up to two times per day. Algae cleaned from the mesh on the inside of the bucket was left in the bucket so there was no effect of cleaning frequency on the final measure of algae in each bucket.

Fish that developed breeding coloration, became bloated, exhibited aggressive behavior, exhibited odd swimming behavior, or died during the course of the experiment were removed and replaced with a fish of the same phenotype and similar size. Fish that were removed during the experiment were killed with an overdose of tricaine methanesulfonate (MS-222; 250mg/L of brackish water with bicarbonate of soda as a buffer at 500mg/L of water). Once all opercular movement ceased for three minutes, indicating death, the spinal cord was severed and brain destroyed to ensure death before the fish was placed in 95% ethanol for preservation for later morphological analysis.

Figure S1. Morphological parameters. (1) total length, (2) standard length, (3) first dorsal spine length, (4) second dorsal spine length, (5) third dorsal spine length, (6) caudal peduncle length, (7) caudal peduncle depth, (8) pelvic spine length, (9) head length, (10) pectoral fin base width, (11) upper lip/jaw length.

Figure S2. Temperature conditions in the heated (red line, circles) and ambient (blue line, squares) mesocosms during the experiment.

Chapter 4

Table S1. Two-way ANOVA results for individual taxa of stomach contents using mesocosms average for response variable using the equation: $y \sim \text{Phenotype} * \text{Temperature} + (\text{Temperature} / \text{Tank})$. *, $p < 0.05$; **, $p < 0.01$.

	All Phenotype Treatments Included					Mixed Phenotype Treatments Excluded				
		Sum Sq	Df	F-value	Pr(>F)		Sum Sq	Df	F-value	Pr(>F)
Algae	Phenotype	0.002988	2	0.7550	0.4771	Phenotype	0.000606	1	0.2382	0.6299
	Temperature	0.000105	1	0.0529	0.8194	Temperature	0.000001	1	0.0003	0.9863
	Phenotype*Temperature	0.002255	2	0.5697	0.5706	Phenotype*Temperature	0.002176	1	0.8557	0.3641
	Temperature*Tank	0.001964	2	0.4964	0.6127	Temperature*Tank	0.002713	2	0.5334	0.5934
	Residuals	0.073214	37			Residuals	0.061030	24		
Amphipods	Phenotype	0.001452	2	0.1685	0.8456	Phenotype	0.000510	1	0.1482	0.7037
	Temperature	0.000267	1	0.0619	0.8050	Temperature	0.000264	1	0.0768	0.7840
	Phenotype*Temperature	0.016403	2	1.9027	0.1635	Phenotype*Temperature	0.014157	1	4.1167	0.0537
	Temperature*Tank	0.006831	2	0.7925	0.4603	Temperature*Tank	0.001720	2	0.2501	0.7808
	Residuals	0.159479	37			Residuals	0.082531	24		
Insects	Phenotype	0.030223	2	6.3549	0.0042 **	Phenotype	0.004783	1	2.0176	0.1683
	Temperature	0.003235	1	1.3606	0.2509	Temperature	0.003204	1	1.3513	0.2565
	Phenotype*Temperature	0.000339	2	0.0714	0.9313	Phenotype*Temperature	0.000049	1	0.0208	0.8867
	Temperature*Tank	0.000070	2	0.0148	0.9854	Temperature*Tank	0.002252	2	0.4750	0.6276
	Residuals	0.087982	37			Residuals	0.056896	24		
Isopods	Phenotype	0.000462	2	0.6489	0.5285	Phenotype	0.0001727	1	0.3295	0.5713
	Temperature	0.000615	1	1.7261	0.1970	Temperature	0.0009208	1	1.7568	0.1975
	Phenotype*Temperature	0.000435	2	0.6109	0.5482	Phenotype*Temperature	0.0001727	1	0.3295	0.5713
	Temperature*Tank	0.001112	2	1.5615	0.2233	Temperature*Tank	0.0017133	2	1.6324	0.2164
	Residuals	0.013179	37			Residuals	0.0125797	24		
Plastic	Phenotype	0.000943	2	0.6747	0.5155	Phenotype	0.0004890	1	0.7589	0.3923
	Temperature	0.000990	1	1.4174	0.2414	Temperature	0.0007352	1	1.1409	0.2961
	Phenotype*Temperature	0.000646	2	0.4622	0.6335	Phenotype*Temperature	0.0006048	1	0.9385	0.3423
	Temperature*Tank	0.000002	2	0.0016	0.9984	Temperature*Tank	0.0002510	2	0.1948	0.8243
	Residuals	0.025845	37			Residuals	0.0154656	24		
Unidentified Materials	Phenotype	0.000167	2	0.0403	0.9606	Phenotype	0.000195	1	0.0928	0.7632
	Temperature	0.000164	1	0.0790	0.7802	Temperature	0.001182	1	0.5637	0.4601
	Phenotype*Temperature	0.002755	2	0.6657	0.5200	Phenotype*Temperature	0.001050	1	0.5005	0.4861
	Temperature*Tank	0.000610	2	0.1474	0.8635	Temperature*Tank	0.000257	2	0.0613	0.9407
	Residuals	0.076558	37			Residuals	0.050341	24		
Total Contents	Phenotype	0.001181	2	0.3324	0.7193	Phenotype	0.000415	1	0.2314	0.6348
	Temperature	0.000000	1	0.0000	0.9961	Temperature	0.001566	1	0.8726	0.3595
	Phenotype*Temperature	0.004885	2	1.3755	0.2653	Phenotype*Temperature	0.000658	1	0.3666	0.5506
	Temperature*Tank	0.004010	2	1.1290	0.3342	Temperature*Tank	0.004850	2	1.3516	0.2778
	Residuals	0.065704	37			Residuals	0.043060	24		

Table S2. Two-way ANOVA results for individual taxa of stomach contents using individual fish for response variable. Equation for analysis including all phenotype mesocosms: $y \sim \text{Partner} * \text{Phenotype} * \text{Temperature} + (\text{Temperature} / \text{Tank})$. Equation for analysis excluding mixed phenotype mesocosms: $y \sim \text{Phenotype} * \text{Temperature} + (\text{Temperature} / \text{Tank})$. *, $p < 0.05$; **, $p < 0.01$.

	All Phenotype Treatments Included					Mixed Phenotype Treatments Excluded				
		Sum Sq	Df	F-value	Pr(>F)		Sum Sq	Df	F-value	Pr(>F)
Algae	Partner	0.000083	1	0.0501	0.8234	Phenotype	0.000696	1	0.3282	0.5689
	Phenotype	0.000545	1	0.3298	0.5672	Temperature	0.000074	1	0.0348	0.8527
	Temperature	0.000895	1	0.5417	0.4636	Phenotype*Temperature	0.000742	1	0.3497	0.5566
	Partner*Phenotype	0.002470	1	1.4949	0.2246	Temperature*Tank	0.001078	2	0.2542	0.7764
	Partner*Temperature	0.000702	1	0.4249	0.5161	Residuals	0.122981	58		
	Phenotype*Temperature	0.000008	1	0.0048	0.9448					
	Temperature*Tank	0.001438	2	0.4351	0.6486					
	Partner*Phenotype*Tank	0.000520	1	0.3148	0.5761					
	Residuals	0.150366	91							
Amphipods	Partner	0.00334	1	0.9606	0.3296	Phenotype	0.000408	1	0.1399	0.70979
	Phenotype	0.00771	1	2.2161	0.1400	Temperature	0.000044	1	0.0149	0.90319
	Temperature	0.00393	1	1.1289	0.2908	Phenotype*Temperature	0.008635	1	2.9590	0.09073
	Partner*Phenotype	0.00161	1	0.4623	0.4983	Temperature*Tank	0.001062	2	0.1820	0.83407
	Partner*Temperature	0.00076	1	0.2178	0.6418	Residuals	0.169258	58		
	Phenotype*Temperature	0.000803	1	2.3102	0.1320					
	Temperature*Tank	0.00643	2	0.9243	0.4005					
	Partner*Phenotype*Tank	0.00373	1	1.0728	0.3031					
	Residuals	0.31649	91							
Insects	Partner	0.00981	1	2.5796	0.111711	Phenotype	0.005617	1	1.2019	0.2775
	Phenotype	0.00003	1	0.0090	0.924794	Temperature	0.002378	1	0.5089	0.4785
	Temperature	0.00038	1	0.1001	0.752422	Phenotype*Temperature	0.000334	1	0.0715	0.7902
	Partner*Phenotype	0.03321	1	8.7309	0.003983 **	Temperature*Tank	0.007419	2	0.7938	0.4570
	Partner*Temperature	0.00015	1	0.0395	0.842968	Residuals	0.271044	58		
	Phenotype*Temperature	0.00001	1	0.0036	0.952412					
	Temperature*Tank	0.00185	2	0.2438	0.784145					
	Partner*Phenotype*Tank	0.00311	1	0.8172	0.368378					
	Residuals	0.34611	91							
Isopods	Partner	0.0000385	1	0.1664	0.6842	Phenotype	0.0001150	1	0.3253	0.5706
	Phenotype	0.000641	1	0.2772	0.5998	Temperature	0.0005612	1	1.5879	0.2127
	Temperature	0.0003608	1	1.5609	0.2147	Phenotype*Temperature	0.0001085	1	0.3071	0.5816
	Partner*Phenotype	0.0002586	1	1.1186	0.2930	Temperature*Tank	0.0013433	2	1.9005	0.1587
	Partner*Temperature	0.0000367	1	0.1588	0.6912	Residuals	0.0204971	58		
	Phenotype*Temperature	0.0000367	1	0.1588	0.6912					
	Temperature*Tank	0.0008031	2	1.7371	0.1818					
	Partner*Phenotype*Tank	0.0001952	1	0.8443	0.3606					
	Residuals	0.0210372	91							
Plastic	Partner	0.000103	1	0.1996	0.65608	Phenotype	0.0005470	1	1.1477	0.2885
	Phenotype	0.000470	1	0.9081	0.34315	Temperature	0.0006980	1	1.4644	0.2311
	Temperature	0.000902	1	1.7438	0.18997	Phenotype*Temperature	0.0005720	1	1.2000	0.2778
	Partner*Phenotype	0.000312	1	0.6031	0.43942	Temperature*Tank	0.0002557	2	0.2682	0.7657
	Partner*Temperature	0.000584	1	1.1286	0.29088	Residuals	0.0276449	58		
	Phenotype*Temperature	0.002870	1	5.5460	0.02067 *					
	Temperature*Tank	0.000041	2	0.0394	0.96136					
	Partner*Phenotype*Tank	0.000010	1	0.0187	0.89145					
	Residuals	0.047086	91							
Unidentified Materials	Partner	0.00054	1	0.1193	0.7306	Phenotype	0.000157	1	0.0311	0.8606
	Phenotype	0.00122	1	0.2677	0.6061	Temperature	0.000313	1	0.0618	0.8046
	Temperature	0.00072	1	0.1587	0.6913	Phenotype*Temperature	0.000003	1	0.0006	0.9813
	Partner*Phenotype	0.00138	1	0.3031	0.5833	Temperature*Tank	0.001349	2	0.1334	0.8754
	Partner*Temperature	0.00777	1	1.7018	0.1953	Residuals	0.293445	58		
	Phenotype*Temperature	0.00785	1	1.7185	0.1932					
	Temperature*Tank	0.00034	2	0.0374	0.9633					
	Partner*Phenotype*Tank	0.00420	1	0.9195	0.3402					
	Residuals	0.41559	91							
Total Contents	Partner	0.00000	1	0.0008	0.9771	Phenotype	0.00059	1	0.0947	0.7594
	Phenotype	0.00119	1	0.2197	0.6404	Temperature	0.00115	1	0.1854	0.6683
	Temperature	0.00112	1	0.2069	0.6503	Phenotype*Temperature	0.00003	1	0.0047	0.9456
	Partner*Phenotype	0.00003	1	0.0057	0.9402	Temperature*Tank	0.01656	2	1.3310	0.2722
	Partner*Temperature	0.00572	1	1.0582	0.3063	Residuals	0.36080	58		
	Phenotype*Temperature	0.00759	1	1.4042	0.2391					
	Temperature*Tank	0.01444	2	1.3351	0.2682					
	Partner*Phenotype*Tank	0.00806	1	1.4912	0.2252					
	Residuals	0.49207	91							

Chapter 4

Table S5. Comparison of morphometric characteristics between completely and incompletely-plated stickleback measured by Mann-Whitney U-tests. *, $p < 0.05$; **, $p < 0.01$.

	Effect Size (r)	Lower Confidence Interval	Upper Confidence Interval	p
Plate Number	0.83	0.801	0.851	0.000**
Total Length	0.0321	-0.145	0.197	0.705
First Dorsal Spine Length	-0.0152	-0.178	0.156	0.858
Second Dorsal Spine Length	0.0277	-0.136	0.195	0.744
Third Dorsal Spine Length	0.151	-0.02	0.316	0.074
Caudal Peduncle Length	0.076	-0.0959	0.242	0.368
Caudal Peduncle Depth	-0.162	-0.318	0.0239	0.055
Pelvic Spine Length	-0.0651	-0.234	0.104	0.441
Head Length	-0.0765	-0.237	0.0917	0.365
Pectoral Fin Base Width	-0.0611	-0.233	0.109	0.470
Upper Lip/Jaw Length	-0.555	-0.677	-0.417	0.000**

CHAPTER

05

Synthesis

Top predators are declining in ecosystems across the world (Ripple et al. 2014). Predator loss can cause changes to overall ecosystem structure and function through trophic cascades - the reciprocal change in predator-prey relationships (Frank et al. 2011; Ripple et al. 2014). In marine ecosystems, trophic cascades are often characterized by a drastic increase in abundance of small predatory fish which are released from predation following declines of top trophic-level piscivorous fish (Eriksson et al. 2009, 2011; Frank et al. 2011). The changes to ecosystem structure are reflected in economic and cultural loss which motivates restoration of historic food-web structure. However, restoration is complicated and made less effective by a lack of understanding of which ecological drivers structure food-webs (Frank et al. 2011). Evolution is an important ecological driver to consider when trying to understand ecosystem dynamics in the face of trophic structure change. Evolution can change population traits and behavior on short timescales and can be expedited by human activity (Hendry et al. 2000). A population's adaptations can modify their functional role and impact on the ecosystem which can promote further environmental change. When ecological and evolutionary processes act on similar timescales simultaneously and influence one another, complex eco-evolutionary dynamics arise (Abrams 2000; Fussman et al. 2007). In the case of marine top predator loss, small predatory fish are faced with high intraspecific competition as the population increases and more individuals fight for the same amount of resources. Consequently, increased competition shifts selective pressure from favoring predation defense traits to favoring efficient foraging traits. Selection for niche differentiation within the population can alter ecosystem function through changing food web dynamics.

In the Baltic Sea, a combination of eutrophication and overfishing of large, piscivorous fish has resulted in a dramatic increase in threespine stickleback (*Gasterosteus aculeatus*), a small-bodied mesopredatory fish (Ljunggren et al. 2010; Eriksson et al. 2011; Bergström et al. 2015). Baltic Sea stickleback are polymorphic for body plate phenotypes, which in other populations are associated with adaptations to different ecological factors (Bentzen et al. 1984; Reimchen 1992; Bergstrom 2002; Reimchen et al. 2002; Schluter 2010; Des Roches et al. 2013). The adaptive capacity of stickleback in the Baltic has not been well-studied. We must understand if plate phenotype is an adaptive trait which leads parts of the population to fill different functional roles in order to predict the full impact of stickleback population expansion and how to restore habitats modified by their increasing numbers. With this thesis I aimed to improve our understanding of the eco-evolutionary dynamics of coastal Baltic Sea threespine stickleback through studies of how different phenotypes interact with resources and whether these are adaptive responses to a changing environment. In Chapters 2 and 3, I analyzed biotic and abiotic data collected from shallow coastal bays along approximately 350 km of the Swedish Baltic coast to quantify associations between stickleback phenotype abundance and other resources. In Chapter 4, I present a mesocosm experiment in which I measured the impacts of different stickleback

phenotypes on lower trophic levels along with the effect of a climate change scenario on these impacts. The main findings are:

- Stickleback body plate counts display a binomial distribution corresponding to completely- and incompletely-plated stickleback. Stickleback classified as incompletely plated were almost exclusively partially plated.
- There is divergence in habitat use, diet, impact on lower trophic levels, and response to climate change between completely- and incompletely-plated stickleback.
- Completely- and incompletely-plated stickleback differentially affect lower trophic levels and these effects depend on water temperature.
- The spatial distribution of stickleback across the coast indicate that there is local adaptation of different plate phenotypes to different habitats, determined by the composition of the vegetation and predator communities.

In some previously studied populations of stickleback, there is no overlap in plate number between phenotypes (Hagen 1967; Cresko et al. 2004) or there is some overlap in number but the pattern is so obvious that the exact number of plates has little influence on classification (Hagen and Gilbertson 1973; Ziuganov 1983; Mäkinen et al. 2008; Le Rouzic et al. 2011). Some of these populations have diverged into multiple species with reproductive isolation (McPhail 1984, 1992; Boughman 2001) and these studies inspired many of the hypotheses investigated in this thesis. Benthic-limnetic stickleback species pairs are well-studied and often exhibit divergent morphology (of other traits in addition to plate morphology), habitat use, and diet, with benthic stickleback being larger, consuming macro-invertebrates, and mating in dense vegetation as compared to limnetic stickleback which consume more plankton and mate in un-vegetated patches at more shallow depths than benthic stickleback (McPhail 1984, 1992). The completely-plated stickleback in the Baltic Sea has a plate pattern which somewhat resembles that of the limnetic form in British Columbian lake populations and like the limnetic form in British Columbian lakes is thought to be better protected against piscivorous fish which are more abundant in limnetic than benthic habitats (McPhail 1994; Peichel et al. 2001). Therefore I formulated my hypothesis to relate the completely-plated phenotype in the Baltic Sea to high abundances of piscivorous fish and limnetic resources. The incompletely-plated stickleback in the Baltic Sea has a plate pattern which resembles that of the benthic form in other populations (McPhail 1994; Peichel et al. 2001). In Chapters 2 and 3 I show that incompletely-plated stickleback dominate the shallow coastal bays sampled for this thesis. All sampled bays are predominantly benthic habitats. Hypotheses therefore also included that the incompletely-plated phenotype in the Baltic Sea predominantly feed on benthic invertebrates and inhabit dense vegetation patches.

The results that I present throughout this thesis suggest the opposite from what I expected; the completely-plated phenotype is more associated with benthic habitats than the incompletely-plated phenotype. In Chapter 2, I show that the relative abundance of incompletely-plated stickleback increases with increasing topographical openness of the sampling sites. I also show that incompletely-plated stickleback consume less amphipods than completely-plated stickleback. Topographical openness is associated with more limnetic habitats and amphipods are common in densely vegetated benthic habitats (Schagerström et al. 2014). The fact that incompletely-plated stickleback are found in more limnetic habitats and consume less benthic prey than completely-plated stickleback suggests that they behave more like the limnetic than the benthic form of other populations. Chapter 3 reinforces these findings as relative abundance of completely-plated stickleback increases with increasing *Fucus* coverage, which is also associated with benthic habitats and benthic stickleback mating sites (Southcott et al. 2013). The conclusion is therefore that completely-plated stickleback exhibit more benthic behavior than do incompletely-plated stickleback. This is somewhat unexpected as all the bays that were sampled are dominated by benthic habitats and incompletely-plated stickleback which appears to be a mismatch if complete plating is the phenotype better adapted to benthic habitats. It is possible that there is a stronger adaptive force which gives incompletely-plated stickleback an advantage over completely-plated stickleback regardless of habitat type. In polymorphic freshwater populations, complete plating has been shown to provide protection against predation by piscivorous fish, and freshwater habitats where these top trophic-level predators are sparse are typically dominated by incompletely-plated stickleback (Reimchen 1992, 2000). My findings in Chapters 2 and 3 reflect this relationship, the relative abundance of incompletely-plated stickleback increased as piscivore biomass decreased in both independent studies. As my co-authors and I showed in Chapter 2, the fish community in the sampling sites were dominated by piscivorous fish or stickleback and because of this dichotomy, sites with enough stickleback to include in analysis of relative abundance of phenotypes all had low piscivorous fish biomass. The advantages that completely-plated stickleback experience in the benthic habitats in our sampling region in the Baltic Sea may not be as great as the advantages experienced by incompletely-plated stickleback in areas with low piscivorous fish biomass. Therefore as long as piscivorous fish populations remain suppressed, incompletely-plated stickleback will dominate.

Dominance of the stickleback community by the incompletely-plated phenotype has implications for our understanding of how the coastal Baltic ecosystem will develop over time. In Chapter 4, I show that through differences in feeding habits the different stickleback plate phenotypes have the potential to affect lower trophic levels differently. Therefore, the lower level communities and trophic status of the habitat may change depending on the phenotype composition of the stickleback community. If stickleback abundance continues to increase, it will be important to note the relative phenotype abundances to understand which resources are likely to face more pressure

from the increased stickleback biomass. If piscivorous fish abundance is indeed the strongest selective force for the stickleback community, as Chapters 2 and 3 suggest, and piscivorous fish biomass continues to decrease (Chapter 2 in this thesis, Eklöf et al. 2020), then the relative abundance of incompletely-plated stickleback can be expected to increase. Alternatively, if restoration of piscivorous fish populations is successful then the relative abundance of incompletely-plated stickleback may decrease. Understanding these dynamics is particularly important in the face of climate change. In the mesocosm experiment (Chapter 4), the climate change treatment affected stickleback behavior differently for the different phenotypes. Different responses by phenotypes to temperature increase implies that climate change can impact which resources will face increased pressure from the stickleback community. Therefore, to predict future trophic states of shallow coastal bays, the stickleback community's response to temperature increase must also be considered.

One of the most pressing questions remaining after the completion of the studies in this thesis is: is there any genetic differentiation between plate phenotypes? Additional questions remain about the demographic history of the phenotypes such as: where does the morphological variation arise from, local adaptation or through introduction of morphotypes from other habitats? I have begun analyses to answer these questions using two complementary approaches: microsatellite analysis and whole genome sequencing (WGS). Using 20 non-neutral microsatellite markers used in comparable studies of Baltic Sea stickleback, I am looking for any population structure that may be related to adaptive traits (Shimada et al. 2011; DeFaveri et al. 2013). Preliminary analysis shows that stickleback collected from the sites sampled for Chapter 2 belong to a genetic cluster identified by DeFaveri et al. (2013) which covers the Bothnian Bay and the majority of the Baltic Proper. Genetic structure within my samples, particularly with respect to the plate phenotypes, is still being examined. Additionally, I have genotyped a subsample of stickleback at a locus called *Eda*. In many other stickleback populations, incompletely-plated stickleback can be further classified as partial or low plated. In Chapter 3, I identified two fish (out of 2038 sampled) that could be unambiguously classified as low plated, and many that could be classified as two different phenotypes based on the plating patterns on each side of the body. A single gene, ectodysplasin (*Eda*), has been identified as contributing up to 75% of the total variation in plate phenotype in some stickleback populations (Colosimo et al. 2004). At markers linked to *Eda*, low-plated sticklebacks are often genotyped as aa, partially plated as Aa, and completely plated as AA. However, this mechanism of control over variation in plating pattern does not explain how fish can be classified as different phenotypes based on each side of their body and many recent studies have found mismatches between *Eda* genotype and plate phenotype (Le Rouzic et al. 2011; Lucek et al. 2012; Pujolar et al. 2017; Liu et al. 2018; Østbye et al. 2018). My results echo this mismatch, only the two unambiguously low-plated individuals were genotyped as Aa while the rest were genotyped as AA. Traits under selection are often highly polygenic, so it is likely that multiple genes are involved in plate phenotype

(Bernatchez 2016; Ferchaud and Hansen 2016). WGS offers a way to see what regions of the genome are differentiated so that I can better understand how many traits are under divergent selection in this population of stickleback. I also hope that WGS will reveal the demographic and evolutionary history of plate phenotypes in coastal Baltic Sea stickleback.

Once we understand the extent of genetic differentiation in Baltic Sea stickleback plate phenotypes, the next research question is to understand the evolutionary consequences of that differentiation. Specifically, if there is spatial or temporal divergence in breeding activity, different degrees of attraction between different phenotypes, hybrid inviability, or any other barriers to reproduction between the phenotypes it could eventually lead to reproductive isolation (Schluter and Price 1993; Boughman 2001; Servedio 2004). Reproductive isolation can further facilitate and accelerate adaptive divergence (Funk 1998; Schluter 2001). On the other hand, if selection favors different phenotypes but there is no reproductive isolation and no fitness disadvantage for intermediates, the adaptations will be recombined and divergent adaptation will not proceed. Studies on morphotype-dependent mate choice and fitness, in combination with the results from the WGS study, are needed to better understand if some extent of reproductive isolation is present. This information can then be used to predict how stickleback phenotype abundances will change over time, which can be informative when predicting the population's effects on the ecosystem over time.

In this thesis I showed that ecological differentiation of stickleback phenotypes is likely an adaptive response to increased intraspecific competition which resulted from declining top predator populations and habitat degradation. My next steps are to determine if ecological differentiation corresponds to expected genetic differentiation. Understanding ecological and genetic differentiation within a population can provide important insights into a population's adaptive capacity. The future likely holds a great deal of change to resource use through management efforts, continued exploitation, and climate change. It will be important to understand populations' adaptive capacities in order to progress towards responsible resource use and ecosystem management.

APPENDIX

Appendix

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English Summary

Nederlandse Samenvatting

Author Affiliations

Acknowledgements

About the Author

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English Summary

The restoration of degraded habitats is a complicated process which needs to consider all ecological drivers of ecosystem structure including evolution. Ongoing evolution can change a population's traits, and therefore functional role, which can modify ecosystem structure. Understanding eco-evolutionary dynamics - the dynamics that arise when ecological and evolutionary processes act simultaneously to constantly influence one another - is of vital importance when the maintaining or restoring habitats based on ecosystem structure. In this thesis, I aimed to provide novel insights into the eco-evolutionary dynamics of the threespine stickleback (*Gasterosteus aculeatus*) in coastal Baltic Sea habitats. A better understanding of the role that stickleback play in these habitats was needed as the population has seen rapid expansion and diversification following changes to trophic structure of offshore and inshore Baltic Sea habitats. I began the thesis with a brief explanation of how the evolution of a single species is affected by and can affect ecosystem structure and function in Chapter 1.

Chapter 2 provides evidence of habitat and dietary differentiation of stickleback plate phenotypes. Utilizing data collected from 32 shallow bays along the central Swedish coast, I show that overall stickleback abundance and relative abundance of incompletely-plated stickleback increases with increasing topographical openness of bays and decreases with increasing piscivorous fish abundance. Using data from stomach contents, I show that completely-plated stickleback consume more amphipods than incompletely-plated stickleback do. I also present data that suggests a change in trophic structure of coastal bays characterized by an increase in stickleback abundance and decrease in juvenile predatory fish abundance.

In Chapter 3, I present further evidence of habitat differentiation between plate phenotypes. This study was conducted across 30 shallow bays similar to those sampled for Chapter 2 to examine ecological differentiation on a microhabitat scale. I show that relative abundance of completely-plated stickleback increases as coverage of bladderwrack increases. I also confirm one of the results of Chapter 2, that relative abundance of completely-plated stickleback increases as biomass of piscivorous fish increases.

Chapter 4 details a mesocosm experiment designed to test whether stickleback communities of different plate phenotype composition had different effects on lower trophic level resources due to dietary differentiation. Using a multifactorial design, a climate change treatment was also included and in this chapter I present evidence that through dietary differentiation and different responses to warming, different plate phenotype compositions may differentially affect ecosystem structure.

Appendix

Chapter 5 is a synthesis of the main results that I present throughout this thesis along with a discussion of the implications of my findings. I also discuss future work which could further improve our understanding of the eco-evolutionary dynamics of the threespine stickleback.

Nederlandse samenvatting

Het herstellen van verarmde habitatten is een gecompliceerd proces dat rekening moet houden met alle ecologische drivers van de structuur van een ecosysteem, inclusief evolutie. Evolutie kan eigenschappen van populaties veranderen en dus ook de functionele rol, wat de structuur van een ecosysteem kan aanpassen. Het begrijpen van de eco-evolutionaire dynamiek – de dynamiek die ontstaat wanneer ecologische en evolutionaire processen tegelijkertijd optreden en elkaar constant beïnvloeden – is van cruciaal belang voor het behoud en herstel van habitatten gebaseerd op de structuur van een ecosysteem. Met deze thesis wilde ik nieuwe inzichten geven in de eco-evolutionaire dynamiek van de driedoornige stekelbaars (*Gasterosteus aculeatus*) in de kusthabitatten van de Baltische zee. Een beter begrip van de rol die stekelbaarzen spelen in deze habitatten was nodig aangezien de populatie een zeer snelle uitbreiding en diversificatie is ondergaan als gevolg van veranderingen in de trofische structuur van Baltische offshore en inshore habitatten. Ik begon deze thesis met een korte uitleg over hoe de evolutie van een enkele soort wordt beïnvloed door en effect kan hebben op de structuur en functie van een ecosysteem in Hoofdstuk 1.

Hoofdstuk 2 laat zien dat er habitat en dieet differentiatie is tussen stekelbaarzen met verschillende beenplaat fenotypes. Aan de hand van data verkregen uit 32 ondiepe baaien langs de centrale Zweedse kust laat ik zien dat zowel de algehele als de relatieve abundantie van stekelbaarzen die niet over hun gehele lichaam beenplaten hebben toeneemt met toenemende topografische openheid van baaien en afneemt bij een stijgende abundantie van visetende vissen. Door het gebruik van data over de maaginhoud laat ik zien dat stekelbaarzen die over hun gehele lichaam beenplanten hebben meer amfipoden consumeren dan stekelbaarzen die gedeeltelijk met beenplaten bedekt zijn. Tevens presenteer ik data die suggereren dat er een verandering heeft plaatsgevonden in de trofische structuur van kustbaaien, gekarakteriseerd door een toename van stekelbaarsabundantie en een afname van juveniele roofvissenabundantie.

In Hoofdstuk 3 geef ik meer bewijs van habitat differentiatie tussen verschillende beenplaat fenotypes. Deze studie werd uitgevoerd in 30 ondiepe baaien, die gelijk zijn aan de baaien die bemonsterd waren voor Hoofdstuk 2, om ecologische differentiatie te onderzoeken op een microhabitat schaal. Ik toon aan dat relatieve abundantie van stekelbaarzen die geheel bedekt zijn door beenplaten stijgt wanneer de bedekking door blaaswier toeneemt. Ik bevestig ook één van de resultaten uit Hoofdstuk 2, namelijk dat de relatieve abundantie van stekelbaarzen die geheel bedekt zijn met beenplaten toeneemt wanneer de biomassa van visetende vissen stijgt.

Hoofdstuk 4 behandelt een mesokosmos-experiment dat ontworpen is om te testen of de stekelbaarsgemeenschappen van verschillende beenplaat fenotype samenstellingen

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verschillende effecten hadden op middelen van lagere trofische levels vanwege dieet differentiatie. Een klimaatverandering-treatment was ook inbegrepen door het gebruik van een multifactorieel ontwerp en in dit hoofdstuk presenteer ik dat, door differentiatie in het dieet en verschillende reacties op opwarming, verschillende beenplaat fenotype samenstellingen op verschillende manieren de structuur van een ecosysteem kunnen beïnvloeden.

Hoofdstuk 5 is een synthese van de belangrijkste resultaten die ik presenteer in deze thesis samen met een discussie van de implicaties van mijn bevindingen. Ik bediscussieer ook toekomstig werk dat ons begrip van de eco-evolutionaire dynamiek van de driedoornige stekelbaars kan verbeteren.

(Translation by Dr. Isabelle van der Ouderaa)

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About the Author

Casey Yanos was born in Pennsylvania, USA on 7 March 1991. Since a young age she loved nature and being outdoors. In high school she decided to pursue a career in science. She began studying marine science in 2009 at College of the Atlantic in Bar Harbor, Maine. She conducted her bachelors thesis on the effects of decomposition on stable isotopic signatures in marine mammal tissue under the supervision of Dr. Sean K. Todd. After receiving her bachelor's degree she worked in freshwater systems

for a few years. In 2014, she worked as a field technician for the U.S. Fish and Wildlife Service in Klamath Falls, Oregon on a pilot study for the propagation of endangered lake sucker fish. She then moved to Toledo, Ohio to pursue a masters in biology at the University of Toledo. Under the supervision of Drs. Christine M. Mayer and Song S. Qian she wrote a master's thesis on the effects of productivity gradients in Lake Erie on fish community structure which required her to build a continuous Bayesian networks model to quantify the relationships between nutrients and all trophic levels of the lake food web. Wanting to return to (at least slightly) marine systems, Casey moved to the Netherlands in early 2017 to study the eco-evolutionary dynamics of the threespine stickleback in the Baltic Sea under the supervision of Drs. Britas Klemens Eriksson and Martine E. Maan. During her studies she joined the Global Consortium for Academic Mental Health which aims to prevent and raise awareness of mental illness in academia. She hopes to use her experiences from the consortium and her knowledge from her studies to work towards a career in outreach and equity in science.